Effect of Iron Supplementation in Pregnancy on Childhood Development: a Long Term Follow-Up of a Randomised Controlled Trial

by

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Abstract

Animal studies have consistently demonstrated that inadequate iron nutrition in pregnancy lead to permanent structural and functional changes in the brain of offspring. However, there are no human intervention trials that have been designed to address the issue of iron nutrition in pregnancy and child development. The aims of the thesis were to determine if improved iron nutrition through routine iron supplementation in pregnancy influences the development of children, and the general health of women. The primary outcome was intelligence quotient (IQ) of the children at 4 years of age. The secondary outcomes were childhood behaviour, general health of the women and outcomes of subsequent pregnancies.

Families who participated in a randomised controlled trial of iron supplementation in pregnancy were invited to participate in a follow up study 4 years after the birth of their children. Anthropometrics, IQ, behaviour and iron status of the children were assessed. IQ was assessed using the Stanford – Binet Intelligence Test and behaviour was assessed using the Strength and Difficulties Questionnaire. Haemoglobin and serum ferritin were assessed as markers of iron status. The general health of women was assessed using the short form (SF)-36. Subsequent pregnancy outcome data were collected from the women's medical records. Outcomes assessments were blinded to intervention.

Seventy percent of families (302/430) from the original trial participated in the follow-up. The mean IQ score of children in the study was 109 ± 11. There were no differences in the group mean IQ (Chapter 5) and iron status (Chapter 4) between children of iron supplemented mothers and those whose mothers were in the placebo groups. The group

mean IQ scores at the 4-year follow-up were109 ± 11 for both groups. At the 4-year follow-up, the group mean haemoglobin concentrations were 123 ± 8g/l vs. 122 ± 6g/l for the iron and placebo, respectively, p=0.36, and the group mean ferritin concentrations (geometric mean ×/÷ SD multiplier) were 17.7 ×/÷1.7 ug/l vs. 19.2 ×/÷1.7 ug/l for the iron and placebo, respectively, p=0.23. However, children from the iron supplemented mothers had higher risk of abnormal behaviour compared with the placebo group (24/151, 16% vs. 12/149, 8% for the iron and placebo, respectively, p=0.037) (Chapter 5). The adjusted odds ratio of having abnormal behaviour for children in the iron group was 2.45, (95% CI: 1.15, 5.41). There were no differences in the SF-36 scores of women or outcomes of subsequent pregnancies between groups (Chapter 6).

In summary, routine iron supplementation in pregnancy has no effect on IQ of children, or the general health of mothers. The study has raised a question on whether routine iron supplementation in pregnancy in well-nourished women in industrialised countries has any adverse effect on long term child development.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Signed:_____ Date: 25/05/2005

Name: SHAO JIA ZHOU

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List of Abbreviations

AMBIT	= Australian Mothers' & Babies' Iron trial
CDC	= Centre for Disease Control
CI	= confident interval
GABA	= gamma-aminobutyric acid
Hb	= haemoglobin
HSQ	= Home Screening Questionnaire
ID	= iron deficiency
IDA	= iron deficiency anaemia
IQ	= intelligence quotient
LBW	= low birth weight
MDI	= Bayley Mental Developmental Index
OR	= odds ratio
PDI	= Bayley Psychomotor Developmental Index
RCT	= randomised controlled trial
RCTs	= randomised controlled trials
SDQ	= Strength and Difficulties Questionnaire
TfR	= transferrin receptor
WHO	= World Health Organisation

Chapter 1. Introduction

Despite decades of research and food fortification programs to combat iron deficiency anaemia (IDA), it remains the most common nutritional deficiency in both the developing and developed world. Young children and pregnant women are at higher risk of developing iron deficiency (ID) and IDA due to their higher iron requirements at these stages of life. According to the World Health Organisation (WHO), the prevalence of IDA in young children is approximately 25%, in pregnant women the prevalence is 52 % in the developing world and 23% in the developed countries [1].

IDA has many health consequences including fatigue, reduced work capacity [2-4], and increased susceptibility to infection [5]. When IDA occur during early childhood and pregnancy there are potentially more serious adverse health outcomes including poor psychomotor development in young children and adverse pregnancy outcomes in pregnant women.

Over the last three decades, numerous studies have linked IDA with impaired cognitive function and school performances in children [6-13]. However, a causal relationship is difficult to establish as IDA is often associated with social disadvantage and malnutrition, which may independently contribute to the poor developmental outcomes in anaemic children. Randomised controlled trials which are designed to establish a causal relationship have been conducted all over the world in an attempt to determine if correction or prevention of IDA in children improves child development.

Therapeutic trials where anaemic children were randomly treated with iron or placebo provide evidence on whether correction of anaemia reverses the developmental deficits associated with IDA. Trials conducted in young children under three years of age have generally shown that iron therapy in anaemic children does not significantly improve developmental test scores compared with placebo treated children [14]. In contrast, therapeutic trials in older children have demonstrated a significant treatment effect of iron on developmental test scores [15-18]. The contrasting results of the trials in early childhood and those in older children have been interpreted by some researchers to indicate that developmental deficits associated with IDA during early development may be irreversible. This view is supported from longitudinal studies showing that children who were anaemic in early childhood continue to perform poorly in developmental tests and school achievement later in life [19-22]. This leads to an important research question on whether prevention of IDA/ID in young children improves developmental outcomes.

Preventative trials have been undertaken where healthy infants were randomly assigned to a feeding regime with or without iron fortified foods or formula from early infancy, and developmental outcomes of the children between the groups were compared after intervention. Results from the preventative trials also fail to demonstrate a clear benefit of preventing IDA in infancy on child development. This finding may be interpreted as that ID alone is not the cause for the developmental deficits observed in anaemic children. However, it has been suggested that there may be a critical period when ID has the most profound effect on child development [23, 24]. The interventions in the preventative trials often start after weaning (4-6 months of age), and it is possible that the critical period in which prevention of IDA may improve child development was missed in these preventative trials.

Animal studies using the rat model have consistently demonstrated that dams fed iron deficient diets during pregnancy and lactation produce pups with reduced brain iron that is irreversible with later iron treatment [25-27]. This in turn is associated with lower learning ability and abnormal behaviour [25], a reduction in activity of some important enzymes involved in memory processing [27], and alteration in myelination [28] in the developing brain. As the mature central nervous system has little or no capacity to remyelinate there is a potential for a permanent effect on brain function of the offspring [23]. Pregnancy and the early postnatal period in the rat model is equivalent to the period of 20-40 weeks gestation in the human fetus [23], which is the most rapid and vulnerable period of brain growth and development.

Collectively both human and animal data imply that inadequate iron nutrition during pregnancy may result in permanent effects on development of the offspring and that the fetal stage might be the critical period for iron nutrition.

This is of particular relevance for humans as IDA is a common problem in pregnancy even in industrialised countries. Although routine iron supplementation in pregnancy has been advocated as a strategy to prevent IDA in pregnancy, it remains controversial and it is not routinely recommended in Australia or the United Kingdom (UK). In part, resistance to implementation of routine iron supplementation during pregnancy is due to lack of strong evidence on its beneficial effects on pregnancy outcomes such as prevention of preterm birth, intrauterine growth retardation and low birth weight [29, 30] or perinatal morbidity or mortality of mothers or babies. However, there are few data relating to outcome measures more traditionally associated with IDA. Furthermore, important outcomes such as the longer- term childhood development have not been explored.

Despite the strong evidence from animal studies suggesting the importance of adequate iron nutrition during pregnancy in normal brain development, there are no randomised controlled trials in humans which have been designed to specifically address the issue of iron nutrition in pregnancy and developmental outcomes of the children. The aims of this thesis were:

1. To determine if improved maternal iron nutrition during pregnancy with routine iron supplementation improves developmental outcomes of the children.
2. To investigate the effects of routine iron supplementation in pregnancy on long term health of the women and outcomes of subsequent pregnancies.

Chapter 2. Literature review

Iron is an essential mineral that plays an important role in the human body. In order to understand the role of iron in health and the functional consequences of iron deficiency, a brief overview of iron biochemistry is summarised first, then the role of iron in neuro-functioning and the effects of iron deficiency on the biochemistry, structure and function of the brain are examined utilising both animal and human studies. Randomised controlled trials with a particular focus on establishing a causal relationship between IDA and poor child development are evaluated. Finally the issue of the critical period of iron nutrition in relation to child development is explored.

2.1 A brief overview of iron biochemistry

Iron can be classified into three categories: functional iron, storage iron and transport iron [31]. These compounds are used as clinical markers of iron status. Approximately two thirds of iron present in the human body is functional iron and is in the form of haemoglobin in red blood cells, which is vital for oxygen transportation [31, 32]. Other functional iron compounds include myoglobin, which stores oxygen in muscles for use during muscle contraction; cytochromes, a medium for electron transfer and energy production within the cells; and other iron containing enzymes that are involved in many biological processes including myelination and synthesis of neurotransmitters [31].

Iron is prioritised for the production and maintenance of functional iron compounds mainly haemoglobin. Surplus iron that is not required for production of functional iron compounds is stored in the body primarily as ferritin, a soluble protein complex which can be utilised

when there is shortage of iron in the system. A small amount of iron is also stored as hemosiderin [31], which is an insoluble iron protein complex formed from the disintegration of ferritin when there is increasing iron accumulation [31]. Iron is transported by transferrin in the circulation [31, 32].

2.2 Iron balance and iron status

The availability of iron in the body is dependent on the balance between iron intake, iron requirements and iron storage of an individual. Inadequate iron intake to meet physiological requirements combined with a lack of iron stores leads to iron deficiency (ID) which can progress to iron deficiency anaemia (IDA) when ID is severe and haemoglobin production is reduced. ID is characterised by a depletion of iron stores as indicated by low serum ferritin and IDA is characterised by low haemoglobin and low serum ferritin (see Table 2.1).

Table 2.1: Markers of iron status across a whole spectrum of iron status (adapted from Yip & Dallman, 1996 [32])

	Iron overload	**Normal iron status**	**Depleted iron stores**	**ID**	**IDA**
Haemoglobin	N	N	N	N	↓
Ferritin	↑	N	↓	↓	↓↓
TFS	↑↑	N	N	↓	↓
MCV	N	N	N	N	↓

Abbreviations: ID: iron deficiency; IDA: iron deficiency anaemia; TFS: transferrin saturation; MCV: mean cell volume; N: normal.

The population at risk of developing ID and IDA include young children and pregnant women due to their higher iron requirements. IDA has been linked with impaired cognitive function in children [7, 13, 21, 33-39] and adverse pregnancy outcomes in women [40-42]. The next section of the review discusses the role of iron on neuro-functioning.

2.3 Iron and neuro-functioning: Evidence from animal studies

Iron is present in many parts of the brain but is highly localised in regions with high levels of neurotransmitters and neuropeptides [43] including the regions of globus pallidus, putamen, thalamus, caudate nucleus and hippocampus [43]. The functions of these regions are primarily related to regulation of muscle movement and information processing [44, 45]. The predominant iron-containing cells in the brain are oligodendrocytes which are involved in myelin production [46]. Iron is a cofactor for a number of enzymes involved in neurotransmitter synthesis, including tryptophan hydroxylase for serotonin synthesis, and tyrosine hydroxylase for noradrenaline and dopamine synthesis, and is essential for electron transfer reactions related to both lipid metabolism and brain energy metabolism [47].

The highly localise nature of iron in the functionally important regions of the brain and the involvement of iron in the synthesis of neurotransmitters indicates a role of iron in neuro-functioning and brain development. Although the mechanism as to how iron affects brain development is not well understood in humans, animal studies in this area provide valuable information for understanding the mechanism.

The following review examines the effect of ID on the biochemistry, structure and function of the brain from animal studies.

2.3.1 Effect of ID on the biochemistry and structure of the brain

It has been consistently demonstrated that ID in developing rats, induced by restriction of iron supply during fetal or early post-natal life, resulted in a reduction of brain non-heam iron which was not completely normalised with iron repletion[46, 48-53]. The reduction in the brain iron concentration was most profound in the regions of hypothalamus, midbrain, thalamus, striatum and hippocampus areas related to information processing, memory and emotion processes [44, 45].

The reduction in the brain iron concentration in young rats was accompanied by a reduction in dopamine receptors and dopamine transporters density [48, 49], which was not completely normalised in some regions after dietary iron repletion. Decreases in cyclic nucleotide phosphohydrase concentration and activity in hindbrain [54] and decreases in gamma-aminobutyric acid (GABA) concentration and activity of the GABA-synthesizing enzyme, glutamate decarboxylase, have also been reported [26]. The GABA system modulates the release of several hormones that are involved in behaviour regulation [26].

Structural changes on the apical dentritic morphology in hippocampus [55], disruption in the generation of oligodendrocytes cells [46] which primarily involves in myelination, and hypomyelination [56, 57] have been reported.

2.3.2 Effect of ID on brain functions

Changes in the brain biochemistry were associated with abnormal behaviour. These included decreased reactivity and stereotype behaviour [56, 57], startle reflex and conditioned avoidance response [58], increased anxiety-like behaviour related to dopamine level [59], poorer ability in spatial navigation [60] and lower performance in learning task [61, 62]. These abnormal behaviours were not completely normalised with iron treatment.

2.3.3 Critical period of iron nutrition in brain development

A study conducted by Kwik-Uribe and colleagues investigated the effect of chronic marginal iron intake during pregnancy and the early postnatal period on the cognitive function of offspring in rats [61]. The offspring from the group with marginal iron intake had attenuated startle responsiveness and lower performance on the Morris water maze (learning) task compared with the iron replete group. These differences in performance were found in association with lower brain iron concentrations. Postnatal iron supplementation did not reverse these disturbances and altered maze learning persisted in the offspring from the marginal iron group compared with the iron replete group. Taneja & co-workers demonstrated that maternal iron deficiency during pregnancy alone or pregnancy and lactation resulted in decreased activity of the GABA pathway in the developing brain of the offspring, while there was no effect of an iron deficient diet fed during lactation alone [26]. Perinatal iron deficiency in rat dams (during pregnancy to 10 days postnatal) has been shown to decrease cytochrome C oxidase activities in selected regions of the neonatal rat brain involved in memory processing [27]. In addition, perinatal brain ID increased the vulnerability of rat hippocampus to hypoxic ischemic insult, which resulted in greater loss of neuronal metabolic activity (cytochrome c oxidase CytOx) and poorer recoverability after insults [52]. Reduced myelination in the developing brain was also

reported in the offspring of dams made ID in pregnancy [28]. As the mature central nervous system has little or no capacity to remyelinate [24] there is a potential for a permanent effect on brain function of the offspring.

2.3.4 Summary of evidence from animal studies

Rat models have been used extensively to study the effect of ID on brain development and functions because of the similarity in the sequence of cell migration, myelination, and cellular differentiation between rats and humans. However, the period of brain development is more compressed in rats than in humans. Pregnancy and the early postnatal period in the rat is equivalent to the period of mid to end of pregnancy in the human fetus [23]. In summary, ID in developing rats resulted in biochemical, structural and functional changes in the brain. These biochemical changes are in turn associated with abnormal behaviour and development, which may or may not be reversible with subsequent iron supplementation. Although extrapolating the results of animal studies to humans needs to be done with caution because the degree of ID induced in experimental animals is more severe than in the case of humans in addition to the differences in the developmental process between species, these results provide biochemical evidence that may underline the mechanisms of iron in neuro-functioning. The subsequent review will examine the evidence from human studies.

2.4 Iron and neuro-functioning: Evidence from human studies

2.4.1 IDA in early childhood

IDA is rare in healthy full term infants before 4 months of age as most healthy infants are born with a generous supply of neonatal iron stores. However, by 6 months of age the storage iron in the liver is depleted unless there is an adequate dietary source of iron. IDA is most prevalent between the age of 6 to 24 months [6, 63] due to the rapid growth and the relatively limited variety of diet in addition to depleted iron stores among this group of young children. This is a period that coincides with the latter part of the brain growth spurt and the development of mental and motor processes [23]. Nutritional deficiencies, such as ID during this period of rapid brain development may interfere with developmental processes that take place at the time of the nutritional insult. There is lack of data on the prevalence of ID or IDA in children from national representative population sample in Australia. In a survey of preschool children in central and southern Sydney, the prevalence of ID and IDA was 5.4% and 1.4%, respectively for children aged between 9-23 months old, and 3.7% and 3%, respectively for children aged between 24-35 months old [64]. The prevalence of ID and IDA in USA is 9% and 3% respectively in children aged between one to three compared with 2% ID and less than 1% IDA in older children [63].

2.4.2 Effects of IDA on child development

Over the last three decades there have been many studies demonstrating that anaemic children have lower test scores on the Bayley mental development index (MDI) [7-9, 11, 65, 66] and psychomotor development index (PDI) [7, 9, 65, 66] compared with non-anaemic iron sufficient children. Although there is a consistent trend showing an association between IDA and lower performances in developmental tests in anaemic children, a causal relationship between IDA and poorer child development has not been established.

It has been suggested that the best way of proving a causal relationship between IDA and poor development is to demonstrate that producing or preventing IDA in children changes child development. Obviously it is unethical and thus not possible to conduct studies that produce IDA in children considering the potential serious health consequences of IDA. Alternatively, randomised controlled therapeutic trials where anaemic children were randomly treated with iron or placebo could provide evidence on whether correction of iron deficiency reverses developmental deficits. Therapeutic trials in this area generally involve iron supplements of children with ID/IDA and developmental outcomes are assessed pre and post treatment. The changes in developmental test scores post intervention give an indication of whether iron treatment improves developmental test scores compared with placebo treated children. Randomised controlled trials allow rigorous evaluation of outcome variables by comparing groups that are comparable in terms of any potential confounding factors [67], and therefore potentially eradicate bias. The advantage of therapeutic trials is that they require less participants and resources than preventive trials [68]. The following review evaluates the evidence from randomised controlled therapeutic trials that investigated the effect of iron therapy on the development of children with iron deficiency, and will then consider preventive trials. I will examine evidence from systematic reviews with meta-analyses first. If quality systematic reviews are not available, then a review of individual randomised controlled trials follows. This approach was taken because of the large literature in this area and the fact that the consideration of postnatal iron supplementation and childhood development is tangential to the question of iron supplementation in pregnancy and child development.

2.4.3 Effects of iron therapy on the development of children with ID/IDA: evidence from therapeutic trials

The aim of this review is to determine if iron treatment improves psychomotor development or cognitive function in children with ID/ IDA.

Selection criteria for published review papers:

I searched for systematic review of randomised controlled trials where children with ID or IDA were randomly allocated to iron treatment or placebo because of the extensive literature relating to ID and development in children. The key outcome assessments of interest were psychomotor or cognitive function.

Search strategy used to identify review papers:

Cochrane library, Medline, Embase and ProQuest electronic databases were searched using the following terms: iron or anaemia or iron deficiency combined with either development or cognitive, mental, psychomotor, Bayley, Stanford – Binet or Wechsler.

Search results:

Eight relevant reviews [14, 68-74] were identified as potentially relevant for inclusion through the electronic databases search and checking the references of identified review papers.

Critique of identified published reviews:

Six of the eight reviews were narrative reviews [69-74], search strategy and selection criteria used to identify trials or number of relevant trials identified were not reported. The review by Grantham-McGregor [68] was recent and extensive. It included case-control and

observational studies, and the quality of relevant studies was also assessed. However, the selection criteria used for including or excluding trials in the review was not reported. The only review that conformed to the QUOROM statement [75, 76], the guidelines for reporting systematic reviews, is the Cochrane review with meta-analysis [14]. Therefore, the results of the Cochrane review [14] are summarised below.

The tile of the Cochrane review:

Iron therapy for improving psychomotor development and cognitive function in children under the age of three with iron deficiency anaemia

Objective of the Cochrane review

To determine if iron therapy given to children under three years of age with IDA improves their performance on tests of psychomotor development or cognitive function.

Selection criteria for the Cochrane review

The review included studies with children under three years of age with IDA. Children had to be randomly allocated to iron (or iron and Vitamin C) or placebo (or vitamin C) and developmental outcomes assessed using standardised tests by observers blinded to treatment allocation.

Type of outcome measure addressed in the Cochrane review:

The outcome measure was the effect of iron therapy on developmental test scores of the children. It was analysed separately for short term iron therapy (≤30 days) and long term iron therapy (>30 days).

The types of iron treatments used in the short term therapy trials were mainly high doses of iron via intramuscularly injection. The doses of iron were calculated based on the iron need to correct anaemia. However, it takes several weeks to achieve the full effect of iron on haemoglobin synthesis [14]. If the effect of IDA on child development is mediated through the reduction in the body's capacity to transport oxygen to tissues, organs and the reduction in tissues' iron stores, then improvements in psychomotor performance may not be seen for at least several weeks after the commencement of the treatment. If this is the case, long term therapeutic trials are required to determine if correction of IDA improves developmental test scores. On the other hand, short term therapeutic trials assess whether improved the function of iron dependent enzymes and the synthesis of neurotransmitters improve developmental test scores.

Results of the Cochrane review:

Five trials of short term iron therapy and two trials of longer term iron therapy were included in the Cochrane review. The results show that short term iron therapy of less than two weeks has no effect on psychomotor or cognitive function in children with IDA under the age of three (see Fig. 2.1). However, there was significant heterogeneity on the treatment effect among the studies, which is indicated by a $I^2 > 50\%$ [77] (see Fig 2.1). Both clinical diversity (such as the severity and the timing of IDA) and methodological diversity (such as different doses and duration of iron treatment) lead to statistical heterogeneity [77]. Therefore, these results need to be interpreted with caution. The effect of long term iron therapy is unclear because there were only two trials with small numbers of children that met the inclusion criteria and the results were discordant (see Fig. 2.2). This led the authors to call for further quality RCTs with long term follow up [14].

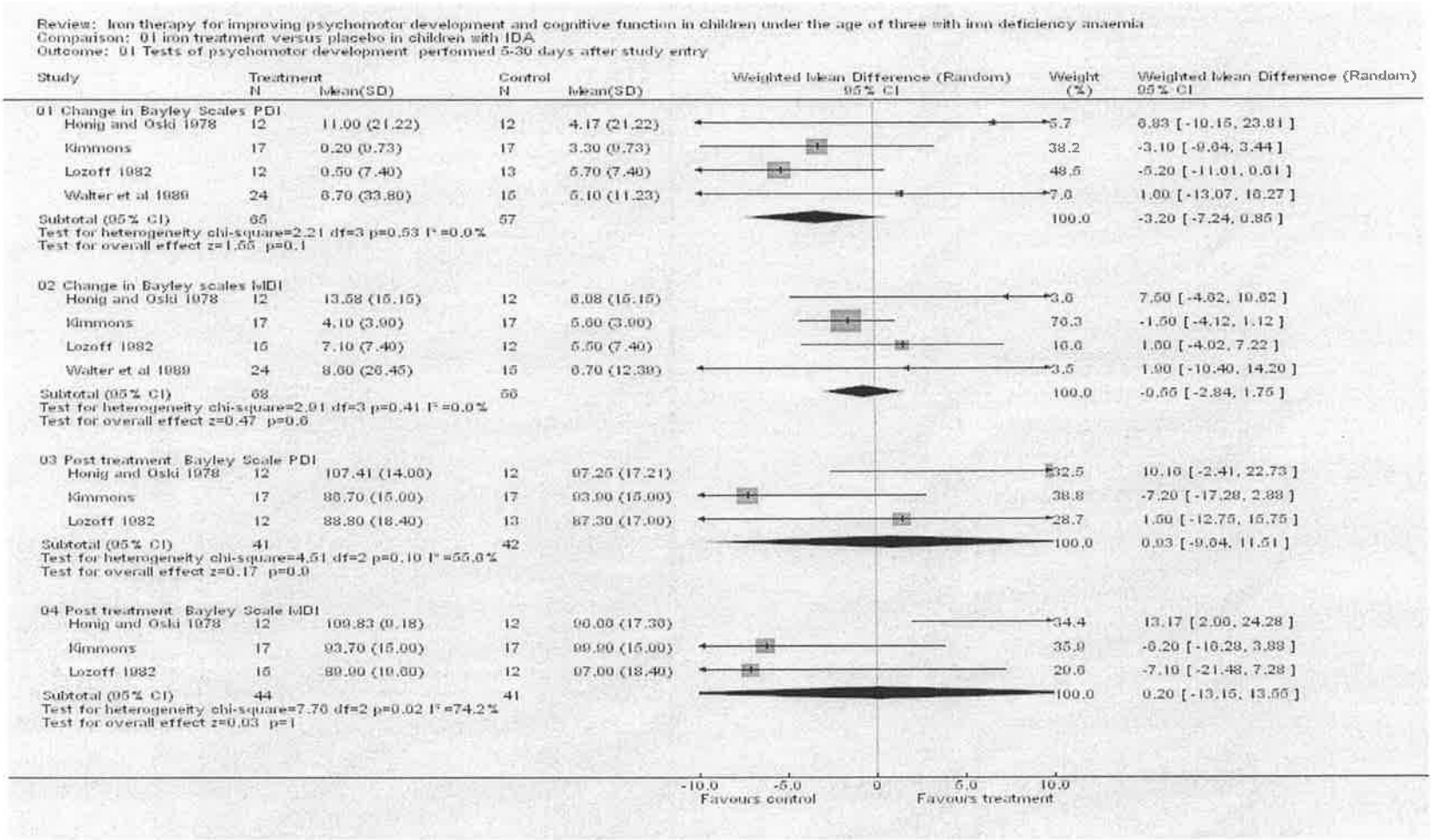


Figure 2.1 Effect of short term iron therapy (≤30 days) on cognitive and psychomotor development of anaemic children under three years of age: results of a meta-analysis (extracted from the Cochrane review [14])

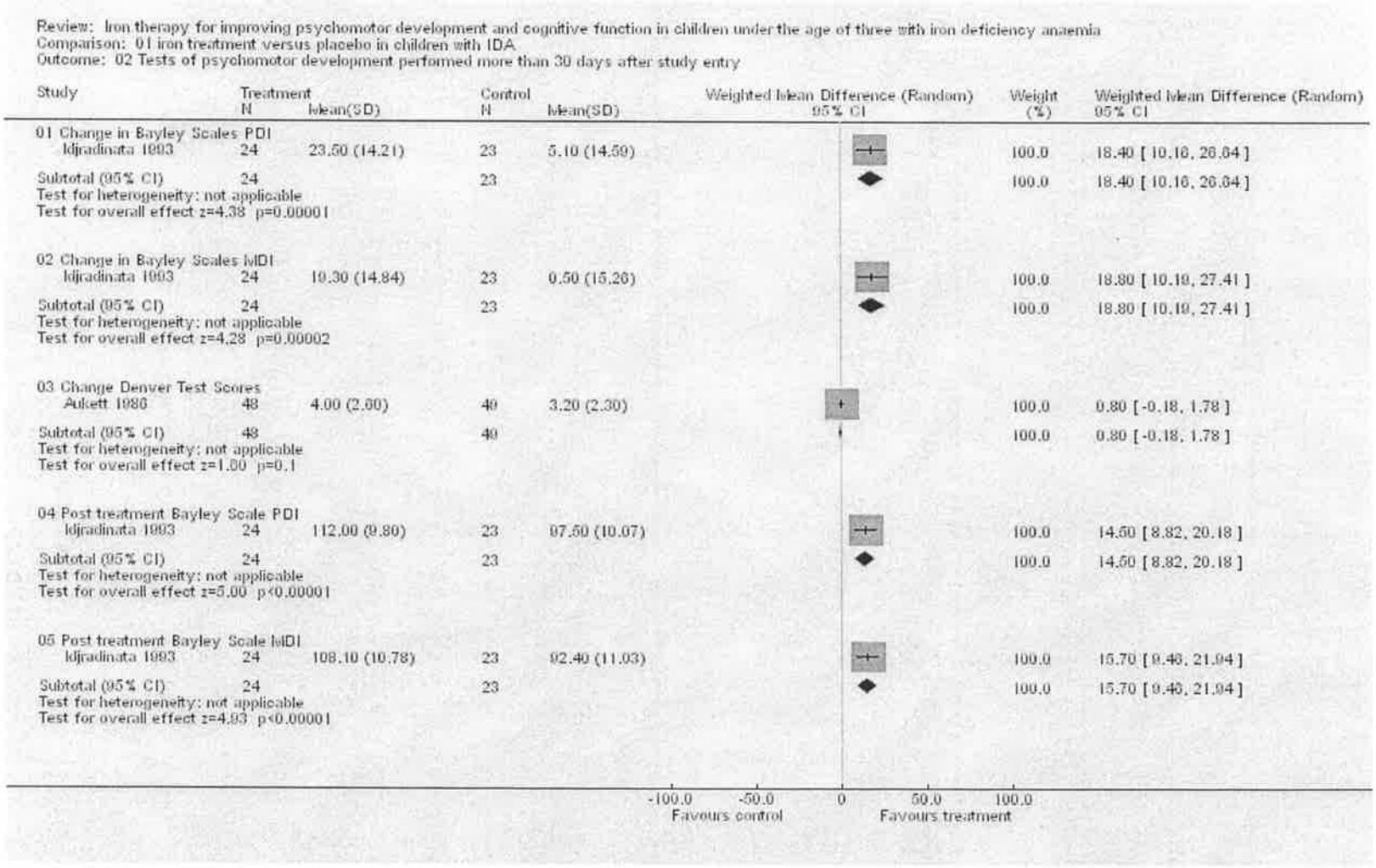


Figure 2.2 Effect of long term iron therapy (>30 days) on cognitive and psychomotor development of anaemic children under three years of age: results of a meta-analysis (extracted from the Cochrane review [14])

The Cochrane review [14] had a clear defined clinical question and selection criteria for studies to be included. The included trials were conducted in both developed and developing countries. All of the trials had low attrition rates (<20%), adequate randomisation concealment and outcome assessments were blinded to intervention, but the sample sizes were relatively small range from 24-110 children with a total of 377 children included. The Cochrane review was limited to children with IDA under 3 years of age and did not include trials in older children. Therefore, additional review of RCTs in children over three years of age is presented below.

Critique of RCTs of iron therapy in children with ID/IDA aged over three years

Using the same search strategy and selection criteria as for identifying review papers except that the type of study was randomised controlled trial rather than review, seven trials from five published papers were identified [15-18, 78] (Table 2.2).

In a well designed double blinded randomised controlled trial in USA [78], 81 adolescent girls with non-anaemia iron deficiency (NAID) were randomly allocated to receive either iron supplements (260mg/day) or placebo for 8 weeks. Cognitive assessment included three attention tests and one multiple verbal and memory test, the Hopkins Verbal Learning Test (HVLT). The results showed that iron supplementation had no effect on three measures of attention, but it improved total recall (memory) performance on the HVLT when compared with placebo treatment.

Soewondo and colleague investigated the effect of iron supplementation on iron status and cognitive function of preschool children in Indonesia [17]. Two hundred and thirty-five

families with preschooler were included in the study. The iron status of the children ranged from IDA (n=49) to iron replete. Half of the children were allocated to iron supplements (50mg/day) for 8 weeks and the other half to placebo. Iron status and cognitive function were assessed before and after the intervention. Cognitive assessments included the Peabody Picture Vocabulary Test (PPVT), discrimination learning and oddity learning which are not standardised tests. It appeared that the improvement in the PPVT score among the children with IDA was higher in the iron group compared with the placebo group (6 points vs. 2 points), but no differences in the discrimination learning or oddity learning scores between groups. However, the results were difficult to interpret because statistical comparisons were based on iron status of the children rather than the intention to treat. In addition, only 139 children had complete data, the number of children lost for each assessment was not reported. The method of randomisation was unclear, and there were differences in baseline cognitive function between the iron and the placebo groups, which indicated potential bias.

Another RCT conducted among preschooler in South Africa, where malaria infection and anaemia are common in children, demonstrated that 10mg/day of iron supplements for 12 months resulted in better language development in the children received iron supplements compared with placebo [18]. In the subgroup of children with Hb<90g/l at baseline, children who received iron supplements also had better motor development. However, the assessments of language and motor development were through self-reported by mothers using a language scale and a motor scale developed by the researchers. This may lead to assessment bias though the mothers were trained to use the tools. Furthermore, the language scale was suitable only for children aged 12-48 months and the motor scale for children aged 12-36 months. Fifty-eight of 417 children within the age group randomised

in the trial were excluded in the analysis because the assessment tools were no longer appropriate for them at 12 months follow up. This may lead to selection bias.

A RCT conducted by Soemantri in Indonesia included 78 children with IDA aged between 10 to 11 years [16]. This trial also used non-standardised cognitive assessments including the Burden-Wisconsin test for concentration and Educational achievement. The results showed that children in the iron group performed better than the placebo group. However, this study also had methodological flaws relating to the method of randomisation, there was difference in baseline cognitive performances between groups. The numbers of children included in the analysis were not reported and analysis of non-verbal IQ was based on iron status rather than by intention to treat.

The report by Seshadri in India included four separate studies [15] (Table 2.2). The first study was not RCT. The other 3 studies were randomised placebo controlled trials, but results of the 4th study were not analysed based on intention to treat but compared treatment effect of anaemic vs. non-anaemic children. Therefore, only the second and third studies are reviewed here. In the second study 28 children (14 matched pairs) with IDA aged five to six were randomly allocated to iron plus folic acid supplements or placebo (contained sugar only) within each pair. The children were matched for growth, iron status and socioeconomic status at baseline. The children who received iron and folic supplements had improved iron status as well as a greater improvement in scores on the Wechsler's Intelligence Scale compared with children in the placebo group post treatment (10 points vs. 5 points for verbal IQ, and 17 points vs. 7 point for performance IQ). This study compared iron and folic acid supplements with placebo. Although the author stated that the improvement in iron status was due to iron supplements and not folic acid, they cited

results from their unpublished study in a similar population. The treatment effect cannot be attributed solely to the iron. The third study from the same paper [15] included 48 boys aged 8-15 years, 16 sets in triplet matched for age, haemoglobin and baseline cognitive function. They were randomly allocated to receive either 30mg iron per day, or 40mg/day or placebo within each set for 60 days. Children in both iron groups had higher cognitive test scores post treatment than the placebo group.

Table 2.2 Summary of randomised controlled trials of iron therapy in older children (>3 years) included in the review

Study	Subjects	Design	Outcome measures	Treatment effect	comments
Bruner et al, 1996, USA	N=81, all NAID, age:13-18 years	DBRCT Iron vs placebo Duration: 8 weeks	3 attention tests Hophins Verbal Learning Test (HVLT): 3 components includes total recall, delayed recall and yes or no recognition	Attention: I = P Total recall of HVLT: I >P 2 other components of HVLT: I=P	8/81 loss. Sample calculation based on a difference in score of 0.5 SD between groups.
Soemantri et al, 1985, Indonesia	IDA: n=78 (43 received iron) NC: n=41 (16 received iron) Aged: 10-11 years	Reported DBRCT Iron vs. placebo Duration: 3 months	Non-verbal IQ: Raven progressive matrices; Concentration: Burden-Wisconsin test; Educational achievement test	Non-verbal IQ: n/a Concentration: I>P for both groups School achievement: IDA group: I>P NC group: I=P	IQ was test pre treatment only, and comparison was based on iron status, not treatment. Method of randomisation unclear, baseline IQ: I > P, No. of subject included in analysis was not reported.
Seshadri 1989, India	2nd study of a series of 4 preschool children aged 5-6 years IDA: n=28 (14 matched pairs for growth, iron status and social class),	DBRCT Iron + folic acid vs. placebo (sugar) Duration: 60 days	Wechsler's Intelligence Scale	I > P	Randomisation by coin tossing within each pair. This trial compared iron plus folic acid supplement to placebo. The improvement in experimental group cannot be attributed solely to iron.
	3rd study School boys aged 8-15 years n=48 (16 matched sets	DBRCT 1: iron 40mg/d 2: iron 30mg/d 3: placebo	Visual recall test Digit-span test Maze test (visual motor) Clerical task	Overall scores: Group 1 > group 3 Group 2 > group 3	Randomisation by coin tossing within each pair.

Table 2.2 Summary of randomised controlled trials of iron therapy in older children (>3 years) included in the review (Con't)

	for age, haemoglobin and cognitive test score)	Duration: 60days			
	4th study School girls aged 8-15 N=166 (83 matched pairs)	DBRCT Iron (60mg/d) vs. placebo Duration: 60 days	Same as above 3 rd study	Analysis based on iron status not intention to treat.	Data available for 65/83 pairs.
Soewondo, et al, 1989 Indonesia	Preschoolers IDA: n=49 (26 in iron, 23 in placebo) ID: n=57 (24 in iron, 33 in Placebo) NC: n=70 (27 in iron, 43 in Placebo)	Reported DBRCT Iron (50mg/d) vs. placebo Duration: 8 weeks	2 discrimination learning (DL), 3 oddity learning (OL), Peabody picture vocabulary (PPVT)	PPVT: I>P (P=0.07). DL: comparison was based on iron status not intention to treat. OL: comparison was based on iron status not intention to treat.	Method of randomisation unknown. Baseline PPVT score: I>P, potential bias. Only 139 children had complete behaviour and haematological data. Whether the No. of subject stated were all children randomised or only those with complete data was unclear. Results difficult to interpret.
Stoltzfus, et al, 2001, South Africa	N=614 preschool children, aged 6 – 59 months	DBRCT Iron (10mg/d) vs. placebo Duration: 1 year	A language development scale (for aged 12-48 months). A motor development scale (for aged 12-36 months). Assessment was self-reporting by the mothers using the scales developed by the researchers.	Language score: I>P Motor score: I>P	Randomisation was by household with stratification by age below or above 3 years. 58/417 were excluded from the analysed because the assessment tools were not appropriate for the age of children at end of intervention.

Abbreviations: NAID: non-anaemia iron deficiency; IDA: iron deficiency anaemia; ID: iron deficiency; IDP: iron depleted; NC: normal control; DBRCT: double blinded randomised controlled trial
I: iron treated group, P: placebo group
Treatment effect: the magnitude of improvement in developmental test score post treatment.

Summary of findings from randomised controlled therapeutic trials

Overall, iron treatment in children with IDA under three years of age showed no clear benefit on psychomotor and cognitive function [14]. In contrast to therapeutic trials in early childhood, trials in older children suggested that iron treatment improved cognitive function and school achievements in children with IDA as well as NAID compared with placebo treated children [15-18, 78]. Although the methodologies in both groups of trials precludes a definitive answer, the contrasting results between therapeutic trials in early childhood and those in older children have been interpreted by some researchers in the field as meaning that developmental deficits associated with IDA in early childhood may not be fully reversible even after correction of IDA [19]. This view is supported from longitudinal studies that children who were anaemic in early childhood continue to perform poorly in developmental tests and school achievements later in life [19, 20, 22]. This leads to the important research question of whether prevention of IDA in young children improves developmental outcomes.

2.4.4 Effects of iron supplements to prevent ID/IDA in infancy on child development: a systematic review and meta-analysis of randomised trials

In the next section of this chapter, I have systematically reviewed data from RCTs aimed at preventing ID/IDA and pooled data in a meta-analysis to determine if prevention of IDA in young children through iron supplementation improves their performance on standardised developmental tests.

Selection criteria for studies to be included in the review:

1. Randomised controlled trials where healthy infants were randomly allocated to either of the following intervention regimes:
    a. iron supplements or placebo
    b. high iron formula or low iron formula
    c. high iron diet with iron fortified foods or conventional feeding practices
2. Intervention began no later than six months of age when IDA is rare or under one year of age and children were free of IDA at trial entry.
3. Outcome assessments included psychomotor or cognitive function using standardised developmental tests.
4. Analyses of outcome data were based on intention to treat.

Search strategy used to identify studies:

I searched the Cochrane library, Medline, Embase and ProQuest electronic databases using the following terms: iron, anaemia, iron deficiency or iron prophylaxis combined with either development or cognitive, mental, psychomotor, Bayley, to identify potentially eligible trials. References from primary trials were checked for potential studies that were missed from the electronic search.

Search results:

Eight RCTs [7, 79-85] were identified as potentially relevant for inclusion. Four trials were excluded after further scrutiny [7, 79-81] (see Table 2.3). The reasons for exclusion included the presence of IDA in some children at baseline [80, 81], analysis was not based on intention to treat [7] and using non-standardised developmental tests [79]. The remaining four trials (see Table 2.4) are discussed further.

Table 2.3 Summary of the preventive trials excluded from the review

Study	Subjects	Design	Outcome	Effect of intervention	Reasons for exclusion
Heywood, et al, 1989 [79]	N=486, age 2 months	RCT to iron (150mg) or placebo (saline) injection at 2months of age. Attention was assessed at 1 year follow up in 119 of the 486 cohort due for follow up over a period of 4 months	Attention: visual-habituation paradigm	Significant interaction between malaria infection and treatment effect. Iron group performed better than placebo only in non-malaria infected children.	Intervention at 2 months but outcome was assessed 10 months after, results complicated by malaria infection. Only followed up 119/486.
Morley, et al , 1999 [79]	N=493 Age: 9 mo old children on cow's milk	RCT: 3 groups Cow's milk (0.05mg/l of iron): n=166 Unfortified formula (0.9mg/l iron): n=165 Iron fortified formula (1.2mg/l iron): n=162 Duration: 9 months	Bayley	No differences in Baley scores between groups	Mean haemoglobin at baseline for the 3 groups ranged from 96-99g/l indicated presence of anaemia.
Walter, et al, 1999 [7]	N=196, 3 months old children	RCT, stratified by feeding regime. For breastfed group: iron fortified cereal vs. unfortified foods Non-breastfed group: iron fortified formula vs. unfortified formula.	Bayley	N/a	Analysis based on iron status not intention to treat.
Williams, et al, 1999 [80]	N=100, age: 5.7-8.6 months, on cow's milk	RCT: to continue with cow's milk or iron fortified formula to 18 mo, follow up to 24 months	Griffiths	No differences in scores at 18 months between groups, but better performance in iron fortified formula groups at 24 months	> 13% of children had IDA at baseline

RCT: randomised controlled trials

Table 2.4 Summary of the preventive trials included in the review

Study	Subjects	Design	Outcome measures	Effect of intervention	comments
Friel, et al, 2003 [84]	N=77, healthy full term 1 month old breastfed children,	RCT Iron (7.5mg/d) vs. placebo Duration: 5 months	Bayley Visual acurity Assessed at 13 months of age	% IDA at 6 months: Iron: 0%, placebo:3/21 (14%. % IDA at 12 months: Iron: 2/23 (9%), Placebo: 1/19 (5%) MDI: I=P PDI: I>P Visual: I>P (not significant, P=0.07)	Children were weaned to iron fortified formula when mothers stopped breastfeed. No sample size calculation.
Moffatt et al, 1994 [81]	N=283, Healthy formula fed infants birth to 2 months	DBRCT Group 1: Iron fortified formula (12.8mg/l of iron) Group 2: Non iron fortified formula (1.1mg/l of iron) Duration: ≥13 months (to 15 months of age)	Bayley	MDI: group 1=group 2 at the end of intervention PDI: group 1=group 2 Infant behaviour: group 1=group 2	Data available for only 154/283. % of ID/IDA between groups at the end of intervention not reported. Sample size calculation based on to detect a 10 points difference in Bayley between groups and not on reduction of ID/IDA in intervention group.

Table 2.4 Summary of the preventive trials included in the review (Con't)

Lozoff, et al, 2003 [82]	N=1657, healthy 6 months old infant free of IDA	RCT stratified according to feeding regime: **Cohort 1:** formula-fed (≥250ml/d of formula) randomly assigned to: Group 1(n=430): high-iron formula (12mg/d) or Group 2 (n=405): low-iron formula (2.3mg/d) **Cohort 2**: formula fed (≥250ml/d of formula) randomly assigned to: Group 3 (n=176): high-iron formula, or Group 4 (n=404): cow's milk plus multivitamin without iron. **Cohort 3**: breastfed (<250ml/d of formula) randomly assigned to: Group 5 (n=112): multivitamin plus iron, or Group 6 (n=130): multivitamin without iron Duration: 6 months	Fagan Bayley	Iron group = group 1+ 2+ 3 + 5 No iron group = group 4 + 6 At the end of the trial: % of IDA: 3% in iron, 23% in no iron Fagan test: shorter mean looking time in iron group (1.39 vs 1.46 seconds), but no difference in novelty preference. Bayley: MDI: iron=non-iron PDI: iron=no iron IBR (behaviour): Iron group was rated better than no iron group in 2/4 aspects. Physical development: non-iron group craw or creep at later age (303 vs 297 days, 1 weeks later).	Combined various intervention groups in the final analyses led to differences in baseline characteristics between the iron and no iron groups included higher % of maternal smoking, higher score on HOME & longer duration of BF in non-iron group. Data for physical development available only for 704/1214 in the iron group and 244/584 in the non-iron group
Yalcin 2000 [83]	N=24, healthy 6 months infants with normal iron status	RCT Iron (1mg/kg/d) vs. no treatment Duration: 3 months	Bayley	% of IDA: 0/7 in iron vs. 2/9 in placebo MDI: iron = no treatment PDI: iron = placebo	Very small No. method of randomisation not reported. 33% (8/24) were excluded for analysis may lead to bias.

MDI: mental developmental index; PDI: psychomotor developmental index; IBR: infant behaviour rating, DBRCT: double blinded randomised controlled trial

Lozoff and co-workers conducted a RCT in 1657 healthy 6 months old infants who were free from IDA at entry to the trial. The infants were recruited over 2 periods of time [83]. During 1991 – 1994, eligible formula fed infants were randomly allocated to high iron formula (12mg/l) or low iron formula (2.3mg/l). During 1994 – 1996 formula fed infants were randomly allocated to high iron formula or cow's milk plus multivitamin supplements with no iron while breastfed infants were randomly allocated to multivitamin plus iron or multivitamin without iron. The duration of intervention was six months and both Bayley and Fagan tests were conducted at the end of intervention. All infants who received iron supplements in their allocated intervention groups were combined to form the iron supplemented group and infants who received no iron supplements in their allocated groups were combined to form the no iron group. At the conclusion of the trial, there was a lower incidence of IDA in the iron group but no differences in the Bayley scores were found. Although infants in the iron group had a shorter time in information processing compared with placebo (1.39 seconds vs. 1.46 seconds), the magnitude was small and there was no difference in novelty preference. The changes of trial protocol half way through the study resulted in six intervention groups with various entrance criteria, and differences in baseline social characteristics of families between the iron supplemented and no iron groups. However, the baseline differences were adjusted in outcome analyses.

Another trial [82] in which 283 formula fed infants aged up to two months were randomised to iron fortified or unfortified formula to 15 months. Although there were no difference in MDI at all points of test, there was a difference in PDI at nine and 12 months. However, the difference was no longer significant at the end of the intervention, and there was a large drop out with only 154/283 (54%) children assessed at 15 months. Sample size calculation was based on the detection of 10 points difference in Bayley score, and may be under

powered to detect any smaller differences. Furthermore, the incidence of ID/IDA at the end of intervention between groups was not reported. Whether intervention led to prevention of IDA in the iron group is not clear.

In a smaller trial conducted by Friel & colleagues [85], 77 healthy infants were randomised to iron supplements or placebo from one month to six months of age. Development was assessed at 13 months using Bayley Infant Developmental Scale. Infants in the iron group were free from IDA at the end of supplementation while 14% of infants in the placebo group had IDA. Children in the iron group had a higher score on the psychomotor developmental index (PDI) compared with children of placebo group, but no difference in the mental developmental index (MDI) between groups was found. This trial had no sample estimation, and the outcome assessment was done 6 months after the end of intervention when the incidence of ID and IDA were similar in both groups.

The last trial [84] included only 24 healthy, iron sufficient six months old infants, and 8/24 (33%) children were excluded from the outcome analyses for reasons included anaemia and non-compliance with treatment. Therefore it is not surprising that there were no differences in Bayley scores between the groups.

Data from the four included trials were pooled in a meta-analysis using statistical package RevMan 4.2 (the Cochrane collaboration, 2003) to determine the effect of iron supplements in non-anaemic children under 1 year of age on developmental test scores. All four trials reported data as mean and SD for outcomes except one [83] where data was reported as mean and standard error (SE). The SD for this trial [83] was calculated based on the SE and the numbers of subjects reported (SD= SE* $\sqrt{N}$). The pooled mean differences

on mental developmental index (Fig 2.3) and psychomotor developmental index (Fig 2.4) between children in the iron and placebo groups were 0.17, 95% CI: -1.17, 1.50, P=0.81 and 0.24, 95% CI: -1.20, 1.68, P=0.75, respectively.

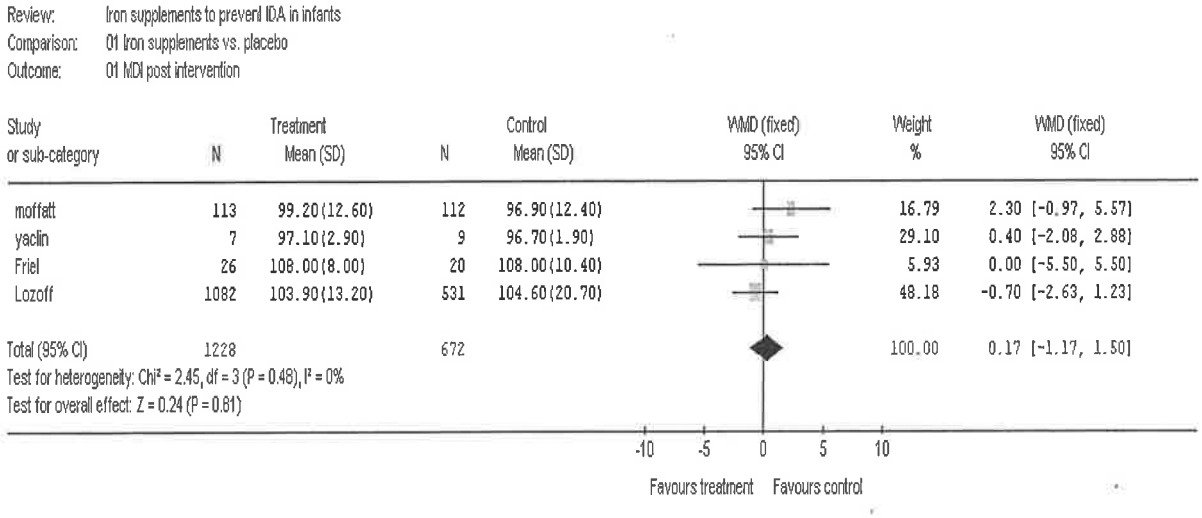


Figure 2.3 Effect of prophylactic iron supplementation in children under 1 year of age on Mental Developmental Index (MDI): results of a Meta-analysis

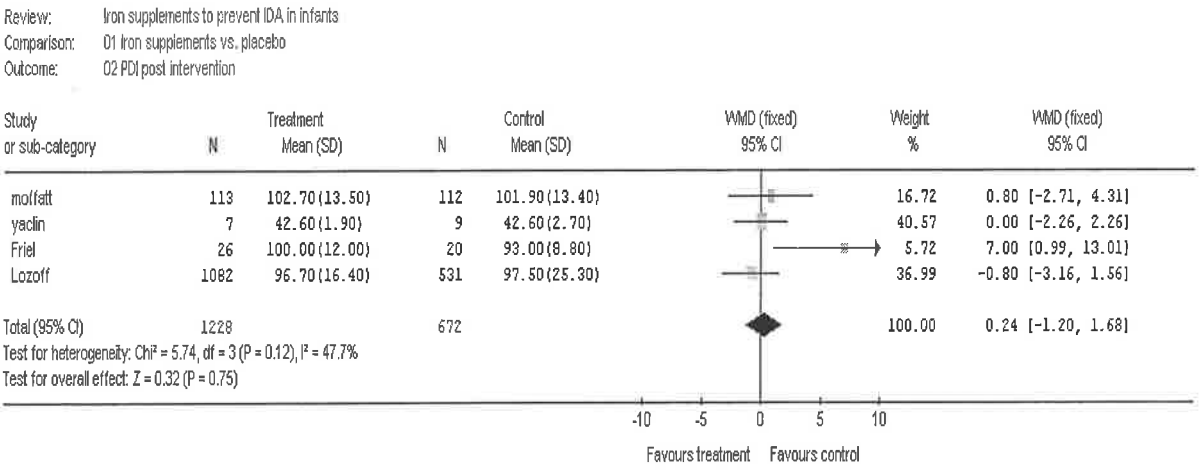


Figure 2.4 Effect of prophylactic iron supplementation in children under 1 year of age on Psychomotor Developmental Index (PDI): results of a Meta-analysis

In summary, there is no strong evidence to support the view that prevention of IDA in infancy improves developmental scores from preventative trials in late infancy and toddlers. This may be interpreted as follow: the lack of iron alone is not the cause for the lower developmental scores associated with IDA or that more specific tests of development are required to determine potential differences. On the other hand, it is possible that the critical period in which prevention of IDA may improve developmental scores, such as fetal stage, was missed in these preventive trials.

2.5 Critical period of iron nutrition in child development

Studies on IDA and childhood development have consistently shown that IDA is associated with poor developmental outcomes. Iron therapy in children with ID/IDA under three years of age has not been universally effective in correcting developmental deficits associated with ID/IDA. There has been a suggestion that the developmental deficits persist. Trials aimed at preventing IDA in infants and toddlers have also shown no strong evidence that prevention of IDA improves developmental outcomes. These results suggest a possibility that there may be a critical period of iron nutrition for child development before infancy. IDA occurring within the critical period may have permanent effects on child development.

Studies using rat models have consistently demonstrated that ID in pregnancy and early lactation leads to permanent changes in brain functions [27, 52, 61]. The pregnancy and early postnatal period in the rat model is equivalent to the period of mid-gestation to term in human development [23]. This is the most rapid and vulnerable period of brain growth and

development. There is a lack of data in humans on the effect of ID/IDA in pregnancy on child development.

Collectively, data from both animal and human studies suggest that the fetal stage might be the critical period for iron nutrition. If this is the case, then ensuring adequate iron nutrition in pregnant women is crucial for long term child development. This can be achieved with a simple inexpensive strategy – routine iron supplementation in pregnancy. The subsequent review focuses on iron nutrition in pregnancy in a general sense. In particular, I examine the effect of routine iron supplementation in pregnancy on clinical outcome measures in the mother and baby with particular focus on the following outcomes:

1. Routine iron supplementation in pregnancy and pregnancy outcomes
2. Routine iron supplementation in pregnancy and iron status of the children
3. Routine iron supplementation in pregnancy and long term growth and development of the children
4. Routine iron supplementation in pregnancy and general health of women.

2.6 Routine iron supplementation in pregnancy: rationale

2.6.1 Iron requirements in pregnancy

Iron requirement during a normal pregnancy has been estimated to be 1000mg that is approximately 3-4 times the amount required for the same period in the non-pregnant state [86]. This is due to the increase in maternal red cell mass, the iron required for fetal growth and placenta, and the basal iron loss [87]. The higher iron requirement during pregnancy is partially met by increasing iron absorption [88] and utilisation of maternal iron stores. It has

been estimated that even with an optimal diet through pregnancy, 500mg of iron would still need to be provided by the body's iron stores to meet the extra demand of iron in pregnancy [86]. However, women of reproductive age often lack sufficient iron stores, even in developed countries [88]. This often results in exhaustion of maternal iron stores during pregnancy, which is reflected in progressive decline in serum ferritin, and may lead to IDA if inadequate exogenous iron supply continues. According to the World Health Organisation, the prevalence of anaemia in pregnancy is 52% in the developing world and 23% in the developed countries and the most common cause of anaemia in pregnancy is iron deficiency [1]. In an Adelaide study the prevalence of IDA in pregnant women was 11% at birth [89].

2.6.2 Classification of iron status in pregnancy

The criteria for defining iron status in non-pregnant women may not be appropriate for pregnant women due to haemodilution, a physiological adaptation in pregnancy that leads to a fall in haemoglobin level often referred to as physiological anaemia [90]. The degree of haemodilution varies at different stages of pregnancy. Maximum haemodilution often occurs between 28 to 32 weeks [87, 91]. The Centre for Disease Control (CDC) and the World Health Organisation (WHO) [1, 92] have established lower cut-offs of haemoglobin and ferritin for the diagnosis of IDA in pregnancy than in non-pregnant women (see table 2.5).

Table 2.5 Classification of iron status in pregnant women by the WHO and the Centre for Disease Control [1, 92]

	WHO	CDC	
	Throughout pregnancy	1st & 3rd trimesters	2nd trimester
Iron sufficient	Haemoglobin ≥110g/l Ferritin ≥12ug/l	Haemoglobin ≥110g/l Ferritin ≥12ug/l	Haemoglobin ≥105g/l Ferritin ≥12ug/l
Iron deficiency	Haemoglobin ≥110g/l Ferritin <12ug/l	Haemoglobin ≥110g/l Ferritin <12ug/l	Haemoglobin ≥105g/l Ferritin <12ug/l
Iron deficiency anaemia	Haemoglobin <110g/l Ferritin <12ug/l	Haemoglobin <110g/l Ferritin <12ug/l	Haemoglobin <105g/l Ferritin <12ug/l

Abbreviations: WHO: World Health Organisation; CDC: Centre for Disease Control.

2.6.3 Consequences of IDA or anaemia in pregnancy

IDA or anaemia during pregnancy has been linked with adverse pregnancy outcomes, such as low birth weight (LBW), preterm birth, perinatal mortality and low iron stores of newborn [1]. The biological mechanisms that may underlie the role of iron on the regulation of fetal growth and parturition (timing of delivery) are largely unknown. It is understood that the major determinant of parturition is the balance between the amount of oestrogen and progesterone present in the circulation [93]. ID may stimulate the production of oestrogen through a cascade of events involving noradrenaline [94], corticotropin-releasing hormone (CRH) [95, 96] and adrenocorticotropic hormone (ACTH) [93]. Parturition begins when the influence of oestrogen is greater than that of progesterone [93]. Furthermore, anaemia can cause chronic hypoxia, a condition known to exist in people living at high

altitude. Birth weight was directly related to calculated maternal arterial oxygen content in women living at high altitude [97] suggesting that chronic hypoxia may adversely affect birth weight. Other possible mechanisms include that IDA may increase oxidative stress and the risk of maternal infection, and both can adversely affect pregnancy outcomes.

Early evidence suggesting an association between anaemia and adverse pregnancy outcomes was mainly from developing countries with high prevalence of anaemia. Anaemia in those countries is not a simple issue of iron deficiency, rather it is a marker of overall malnutrition. It is not surprising that such association exists between anaemia and adverse pregnancy outcomes, as adequate nutrition in pregnancy is necessary for achieving optimal pregnancy outcomes.

More recent large prospective cohort studies have shown an U-shape relationship between the haemoglobin concentration in pregnancy and the incidence of LBW and preterm birth with haemoglobin values at both the low and the high ends associated with poorer outcomes [98-101]. However, the levels of haemoglobin in pregnancy associated with minimum risk of LBW, preterm birth and perinatal mortality varied among the studies and among different ethnics groups [99]. Interestingly, the haemoglobin levels associated with minimum rate of LBW or preterm birth included the current cut-off for anaemia in pregnancy in two of the large cohort studies in USA [98] and in Britain [99].

Results of observational studies are difficult to interpret because complications of pregnancy which may be independently associated with adverse pregnancy outcomes were often not controlled, and assessments of iron status were done at various stages of pregnancy. With maximum haemodilution occurring between 28 and 32 weeks of gestation

[91], preterm birth is inevitably linked to lower maternal haemoglobin at birth. Therefore, a causal relationship between anaemia or IDA in pregnancy and pregnancy outcomes cannot be established. The following section examines evidence from RCTs of iron supplementation in pregnancy to determine the effect of routine iron supplementation in pregnancy on pregnancy outcomes.

2.7 Routine iron supplementation in pregnancy: effect on pregnancy outcomes

The aim here is to determine if prevention of IDA in pregnancy through routine iron supplementation improve pregnancy outcomes. There are numerous RCTs of prophylactic iron supplementations in pregnancy. Majority of them have focused on haematological outcomes and few have assessed clinical outcomes. There is clear evidence that prophylactic iron supplementation in pregnancy is effective for the prevention of ID and IDA in pregnancy. Therefore, the following review focuses on effects of iron supplementation in pregnancy on pregnancy outcomes including birth weight, the single biggest determinant of mortality in the first year of life [102], gestational age at birth or preterm birth, neonatal mortality or morbidity. Pregnancy outcome is often a secondary outcome of RCTs on iron supplementation in pregnancy, and individual RCTs are often underpowered to determine the effect of iron supplementation on pregnancy outcomes. Therefore, this review focuses on systematic reviews of RCTs on iron supplementation in pregnancy with meta-analyses. If quality systematic reviews are not available, then I will examine the results of individual RCTs.

Search strategy used to identify published review papers:

Cochrane library, Medline, Embase and ProQuest electronic databases were searched using the following terms: anaemia, iron deficiency or iron supplementation combined with birth weight, preterm birth, prematurity, LBW, SGA (small for gestation age). A filter was applied to the type of study as "review" to locate review paper. The search was limited to papers published in English language due to the difficulty of understanding other languages. It has been shown that results of meta-analyses which included only studies reported in English have little or no difference compared with language- inclusive meta-analyses [75].

Selection criteria for inclusion of published review papers

- Systematic review of RCTs where pregnant women were randomly allocated to receive either iron supplements or placebo or no iron.
- Outcome assessments included at least one of the following: birth weight, LBW, SGA, gestation age, preterm birth, neonatal complications and fetal or neonatal mortality or morbidity.

Search results:

Seven reviews [29, 30, 40, 42, 93, 103, 104] were identified as potentially relevant for inclusion through the electronic databases search and checking the references of identified trials and review papers. The review by Scholl & Hediger did not include RCTs [104]. Another four reviews were narrative and did not report search strategy or inclusion criteria used to identify eligible trials [29, 40, 42, 93]. The review paper by USTF [29] was comprehensive, but it did not report the strategy for identifying relevant trials or the selection criteria for trials to

be included in its review. Therefore these five reviews are not discussed further. The other two reviews [30, 103] were systematic with transparent methodology and are discussed below.

Critique of the two systematic reviews identified:

1. The review conducted by Rasmussen titled: Is There a Causal Relationship between Iron Deficiency or Iron-Deficiency Anemia and Weight at Birth, Length of Gestation and Perinatal Mortality? [103].

The aim of Rasmussen's review was to determine whether ID, IDA and anaemia in pregnant women cause LBW, preterm birth or perinatal mortality. It included both RCTs and observational studies. A thorough search of the literature from 1966 to 1999 in English, French and Spanish languages was conducted and 23 RCTs were reported in the review. The review not only included trials that compared iron versus placebo, but also included trials that compared iron with iron and folic acid or compared iron with iron, folate and vitamin B12. The common weaknesses of the trials highlighted by the author were inadequate sample sizes or failing to correct anaemia which tends to produce false-negative findings. Although the search strategy was reported in detail, the criteria for inclusion or exclusion of trials for the review were not stated and no information was provided on any potential relevant trials identified but was subsequently excluded. The author concluded that supplementation of pregnant women with iron or folic acid or both does not appear to increase birth weight or the duration of gestation.

2. The Cochrane review with meta-analysis titled: Iron supplementation in pregnancy [30].

Aim of the Cochrane review:

The aim of the Cochrane review was to assess the effect of iron supplementation on haematological parameters and pregnancy outcomes.

The selection criteria for studies included in the Cochrane review:

All RCTs of iron supplementation versus no iron or placebo, or routine iron supplementation versus selective supplementation for approximately 16 weeks duration with satisfactory randomisation of allocation to the study or the control group. Trials with attrition rates of greater than 25% or outcome variables presented as mean only without SD were excluded.

Type of outcome measures addressed in the Cochrane review:

- Haematological parameters.
- Substantive measures of pregnancy outcomes and other clinical information including side effects.

Results of the Cochrane review:

Twenty of the sixty trials identified were included in the Cochrane review. The other 40 trials were excluded mainly due to high attrition rate, inadequate randomisation or the data were not usable for meta-analysis.

There was no difference in birth weight between the iron supplemented and placebo groups (Table 2.6). No other pregnancy outcomes were available for meta-analysis from the included trials. In the analysis comparing routine iron supplementation with selective iron supplementation when women were anaemic, there was no difference in the incidence of

preterm birth (see Table 2.6) or small for gestational age (see Table 2.6), but the odds ratios for stillbirth and convulsion in childhood were lower for the selective iron supplementation compared with routine iron supplementation (see Table 2.7).

However, the majority of the included trials in the meta-analyses were for the assessment of haematological outcomes. Data for pregnancy outcome or other clinical outcome were from single trials. This led the author to conclude that there was very little information on clinical outcomes for either the mother or baby, and no conclusion can be drawn on the effects of iron supplementation in pregnancy on pregnancy outcomes [30].

Table 2.6 Summary of the meta-analysis results related to clinical outcomes from Cochrane review: compared routine iron versus placebo or no iron in pregnancy (adapted from the Cochrane review [30]).

Outcomes	**No. of studies**	**No. of participants**	**Peto Odds Ratio/ Weighted Mean Difference (95% CI)**
Birth weight (g)	1	197	30.00 (-89.99, 149.99)
Intrauterine growth retardation/small for dates	0	0	Not estimable
Congenital malformation	0	0	Not estimable
Caesarean section	0	0	Not estimable
Pregnancy hypertension	0	0	Not estimable
Urinary tract infection	0	0	Not estimable

Table 2.7 Summary of the meta-analysis results related to clinical outcomes from Cochrane review: compared selective versus routine iron supplementation in pregnancy (adapted from the Cochrane review [30])

Outcomes	No. of studies	No. of participants	Peto Odds Ratio 95% CI
Preterm delivery	1	2694	1.41 (0.94, 2.12)
Post term pregnancy	1	2694	0.55 (0.19, 1.58)
Low birthweight	1	2694	1.12 (0.72, 1.75)
Small for gestational age	1	2690	1.10 (0.79, 1.52)
Admission to neonatal unit	1	2694	1.06 (0.80, 1.40)
Infection in infant	1	2694	0.75 (0.48, 1.18)
Stillbirth >28 weeks of gestation and death in the first week of life	1	2694	0.33 (0.11, 0.99)*
Convulsion in childhood	1	2682	0.45 (0.25, 0.79)*
Congenital malformations	1	2694	1.01 (0.77, 1.33)

* $P<0.05$

Both of the systematic reviews [30, 103] discussed above included RCTs published up to 1999. Therefore, an additional search was conducted to identify individual RCTs published after 1999 using the same search strategy for identifying review paper except that the filter applied to the type of the study was "randomised controlled trial" rather than review. The selection criteria for trials to be included were random allocation of pregnant women to iron or placebo group and outcome assessments included pregnancy outcomes or other clinical outcomes. Two RCTs were identified [89, 105] and are discussed below.

Cogswell & co-workers conducted a double blinded randomised controlled trial of iron supplementation in pregnancy in USA [105]. Pregnant, non-anaemic iron replete women were randomly allocated to receive iron supplements of 30mg/day or placebo before 20 weeks of gestation and continued to 28 weeks of gestation. Iron status was retested at 28 weeks gestation and women who were iron replete continued to take their allocated supplements whereas women with iron depletion, ID or IDA were given iron supplements at doses ranged from 30mg to 120mg depending on the degree of iron deficiency. Data on the birth weight of the babies were available for only 117/146 (80%) women randomised in the iron group and 96/129 (74%) in the placebo group. The mean birth weight was higher (3277 ± 501g vs. 3072 ± 635g, P=0.01) and the incidence of LBW was lower in the iron group compared with the placebo group, despite the fact there were no differences in any iron parameters or incidence of ID, IDA or anaemia at 28 weeks gestation between groups.

Another larger double blinded randomised controlled trial of iron supplementation in pregnancy was conducted by Makrides & colleagues in Australia [89]. Four hundred and thirty non-anaemic pregnant women were randomly allocated to iron supplements (20mg/day) or placebo from 20 weeks gestation to the end of pregnancy. All women received standard obstetric care and if obstetricians considered women to be anaemic they were advised to take 80mg iron/day in addition to their allocated trial supplements. At the end of pregnancy, women in the iron group had higher haemoglobin and serum ferritin level, and lower incidence of ID and IDA compared with women in the placebo group. However, there were no differences in mean birth weight, incidence of LBW or preterm birth between the iron and placebo groups.

These two RCTs were similar in design, but the outcomes were discordant. Compared with the trial of Cogswell et al [105], Makrides et al [89] had larger sample size (430 compared with 275) and complete birth weight data for all women randomised. I included these two RCTs [89, 105] and the only RCT [106] with birth weight outcome included in the Cochrane review [30] in a meta-analysis to determine the effect of maternal iron supplementation on birth weight of the infants. MevMan 4.2 (The Cochrane Collaboration, 2003) was used for the meta-analysis. There was significant heterogeneity among the studies in the fixed effect estimate model with a P-value for the Chi-square test equal to 0.04 and I^2 =69% (see Fig 2.5). Therefore, a random effect estimate model was used to repeat the meta-analysis [77] (see Fig. 2.6). The random effect estimate model addresses the question on what is the average treatment effect whereas the fixed effect model addresses the question on what is the best estimate treatment effect [77]. Results from both analyses showed that the weighed mean differences in birth weight between the iron and placebo groups were very small (<0.5 SD) and the P-values were greater than 0.1 (see Fig 2.5 & Fig 2.6). This result needs to be interpreted with caution because of large variability between studies included in the analysis.

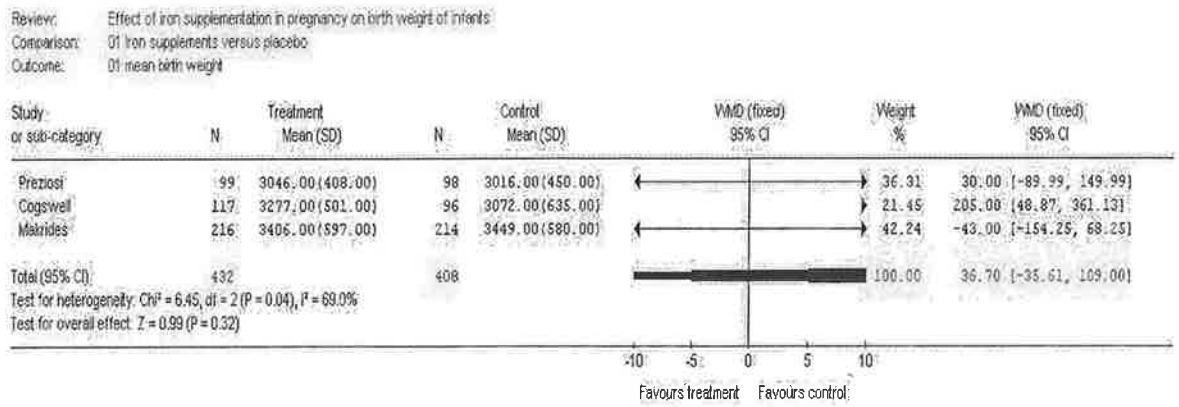


Figure 2.5 Effect of iron supplementation in pregnancy on birth weight of the newborns: results of a meta-analysis using a fix effect estimate model.

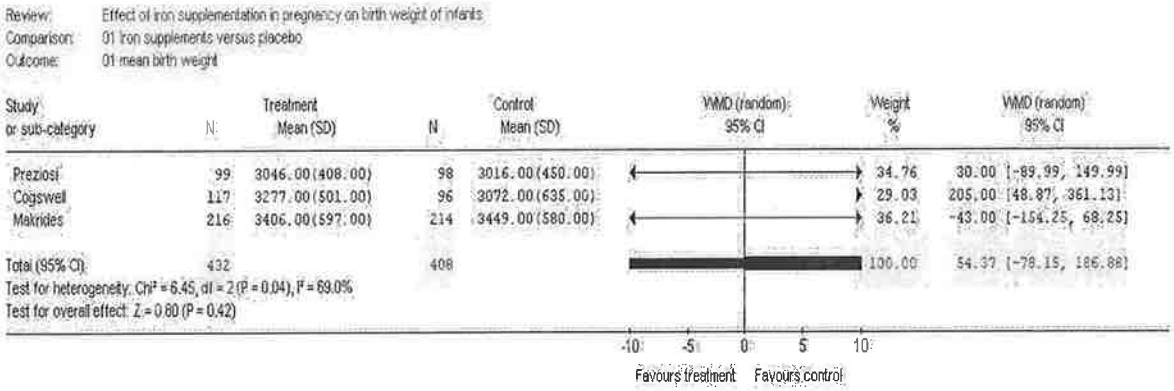


Figure 2.6 Effect of iron supplementation in pregnancy on birth weight of the newborns: results of a meta-analysis using a random effect estimate model

In summary, the results of published systematic reviews and meta-analyses suggest that iron supplementation in pregnancy has little or no effect on the pregnancy outcomes. The next section examines the relationship between maternal iron status and the iron status of newborns and infants.

2.8 Routine iron supplementation in pregnancy: effect on the iron status of infants

Iron stores of a newborn are derived from the mother via the placenta. The general perception is that the fetus gets preferential treatment for iron and maternal iron status does not affect fetal iron supply unless there is severe maternal iron deficiency. This view is largely based upon observational studies which assessed the relationship between maternal iron status and haematological parameters in cord blood samples. It has not been rigorously tested in well-designed clinical trials. In order to determine if and how maternal iron status affects the supply of iron to the fetus, one needs to understand how iron is transferred from mother to the fetus.

2.8.1 Iron transfer from mother to fetus

The majority of fetal iron accretion occurs during the third trimester of pregnancy with a daily accretion of 1.6-2.0mg/kg. The regulation of fetal iron accretion appears to be dependent on fetal iron demand [107]. Iron is transported actively across the placenta by a series of iron transporters[108, 109]. It is believed that the placental syncytiotrophoblast is responsible for the uptake of transferrin from maternal serum via transferrin receptor (TfR). The transferrin and TfR complex is then incorporated into endosomes and the iron is subsequently transported out of endosomes and across the basal membrane into the fetal circulation [108, 110]. The regulation of iron transfer from mother to fetus has mainly been studied in animal models. This iron transfer process is regulated by two factors: the number of TfR on the apical (maternal) side of placental cells and the concentration of ferritin in the cells [110]. The number of TfR is regulated by iron regulatory protein-1 [111]. Its activity increases and the number of TfR increases if cellular iron is low and vice versa. It has been shown that iron regulatory protein-1 activity was increased in the placenta with iron deficiency state in human (low cord SF and lower placental non-haem iron). Although mild maternal ID does not appear to affect fetal iron acquisition, moderate maternal ID may decrease the iron transfer to the fetus and result in lower iron stores in early infancy of the children [40].

2.8.2 Influence of maternal iron status on fetal and infant iron status

Studies that compared the haemoglobin concentration in cord blood between anaemic and non-anaemic pregnant women [25, 112-117] generally showed no difference in cord haemoglobin between groups with the exception of a few studies [113, 116]. This is no surprise as iron is prioritised for haemoglobin synthesis, and one would not see the change in

haemoglobin unless iron supply is severely restricted. Although there were no differences in cord haemoglobin concentration between anaemic and non-anaemic women in most studies, the haemoglobin levels were consistently lower and the incidences of IDA were consistently higher during the first year in infants born to anaemic mothers even after feeding practices and social status were controlled [25]. Similar results were found in a Canadian study [118] which showed a positive correlation between maternal haemoglobin at third trimester and haemoglobin of infants at nine months of age. Maternal IDA at birth has been shown to increase the risk of developing ID in infancy [119]. This led to a call for prevention of IDA in pregnancy as a preventive strategy for controlling IDA in infancy [119].

Studies that compared serum ferritin concentration in cord blood between women with ID and those without ID at birth [86, 116, 120-125] showed lower cord ferritin concentration or low iron stores in women with ID compared with women who were iron sufficient. Fetal liver iron storage has been shown to be affected by maternal ID [113]. Singla & colleagues [113] examined the relationship between maternal plasma iron and storage iron in the fetal liver collected from aborted fetus or stillborn babies [113]. Fetal liver storage iron concentration and total storage iron was lower in fetus from mothers with ID (plasma iron<10umol/l) compared with mothers who were iron sufficient. The iron concentration in the liver is a good indicator of the fetal/infant iron status as liver is a major organ for storage iron.

Studies that investigated the relationship between maternal iron status in pregnancy and iron status of infants beyond birth [25, 86, 106, 115, 118, 119, 121] have consistently demonstrated a positive association between maternal iron status at birth and iron status of infants in first year of life, despite the absence of such association between maternal iron status and cord blood iron indices at birth in some of the studies. This suggests that differences in iron

stores probably exist at birth between newborns from iron deficient and iron replete mothers even though the iron indices in cord blood failed to show the differences. It raises an important question on whether cord blood is a good indicator for infants iron status because cord blood is a mixture of maternal and fetal blood.

2.8.3 Effect of routine iron supplementation in pregnancy on infant iron status: evidence from RCTs

Five randomised controlled trials that investigated the relationship between iron supplementation during pregnancy and iron status of infants were identified [106, 126-129]. While iron supplementation improved maternal iron status in all 5 studies, its impact on infant iron status was less clear cut.

Four [106, 126, 128, 129] of the five trials compared iron supplementation (from mid-pregnancy at a doses range from 66mg/day to 120mg/day) with placebo. Two of them [106, 128] showed better iron status in infants from the iron supplemented group compared with the placebo group in terms of higher serum ferritin in cord blood [128] and in infants' blood at three and six months of age [106]. Another two studies [126, 129] showed no differences in cord haemoglobin and ferritin between the iron supplemented and placebo groups. However, iron parameters in cord blood were only available for 80/154 of women in one of the studies [129]. The high attrition rate may result in bias. The other study [126] had a relatively small sample size (n=97 in total) in a well-nourished population (all women had haemoglobin>100g/l). Therefore it is unlikely that it had sufficient statistical power to detect any difference in cord serum ferritin levels if it exists.

A RCT by Guldholt & colleagues [127] compared iron status of infants whose mothers received high dose iron supplements in pregnancy with those who received low dose iron supplements. It also showed no difference in cord serum ferritin level between groups. This may reflect a ceiling effect, in that low dose iron supplements may adequately replenish maternal iron stores in terms of providing optimal iron supply for the fetus.

Overall the results from both observation and intervention studies were inconclusive in terms of the relationship between maternal iron status and the iron indices of cord blood or infant blood. Most of the studies had a relatively small sample size with a high attrition rate and no sample size calculation. Factors which can influence the iron status of mothers or infants such as gestational age at birth, birth weight and infection, had not been considered or controlled in most studies. All of these may have contributed to the apparent conflicting results. Overall, it appears that lower maternal iron stores lead to lower iron stores in the infants. Studies in populations with high prevalence of IDA and ID were more likely to find an association between maternal and infant iron status compared with studies in well-nourished populations. Furthermore, there is clear evidence that fetal iron stores in vital organs such as the liver and kidneys are reduced by maternal iron deficiency. Collectively these data suggest that infants born to mothers with IDA or ID may have lower iron stores and may be at risk of developing ID in early infancy or early life, but further work is needed to substantiate these data.

2.9 Routine iron supplementation in pregnancy: effect on child development

Despite evidence from animal studies suggesting the importance of adequate iron nutrition in pregnancy on brain development of offspring, there are no human intervention trials that have assessed child development in relation to iron supplementation in pregnancy. The only relevant human study, which was designed to investigate the developmental outcomes of the children born small for gestational age [130], found that children with cord ferritin levels in the lowest quartile scored lower on some mental and psychomotor tests at 5 years than children with cord ferritin levels in the 2 middle quartiles. However, children with cord ferritin in the highest quartile also scored lower on the tests. The authors suggested that the children with cord ferritin in the highest quartile might have falsely elevated ferritin level due to possible maternal infections. This finding suggests that developmental outcomes of the children may be associated with iron status of the mother at birth or during pregnancy. However, it is not possible to assess whether this association is causal with this observational study because there are many potential confounding factors of child development as well as iron status.

2.10 Routine iron supplementation in pregnancy: effect on general health of women

There are few data relating to iron supplementation in pregnancy and health outcomes of women more traditionally associated with ID/IDA such as work capacity, general well-being and fatigue. To the best of my knowledge, there is only one published study that has assessed the long term health of women in relation to iron supplementation during pregnancy beyond 6 months post-partum. Hemminki & colleagues conducted a multi-centre randomised trial of iron supplementation in pregnancy in Finland involving 2694 pregnant women[131]. In that study, pregnant women were randomly allocated to a routine

iron supplementation group or a selective iron supplementation group. Women in the routine iron group were advised to take a single daily dose of 100mg of iron from their first antennal visit (no later than 17 weeks of gestation) throughout the pregnancy. Women in the selective supplementation group were prescribed iron supplements only when haemoglobin was less than 105g/l on two consecutive visits after 14 weeks of gestation. In a seven year follow up [132], the outcomes of subsequent pregnancies and the health of women were evaluated from data in the population register, cause of death register, hospital discharge register and birth register. Their results showed no differences in maternal death, the numbers or timing or reasons for hospitalisation between the groups. The outcomes assessed in Hemminki's study were of major morbidity and mortality. However, the general health and well-being of the women may be more relevant to iron nutrition.

2.11 Summary of the literature review and the rationale for the study presents in this thesis

There is strong evidence which points to an association between IDA and poor development in children. Iron interventions in late infancy and the second year of life have not been universally effective at improving childhood development in either anaemic or non-anaemic children. The most recent Cochrane systematic review of trials where anaemic children were randomly treated with iron or placebo demonstrated no clear benefit of iron on development and makes a strong case for further research [14]. Trials aimed at preventing IDA in children during late infancy and the second year of life have also failed to consistently show that prevention of IDA improves developmental outcome [80, 133]. Together these findings support the hypothesis generated from animal studies that the fetal

stage might be the critical period for iron nutrition. If the critical period does occur at the fetal stage, then optimal iron nutrition during pregnancy could potentially benefit the development of a child in the long term. Prevention of low iron intake in pregnancy can be simple, inexpensive and may offer a low risk strategy to improve the outcome of children. This might have an important public health implication in the recommendation of routine iron supplementation during pregnancy.

However, there are no human intervention trials that have been designed to specifically address the issue of iron nutrition in pregnancy and the developmental outcomes of the children. This is an area that has been highlighted as a priority for research [134, 135] and the study presented in this thesis aimed to fill the knowledge gap in this field.

Chapter 3. General Methods

3.1 Introduction

This thesis presents a long-term follow-up study of a randomised controlled trial of iron supplementation in pregnancy called "Australian Mothers' and Babies' Iron Trial" (AMBIT) [88]. There are three aspects to the long term follow-up including iron status (Chapter 4), IQ, behaviour and growth of the children (Chapter 5), and the general health and subsequent pregnancy outcomes of mothers (Chapter 6). A brief summary of the AMBIT is presented first in this chapter to provide background information for the follow-up in the subsequent Chapters. The remainder of this chapter describes the common features of the study design, the trial participants and recruitment process for all three aspects of the follow-up. Methods that are specific to each aspect of the follow-up are described in the relevant chapters separately.

3.2 Background information of the AMBIT

The AMBIT was a double blinded randomised controlled trial which was designed to assess the effects of low dose iron supplementation during pregnancy on maternal iron status, health and well-being [88]. It was conducted by Makrides and colleagues during 1997-1999 at the Women's and Children's Hospital in Adelaide, Australia. Pregnant women were recruited from the antenatal clinic in the hospital and 430 pregnant women with gestational age less than 20 weeks and no anaemia (haemoglobin $\geq$ 110g/l) were enrolled in the trial. They were randomly allocated to receive either iron (equivalence to 20mg/day

of elemental iron in the form of $FeSO_4$) or placebo from 20weeks gestation to the end of pregnancy, and were followed up to 6 months post-partum. All women received standard obstetric care and if obstetricians considered women to be anaemic they were advised to take 80mg iron/day in addition to their allocated trial supplements.

The dose of supplemental iron used in the trial was aimed at increasing the women's iron intake to meet the minimum recommended dietary iron intake for pregnant women in Australia, which is 22mg/d [136]. Compliance with taking trial supplements was measured with monthly phone call to assess the average number of tablets not taking during the previous months. Women were supplied with excess trial tablets and back-count of the number of tablets returned was also used to assess compliance. The compliance rate was 86% from back-count of tablets and there was a strong correlation between compliance assessed based on tablets back-count and those based on the monthly phone call ($r=0.86$, $P<0.001$) [89]. Women in the iron group had higher mean haemoglobin (127 ± 13g/l vs. 120 ± 12g/l, $P<0.001$) and higher mean serum ferritin (21 ± 18ug/l vs 14 ± 10ug/l, $P<0.001$) concentration, and lower incidences of IDA (6/193, 3% vs. 20/185, 11%, $P<0.05$) and ID (65/186, 35% vs 102/176, 35%, $P<0.001$) at the end of pregnancy compared with women in the placebo group [89]. ID and IDA were classified according to the WHO criteria [1]. For the remainder of the thesis, children are referred to as the iron group or the placebo group according to their mothers' group allocation in the AMBIT.

3.3 The design of the follow-up study

All three aspects of the follow-up are prospective and were conducted 4 years after birth except that the iron status of the children (Chapter 4) was assessed at both 6 months and 4 years after birth.

Primary objective of the thesis:

- To compare IQ of the children at 4 years between the iron and placebo groups (Chapter 5).

Other objectives of the thesis:

- To compare iron status of the children at 6 months and 4 years of age between the iron and placebo groups (Chapter 4)
- To compare childhood behaviour and growth of the children between the iron and placebo groups (included in Chapter 5)
- To compare the general health and subsequent pregnancy outcomes of mothers between the iron and placebo groups (Chapter 6)

3.4 Subjects

All families that did not withdraw their consent for follow up were eligible to participate in the follow up study (see Appendix 1). There were no specific exclusion criteria. The number of eligible families at 4-year follow-up was 382 with 385 children (including 3 sets of twins). Some families that took part in the follow-up only consented to certain aspects of the study, for example, a child participated in the IQ assessment may not have participated in the assessment of iron status, or a mother participated in the follow-up but her child did not take part in the study and vice versa. Therefore, a participant flow chart for each aspect of the follow-up is presented individually in the relevant chapter to provide a simplified illustration.

3.5 Recruitment at the 4-year follow-up

The children were born between May 1998 and September 1999. During May 2002-December 2003 all eligible families were invited to participate in the 4-year follow up when their children were 4 years of age. An invitation letter was sent to each family 6 weeks before the child's 4th birthday with an information sheet included. A reply-paid envelope was provided for families to register their interest. A follow up phone call was made to the families who expressed their interest to explain the study. Families who did not reply were also followed up with a phone call to ascertain that they had received the letter. For families whose contact details were outdated, active searches for current contact details were conducted (see below 3.6). Mothers were given the opportunity to discuss the study with their family and their doctor. With consent the mother and her child were enrolled in the study. After enrolment, an appointment for the mother and her child to come to the Women's & Children's Hospital around the time of the child's 4th birthday ± 2 weeks was made and posted. A reminding phone call was made 1- 2 days before the scheduled appointment.

3.6 Strategies to improve participation

The primary objective of the thesis was to compare the IQ of the children between the iron and placebo groups (Chapter 5). The following strategies were used to ensure as many children were assessed for IQ as possible to achieve adequate statistical power.

In the AMBIT, an alternative contact person was recorded to help locate participants in the follow up. Since the completion of the AMBIT, yearly contact with all families was

maintained through Christmas cards and newsletters. Each mail-out offered the opportunity to update the contact details of families whose letters were returned due to change of address. Families were contacted using the contact details collected in the AMBIT first. If this failed, phone book, online Whitepages (Australia), medical records and the Electoral Roll were subsequently searched for updated contact details.

If the families were not able to attend the hospital clinic (including families who had moved interstate), a neutral location near the child's home was arranged for assessments to take place. This included a private room at the childcare centre or the kindergarten where the child was attending or a private room at a medical centre. For families who had moved to interstate or to the country (5/153, 3% in the iron group vs 6/149, 4% in the placebo group), appointments were arranged for the children to be assessed in their local area by a qualified experienced psychologist.

One of the objectives of the thesis was to assess the iron status of the children (Chapter 4). This required a small blood sample to be taken from the children. If parents did not want their children to have a blood sample taken but consented to other assessments, the blood test was waived to maximise the follow up coverage of the children.

3.7 Blinding

The student and other staff involved in the data collection of the follow-up study were not involved in the intervention phase and were blinded to the mothers' group assignment in the AMBIT until primary outcome analysis had been completed. The mothers were also unaware of their group assignment in the AMBIT. They were informed of the outcome of

the AMBIT when the preliminary results were available, and were also advised that their group assignment would be revealed after the completion of the follow-up study.

3.8 Power estimation

The sample size calculation was based on the primary objective of the thesis. It was aimed at achieving at least 80% power to detect a minimum difference in IQ score between the iron and the placebo group that is considered clinically important. The major factor in the sample size calculation was the expected or hypothesised difference in IQ of the children between the iron and placebo group.

RCTs of neonatal feeding practices have shown that preterm infants fed banked human milk [137] or formula supplemented with long chain fatty acids [138] have a 5-8 point developmental advantage over children fed standard formulas. Similarly, children exposed to lead in early life have cognitive scores that are 5-7 points lower than unexposed children [139]. Differences of this magnitude have prompted public health authorities to promote human milk feeding, recommend the use of formulas supplemented with long chain fatty acids over those not supplemented for preterm infants and to guard against lead exposure.

Differences in developmental scores reported between anaemic and non-anaemic children range from 8 points [7] to 15 points [9, 133]. If there is a difference in the developmental test scores of the children between the iron and placebo groups in the follow-up study, it was expected to be of the same magnitude or smaller. The minimal difference was assumed to be similar to that found or hypothesised for other nutrients and those between breastfed and formula fed infants.

It was anticipated that the majority of 385 children remaining in the study at 6 month follow-up would participate in the 4-year follow-up. Assuming a loss to follow up of 10%, 346 children (173 per group) would complete the follow-up phase of the study which had 82% power (α=0.05) to detect a 5-point minimal difference in the Stanford-Binet scores between groups assume SD of 16. The sample size calculation was re-evaluated half way through the study. The SD for the mean IQ of the children in my study sample was 12. The smaller SD than assumed means a smaller sample size was needed to achieve the same statistical power. It was then estimated, 106 children per group was required to detect a minimal 5 IQ points difference between the iron and placebo groups with 85% power. With 302 children in the study it has 95% power to detect the same difference.

3.9 Data collection

I carried out the 4-year follow-up study and collected all the data at 4-year follow-up assessments. Makrides & colleagues collected the raw data on iron status of the children at 6 months of age, which formed parts of the raw data in Chapter 4.

Participating families were asked to attend a clinical appointment for assessment. During the clinical appointment, the IQ was assessed first by the psychologist before other assessments to minimise distraction like the discomfort from blood test. The anthropometrics were then measured by the student using standardised procedures and blood sample was taken at the end of the visit by an experienced nurse.

Information on birth details of the children including gestational age at birth, birth weight, length, head circumference, birth order of the children were obtained from the AMBIT data set collected (from the medical records) by Makrides & colleagues [89]. Data on duration of breastfeeding were collected in the AMBIT when the children were 6 months of age as well as at the follow-up when they were 4 years of age. Both data were compared to check for discrepancies in maternal recall. There was a strong correlation between the data collected at 6-month and at the 4-year follow-up ($r = 0.89$, $P < 0.0001$). Therefore, the data collected at 4-year follow-up was used in all analyses because the data collected at 6 months did not provide information on breastfeeding beyond 6 months of age. Duration of breastfeeding was reported by the mothers and was defined as the total duration of any breastfeeding. Preterm birth was defined as gestational age at birth less than 37 weeks and full term birth was defined as gestational age at birth greater than or equal to 37 weeks and less than or equal to 42 weeks. Maternal smoking during pregnancy, parental education levels and occupations were also obtained from the AMBIT data set. The study was approved by the Human Research Ethics Committee at the Women's & Children's Hospital in Adelaide, Australia. Informed consent was obtained from all participants.

3.10 Data analysis

The majority of data analyses were performed using SPSS (SPSS, version 10.0, Chicago, IL, USA) except for the regression analyses, which were conducted using Stata (Intercooled Stata 8.0 for Window, USA). Frequency distributions of all outcomes variables were inspected. Variables with non-normal distributions were transformed in order to achieve a near normal distribution when possible (eg. serum ferritin). Parametric tests were used for variables with normal distribution and non-parametric tests were used

for variables that were not normally distributed. However, non-parametric tests generally have lower statistical power and lack of details of descriptive statistics data for continuous variables. It is well recognised that parametric tests are relatively robust and with a sample size of greater than 30 per group, it is reasonable to use parametric tests for outcome variables that are not normally distributed [140]. Therefore, both parametric and non-parametric tests were conducted and the P-values were compared in the case of outcomes that are not normally distributed. Analyses were 2-sided and statistical significance was set at a P-value of less than 0.05 for all statistical analyses. The baseline characteristics between the participants and non-participants or between the iron and placebo groups were compared using t-tests for continuous variables and chi-square tests for dichotomous variables to assess potential bias as a result of attrition. Primary analysis was based on the intention to treat, which is to compare outcomes between all the iron and placebo groups.

Chapter 4. Effect of iron supplementation in pregnancy on iron status of the children at 6 months and 4 years of age: a long-term follow-up

4.1 Introduction

Iron deficiency (ID) and iron deficiency anaemia (IDA) remain the most common nutritional deficiency during infancy and early childhood due to rapid growth while dietary iron intake is relatively limited, particularly in exclusively breastfed infants. Iron stores of infants are utilised to meet the high iron demand and are one of the important factors in maintaining iron balance and preventing ID and IDA during early infancy. Infants born with higher iron reserves at birth may have more storage iron to spare and are at lower risk of developing ID and IDA in infancy [40, 140], a condition that has been linked to impaired psycho-social development in infants and young children [7, 19, 20].

Iron stores of newborns are derived from the mothers via their placenta. The extent to which maternal iron status influences the iron status of infants is not well understood. In past decades, studies that evaluated the effect of maternal iron status on the iron status of the children were often cross sectional and compared iron status of children between anaemic and non-anaemic mothers, or examined the correlation between maternal and infant iron status. While some studies showed that children born to anaemic mothers or mothers with ID at birth had lower iron status at birth or during infancy compared with infants of non-anaemic mothers [25, 105, 111, 113, 121, 122], other studies did not support that finding [124, 128, 141]. This type of study has a limited role in determining the relationship between maternal and infant iron status because it is difficult to control for all potential confounders.

Randomised trials of iron supplementation in pregnancy aimed at preventing ID/IDA in pregnant women provide a better alternative to examine the relationship between maternal iron nutrition and infant iron status while limiting the influences of potential confounders. However, the majority of these studies have focused on the iron status of mothers and few assessed the iron status of infants [106, 126, 128, 129]. Furthermore, randomised controlled trials with long-term follow up beyond birth are scarce. The aim here was to examine the iron status of the children at 6 months and 4 years of age whose mothers participated in a randomised controlled trial of iron supplementation in pregnancy, the AMBIT [89].

4.2 Subjects and Methods

The follow-up of the iron status of the children was conducted when the children were 6 months and 4 years of age, and was a secondary outcome of the long-term follow-up of the AMBIT (the primary outcome is IQ of the children presents in Chapter 5). Therefore the blood tests were optional in order to have as many children assessed for the primary outcome as possible. If families consented to the blood test, an appointment was arranged for the children to have the blood test at the Women's & Children's Hospital. There were 433 live births (including 5 sets of twins) in the AMBIT. At the 6-month follow-up, families of 48 children declined to be followed up, and another 49 children had unsuccessful venipuncture or did not consent for the blood test. This resulted in 336 children at the 6 month assessment. At the 4-year follow-up, parents of 145 children declined follow-up or blood test, and another 27 children were lost to follow-up. The number of children from the cohort assessed at 4-year follow-up was 213 (see Fig 4.1).

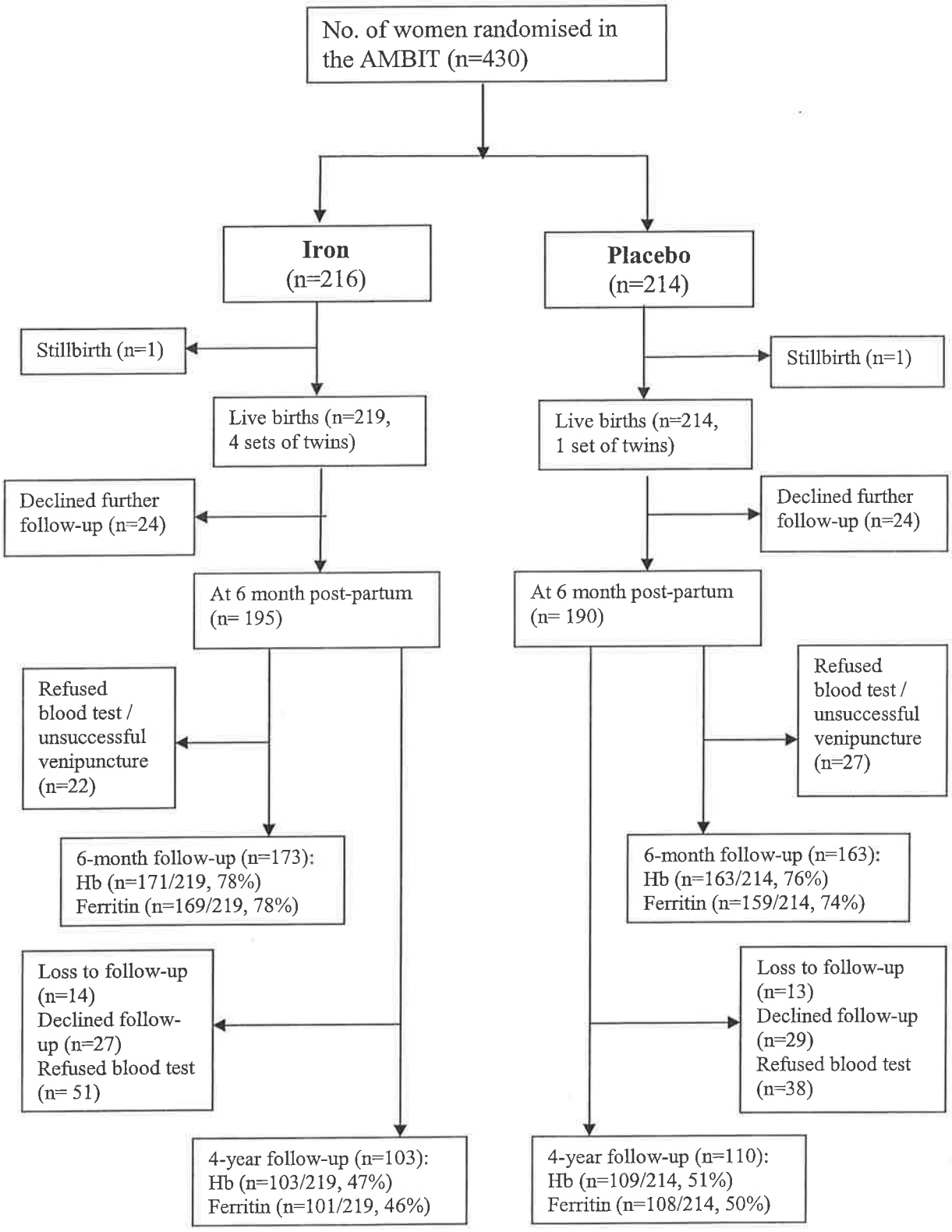


Figure 4.1 Participants flow diagram: follow-up of iron status of children

Demographic characteristics of families including maternal age, parental education, maternal smoking during pregnancy, and the birth details of the children were extracted from the AMBIT data set (see Chapter 5.2.2 other assessments for details of collection method).

Up to 2 mL of non-fasting blood was taken from each child by venipuncture for assessment of iron status. Haemoglobin and serum ferritin, the most commonly used biochemical markers for iron status, were measured. Ferritin is an acute phase reactant and its value can be elevated up to 2 weeks following minor infection [143, 144]. Therefore, parents were asked if their children had a cold or other infectious diseases, or immunisations in the last 2 weeks before a blood sample was taken. If this was the case, the blood test was delayed for 2 weeks to minimise false elevation of serum ferritin.

The analyses of haemoglobin and ferritin were conducted by the Department of Haematology at the Women's and Children's Hospital, which is a NATA accredited laboratory. Haemoglobin concentration was measured spectrophotometrically using an Abbott Cell Dyn 4000 analyser. Serum ferritin was determined by a microparticle enzyme immunoassay on an Abbott AxSYM Automated Analyser. Analyses were completed within 3 hours of collection. Quality controls were used to check the accuracy of the analytical methods. Mean ±SD for haemoglobin (Abbott Cell Dyn 26 tri-level haematology control, lot CD091) and ferritin (Biorad Immuno Plus Control) quality controls were 135±0.96g/L for haemoglobin and 53±3.4μg/L for ferritin compared with the certified values of 137±3.2g/L and 57±5.0μg/L for haemoglobin and ferritin, respectively. IDA and ID was defined according to the diagnostic criteria of the laboratory where the tests were conducted (see Table 4.1).

Table 4.1 Diagnostic criteria for IDA and ID based on haemoglobin and ferritin in this study

Age of children	IDA	ID
6 months	Hb <100g/l & ferritin <10ug/l	Ferritin <10ug/l
2 – 4 years	Hb <101g/l & ferritin <10ug/l	Ferritin < 10ug/l
> 4 years	Hb <116g/l & ferritin <10ug/l	Ferritin < 10ug/l

IDA: iron deficiency anaemia; ID: iron deficiency; Hb: haemoglobin

4.3 Statistical analysis

Serum ferritin data was positively skewed and was log transformed for statistical analysis, and the results were re-transformed into antilogarithms and are reported as geometric means. Independent t-tests were used to compare haemoglobin and ferritin concentrations of the children between the iron and placebo groups. This analysis was repeated in the subgroup of children who were born full term because most preterm children received some forms of iron supplementation in the neonatal period. Linear regression analyses were conducted to examine potential predictors of iron status of children at 6 months and 4 years of age. Exploratory analyses were performed to compare iron status of children between mothers who had IDA/ID at birth and those who did not while the intention to treat analysis showed no effect of intervention on the iron status of children.

4.4 Results

The rate of follow-up for iron status assessment was 78% (336/433) at 6 months and 49% (213/433) at 4 years. There were no differences in baseline characteristics of the families between participants and non-participants at either 6-month or 4-year follow-up with the exception that children who did not take part in the follow-up had mothers who were more likely to smoke in pregnancy (29% vs. 17%, P=0.01 at 6 months, 23% vs. 16%, P=0.07 at 4 years) (see Table 4.2).

4.4.1 Demographic characteristics of children

The ages of the children were 26 ± 2 weeks (6.1 ± 0.4 months) at the 6 month assessment (49% boys) and 4.2 ± 0.2 years at 4 year assessment (51% boys). The follow-up rate did not differ between the iron and placebo groups at either 6 months (79% vs. 76%, P=0.47) or 4 years (47% vs. 51%, P=0.33). There were no differences in baseline characteristics or birth details of the children who participated between the iron and placebo groups at either the 6-month or 4-year follow-up (see Table 4.3). Maternal haemoglobin and serum ferritin at birth were significantly higher in the iron group compared with the placebo group as a result of the intervention in the AMBIT (see Table 4.3). The duration of breastfeeding reported at the 4-year assessment was longer than at the 6-month assessment because 42% of the children were still breastfed at the 6-month assessment.

Table 4.2 The baseline characteristics of the families between participants and non-participants at 6-month and 4-year follow-up[a].

	At 6-month follow-up			At 4-year follow-up		
	Participants (n=336)	**Non-participants (n=96)**	**P-value**	**Participants (n=213)**	**Non-participants (n=220)**	**P-value**
Maternal age (year)	28.8 ± 4.9	28.4 ± 5.5	0.48	28.8 ± 5.1	28.8 ± 5.1	0.94
Smoking in pregnancy	56/336 (17%)	29/99 (29%)	0.01	35/213 (16%)	51/222 (23%)	0.07
Mother's education			0.99			0.32
Year 12 or below	263/336 (79%)	72/93 (78%)		170/213 (80%)	167/220 (76%)	
Diploma or degree	72/336 (21%)	20/93 (22%)		43/213 (20%)	53/220 (24%)	
Father's education			0.74			0.26
Year 12 or below	252/325 (78%)	66/87 (76%)		162/204 (79%)	151/202 (75%)	
Diploma or degree	73/325 (22%)	21/87 (24%)		42/204 (21%)	51/202 (25%)	

a: data are mean ± SD or number (%) unless otherwise specify

Table 4.3 The demographic characteristics of the children between the iron and placebo groups at 6-month and at 4-year follow-up[a]

	At 6-month follow-up			At 4-year follow-up		
	Iron (173)	**Placebo (163)**	**P-value**	**Iron (n=103)**	**Placebo (n=110)**	**P-value**
Baseline characteristics						
Maternal age (year)	28.8 ± 5.1	28.9 ± 4.8	0.86	28.6 ± 4.9	29.1 ± 5.1	0.46
Smoking in pregnancy	29/173 (17%)	27/163 (17%)	0.96	17/103 (17%)	18/110 (16%)	0.97
Mother's education			0.62			0.27
Year 12 or below	134/173 (77%)	129/163 (80%)		78/103 (76%)	90/110 (82%)	
Diploma or degree	39/173 (23%)	34/163 (21%)		25/103 (24%)	20/110 (18%)	
Father's education			0.08			0.79
Year 12 or below	136/167 (81%)	116/158 (73%)		71/98 (72%)	77/104 (74%)	
Diploma or degree	31/167 (19%)	42/158 (27%)		27/98 (28%)	27/104 (26%)	
Other characteristics						
Gestational age (week)	39.5 ± 1.8	39.3 ± 1.9	0.22	39.7 ± 1.3	39.3 ± 1.8	0.07

Table 4.3 The demographic characteristics of the children between the iron and placebo groups at 6-month and at 4-year follow-up (con't)

Preterm birth (GA<37weeks)	10/173 (6%)	15/163 (9%)	0.23	4/103 (4%)	11/109 (10%)	0.07
Birth wt (g)	3438 ± 554	3473 ± 542	0.55	3531 ± 488	3444 ± 547	0.22
Sex: Male	88/173 (51%)	76/163 (47%)	0.437	54/103 (52%)	54/110 (49%)	0.58
Maternal Hb at birth (g/l)	128 ± 11	121 ± 12	<0.01	128 ± 13	119 ± 11	<0.01
Maternal ferritin at birth (ug/l)[b]	16.1 ×/÷ 2.0	11.1 ×/÷1.8	<0.01	15.4 ×/÷ 2.0	11.1 ×/÷1.8	<0.01
Length of breastfeeding (week)	15 ± 11	15 ± 11	0.68	33 ± 38	26 ± 29	0.15
% Breastfeeding at discharge	148/173 (86%)	140/163 (86%)	0.92	89/103 (86%)	94/110 (85%)	0.84
% Breastfed >3 months	98/172 (57%)	97/163 (60%)	0.63	63/103 (61%)	58/110 (53%)	0.21
% Breastfed >6 months	72/172 (42%)	70/163 (43%)	0.84	46/103 (45%)	47/110 (43%)	0.77
Age at assessment[c]	6.1 ± 0.4	6.1 ± 0.4	0.26	4.2 ± 0.2	4.1 ± 0.2	0.19

a: data are mean ± SD or number (%) unless otherwise specified

b: ferritin values are geometric means ×/÷ SD multipliers

c: age at assessment are reported in months at 6-month follow-up and in years at 4-year follow-up

4.4.2 Iron status of children

The percentage of ID was 5% (17/328) at 6 months and 11% (24/209) at 4 years assessments. None of the children had IDA at 6 months assessment and three children had IDA at 4 years (3/213, 1.4%). Although the haemoglobin cut off for classifying IDA in my study differ for children aged 2-4 years and children aged above 4 years (see Table 4.1), which may introduce potential bias, no children aged under 4 years at assessment had a Hb<116g/l except one who did not have ID. Therefore, all children in the study classified as ID or IDA were subjected to the same criteria. Haemoglobin of the children at 4 years of age was significantly correlated with haemoglobin of the children at 6 months of age ($r=0.31$, $P<0.01$, Fig. 4.2). There were no associations between ferritin at 6 months and at 4 years, or between haemoglobin and ferritin at either 6 months or 4 years.

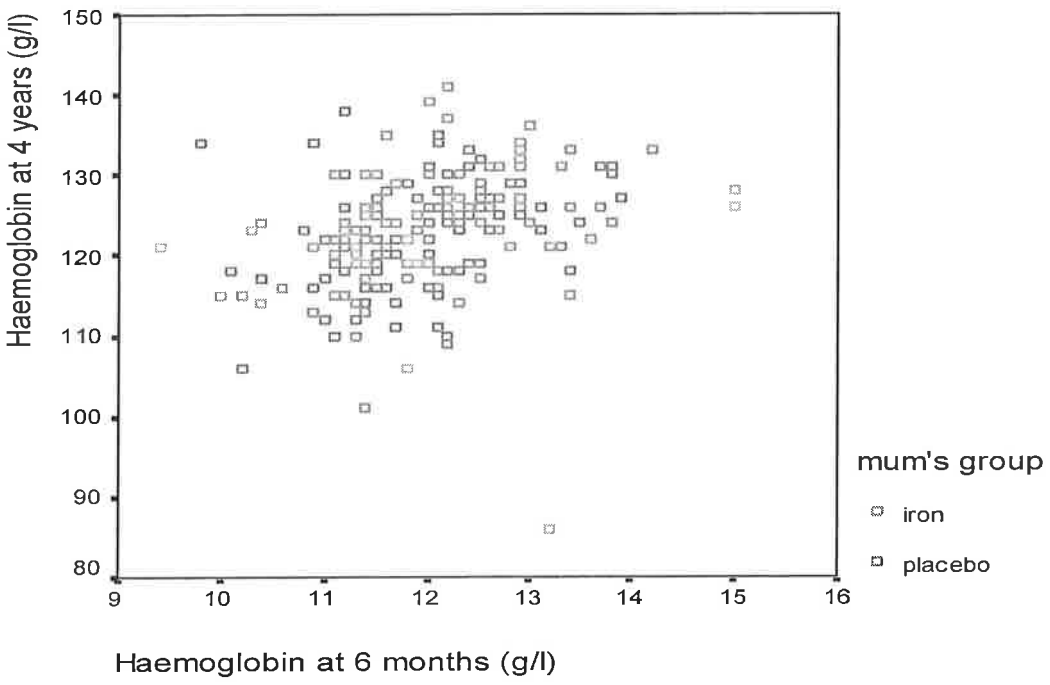


Figure 4.2 Correlation between the haemoglobin concentration of the children at 4 years of age and at 6 months of age

Analysis by intention to treat

There were no differences in haemoglobin and serum ferritin concentration, or percentage of ID and IDA of the children between the iron and placebo groups at either 6 months or 4 years assessments (see Table 4.4). One child in the placebo group had serum ferritin of 285ug/l at 6 months assessment who had an infection confirmed clinically after the blood test. Excluding this child or twins from the analyses did not change the outcomes.

Subgroup analysis of the children who were born full term

In the subgroup of children who were born full term, mean haemoglobin at 6 months of age was marginally higher in the iron group compared with the placebo group (121 ± 7g/l vs. 119 ± 7 g/l, MD: 1.9, 95% CI: -0.02, 3.86, P=0.05). There were no other differences in any of the iron indices between the iron and placebo groups at either 6 months or 4 years assessment in the subgroup analysis (see Table 4.5).

Linear regression analysis

Having found that there was no effect of the intervention on the iron status of the children, regression analyses were conducted to further examine potential predictors of haemoglobin and ferritin concentrations of children treating the data as if they were obtained in an observational study. Log ferritin was used in the regression analyses. Potential predictors examined in the regression analyses including parental education levels, smoking during pregnancy, gestational age at birth, birth weight, length of breastfeeding, gender of the children and the mother's group allocation in the AMBIT. These predictor variables were

chosen either because they have been shown to influence iron status of children in the literature or they were correlated with the haemoglobin or ferritin in simple regression analyses with a p-value <0.2. The regression for iron status at 6 months was conducted among the subgroup of children who were born full term because most fetal iron accretion occurs during the last trimester of pregnancy and full term infants have the full benefit of fetal iron supply from the mothers. At 4 years the regression analyses were conducted with all children included because there was no difference in iron status at 4 years between the iron and placebo groups regardless of whether all children were included in the analyses or only the subgroup of the children who were born full term.

1. Predictors of haemoglobin at 6 months

   Length of breastfeeding was the only variable that was significantly correlated with the haemoglobin of the children at 6 months. Haemoglobin at 6 months was decreased by 0.11g/l for every week of breastfeeding (see Table 4.6). The unadjusted regression coefficient ± SE for the iron group was 1.92 ± 0.98, P=0.05 (see Table 4.6). After adjusting for the other potential predictors (Table 4.6), children in the iron group had mean haemoglobin that was 2g/l higher than the placebo group. The adjusted regression coefficient was 2.00± 1.01, P=0.05.

2. Predictors of ferritin at 6 months

   Birth weight and gender of the children were significantly correlated to ferritin of the children at 6 months of age (see Table 4.7). Although statistically significant, the effects of gender and birth weight on ferritin were both small. Ferritin level was increased by 1.4ug/l for every kilogram increased in birth weight. Compared with girls, boys had ferritin concentration that was 1.4ug/l lower than girls (see Table 4.7). After

adjusting for the other potential predictors (Table 4.7), the mother's group allocation in the AMBIT had no effect on the ferritin of the children at 6 months of age. The unadjusted and adjusted regression coefficient ×/÷ SE multiplier for the iron group were 1.07 ×/÷1.08, P=0.34 (see Table 4.7) and 1.21 ×/÷ 1.08, P=0.22, respectively.

3. Predictors of haemoglobin at 4 years

   Gender of the children was the only factor that influenced the haemoglobin of the children at 4 years of age. The adjusted regression coefficient was –2.27 for boys when compared with girls (see Table 4.8). This indicated that girls had haemoglobin 2.27 g/l higher than boys at 4 years assessment. The mothers' group assignment in the AMBIT had no effect on the haemoglobin of the children at 4 years of age after adjustment for the other potential predictors (Table 4.8). The unadjusted and adjusted regression coefficient ± SE for the iron group was 0.83 ± 0.95, P=0.40 (see Table 4.8) and 1.03 ± 1.05, P=0.32, respectively.

4. Predictors of ferritin at 4 years

   None of the potential predictors were significantly correlated to the ferritin concentration at 4 years (see Table 4.9). After adjusting for the other potential predictors, the mother's group allocation in the AMBIT had no effect on the ferritin of the children at 4 years of age. The unadjusted and adjusted regression coefficient ×/÷ SE multiplier for the iron group was -1.08 ×/÷ 1.08, P=0.27 and -1.08 ×/÷ 1.09, P=0.34 respectively (see Table 4.9).

Exploratory analyses

There were no differences in haemoglobin and ferritin concentrations, or percentages of ID and IDA at either 6 months or 4 years of age between children whose mothers had ID/IDA at birth and those who did not, with the exception that the percentage of IDA at 4years was higher in children whose mothers had IDA at birth compared with those who did not (1/13, 8% compared with 2/200, 1%, P=0.04) (Table 4.10). However, there were only three children with IDA at 4 years, this difference is likely to be a result of chance finding.

Table 4.4 Iron status of the children between the iron and placebo groups: analysis by intention to treat[a]

	At 6 months assessment			At 4 years assessment		
	Iron (n=173)	**Placebo (n=163)**	**P -value**	**Iron (n=103)**	**Placebo (n=110)**	**P**
Hb (g/l)	121 ± 9	119 ± 9	0.10	123 ± 8	122 ± 6	0.36
Ferritin (ug/l)[b]	32.5 ×/÷ 2.0	30.8 ×/÷ 2.0	0.48	17.7 ×/÷ 1.7	19.2 ×/÷ 1.7	0.23
% ID[c]	11/169 (7%)	6/159 (4%)	0.26	12/101 (12%)	12/108 (11%)	0.86
% IDA[d]	0 /173 (0%)	0/163 (0%)	n/a	2/103 (2%)	1/110 (1%)	0.61[e]

a: data are mean ± SD or number (%) unless otherwise specified

b: ferritin value are geometric mean ×/÷ SD multiplier

c: iron deficiency

d: iron deficiency anaemia

e: P-value of Fisher's exact test

Table 4.5 Iron status of the children between the iron and placebo groups: subgroup analyses of the children born full term[a]

	At 6 months assessment			At 4 years assessment		
	Iron (n=164)	**Placebo (n=148)**	**P-value**	**Iron (n=99)**	**Placebo (n=98)**	**P-value**
Sex: Male	82/164 (50%)	69/148 (47%)	0.55	52/99 (53%)	48/98 (49%)	0.61
Hb (g/l)	121 ± 9	119 ± 8	0.05	123 ± 8	122 ± 6	0.44
Ferritin (ug/l)[b]	33.2 ×/÷ 2.0	31.3 ×/÷ 2.0	0.45	17.5 ×/÷ 1.8	19.8 ×/÷ 1.7	0.13
% ID[c]	10/158 (6%)	5/144 (3%)	0.25	12/95 (13%)	9/96 (9%)	0.47
% IDA[d]	0/161 (0%)	0/147 (0%)	a/a	2/97 (2%)	1/98 (1%)	0.55[e]

a: data are mean ± SD or number (%) unless otherwise specified (as in b)

b: ferritin values are geometric mean ×/÷ SD multiplier

c: iron deficiency

d: iron deficiency anaemia

e: P-value of Fisher's exact test

Table 4.6 Estimated regression coefficients in haemoglobin concentration (g/l) of children at 6 months of age for potential predictors

	Simple regression (unadjusted)		Multivariate regression (adjusted for others variables)	
Predictor variable (referent category)[a]	**Coefficient ± SE**	**P-value**	**Coefficient ± SE**	**P-value**
Length of breastfeeding /week	-0.13 ± 0.04	<0.01	-0.11 ± 0.04	0.01
Gestational age /week	-0.43 ± 0.41	0.30	-0.01 ± 0.45	0.98
Birth weight /kg	-1.40 ± 1.01	0.16	-0.78± 1.12	0.48
Sex (female): **Male**	-1.40 ± 0.98	0.15	-1.23 ± 1.03	0.23
Smoking in pregnancy (No):				
Yes	0.95 ± 1.35	0.48	-0.50 ± 1.43	0.72
Mother's education (≤Year 12):				
Diploma /degree	-0.69 ± 1.20	0.56	0.12 ± 1.30	0.92
Father's education (≤Year 12):				
Diploma/degree	-1.46 ± 1.21	0.23	-0.67 ± 1.31	0.61
Group (placebo): **Iron**	1.92 ± 0.98	0.05	2.00 ± 1.01	0.05

a: categories in bracket are the referent categories, eg: Group (placebo): Iron, indicates that the reference group is the placebo

Table 4.7 Estimated regression coefficients in ferritin concentration (ug/l) of children at 6 months of age for potential predictors[a]

	Simple regression (unadjusted)		Multivariate regression (adjusted for others variables)	
Predictor variable (referent category)[b]	**Coefficient** ×/÷ **SE**	**P-value**	**Coefficient** ×/÷ **SE**	**P-value**
Length of breastfeeding /week	-1.00 ×/÷1.00	0.82	- 1.00 ×/÷1.00	0.66
Gestational age /week	1.04 ×/÷ 1.03	0.16	1.01 ×/÷1.03	0.69
Birth weight /kg	1.32 ×/÷ 1.08	<0.01	1.40 ×/÷1.09	<0.01
Sex (female): **Male**	-1.34 ×/÷1.07	<0.01	-1.42 ×/÷1.08	<0.01
Smoking in pregnancy (No):				
Yes	-1.14 ×/÷1.11	0.20	-1.06 ×/÷1.11	0.56
Mother's education (≤Year 12):				
Diploma /degree	-1.03 ×/÷1.10	0.74	-1.00 ×/÷1.10	0.96
Father's education (≤Year 12):				
Diploma/degree	-1.03 ×/÷1.10	0.73	-1.03 ×/÷1.10	0.75
Group (placebo): **Iron**	1.07 ×/÷1.08	0.34	1.21 ×/÷1.08	0.22

a: log ferritin was used in analyses

b: categories in bracket are the referent categories, eg: Group (placebo): Iron, indicates that the reference group is the placebo

Table 4.8 Estimated regression coefficients in haemoglobin concentration (g/l) of children at 4 years of age for potential predictors

	Simple regression (unadjusted)		Multivariate regression (adjusted for others variables)	
Predictor variable (referent category)[a]	**Coefficient ± SE**	**P-value**	**Coefficient ± SE**	**P-value**
Length of breastfeeding /weeks	-0.01 ± 0.01	0.69	-0.004 ± 0.01	0.78
Gestational age /weeks	-0.31 ± 0.26	0.22	-0.19 ± 0.29	0.50
Birth weight /kg	-1.20 ± 0.95	0.20	-0.61 ± 1.10	0.57
Gender (female): **Male**	-2.72 ± 0.97	<0.01	-2.27 ± 1.06	0.03
Smoking in pregnancy (No):				
Yes	-1.29 ± 1.35	0.33	-1.78 ± 1.46	0.22
Mother's education (≤Year 12):				
Diploma /degree	-1.05 ± 1.247	0.40	-0.90 ± 1.37	0.51
Father's education (≤ year 12):				
Diploma/degree	-1.31 ± 1.263	0.30	-0.72 ± 1.36	0.59
Group (placebo)**: Iron**	0.83 ± 0.95	0.40	1.03 ± 1.05	0.32

a: categories in bracket are the referent categories. eg: Group (placebo): Iron, indicates that the reference group is the placebo

Table 4.9 Estimated regression coefficients in ferritin concentration (ug/l) of children at 4 years of age for potential predictors[a]

	Simple regression (unadjusted)		Multivariate regression (adjusted for others variables)	
Predictor variable (referent category)[b]	**Coefficient** ×/÷**SE**	**P-value**	**Coefficient** ×/÷**SE**	**P-value**
Length of breastfeeding /weeks	-1.00 ×/÷1.00	0.91	1.00 ×/÷1.00	0.71
Gestational age /weeks	-1.00 ×/÷1.02	0.84	-1.00 ×/÷1.02	0.93
Birth weight /kg	-1.00 ×/÷1.07	0.97	1.03 ×/÷1.09	0.76
Gender (female): **Male**	-1.03 ×/÷1.08	0.66	-1.02 ×/÷1.09	0.83
Smoking in pregnancy (No):				
Yes	1.18 ×/÷1.11	0.09	1.19 ×/÷1.12	0.11
Mother's education (≤Year 12):				
Diploma /degree	-1.04 ×/÷1.10	0.64	1.02 ×/÷1.11	0.86
Father's education (≤ year 12):				
Diploma/degree	-1.42 ×/÷1.10	0.17	-1.11 ×/÷1.11	0.30
Group (placebo): **Iron**	-1.08 ×/÷1.08	0.27	-1.08 ×/÷1.09	0.34

a: log ferritin was used in the regression analyses

b: categories in bracket are the referent categories. eg: Group (placebo): Iron, indicates that the reference group is the placebo

Table 4.10 Comparison of the iron status of the children between mothers who had ID/IDA at birth and those who did not[a]

	Maternal ID at birth			Maternal IDA at birth		
	Yes	**No**	**P**	**Yes**	**No**	**P**
Iron status at 6 months	N=139	N=194		N=23	N=310	
Hb (g/l)	120 ± 10	120 ± 9	0.95	122 ± 10	120 ± 9	0.33
Ferritin (ug/l)[b]	33.8 ×/÷2.1	30.3 ×/÷ 1.9	0.17	32.4 ×/÷ 2.0	31.6 ×/÷ 2.0	0.87
% ID[c]	7/137 (5%)	10/192 (5%)	0.96	1/23 (4%)	16/306 (5%)	0.85
% IDA[d]	0/139 (0%)	0/194 (0.5%)	n/a	0/23 (0%)	0/310 (0.3%)	n/a
Iron status at 4 years	N= 95	N=118		N=14	N=200	
Hb (g/l)	123 ±7	122 ± 7	0.15	121 ± 6	123 ± 7	0.27
Ferritin (ug/l)[b]	17.8 ×/÷ 1.7	18.9 ×/÷ 1.7	0.43	21.2 ×/÷ 1.7	18.3 ×/÷ 1.7	0.80
% ID[c]	14/92 (15%)	10/117 (9%)	0.13	2/13 (15%)	22/196 (11%)	0.64
% IDA[d]	2/94 (2%)	1/118 (1%)	0.58[e]	1/13 (8%)	2/200 (1%)	0.04[e]

a: data are mean ± SD or number (%) unless otherwise specified
b: ferritin values are geometric mean ×/÷ SD multiplie
c: iron deficiency
d: iron deficiency anaemia
e: P-value of Fisher's exact test

4.4.3 Summary of results

There were no differences in the mean haemoglobin, serum ferritin concentrations or percentage of ID/IDA between the iron and placebo groups at either 6 months or 4 years of age. However, subgroup analyses of the children who were born full term (37 weeks ≤ GA ≤ 42 weeks) showed children in the iron group had higher haemoglobin at 6 months of age after adjusting for the length of breastfeeding. Length of breastfeeding and gender of the children were the main factors that influenced the iron status of the children. Haemoglobin at 6 months was decreased by 0.11g/l for every week of breastfeeding. Compared with boys, girls had higher serum ferritin at 6 months and higher haemoglobin at 4 years of age.

4.5 Discussion

This long term follow up study showed that iron status of the children did not differ at 6 months or 4 years of age between children whose mothers received routine iron supplements in pregnancy and those whose mothers were in the control group in the AMBIT [89].

To the best of my knowledge, there is only one randomised placebo controlled trial of iron supplementation in pregnancy that had followed up the iron status of the children beyond birth published in the English language [106]. In the study conducted by Preziosi and colleagues [106], pregnant women were randomly allocated to receive either 100mg/d of iron or placebo from 6 months of pregnancy to the end of pregnancy. Although there were no differences in iron indices in cord blood between the iron and placebo groups, infants of iron supplemented mothers had higher serum ferritin at 3 and 6 months of age compared

with infants whose mothers were in the placebo group. The different findings between this study and my study may be partly due to the differences in the severity and prevalence of ID between the two different study populations. Preziosi's study was conducted in a developing country (Niger) where ID and anaemia in pregnancy were more severe and prevalent. Sixty-five percent of the women in that study were anaemic at entry to the trial (6 months of pregnancy), whereas women in my study were non-anaemic at baseline (20 weeks of gestation). It has been demonstrated from observational studies that mild anaemia in pregnancy did not affect the iron status of newborns whereas more severe anaemia did [113, 114].

There is a lack of data on haemoglobin and ferritin levels, or prevalence of ID/IDA in national representative samples of infants or preschool children in Australia. In a study that assessed the iron status of 678 children aged 9 to 62 months in central and southern Sydney in Australia, the overall prevalence of ID was 2.8% with the highest rate of 5.4% among children aged 9 to 23 months [64]. The mean haemoglobin and ferritin of the children in my study are compatible with the data from the 1995 British National Diet and Nutrition Survey [145], where mean haemoglobin and ferritin were 120g/l and 21ug/l respectively for children age between 1.5 to 2.5 years, and 124g/l and 25ug/l respectively for children age between 3.5 to 4.5 years. The prevalence of ID was 20% in the British children age between 1.5 to 4.5 years [145], and 9% in children aged one to two years and 3% in children aged three to five years in USA [146]. The rate of ID among the children in my study is lower than the British data [145] and higher than the USA [146]. This difference may be partly due to the different criteria used to diagnose ID and the different ages of children included in the different studies because the prevalence of ID is highest among toddlers than other age groups of children [146]. Whether there are differences in iron intakes of children or

strategies to prevent ID among the different populations, which may contribute to the different prevalence of ID, was beyond the scope of my study. Although the haemoglobin of the children at 6 months in the iron group was higher than the placebo group after adjusting for the length of breastfeeding, the effect was small and there were no differences in iron stores at 6 months or iron status of the children at 4 years between the groups. It is unlikely that this finding has any clinical significance.

The gender differences in iron status found in my study is in agreement with the literature. Lower haemoglobin in male infants at 4, 6 and 9 months of age among 121 Swedish and 142 Honduran [147], and in 1175 British toddlers at 18 months [148] have been reported. It has also been shown that ferritin concentrations in the cord blood of male infants was lower compared with those of female infants [149]. The biological basis for this phenomenon is not well understood. It has been suggested that differences in hormone [147], iron stores at birth [150] and growth rates between girls and boys may contribute to the gender differences in iron status. This has raised a question about whether there is a need for sex specific diagnostic criteria for ID and IDA in children. Whether this gender difference in iron status has any functional consequences has not been explored.

It is generally agreed that full term infants are born with generous iron stores which protect them from developing ID in the first few months of life [146]. After that infants are increasingly dependent on dietary iron supply to meet the high iron demand for rapid growth. Despite its high bioavailability, the iron content of breast milk declines as lactation progresses and it may be inadequate to meet the requirement for optimal production of haemoglobin and growth of the older infants [151], and other sources of dietary iron from solid foods are needed to ensure adequate iron intake is achieved.

The limitation of this study is the high attrition rate at 4-year follow-up. There are many potential confounders of iron status of children including socio-economic status, infection, feeding practice and dietary iron intake to name a few. Although there were no significant differences in many social outcome variables between participants and non-participants, there was a higher rate of maternal smoking in pregnancy in the non-participants. This may lead to selection bias as smoking is often an indicator of dietary and lifestyle practices, which may also influence the iron status of the children. The extent of selection bias is difficult to determine, but there were no differences in baseline characteristics between the iron and placebo groups at either 6-month or 4-year follow-up. This may suggests that the difference between the participants and non-participants may not have significantly influenced the outcomes.

4.6 Conclusion

Within the scope of this follow-up, routine iron supplementation in pregnancy had no clinically important effect on the iron status of children in this well nourished population.

Chapter 5. Effect of maternal iron supplementation in pregnancy on IQ, behaviour and growth of the children at 4 years of age

5.1 Introduction

Iron is essential for brain growth and function. Evidence from animal studies has consistently demonstrated that inadequate iron nutrition during pregnancy leads to permanent structural and functional changes in the brain of offspring. However, there are no human intervention trials that have been specifically designed to examine the effect of iron nutrition in pregnancy and childhood neurodevelopment.

The aim of this study was to determine if improved maternal iron nutrition during pregnancy through routine iron supplementation improves neurodevelopment of the children. The primary objective was to assess the IQ of children at 4 years of age whose mothers participated in the AMBIT [88].

5.2 Subjects & Methods

5.2.1 Subjects

Of the 386 eligible families who completed the AMBIT (see Chapter 3.4), 27 of them could not be found at the 4-year follow-up. The remaining 359 families were contacted and 302 children (included 3 sets of twin) participated in the follow-up study with parental consent (see Appendix 1 & Fig. 5.1). This is equivalent to 78% of eligible children and 70% of all enrolled in the AMBIT.

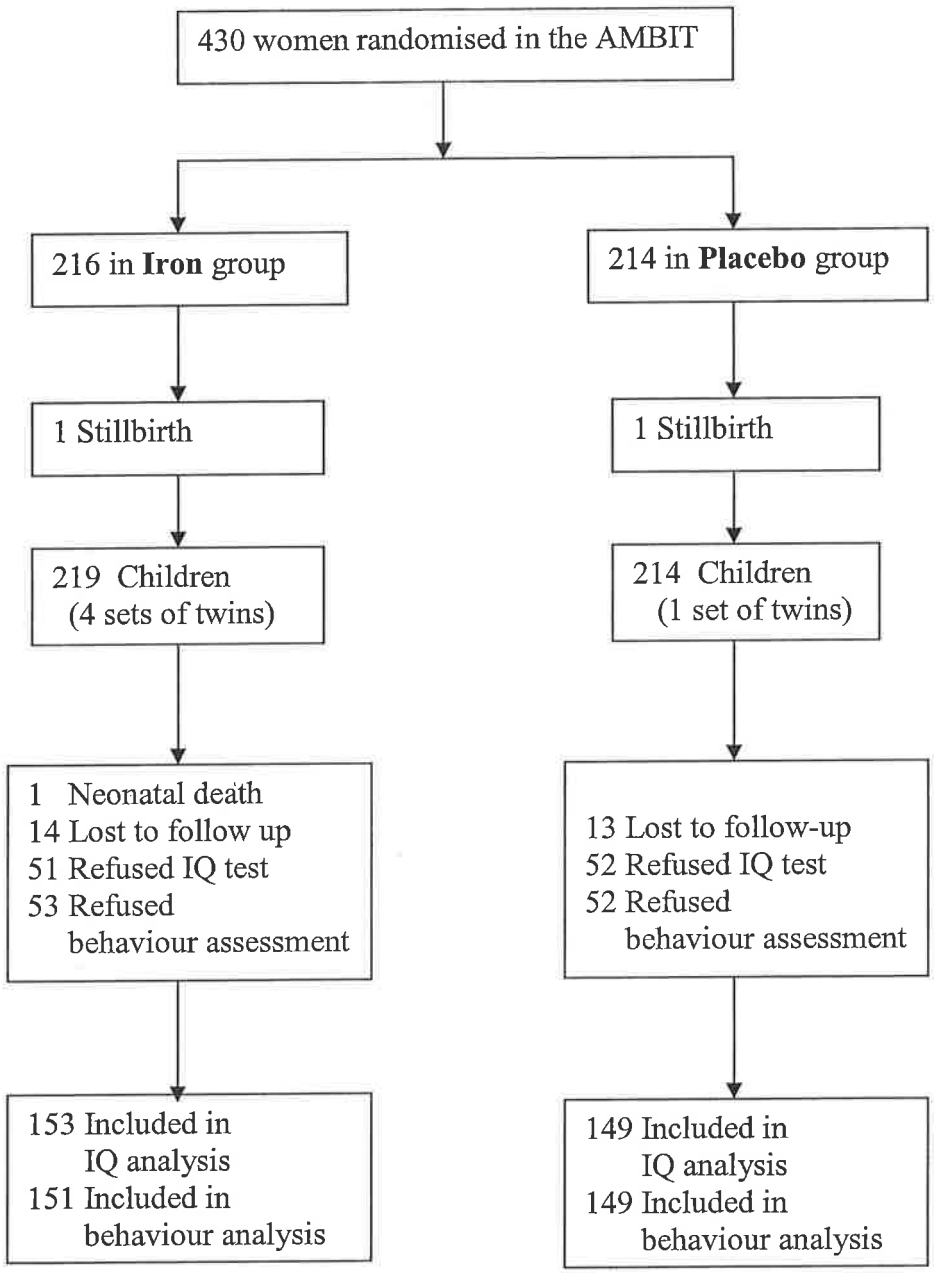


Figure 5.1 Participants flow diagram: follow-up of IQ and behaviour of children

Primary outcome:

- IQ at 4 years of age as assessed by the Stanford – Binet Intelligent Test

Secondary outcomes:

- Weight, height & head circumference at 4 years of age
- Behaviour at 4 years of age as assessed by the Strength and Difficulties Questionnaire (SDQ) [152].

5.2.2 Assessments

1. Growth

Height, weight and head circumference were assessed as an index of growth and nutritional well-being of the children. Weight was measured using an electronic scale with an error of ±10g. Height was measured using a stadiometer with an error of ± 0.5cm. The child without shoes, stood straight and looked straight ahead with the back and heels against the stadiometer. Head circumference was measured around the occipitofrontal circumference using a non-stretching tape with an error of ± 0.5cm. All anthropometrics measures were conducted by the research student who was blinded to the mothers' group allocation in the AMBIT.

2. Child development

Child development was assessed using the Stanford – Binet Intelligent Test (4^{th} Ed) [153]. Previous studies suggested that the specific areas of cognitive deficit associated with IDA were short-term memory and attention span. The Stanford – Binet Intelligent Test provides

a rating of overall cognitive ability (composite IQ score) with separate subscales of IQ for short-term memory as well as verbal, visual and quantitative reasoning. It is an international standardised tool for global developmental assessment, and it has been used widely in studies that examine the effects of IDA on child development. The Bayley Infant Developmental Scale is another common assessment tool used in this area of research. However, it is only suitable for children up to the age of two and a half years while the Stanford – Binet Intelligent Test is suitable for children from two and half years of age to adult.

The Stanford – Binet Intelligent Test assesses three levels of cognitive abilities with 15 categories of testing [153]. The first level of cognitive ability is the general ability, the cognitive process that an individual uses to organise adaptive strategies for solving novel problems. The second level of cognitive ability reflects the cognitive skills for acquiring and using information on verbal and quantitative concepts to solve problems and problems involving nonverbal stimuli. The second level of cognitive ability also consists of short-term memory. The third level of cognitive ability includes reasoning, quantitative and visual reasoning. It involves showing picture cards, repeat sentences, copying and building certain patterns using blocks and beads, and some comprehension tests. It takes approximately 1 hour to complete the test for a 4 years old child. Raw scores on all tests are converted to age standardised scores that have a mean of 100 and a SD of 16 [153]. All children had their development assessed first to ensure that they were not upset or unsettled by other assessments, for example having blood taken.

The majority of tests were administrated by two qualified psychologists who had experience in conducting Stanford – Binet assessment in young children. They were also

trained before the commencement of the study to ensure the consistency in conducting and scoring the assessment. The assessments were conducted primarily at a hospital clinic (244/302, 81%). If the family was unable to attend a hospital appointment, the assessment was done at a private room where the child attended a child care centre (47/302, 15%). For a small number of children (11/302, 4%) whose families had moved interstate, the assessment was done by a qualified experienced psychologist in his or her private clinic in their local area. The costs associated with the test were paid for by the study team. Overall, there were no differences between the iron and placebo group in the percentage of children who had IQ assessment done at child care centre (23/153, 15% vs 24/149, 16% for the iron and placebo group, respectively, p=0.797) or interstates (5/153, 3% vs 6/149, 4% for the iron and placebo group, respectively, p=0.08).

3. Behaviour

The behaviour of the children was assessed using the "Strength and Difficulties Questionnaire-parent report form" (SDQ) [152]. There are several versions of SDQ, the P3/4 was used in the study which is a parent-reported form for children aged between 3 and 4 years. The questionnaire was mailed to the parents to complete and returned it back when they attended the clinical appointment.

The SDQ is a 25 items behaviour screening questionnaire for assessing the psychological adjustment of children. It has been validated internationally including Australia [154] and has been shown to highly correlate with the Child Behaviour Checklist established by Achenbach but is less time consuming (less than one quarter of the length of the Child Behaviour Checklist) [155].

The 25 items generate a total difficulties score and scores on 5 subscales including: emotional symptoms, conduct problems, hyperactivity, peer problems and prosocial behaviour [156]. The total difficulties scores range from 0 – 40. For each SDQ score, the extreme 10% of a community population is defined as a high risk group of having behaviour problems. For all SDQ scores the high risk group comprise the 10% of the population with the highest scores except for prosocial scale, where the high risk group comprises 10% of the population with the lowest scores [156].

The SDQ scores can also be classified into 3 categories as normal, borderline and abnormal behaviour. The range of reference values is presented in Table 5.1

Table 5.1 The cut-off score for diagnosis of abnormal behaviour based on the "Strength and Difficulties Questionnaire (SDQ)" score [152]

Scale	Normal	Borderline	Abnormal
Total difficulties	0-13	14-16	17-40
Emotional symptoms	0-3	4	5-10
Conduct problems	0-2	3	4-10
Hyperactivity	0-5	6	7-10
Peer problem	0-2	3	4-10
Prosocial behaviour	10-6	5	4-0

4. Other assessments

1) Home environment

Home environment, a factor known to influence child development, was assessed using "The Home Screening Questionnaire" (HSQ) [157]. It is a simplified form of the "Home Observation for Measurement of the Environment (HOME) Inventory" [158] and was designed to identify children at risk of developmental delays due to negative environmental influences. It provides information on the quality and quantity of cognitive, social and emotional support available to each child in the home environment.

There are two forms of the HSQ, one for use in children age between 0-3 years and the other for children age between 3-6 years. The latter form was used in the study. It is a parent-answered questionnaire which takes approximately 10 minutes to complete. The questionnaire was mailed to the families with the appointment notification and parents were asked to bring the completed questionnaire with them to the appointment. The questionnaire was scored according to the Home Screening Questionnaire Reference Manual [157]. Higher scores indicate a better home environment for child development. The maximum score that can be derived from the HSQ is 56. A score of 41 or below indicates potential risk environment for developmental delays.

2) Maternal/Parental characteristics:

This included maternal age, parental education levels and occupations. Education level was defined as the highest education level completed and was classified into two categories as year 12 or below (including trade certificate) and diploma or degree for all primary analyses. Occupation was scored according to the "Daniel Scale of Prestige of Occupation in Australia" [159]. A higher score indicates lower skilled occupation, which is generally associated with lower socio-economic status. Maternal iron status at birth and smoking

during pregnancy were attained from the AMBIT. IDA in pregnancy was classified as haemoglobin<110g/L and serum ferritin< 12ug/l, and ID was classified as ferritin < 12ug/l according to the CDC criteria [92].

3) Characteristics of the children:

Information on birth order and gestational age at birth were extracted from the AMBIT data set collected from the medical record. Length of breastfeeding was collected in this follow-up study when the children were 4 years old as well as in the AMBIT when the children were six months of age. The data collected at the 4-year follow-up was used in all analyses because the data collected at 6 months did not provide information on breastfeeding beyond 6 months of age. Length of breastfeeding was reported by the mothers and was defined as the total duration of any breastfeeding. Iron status of the children was assessed as part of the follow-up study at 6 months and at 4 years of age (see Chapter 4).

5.2.3 Data analysis

1. Primary analyses

Primary comparisons were based on intention to treat for all outcome measures. Independent sample t-tests were used to compare IQ, SDQ scores, weight, height and head circumference between the iron and the placebo group. SDQ scores were not normally distributed and were not normalised with common transformations. Therefore, Mann-Whitney U tests were performed to compare the SDQ scores between groups in addition to the t-tests. Percentage of children with IQ below 1SD or below 2SD, maternal smoking

during pregnancy, parental education levels between the iron and the placebo groups were compared using chi-square tests.

2. Secondary analyses /Linear regression

Linear regression analyses were conducted to examine potential predictors of IQ while the intention to treat analyses showed no effect of iron supplementation on IQ of the children. Potential predictors included in the regression analyses were chosen based on one of the two criteria: either they have been shown to influence child development in the literature or they were correlated with IQ score in simple regression analyses with a p-value < 0.2.

3. Exploratory analyses

When results of the regression analyses indicated that maternal haemoglobin at birth had a negative association with IQ of the children, exploratory analyses were undertaken with iron status of mothers at birth as the explanatory variables for IQ and SDQ. The aims were to investigate how maternal iron status at the lower end of the spectrum (IDA/ID) and relatively high haemoglobin (>130g/l) at the other end of the spectrum influence IQ of the children. The cut off of 130g/l for high haemoglobin level was used because it has been suggested that a haemoglobin level above 130g/l in pregnancy was associated with adverse pregnancy outcomes [100]. To investigate the effect of maternal IDA/ID at birth on IQ of the children, two groups of the children were created based on whether their mothers had IDA/ID at birth, and t-tests were used to compare mean IQ between the groups. To examine the effect of relatively high haemoglobin in pregnancy on IQ of the children, the

children were grouped according to maternal haemoglobin at birth above or below (included equal to) 130g/l.

Exploratory analyses were also conducted with the children's iron status at 6 months and at 4 years as the explanatory variables because IDA in children has been linked to lower IQ and abnormal behaviours in observational studies [10, 11, 160]. The grouping variable in each of the exploratory analysis was IDA/ID at 6months of age or at 4years of age.

It has been shown that anaemic children were fearful and less exploratory in play situations [10] and some researchers have suggested that these behaviours may contribute to the lower IQ/DQ in their developmental assessment. Therefore, exploratory analyses were undertaken to examine the association between iron status and SDQ score, and between SDQ score and IQ of the children.

The objectives of the exploratory analyses were:

- to compare IQ and SDQ score between children whose mothers had IDA/ID at birth and those who did not.
- to compare IQ between children whose mothers had haemoglobin at birth above or below 130g/l.
- to compare IQ and SDQ score between children who had IDA/ID at 6 months and those who did not.
- to compare IQ and SDQ score between children who had IDA/ID at 4 years and those who did not.
- to compare IQ between children who had an abnormal SDQ score and those who did not.

5.3 Results

The baseline characteristics of the families were not different between those who participated in the follow up study and those who did not, except that the percentage of maternal smoking during pregnancy was higher in the non-participating families (see Table 5.2).

The mean age of the children in the study was 4.2 ± 0.2 years (3.9 – 5.3 years) and half of the children were male (150/302, 50%). Ninety-three percent (280/302) of the children were assessed before 4.5 years of age and 2% (6/302) were over 5 years of age at assessment. The demographic characteristics of the children between the iron and the placebo group did not differ, except that children of the placebo group had lower HSQ score compared with the iron group (see Table 5.3). This difference, although statistically significant, was small in magnitude (MD:1.14, 95% CI: 0.18, 2.10).

Table 5.2 The baseline characteristics of the families between participants and non-participants in the follow-up study[a]

	Participants (302)	Non-participants (133)	P-value
Age of the mothers (years)	28.9 ± 5.0	29.1 ± 5.0	0.66
No. of children in the families	1.8 ± 0.9	1.9 ± 1.0	0.57
Mother's education level			0.56
Year12 or below	235/302 (78%)	102/127 (80%)	
Diploma or Degree	67/302 (22%)	25/127 (20%)	
Father's education level			0.72
Year12 or below	226/291 (78%)	92/121 (76%)	
Diploma or degree	65/291 (22%)	29/121 (24%)	
Mother smoked in pregnancy	50/299 (17%)	35/129 (27%)	0.01

a : Data are mean & SD (n) or number (%) unless stated otherwise

Table 5.3 Demographic characteristics of the children between the iron and placebo groups in the follow-up study[a].

	Iron (153)	Placebo (149)	P-value
Age of the mothers (years)	28.41 ± 4.74	28.40 ± 5.10	0.98
Mother's education level			
Year 12 or below	116/153 (76%)	119/149 (80%)	0.39
Diploma or Degree	37/153 (24%)	30/149 (20%)	
Father's education level			
Year 12 or below	121/147 (82%)	105/144 (73%)	0.05
Diploma or degree	26/147 (18%)	39/144 (27%)	
Mother's occupation score[b]	4.86 ± 0.82	4.83 ± 0.87	0.73
Father's occupation score[b]	4.59 ± 1.05	4.58 ± 1.17	0.94
Mother smoked in pregnancy	25/153 (16%)	25/149 (17%)	0.91
HSQ score[c]	45 ± 4	44 ± 4	0.02
Age of the children at assessment (years)	4.2 ± 0.2	4.2 ± 0.2	0.12
Gender of the children: Male	80/153 (52%)	70/149 (47%)	0.35
Birth order of the children			0.78
1st	75/153 (49%)	68/149 (46%)	
2nd	55/153 (36%)	55/149 (37%)	
≥ 3rd	23/153 (15%)	26/149 (17%)	
Gestation age at birth (weeks)	39.5 ± 1.4	39.4 ± 1.7	0.74
Length of breastfeeding (weeks)	32.1 ± 35.2	27.1 ± 29.0	0.18
% of breastfeed	125/153 (82%)	128/149 (86%)	0.32
% of breastfeed ≥ 6 months	73/153 (48%)	67/149 (45%)	0.63

a : Data are mean & SD (n) or number (%) unless stated otherwise.
b: Based on the scale published by Daniel: "privilege and prestige: occupations in Australia". The higher the score, the lower the skilled occupation.
c: Score from the Home Screening Questionnaire [157]

5.3.1 Growth

There were no differences in mean weight, height and head circumference between the iron and placebo groups (see Table 5.4). Adjustment for gender and age of the children at assessment using ANCOVA showed that neither gender nor age of the children at assessment had a significant effect on the weight of the children ($F=2.47$, $p=0.11$ for gender, $F=0.193$, $p=0.66$ for age, Table 5.5). In contrast, the age of the children at assessment had a significant effect on the height of the children ($F=9.33$, $P<0.01$, Table 5.5) and the gender of the children also had a significant effect on the head circumference of the children ($F=23.08$, $P<0.01$, Table 5.5). Although statistically significant, the effect of age on height ($Eta^2 =0.03$, indicate age explained only 3.1% of variance in height) or gender on head circumference ($Eta^2=0.07$, gender explained 7.5% of variance in head circumference) were both small (see Table 5.5). After adjusting for age of the children at assessment and the gender of the children, the mothers' group in AMBIT had no effect on weight, height or head circumference of the children (see Table 5.6).

Table 5.4 Weight, height & head circumference of the children between the iron and placebo groups[a]

	Iron (n=148)	Placebo (n=144)	MD (95%CI)[b]	P-value
Age at assessment (years)	4.2 ± 0.2	4.1 ± 0.2	0.03 (-0.02, 0.08)	0.19
Gender: male	78/148 (53%)	67/144 (47%)	n/a	0.29
Weight (kg)	18.0 ± 2.4	17.9 ± 2.5	0.13 (-0.44, 0.70)	0.65
Height (cm)	105.0 ± 4.5	104.6 ± 3.6	0.36 (-0.62, 1.33)	0.47
Head circumference (cm)	51.0 ± 1.4	51.0 ± 1.4	0.06 (-0.27, 0.39)	0.71

a: Data are mean ± SD, or number (%) unless otherwise specify

b: Mean difference with 95% confidence interval

Table 5.5 Effect of mothers' group in the AMBIT, gender and age of the children at assessment on weight, height and head circumference of the children at the 4-year follow up: summary of ANCOVA

Independent	**Weight**			**Height**			**Head Circumference**		
variables	**F**	**P**	**Eta^2**	**F**	**P**	**Eta^2**	**F**	**P**	**Eta^2**
Age	0.19	0.66	0.001	9.33	<0.01	0.031	0.09	0.75	0
Mothers' group	0.11	0.73	0.000	0.18	0.66	0.001	0.01	0.94	0
Gender	2.47	0.11	0.009	0.80	0.37	0.003	23.08	<0.01	0.075

Eta^2: Indicates effect size of the independent variables (age, gender of the children and mothers' group in AMBIT) on the dependent variable (weight, height & head circumference).

Table 5.6 Adjusted weight, height & head circumference of the children between the iron and placebo groups after adjusting for the age of the children: results from the ANCOVA

Growth	**Iron (n=148)[a]**	**Placebo (n=144) [a]**
Weight (kg)	18.0 ± 0.2	17.9 ± 0.2
Height (cm)	104.9 ± 0.3	104.7 ± 0.3
Head circumference (cm)	51.0 ± 0.1	51.0 ± 0.1

a: Results are mean ± SE, and evaluated at age of the children=4.15 years in the model

5.3.2 IQ (Intelligence Quotient)

The composite IQ scores were 109 ± 11 for both the iron and placebo groups. There were no differences in the mean composite IQ score or any of the subscales of IQ between the two groups (see Table 5.7). The percentage of the children with IQ below 1SD or below 2SD between the iron and placebo groups did not differ (see Table 5.7).

Table 5.7 IQ of the children between the iron and placebo groups[a]

IQ scores	Iron (n=153)	Placebo (n=149)	MD/RR (95% CI)[b]	P-value
Composite	109 ± 11	109 ± 11	-0.03 (-2.47, 2.40)	0.98
Verbal reasoning	109 ± 12	109 ± 11	-0.22 (-2.88, 2.44)	0.86
Visual reasoning	103 ± 10	104 ± 11	-0.64 (-3.00, 1.72)	0.59
Quantitative reasoning	114 ± 12	114 ± 12	-0.37 (-3.09, 2.35)	0.79
Short-term memory	104 ± 12	104 ± 12	0.64 (-2.15, 3.44)	0.65
Composite IQ < 1 SD[c]	19/153 (12%)	20/149 (13%)	0.91 (0.47, 1.79)	0.79
Composite IQ < 2 SD	4/153 (3%)	3/149 (2%)	1.3 (0.29, 5.94)	0.72

a: data are mean ± SD, or number (%)

b: mean difference or relative risk with 95% confidence interval

c: included children with IQ< 2SD

Potential predictors examined in the regression analyses including maternal age, parental education levels, birth order and gender of the children, gestation age at birth, length of breastfeeding and HSQ score. Among them gender and birth order of the children, parental education levels and HSQ score were all associated with IQ of the children, whereas

maternal age, gestational age at birth and length of breastfeeding were not associated with IQ of the children. The unadjusted regression coefficient (simple regression between IQ and a single predictor) and the adjusted regression coefficient (multivariate regression adjusting for the other predictors) are presented in Table 5.8. Children who were the firstborn child in a family had 5 IQ points higher compared with those who were third or subsequent born child. Compared with boys, the adjusted regression coefficient for girls was 3.33 ±1.25 (SE) ($P<0.01$, Table 5.8). This indicates girls had 3.33 ±1.25 points higher IQ than boys. As expected education levels of both parents were significantly associated with IQ of the children in simple regression models. However, after adjusting for other predictors the association between the mothers' education level and IQ of the children was no longer statistically significant. Children whose fathers had a diploma or higher degree had 4 points higher IQ compared with children whose fathers did not complete year 12 (the adjusted coefficient was 3.93 ± 1.83, $P=0.03$, Table 5.8). HSQ score was positively correlated with IQ of the children and for every point increase in HSQ score, there was an increase of 0.6 IQ score (the adjusted coefficient was 0.58 ± 0.15, $P<0.01$, Table 5.8). After adjustment for the other predictors listed above, the mothers' group assignment in the AMBIT had no effect on the IQ of the children (compared with placebo, the adjusted coefficient for iron was -0.70 ± 1.25, $P=0.57$, Table 5.8).

Table 5.8 Estimated regression coefficient in IQ point of the children for potential predictors

	Simple regression (ignoring other variables)			Multivariate regression (adjusting for other variables)		
Variable (referent category)	**Coefficient±SE**	**Pr(>|t|)**	**Pr(>F)**	**Coefficient±SE**	**Pr(>|t|)**	**Pr(>F)**
Gender (male)			0.02			<0.01
female	2.72±1.23	0.02		3.33±1.25	<0.01*	
Birth-rank (1)			0.02			0.03
2	-1.86±1.35	0.16		-1.42±1.38	0.30	
3+	-4.70±1.76	<0.01		-4.85±1.90	0.01*	
Gestational age (<36 wks)			0.69			0.92
36-38wks	5.12±3.85	0.18		2.44±3.80	0.52	
39wks	5.38±3.70	0.14		2.97±3.65	0.41	
40wks	5.05±3.62	0.16		2.23±3.56	0.53	
41wks	6.10±3.63	0.09		3.58±3.61	0.32	
>41wks	5.96±3.74	0.11		2.78±3.70	0.45	
Breast feeding (none)			0.57			0.91
1 to 5 wks	1.75±2.57	0.49		1.44±2.60	0.58	
6 to 15 wks	0.21±2.10	0.91		-0.73±2.13	0.73	
16 to 35 wks	1.48±2.06	0.47		-0.66±2.10	0.75	

Table 5.8 Estimated regression coefficient in IQ point of the children for potential predictors (con't)

>36 wks	2.64±1.89	0.16		0.12±1.98	0.95	
Maternal age (<22)			0.24			0.07
22 to 25	-3.89±2.58	0.13		-4.21±2.62	0.11	
26 to 29	-0.68±2.43	0.78		-0.92±2.48	0.71	
30 to 35	-0.11±2.48	0.96		1.16±2.61	0.65	
>35	-1.96±2.98	0.51		-2.85±3.11	0.35	
Mother's education (<Yr12)			<0.01			0.09
Yr 12 &TAFE	0.49±1.39	0.72		-1.47±1.49	0.32	
Diploma+	4.98±1.61	<0.01		2.10±1.81	0.24	
Father's education (<Yr12)			<0.01			0.16
Yr 12 & TAFE	3.58±1.45	0.01		2.22±1.55	0.15	
Diploma+	5.40±1.64	<0.01		3.93±1.83	0.03*	
missing	-1.30±3.37	0.70		-0.57±3.60	0.87	
Home Screening score	0.70±0.14	<0.01	<0.01	0.58±0.15	<0.01	<0.01
Treatment (Placebo)			0.97			0.57
Iron	-0.03±1.24	0.97		-0.70±1.25	0.57	

5.3.3 Behaviour

The median (interquartile range) for total SDQ score of children in the study was 9 (6, 13). There were no differences in the median total SDQ score or any of the five subscales of SDQ scores between children in the iron group and those in the placebo group regardless of whether parametric or non-parametric tests were used (see Table 5.9).

The percentages of children with SDQ scores in the abnormal ranges for all 5 subscales did not differ between the iron and placebo groups. However, the percentage of children with a total SDQ score in the abnormal range was higher in the iron group compared with the placebo group (16% vs 8%, P=0.03, see Table 5.10). Similar findings were confirmed when the SDQ scores were classified into high risk for behaviour problems based on the extreme 10% of the children in the study. However, when the SDQ scores were reanalysed into three categories as normal, borderline and abnormal, the differences in the percentage of children in each category between the iron and placebo groups were no longer statistically significant (see Table 5.11).

Table 5.9 The "Strength and Difficulties Questionnaire" (SDQ) scores between the iron and placebo groups[a]

SDQ[b] scores	Iron (n=151)	Placebo (n=149)	P (Mann-Whitney U test)	P-value (t-test)
Total difficulties	9 (6, 13)	9 (6, 12.5)	0.89	0.65
Emotion symptoms	1 (0, 3)	1 (0, 2)	0.50	0.64
Conduct problems	3 (1, 4)	3 (1, 4)	0.78	0.92
Hyperactivity	4 (2, 5)	3 (2, 5)	0.78	0.94
Peer problems	1 (0, 3)	1 (0, 2)	0.08	0.06
Prosocial behaviour	7 (6, 9)	8 (6, 9)	0.53	0.54

a data are median (interquartile range)

b: the Strength and Difficulties Questionnaire [156]

Table 5.10 Percentages of children with SDQ[a] scores above the cutoff for abnormal behaviour or SDQ scores in the high risk of having behaviour problems between the iron and placebo groups[b]

	Abnormal behaviour scores				High risk of behaviour problems			
SDQ scores[a]	**SDQ Cutoff[c]**	**Iron (n=151)**	**Placebo (n=149)**	**P-value**	**SDQ Cutoff[d]**	**Iron (n=151)**	**Placebo (n=149)**	**P-value**
Total dificulties	≥ 17	24/151 (16%)	12/149 (8%)	0.03	≥17	24/151 (16%)	12/149 (8%)	0.03
Emotion	≥ 5	12/151 (8%)	14/149 (9%)	0.65	≥ 4	24/151 (16%)	21/149 14%)	0.66
Conduct	≥ 4	45/151 (30%)	53/148 (36%)	0.28	≥ 5	26/151 (17%)	27/149 (18%)	0.83
Hyperactive	≥ 7	21/151 (14%)	18/149 (12%)	0.63	≥ 7	21/151 (14%)	18/149 (12%)	0.63
Peer problem	≥ 4	23/151 (15%)	14/149 (9%)	0.12	≥ 4	23/151 (15%)	15/149 (10%)	0.17
Prosocial	≤ 4	6/151 (4%)	6/149 (4%)	0.98	≤ 5	24/151 (11%)	21/149 (14%)	0.66

a: Behaviour scores from the Strength and Difficulties Questionnaire [156]

b: Data are number (%)

c: Cutoff scores for abnormal behaviour (see Table 5.1)

d: The high risk group was defined as children with SDQ scores in the top 10 percentile for all scales except for prosocial which was the bottom 10 percentile of the children

Table 5.11 Percentage of children with SDQ[a] score in normal, borderline and abnormal range between the iron and placebo groups[b]

	Normal		Borderline		Abnormal		
SDQ scores[a]	**Iron**	**Placebo**	**Iron**	**Placebo**	**Iron**	**Placebo**	**P-value**
Total difficulties	116/151 (77%)	120/149 (81%)	11/151 (7%)	17/149 (11%)	24/151 (16%)	12/149 (8%)	0.06
Emotion	127/151 (84%)	128/149 (86%)	12/151 (8%)	7/149 (5%)	12/151 (8%)	14/149 (9%)	0.48
Conduct	64/151 (42%)	65/149 (44%)	42/151 (28%)	31/149 (21%)	45/151 (30%)	53/149 (36%)	0.31
Hyperactivity	122/151 (81%)	122/149 (82%)	8/151 (5%)	9/149 (6%)	21/151 (14%)	18/149 (12%)	0.87
Peer problem	109/151 (72%)	123/149 (83%)	19/151 (13%)	11/149 (7%)	23/151 (15%)	15/149 (10%)	0.09
Prosocial	127/151 (84%)	128/149 (86%)	18/151 (12%)	15/149 (10%)	6/151 (4%)	6/149 (4%)	0.87

a: Strength and Difficulties Questionnaire [156]

b: Data are number (%)

Home environment is a factor known to influence child development. Children with normal/borderline SDQ scores had a higher HSQ score compared with children who had an abnormal SDQ score (44.4 ± 4.0, n=261 vs 42.1 ± 5.5, n=35, MD: 2.3, P<0.01, 95% CI: 0.79, 3.74). In addition, the difference in HSQ score between the iron and placebo groups was also statistically significant (see Table 5.3). Therefore, logistic regression analyses were conducted to examine if the higher rate of abnormal total SQD score in the iron group still holds after adjusting for the HSQ score, age, gender and birth order of the children, and parental education levels. The adjusted odds ratio of having abnormal total SDQ score was 2.45 in the iron group compared to the placebo group (see Table 5.12). The HSQ score was a predictor of abnormal total SDQ score with OR of 0.88 (see Table 5.12). Neither age nor gender of the children was a significant factor in the model (see Table 5.12).

Logistic regression analyses to adjust for the effect of HSQ score, age and gender of the children were also conducted for all 5 subscales of SDQ score as outcomes (see Table 5.13). Overall the OR of having abnormal SDQ scores did not differ between the iron and placebo groups for any of the five subscales of SDQ. HSQ score was a predictor for emotional problem (OR: 0.90, P=0.02, Table 5.13), conduct problem (OR: 0.89, P<0.01, Table 5.13) and peer problem (OR: 0.89, P<0.01, Table 5.13). Again, neither gender nor age of the children was a significant factor in predicting abnormal behaviours.

Table 5.12 Odds ratio of having abnormal total SDQ score for children in the iron group after adjusting for age, gender and HSQ score: results of logistic regression

	OR (95% CI)[a]	P-value
Mother's group in AMBIT: iron	2.45 (1.15, 5.41)	0.02
Age	0.84 (0.14, 5.01)	0.85
Gender: female	0.56 (0.27, 1.19)	0.13
HSQ score[b]	0.88 (0.82, 0.95)	<0.01

a: Odds ratio (95% confidence interval)

b: Score of Home Screening Questionnaire [157]

Table 5.13 The adjusted odds ratios of having abnormal SDQ score in five subscales for children in the iron group: results of logistic regression

	Emotion		**Conduct**		**hyperactive**		**Peer problem**		**Prosocial**	
Variable (referent category)	**OR (95% CI)** [a]	**P-value**	**OR (95% CI)** [a]	**P-value**	**OR (95% CI)** [a]	**P**	**OR (95% CI)** [a]	**P**	**OR (95% CI)** [a]	**P**
Mother's group (placebo): iron	0.89 (0.38, 2.06)	0.78	0.85 (0.51, 1.34)	0.51	1.26 (0.63, 2.53)	0.50	2.02 (0.96, 4.25)	0.064	0.97 (0.30, 3.16)	0.965
Age	4.05 (0.94, 17.38)	0.06	1.03 (0.34, 3.16)	0.95	0.72 (0.13, 3.90)	0.70	0.16 (0.01, 1.86)	0.144	0.58 (0.3, 12.09)	0.726
Gender (male): **female**	0.49 (0.21, 1.17)	0.10	0.92 (0.56, 1.51)	0.73	0.52 (0.26, 1.05)	0.06	0.59 (0.28, 1.23)	0.161	0.48 (0.14, 1.65)	0.245
HSQ score [b]	0.90 (0.83, 0.99)	0.02	0.89 (0.84, 0.95)	<0.01	0.94 (0.87, 1.01)	0.09	0.89 (0.82, 0.96)	0.002	1.0 (0.87, 1.15)	0.995

a: Odds ratio (95% confidence interval)

b: Score of home screening questionnaire [157]

5.3.4 Influences of Maternal iron status on IQ and behaviour of the children

Exploratory analysis on the IQ of children between anaemic and non-anaemic mothers showed that children born to anaemic mothers (at birth) had a higher IQ than children born to non-anaemic mothers (113 ±12 and 108 ±11 respectively, p=0.03) despite a lower mean HSQ scores and less skilled occupations of the anaemic mothers (see Table 5.14). Therefore, multivariate regression analyses were conducted to explore the relationship between maternal haemoglobin at birth and IQ of the children at 4 years. Maternal haemoglobin at birth was negatively associated with IQ of the children (Fig 5.2). The unadjusted coefficient in IQ point for haemoglobin concentration (g/l) at delivery was –0.062, P=0.29. The adjusted coefficient was -1.13 ± 0.54, P=0.038 after adjusting for gestational age at birth, gender and birth order of the children, parental education levels and the Home Screening Questionnaire scores. There was no difference in IQ of the children between mothers with or without ID at birth (109 ± 12, n=123 vs 109 ± 10, n=155, P=0.78, 95% CI: -2.95, 2.24), or mothers with haemoglobin above or below 130g/l at birth (108 ± 10, n=88 vs 109 ± 11, n=202, P=0.41, 95% CI: -3.83, 1.57).

Also there were no differences in SDQ score or percentages of children with abnormal SDQ score between children of anaemic and non-anaemic mothers, or between children whose mothers had ID at birth and those who did not, or between children whose mothers had haemoglobin above or below 130g/l (Table 5.15).

Table 5.14 Demographic characteristics and IQ of the children between anaemic mothers (at birth) and non-anaemic mothers[a]

	Maternal anaemia at birth			
	Yes (25)	**No (265)**	**MD (95% CI)[b]**	**P**
Age of the mothers in the RCT (years)	27.4 ± 5.0	29.1 ± 4.9	-1.67 (-3.68, 0.33)	0.101
Mother's education level				0.91
< Yr 12	10/25 (40.0%)	97/265 (36.6%)	n/a	
Yr12 or trade certificate	10/25 (40.0%)	106/265 (40.0%)		
Diploma or degree	5/25 (20.0%)	62/265 (23.4%)		
Father's education level				0.74
< Yr 12	9/23 (39.1%)	80/255 (31.4%)	n/a	
Yr12 or trade certificate	9/23 (39.1%)	110/255 (43.1%)		
Diploma or degree	5/23 (21.7%)	65/255 (25.5%)		
Mother's occupation score	5.2 ± 0.7	4.8 ± 0.8	0.361 (0.16, 0.71)	0.04
Father's occupation score	4.6 ± 1.2	4.6 ± 1.1	0.06 (-0.40, 5.3)	0.78
HSQ score	42.6 ± 3.9	44.3 ± 4.3	-1.64 (-3.38, 0.10)	0.06
Mother smoked in pregnancy	5/25 (20.0%)	42/265 (15.8%)	n/a	0.57
Mother's haemoglobin at birth (g/l)	100 ± 11 (25)	127 ± 10 (265)		<0.01
Mother's serum ferritin at birth (ug/l)[c]	8.5 ×/÷ 1.7 (22)	14.5 ×/÷ 2.0 (253)		<0.01
Age of the children (years)	4.11 ± 0.12	4.18 ± 0.24	-0.07 (-0.17, 0.28)	0.16
Gender of the children: Male	12/25 (48%)	132/265 (50%)	n/a	0.86
Birth order of the children				0.80
1st	13/25 (52%)	124/265 (47%)	n/a	
2nd	9/25 (36%)	97/265 (37%)		
≥ 3rd	3/25 (12%)	44/265 (16%)		
Gestation age at birth (weeks)	39.4 ± 1.5	39.5 ± 1.6	-0.74 (-0.72, 0.57)	0.82
Length of breastfeeding (weeks)	24.4 ± 30.0	30.1 ± 32.5	-5.72 (-19.03, 7.59)	0.39
IQ : Composite	113 ± 12	109 ± 11	4.71(0.31, 9.10)	0.03
Verbal	112 ± 12	108 ± 12	3.98 (-0.80, 8.8)	0.10
Visual	107 ± 14	103 ± 10	3.21(-1.04, 7.47)	0.13
Quantitative	118 ± 13	114 ± 12	4.33 (-.060, 9.27)	0.08
Short-term memory	108 ± 12	104 ± 12	4.12 (-0.89, 9.13)	0.10

a : Data are mean ± SD or number (%) unless otherwise stated.
b: Mean difference & 95% confidence interval
c: Ferritin values are geometric mean & SD multiplier.

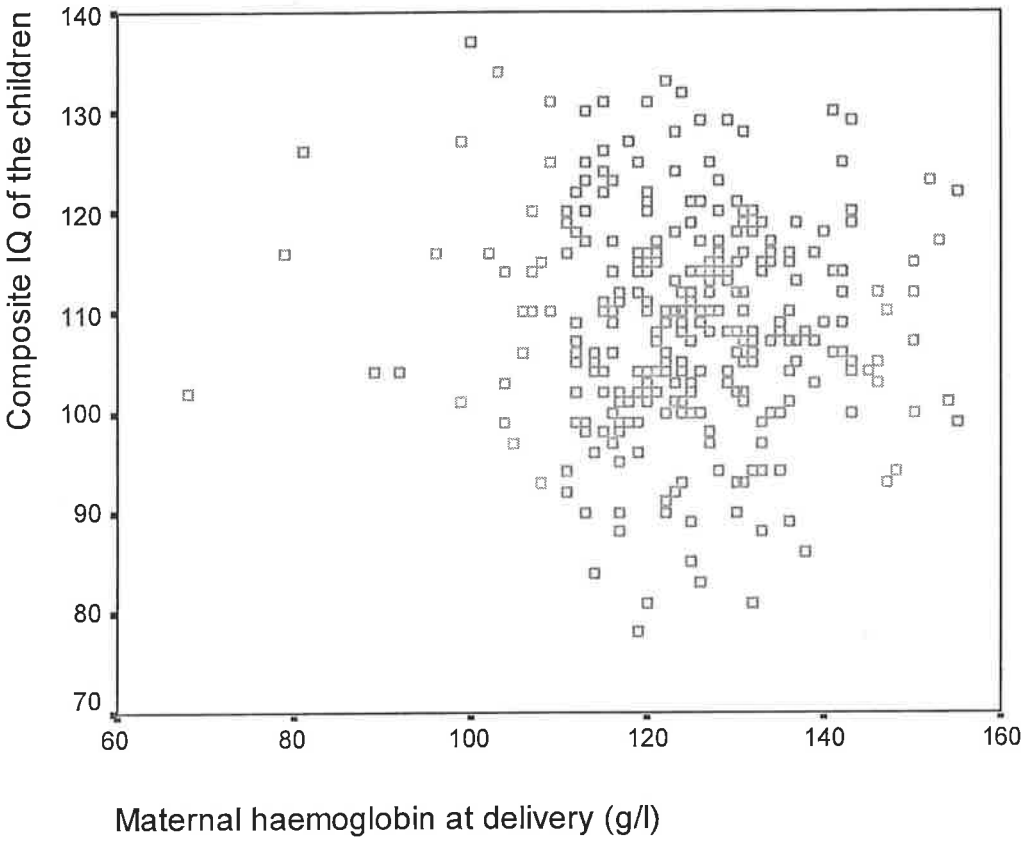


Figure 5.2 The correlation between maternal haemoglobin at delivery and IQ of the children at 4 years

Table 5.15 Comparison of the total SDQ[a] score and percentage of children with abnormal total SDQ score in relation to maternal iron status at birth

SDQ score[a]	**Maternal anaemic at birth (haemoglobin< 110g/L)**			**Maternal ID[b] at birth (ferritin<12ug/L)**			**Maternal haemoglobin at birth >130g/l**		
	Yes (n=25)	No (n=263)	P	Yes (n=132)	No (n=154)	P	Yes (n=88)	No (n=200)	P
Mean rank[c]	166	142	0.17	134	141	0.43	137	147	0.34
% of abnormal SDQ[d]	2/25 (8%)	33/263 (13%)	0.50	16/122 (13%)	19/154 (12%)	0.84	11/88 (13%)	24/200 (12%)	0.90

a: The total difficulties score from the Strength & Difficulties Questionnaire [152]

b: Iron deficiency

c: Results of Mann-Whitney tests

d: Results of Chi-square tests

5.3.5 Iron status of the children and IQ and behaviour score at 4 years of age

There were no differences in composite IQ score or total SDQ scores between children with or without ID at 6 months (see Table 5.16). Children without ID at 4 years had higher SDQ scores compared with those who had ID (11± 5 vs. 8± 6, MD: 2.88 (0.69, 5.06, P=0.01, Table 5.16) though the mean SDQ scores were within the normal range for both groups. No children had IDA at 6 months of age and only three children had IDA at 4 years assessment. Meaningful statistical comparison of IQ between children with or without IDA was not possible. When children were grouped into four groups based on quartiles of haemoglobin or serum ferritin levels at 6 months or 4 years of age, there were no differences in IQ or SDQ scores between various iron status at either 6 months or 4 years (Table 5.17) except the SDQ score between various ferritin levels at 4 years (Table 5.18).

There was no difference in the percentage of children with abnormal SDQ scores between children who had ID at 6months and those who did not (2/14, 14% vs 32/249, 13%, P=0.86). At 4 year follow-up, of the 208 children who had their serum ferritin measured, 26 of them had an abnormal SDQ scores. None of the 26 children had ID at the 4-year assessment and only 2 of them had ID at 6 months.

Table 5.16 Comparison of composite IQ and total SDQ[a] scores between children with ID[b] and those without ID at 6 months or 4 years.

	ID at 6months			ID at 4years		
	Yes	**No**	**P**	**Yes**	**No**	**P**
	(n=14)	**(n=250)**		**(n=24)**	**(n=184)**	
Mean IQ	107 ± 9	109 ± 11	0.47	109 ± 11	109 ± 11	0.56
Mean SDQ	9 ± 4	10 ± 5	0.38	8 ± 6	11± 5	0.01
Mean rank SDQ[c]	119	133	0.52	75	108	0.01

a: The total difficulties score from the Strength and Difficulties Questionnaire [152]

b: Iron deficiency

c: Mann-Whitney tests

Table 5.17 Mean composite IQ of the children according to quartiles of haemoglobin and ferritin

	1st quartile	2nd quartile	3rd quartile	4th quartile	P-value
Iron status of children at 6 months					
Haemoglobin[1]	108 ± 10 (n=79)	108 ± 10 (n=62)	112 ± 12 (n=66)	108 ± 12 (n=60)	0.09
Ferritin[2]	109 ± 11 (n=72)	109 ± 11 (n=60)	108 ± 11 (n=64)	111 ± 11 (n=68)	0.24
Iron status of children at 4 years					
Haemoglobin[3]	108 ± 11 (n=59)	110 ± 11 (n=52)	108 ± 10 (n=51)	109 ± 12 (n=50)	0.68
Ferritin[4]	107 ± 10 (n=59)	109 ± 10 (n=46)	111 ± 10 (n=54)	108 ± 12 (n=50)	0.21

1. quartile values of haemoglobin at 6 months (g/l): 110 ± 5; 117 ± 2; 123 ± 1; 132 ± 6.
2. quartiles values of ferritin (geometric mean ×/÷ SD multiplier) at 6 months (ug/l): 15 ×/÷ 5; 26 ×/÷ 3 ; 40 ×/÷ 6; 83 ×/÷ 38
3. quartile values of haemoglobin at 4 years (g/l): 114 ± 5; 121 ± 1; 125 ±1; 132 ±3
4. quartile values of ferritin (geometric mean ×/÷ SD multiplier) at 4 years (ug/l): 10 ×/÷ 3; 16 ×/÷1; 22 ×/÷ 2; 39 ×/÷ 13

Table 5.18 Mean total SDQ[a] scores of the children according to quartiles of haemoglobin and ferritin

	1st quartile	2nd quartile	3rd quartile	4th quartile	P-value
Iron status of children at 6 months					
Haemoglobin	10 ± 5 (n=78)	10 ± 6 (n=62)	10 ± 5 (n=66)	10 ± 6 (n=60	0.93
Ferritin	10 ± 5 (n=72)	10 ± 6 (n=60)	10 ± 6 (n=63)	9 ± 5 (n=68)	0.61
Iron status of children at 4 years					
Haemoglobin	11 ± 6 (n=59)	11 ± 5 (51)	10 ± 5 (n=51)	10 ± 5 (n=50)	0.51
Ferritin	8 ± 5 (n=59)	12 ± 5 (n=45)	10 ± 5 (n=54)	11 ± 5 (n=50)	0.003[b]

a: Behaviour difficulty scores from the Strength and Difficulties Questionnaire [152]

b: The mean SDQ scores were significant different between the1st quartile & the 2nd quartile (p=0.008), and between the1st quartile & the 4th quartile (p=0.018), but no differences in the mean SDQ scores between other group comparisons.

5.3.6 Behaviour score and IQ of the children

Total SDQ scores were negatively correlated with IQ of the children (Spearman's correlation coefficient r=-0.237, P<0.01, Fig 5.3). Children with total SDQ scores in the abnormal range had lower IQ and higher percentage with IQ below 1SD compared with children whose SDQ scores were in the normal range (see Table 5.19). Similar results were found when SDQ score was reclassified as normal or borderline/ abnormal range. Compared to children with a normal SDQ score, the OR of having IQ below 1SD were 1.9 ± 1.0 (SE) (P=0.22, 95% CI: 0.67, 5.54) for children with a borderline SDQ score, and 3.6 ± 1.5 (SE) (P<0.01, 95% CI: 1.52, 8.27) for children with an abnormal SDQ score. Inclusion of the SDQ score as a categorical variable in the regression model of IQ and confounders (described above in Table 5.8) did not change the outcome that the mothers' group in ABMIT had no effect on IQ of the children.

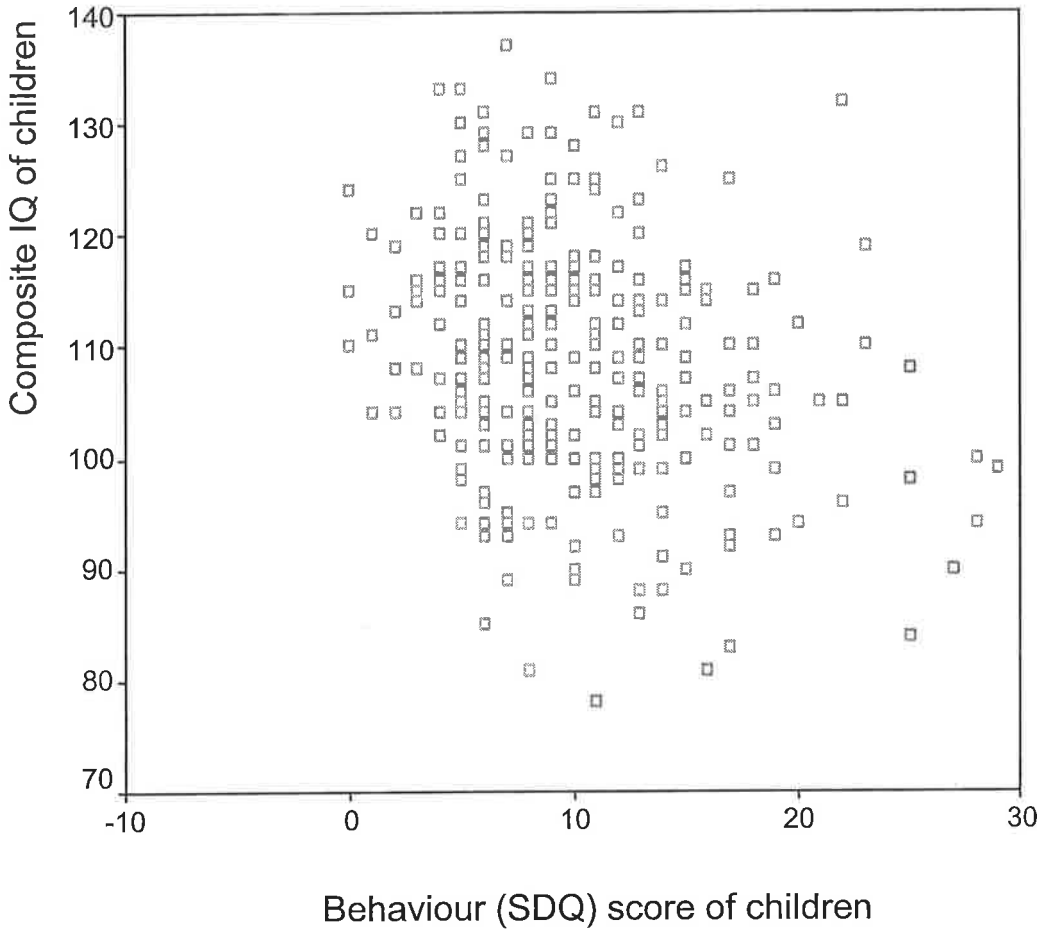


Figure 5.3 The relationship between behaviour score (SDQ) and IQ score of the children

Table 5.19 IQ scores between children with normal/borderline and abnormal behaviour according to the SDQ score[a]

IQ scores	Normal/ borderline (n=264)	Abnormal (n=36)	MD/ RR (95% CI)[b]	P-value
Composite	110 ± 11	104 ± 11	6.09 (2.39, 9.80)	<0.01
Verbal reasoning	109 ± 11	102 ± 13	7.00 (2.96, 11.04)	<0.01
Visual reasoning	104 ± 10	100 ± 9	3.90 (0.30, 7.50)	0.03
Quantitative reasoning	114 ± 12	110 ± 12	4.88 (0.73, 9.04)	0.02
Short-term memory	105 ± 12	100 ± 12	4.82 (0.54, 9.10)	0.02
Composite IQ< 1SD	29/264 (11%)	10/36 (28%)	0.39 (0.21, 0.74)	0.01[c]
Composite IQ< 2SD	5/264 (2%)	2/36 (6%)	0.34 (0.07, 1.69)	0.20[c]

a: Data are mean ± SD, or number (%)

b: Mean difference or relative risk (95% confidence interval)

c: P-value of Fisher's exact tests

5.4 Discussion

This study has shown that iron supplementation in pregnancy has no beneficial effect on the IQ, behaviour and growth of the children at 4 years of age in this study population. It has also raised a concern on whether routine iron supplementation in pregnancy in well-nourished women has an adverse effect on behaviour of the children. To the best of my knowledge, this is the first human intervention trial that was specifically designed to prospectively evaluate the effect of iron supplementation in pregnancy on long term child development. The unique feature of this study is the long term follow-up of a double blinded randomised controlled trial.

This result is in contrast to the evidence from animal studies which show deficiency in iron during pregnancy results in biochemical, structural and functional changes in the brain of offspring which are not fully reversible with latter iron repletion [47, 49, 50, 61]. The absence of the beneficial effect of perinatal iron supplements on IQ of the children in our study may be explained by the severity of ID during pregnancy. In the animal studies, ID induced in the experimental animals was more severe with extreme dietary iron restriction. The differences in iron intake between experimental animals and control animals are often 10 to 20 fold, and in some cases the experimental animals were totally deprived of dietary iron. In contrast IDA in our placebo women was mild and an adequate iron supply to the developing fetus may have been achieved. The dose of supplemental iron used in the AMBIT was low (20mg/day) compared with most common iron supplements used in pregnancy. However, the 3 fold difference in iron intake between the iron and the placebo mothers in the AMBIT [89] resulted in a change of maternal haemoglobin and ferritin that were similar to other iron supplementation trials with high doses iron of around 100mg/day

[30]. Therefore it is unlikely that higher doses of iron supplements in pregnancy would benefit child development. Furthermore, child development is a result of complex interaction between genetic potential and environmental enrichment [161]. A positive child-rearing environment may overshadow any small effect of perinatal iron supplementation on child development whether it is beneficial or detrimental. This was clearly highlighted from the regression analyses that higher parental education levels and higher HSQ score, which generally indicate better socio-economic status, were significantly associated with higher IQ score of the children. Other studies have consistently demonstrated the influences of environmental factors on child development.

Although there are no existing studies that examine the relationship between maternal iron status in pregnancy and child development, Tamura and co-workers have examined the relationship between cord ferritin levels and IQ of the children at 5 years of age [130]. In a study that was designed to investigate the developmental outcomes of the children born small for gestational age [130], they found that children with cord ferritin levels in the lowest quartile scored lower on some mental and psychomotor tests than children with cord ferritin levels in the 2 middle quartiles. However, children with cord ferritin in the highest quartile also scored lower on the tests. The authors suggested that the children with cord ferritin in the highest quartile might have falsely elevated ferritin level due to possible maternal infections. It is not possible to directly compare my study results with the results of Tamura's study as maternal iron status was not reported in their study.

Interestingly, children born to anaemic mothers had a higher IQ scores compared with children of non-anaemic mothers despite a less favourable environment as indicated by lower HSQ score and less skilled occupation of the anaemic mothers. The higher IQ in the

children born to anaemic mothers in my study and the non-linear relationship between cord ferritin levels and IQ of the children in Tamura's study should be interpreted with great caution as there are many possible confounders of child development. However, these results may suggest that there may be a range of maternal haemoglobin levels in pregnancy that are optimal for the developing fetus. This view is supported by several large cohort studies [98-101] that showed a U-shaped relationship between maternal haemoglobin levels and pregnancy outcomes. Maternal haemoglobin levels at both low and high ends were associated with adverse pregnancy outcomes. Interestingly, the haemoglobin levels associated with optimal pregnancy outcomes were below the current cutoff for the diagnosis of anaemia in pregnancy in some of these studies.

The lack of correlation between iron status and IQ of the children in my study may be due to the low rate of ID among the children in this study. They were relatively well-nourished and IDA was rare. There is a well established link between IDA in infancy and poor cognitive development [69, 70]. I chose to follow up the children at 4 years of age rather than in infancy when IDA is more prevalent because it has been suggested that IQ in older children correlates well with school achievements and later achievements in adults than developmental assessment results early in infancy.

The mean IQ of the children in my study was slightly higher than the American norm, where the standardisation of the Stanford – Binet Intelligent Test score is based. This is not uncommon as a validation study in Australian population has shown that Australian children have higher IQ than the American norm using this assessment tool particularly at a younger age [153]. This does not affect the validity of the study as all children were assessed in the same way with the same assessment tool, and the outcomes of interest was

a group mean score rather than an individual score. Furthermore, a recent American study conducted on over 200 four years old urban children also showed a higher mean IQ than the standardised mean and their mean IQ was strikingly similar to my result (John Colombo, 2004, unpublished data from personal communication). The higher IQ in girls compared with boys is consistent with the literature that girls perform better in cognitive tests at younger ages, but the difference no longer exist when they are teenagers.

The mean SDQ scores are similar to a validation study [154] among 1359 Australian children (802 of them were aged between 4-6 years) except that the score for conduct behaviour was slightly higher. The assessment of conduct behavioural problems related to whether a child often has temper tantrums, fights with other children or is disobedient. The higher score for conduct behaviour in my study may reflect the difference in the perception of the problem between the parents in this study and the parents in the validation study from different states within Australia. However, the higher risk for abnormal behaviour in the iron group even after adjusting for confounders was unexpected. Animal studies have shown that iron deficiency in young rats leads to increased anxiety-like behaviour and decreased the ability to navigate [56, 61]. Iron deficiency in children has also been linked with alteration in behaviours including anxiety and melancholy and less attentive to instruction [8, 10]. However, there is no data on the effects of perinatal iron nutrition and behaviour of the children later in life. Furthermore, behaviour was a secondary outcome of my study and the results need to be interpreted with caution. It may due to chance as there was only a small number of children with abnormal scores. It is also possible that excess iron during the perinatal period may be detrimental. Whether routine iron supplementation in pregnancy in industrialised countries has any adverse effects on the behaviour of the children requires further investigation.

Although families who did not participate in the follow up study had higher rate of maternal smoking during pregnancy, there was no difference in the rate of maternal smoking or any other baseline characteristics between the iron and placebo groups in the follow-up. Therefore it is unlikely that the results would bias towards the null hypothesis. However, results from my study may not be generalised to other populations where iron deficiency and anaemia are more prevalent and more severe, or where malnutrition is common.

5.5 Conclusion

This study showed that routine iron supplementation in pregnancy in otherwise well-nourished women has no beneficial effects on the long term growth and development of the children. Further research is needed in well-nourished women in industrialised countries.

Chapter 6. Effect of iron supplementation in pregnancy on long term health of women

6.1 Introduction

Routine iron supplementation in pregnancy is a strategy adopted in many countries to prevent ID and IDA in pregnant women, a common problem in both the developing and developed world. While routine iron supplementation has proved effective in preventing ID and IDA in pregnancy [30], the functional consequences of preventing ID and IDA in pregnancy are not well understood. Whether routine iron supplementation in pregnancy influences the long term health of women is largely unknown. The aims of this study were to examine the effect of iron supplementation in pregnancy on the long term health of women and outcomes of subsequent pregnancies.

6.2 Subjects and Recruitment

Women who participated in the randomised controlled trial of iron supplementation in pregnancy (AMBIT) during 1997 – 1999 [88] were assessed during 2002-2003 in this follow-up study. Details for the recruitment process see Chapter 3.5.

6.3 Methods

The general health of the women since completion of the AMBIT was assessed using the MOS 36-items Short-Form Health Survey (SF-36) [162]. The SF-36 questionnaire is a

standardised tool for assessing quality of life and it has been validated internationally including Australia. The questionnaire was posted to the women to complete and was returned to the researcher when they attended an appointment for their children's developmental assessment (see Chapter 3.5). The self-completed SF-36 assesses eight areas of health including:

1. Physical Functioning: assesses limitation in physical activities due to health.
2. Role Functioning – Physical: assesses the impacts of physical health on work and other daily activities.
3. Role Functioning – Emotion: assesses the impacts of any emotional problem on work and other daily activities.
4. Social Functioning: assesses impacts of physical health or emotional problems on normal social activities.
5. Bodily Pain: assesses severity of any pain and their impacts on usual work and home duty.
6. Mental Health: assesses general mental health and emotional control.
7. Vitality: assesses energy levels and level of fatigue.
8. General Health Perception: perception of one's own health.

Scores for all 8 areas are expressed on a scale of 0-100, a higher score indicates a better state of health or well-being. High scores in the areas of physical functioning, role functioning-physical and emotion, social functioning and pain indicate the absence of limitations or disabilities. These scores are typically higher than scores for the areas of general health, vitality & mental health, where a high score indicates positive well-being (for these 3 aspects, a score in the mid-range indicates that a person has reported no

limitations or disabilities). The results of SF-36 were scored and the raw scores were transformed according to the SF-36 Scoring Rules [163].

Additional information on non-pregnancy related health problems or hospital admissions since the completion of the AMBIT were also collected. This information was collected via a structured interview with open-ended questions. Health problem is defined as any non-pregnancy related problems detected since the completion of the AMBIT that required medical treatment, where consultation with health care professionals and subsequent treatment were required. Hospital admission was defined as any hospital admissions that required overnight stay due to non-pregnancy related medical conditions both pre-existing and diagnosed after the completion of the AMBIT. If tubal ligation was the sole reason for hospital admission, it was not counted as an admission. Specific information about whether iron deficiency anaemia had been diagnosed since the completion of the AMBIT was also collected by self-report.

If the women had any subsequent pregnancies since the AMBIT, information on outcomes of all subsequent pregnancies was collected from their medical records or their children's health booklets issued by the hospitals where the children were born. This included gestational age at birth, mode of delivery, birth weight, length, head circumference, Apgar score at 5 minutes, and any neonatal complications. Women were also asked if they took any iron supplements or multivitamins containing iron during any subsequent pregnancies and if they had IDA in any subsequent pregnancies.

Data analyses were performed using SPSS (SPSS, version 10.0, Chicago, IL, USA). Primary analyses were based on intention to treat, which is to compare all outcome

variables between the iron supplemented and placebo groups as they were originally randomised in the AMBIT. Independent sample t-tests were used to compare the SF-36 scores, the number of all subsequent pregnancies, gestational age at birth, birth weight, length, head circumference and Apgar score of all subsequent pregnancies between the iron and placebo groups. Health problems and hospital admissions, IDA in any subsequent pregnancies were compared using Chi-square tests.

6.4 Results

Of the 430 women randomised in the AMBIT, 300 of them provided information on outcomes of subsequent pregnancies and 299 completed the SF-36 (see Fig 6.1).

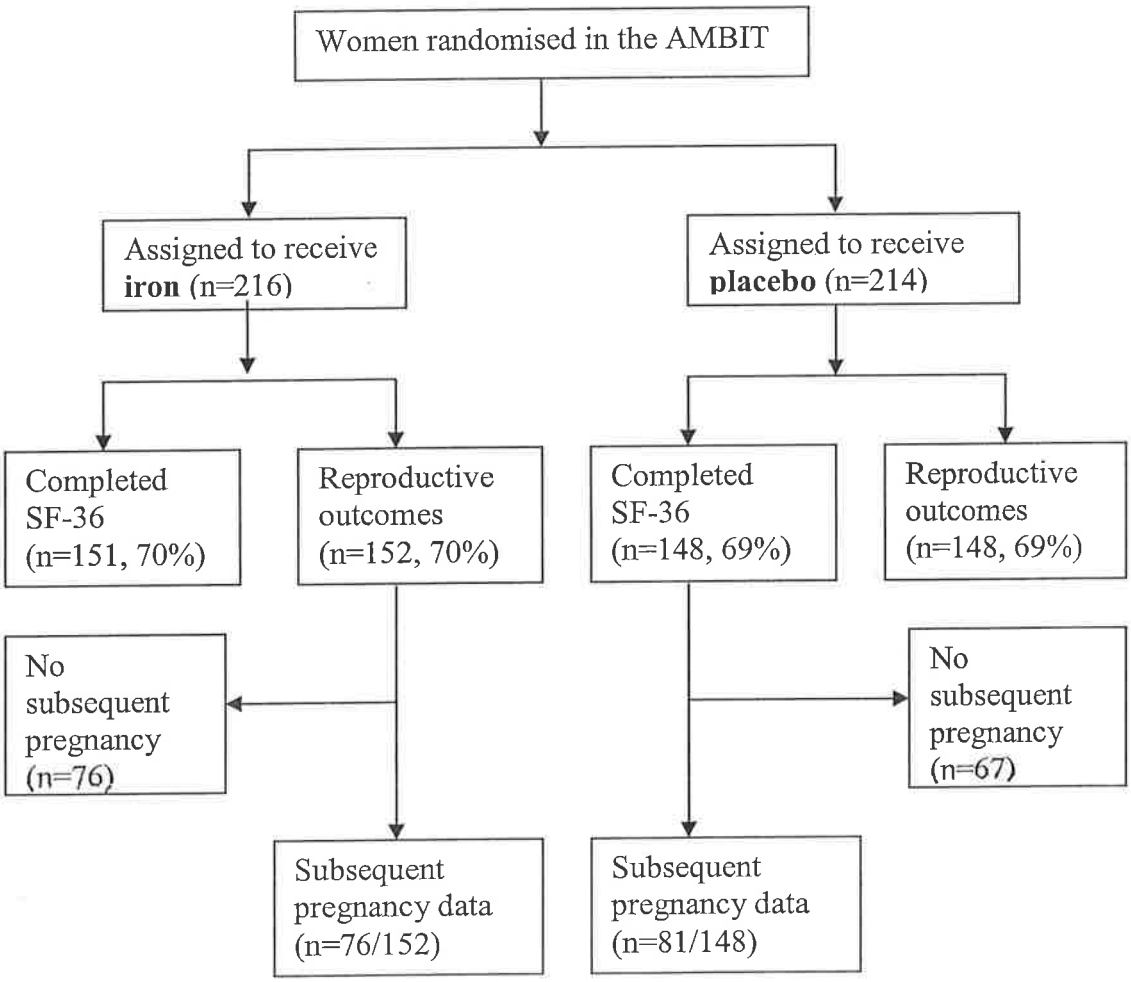


Figure 6.1 Participants flow diagram: follow-up of women

The mean age of women at follow up was 33.0 ± 4.8 years. The baseline characteristics between women who participated and those who did not were similar in terms of age, education level, occupation score and number of previous births. However, women who did not participate had higher rates of smoking during pregnancy and higher mean haemoglobin at entry to the AMBIT (see Table 6.1). There was one death in the iron group during the follow up period (occurred more than 3 years after the AMBIT). The cause of the death is unknown, as the family did not wish to participate in the follow up study. Therefore I did not have permission to seek further information from appropriate government agencies.

Table 6.1 The baseline characteristics of women between participants and non-participants

	Participants (299)	**Non-participants (131)**	**P-value**
Age (years)	28.8 ± 5.0	28.6 ± 5.3	0.63
No. of previous births	0.8 ± 0.9	0.9 ± 1.0	0.56
Education			
Year 12 or below	233/299 (78%)	101/125 (81%)	0.50
Diploma or Degree	66/299 (22%)	24/125 (19%)	
Occupation scores	4.9 ± 0.9	5.1 ± 0.8	0.11
Smoke in pregnancy	50/299 (17%)	34/127 (27%)	0.01
Hb (g/l)	129 ± 8	131 ± 8	0.01
MCV (fl)	89 ± 4	89 ± 4	0.88

There were no differences in the demographic characteristics of the women between the iron and placebo groups at the follow up (see Table 6.2).

Table 6.2 The demographic characteristics of the women in the follow up study between the iron and placebo groups

	Iron (151)	**Placebo (148)**	**P-value**
Age (years)	33.0 ± 4.8	33.0 ± 5.1	0.96
≤ 30	43/151 (28%)	45/148 (30%)	
30 - 40	93/151 (61%)	88/148 (59%)	
> 40	15/151 (10%)	15/148 (10%)	
Number of children	1.7 ± 0.9	1.8 ± 1.0	0.65
1	73/1531(48%)	70/148 (47%)	
2	54/151 (36%)	49/148 (33%)	
≥3	24/151 (16%)	29/148 (20%)	
Education			0.45
Year 12 or below	115/151 (76%)	118/148 (80%)	
Diploma or Degree	36/151 (24%)	30/148 (20%)	
Occupation scores	5.0 ± 0.8	4.9 ± 0.9	0.68
Smoke in pregnancy	25/151 (17%)	25/148 (17%)	0.93

6.4.1 General health

The percentage of women who had been diagnosed with IDA (not pregnancy related) by their doctors since the completion of the AMBIT was 3.6% (11/298). There were no differences in the percentage of women who had IDA, health problems requiring medical treatment or hospital admissions since the completion of the AMBIT between the iron and placebo groups (see Table 6.3). The health problems were summarised into three categories: 1) reproductive / gynaecological problem: included abnormal PAP smear, cervical cancer, ovular cyst and endometriosis; 2) mental health problem: included anxiety, depression and break down; 3) general medical problem: included arthritis, diabetes, ulcerative colitis, chronic fatigue, celiac, hypertension, infectious disease, back pain and injury.

Table 6.3 Percentage of IDA[a], health problems and hospital admissions occurring since the completion of the AMBIT between the iron and placebo groups

	Iron	Placebo	P-value
IDA[a]	7/151 (5%)	4/147 (3%)	0.39
Hospital admissions	22/151 (15 %)	26/147 (18%)	0.49
Reproductive/Gynaecological	6/22 (27%)	7/26 (27%)	
Non-reproductive surgery/injury	3/22 (14%)	7/26 (27%)	
General medical	13/22 (59%)	12/26 (46%)	
Health problems	18/151 (13%)	21/147 (14%)	0.75
Reproductive/Gynaecological[b]	6/18 (33%)	5/21 (24%)	
Mental[c]	3/18 (17%)	5/21 (24%)	
General medical[d]	9/18 (50%)	11/21 (50%)	

a: iron deficiency anaemia

b: including: abnormal PAP smear, cervical cancer, ovular cyst and endometriosis.

c: including: anxiety, depression and break down.

d: including: arthritis, diabetes, ulcerative colitis, chronic fatigue, celiac, hypertension, pneumonia, kidney infection, back pain and injury.

6.4.2 SF-36

There were no differences in either raw or transformed scores between the iron and placebo groups in any of the 8 areas of health assessed (Table 6.4 & Table 6.5). The scores for women in this study were comparable to the population norm from the 1995 National Health Survey (see Table 6.5). In the areas of physical functioning, role functioning physical, bodily pain and general health perception, the scores were relatively higher for women in this study compared to the population norm (Table 6.5 & Fig 6.2), which indicated better health status in these areas. However, the effect size were all less than 0.5 of the standard deviation of National Health Survey, and it is not considered clinical significant [164].

Table 6.4 The mean raw scores on eight health concepts of SF-36 between the iron and placebo groups

Areas of health	**Iron (n=151)**	**Placebo (n=148)**	**P-value[a]**
Physical functioning	29.0 ± 3.4	29.0 ± 3.1	0.83
Role functioning-physical	8.0 ± 1.3	8.0 ± 1.2	0.97
Role functioning-emotion	6.0 ± 0.9	6.0 ± 0.9	0.63
Social functioning	10.0 ± 1.6	10.0 ± 1.5	0.93
Bodily pain	11.4 ± 2.1	10.5 ± 1.9	0.89
Mental health	24.0 ± 3.5	25.0 ± 3.6	0.61
Vitality	17.0 ± 3.9	16.0 ± 3.8	0.18
General health perceptions	21.4 ± 3.6	21.4 ± 3.7	0.40

a: P-value of Mann-Whitney U tests for mean rank between the iron and placebo groups

Table 6.5 The transformed scores on eight health concepts of SF-36 between the iron and placebo groups with reference to 1995 National Health Survey (NHS) [165]

	Iron (n=151)	**Placebo (n=148)**	**All (n=299)**	**NHS (n=9,000)[b]**	**Effect size[a]**
Physical functioning	88.4 ± 17.0	88.9 ± 15.4	88.7 ± 16.2	81.1 ± 24.3	0.31
Role functioning-physical	82.1 ±33.5	84.0 ± 29.9	83.0 ± 31.7	78.8 ± 36.0	0.12
Role functioning-emotion	85.0 ± 30.5	83.6 ± 31.5	84.3 ± 30.9	81.6 ± 33.6	0.08
Social functioning	84.5 ± 20.5	85.6 ± 18.6	85.1 ± 19.6	84.1 ± 22.9	0.04
Bodily pain	82.1 ± 20.5	82.7 ± 18.9	82.4 ± 19.7	75.7 ± 25.4	0.26
Mental health	76.0 ± 14.2	76.5 ± 14.1	76.0 ± 14.2	74.6 ± 17.3	0.08
Vitality	59.2 ± 19.1	60.6 ± 19.3	59.2 ± 19.1	62.5 ± 20.1	0.16
General health perceptions	77.9 ± 18.2	78.8 ± 17.9	77.9 ± 18.2	72.0 ± 20.3	0.29

a: effect size = |mean score of all participants – mean score of NHS| / SD of NHS
b: mean and SD were extracted from Table 2 of 1995 National Health Survey: SF-36 Population Norms [165]

The SF-36 scores of the women in this study (4-year follow-up) were also significantly correlated with the SF-36 scores at 6 months post-partum assessment [89] for all areas of health (see Table 6.6). The Spearman's correlation coefficients ranged from r=0.132 for role functioning-emotion to r=0.613 for general health perceptions (see Table 6.6). There was a trend towards decreasing in SF-36 scores at the 4-year follow up compared with scores at 6 months post-partum (see Fig 6.2.). However, the decreases did not reach statistical significant except for mental health (75.9 ± 14.2 vs. 78.5 ± 15.8, MD: -2.52, 95% CI: -4.41, -0.62, P=0.01) and general health perception (77.9 ± 18.0 vs. 81.7 ± 17.2, MD: -3.75, 95% CI: -5.58, -1.91, P<0.01).

Table 6.6 Spearman's correlation coefficient between SF-36 scores at the 4-year follow-up and at the 6-month follow-up

	Correlation coefficient	P
Physical functioning	0.467	<0.01
Role functioning-physical	0.329	<0.01
Role functioning-emotion	0.132	0.02
Social functioning	0.296	<0.01
Bodily pain	0.391	<0.01
Mental health	0.430	<0.01
Vitality	0.465	<0.01
General health perceptions	0.613	<0.01

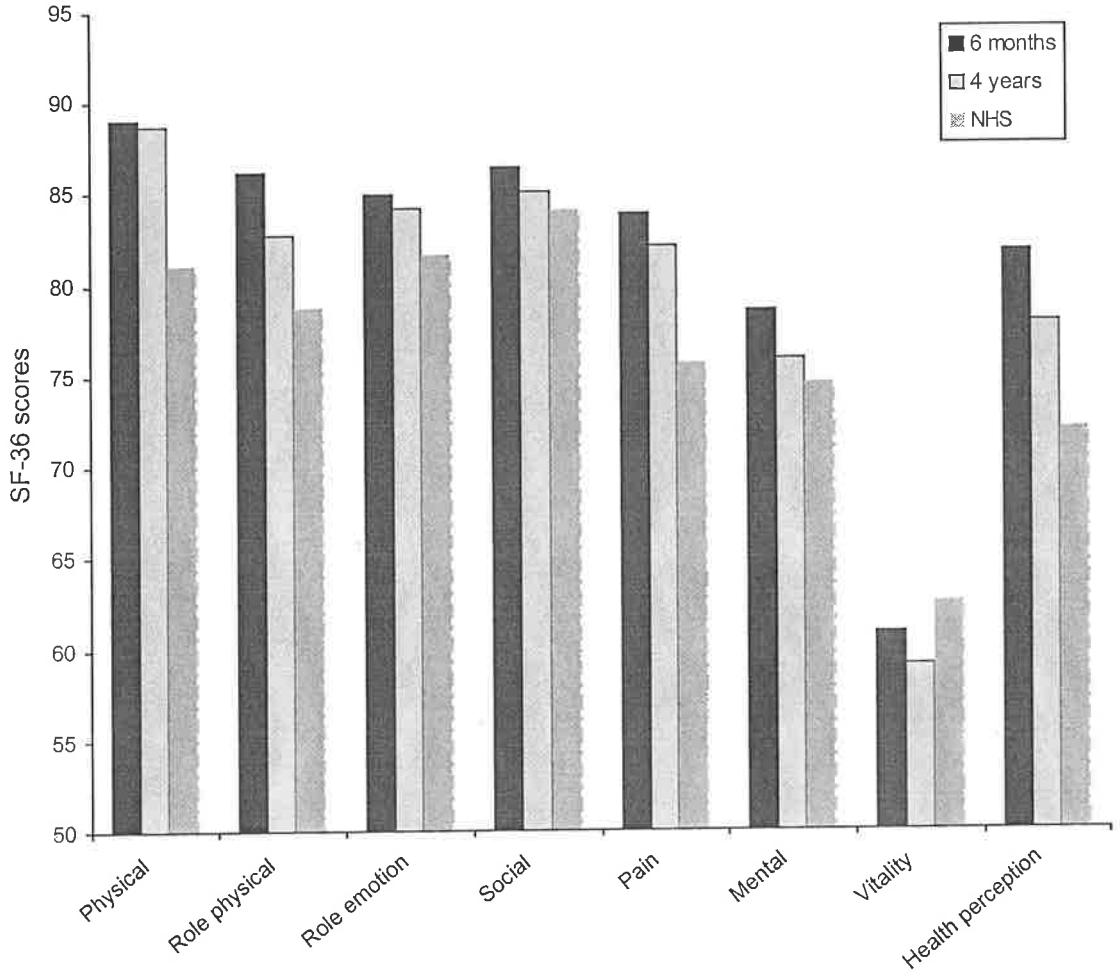


Figure 6.2 The SF-36 scores of the women at 6 months post-partum[89] and at the 4-year follow-up compared with results of 1995 National Health Survey (NHS)[165]

6.4.3 Pregnancy outcomes

There were no differences in the percentage of women who had one subsequent pregnancy (76/152, 50% vs. 81/148, 55%, P=0.69), or two subsequent pregnancies (18/152, 12% vs. 13/148, 9%, P=0.26) between the iron and placebo groups (see Table 6.7). No differences in the outcomes of the first or second subsequent pregnancy were found (see Table 6.7 & Table 6.8). There were only four women in the study who had a third subsequent pregnancy, two in each group. In the iron group, one pregnancy resulted in full term birth, the other pregnancy was terminated by choice. In the placebo group, both pregnancies resulted in miscarriage. There were no differences in the percentage of women who had IDA in subsequent pregnancies between the iron and placebo groups (see Table 6.8).

Table 6.7 Outcomes of subsequent pregnancies between the iron and placebo groups

Pregnancy outcomes	**Iron**	**Placebo**	**P**
1st subsequent pregnancy	N=76	N=81	0.69
Live birth	64/76 (84%)	68/81 (84%)	
Miscarriage	6/76 (8%)[a]	6/81 (7%)	
Abortion	5/76 (7%)	4/81 (5%)	
Still birth	1/76 (1%)	1/81 (1%)	
Were pregnant at assessment	0/76 (0%)	2/81 (2%)	
2nd subsequent pregnancy	N=18	N=13	0.26
Live birth	9/18 (50%)	10/13 (77%)	
Miscarriage	1/18 (6%)	1/13 (8%)	
Abortion	4/18 (22%)	0/18 (0%)	
Still birth	0/18 (0%)	0/18 (0%)	
Were pregnant at assessment	4/18 (22%)	2/13 (15%)	

a: included one atopic pregnancy

Table 6.8 Birth details of subsequent pregnancies (live births) between the iron and placebo groups

	1st subsequent pregnancy			2nd subsequent pregnancy		
	Iron (n=64)	**Placebo (n=68)**	**P**	**Iron (n=9)**	**Placebo (n=10)**	**P**
Gestation age (weeks)	39.5±1.4	39.5±1.7	1.00	39.4±1.7	39.6±1.3	0.82
Weight (g)	3585±497	3624±621	0.69	3426±688	3481±457	0.83
Length (cm)	50.7±2.3	50.8±3.1	0.79	50.4±3.7	50.4±2.1	0.98
Head circumference (cm)	34.9±1.5	34.8±2.0	0.76	34.2±2.0	34.3±1.0	0.91
Mode of delivery			0.57			0.75
NVD[a]	45/64 (70%)	53/68 (78%)		6/9 (67%)	8/10 (80%)	
Instruments	3/64 (5%)	3/68 (4%)		2/9 (22%)	1/10 (10%)	
Caesarean	16/64 (25%)	12/68 (18%)		1/9 (11%)	1/10 (10%)	
Gestation <37 weeks	1/64 (2%)	0/68 (1%)	0.96	1/9 (11%)	0/10 (0%)	0.27
Birth weight<2500g	1/64 (2%)	2/68 (3%)	0.30	1/9 (11%)	0/10 90%)	0.30
% of IDA[b] in pregnancy	7/64 (11%)	6/68 (9%)	0.68	4/9 (44%)	2/10 (20%)	0.25
% took iron supplements	22/64 (34%)	23/68 (34%)	0.99	5/9 (56%)	5/10 (50%)	0.80
% of neonatal complication	4/64 (6%)	2/68 (3%)	0.37	1/9 (11%)	0/10 (0%)	0.279

a: normal virginal delivery;
b: iron deficiency anaemia

6.5 Discussion

This study found no differences in long term health and outcomes of subsequent pregnancies between women who received routine iron supplements during pregnancy and those who were in the control group in the AMBIT [89].

There are numerous studies investigating the effects of prophylactic iron supplementation in pregnancy over the last decades. Most studies assessed short term effects, which focused on haematological outcomes in late pregnancy and at birth. Few studies assessed clinical outcomes such as pregnancy outcomes [89, 105, 106, 166] and general health and well-being [89, 131]. Furthermore, evidence on the effect of iron supplementation in pregnancy on long-term health of women is scarce. There is only one published study in this area by Hemminki & Co-workers [132].

Hemminki's study compared the morbidity and mortality of mothers and their children between women who received routine iron supplementation in pregnancy with those who received iron supplementation only if they had become anaemic during pregnancy. The dose of supplemental iron used in Hemminki's study was 100mg per day in contrast to the 20mg used in the AMBIT [89]. Despite the differences in the study designs, both studies suggest that routine iron supplementation in pregnancy has no long term health benefits for women compared with no iron supplementation [89] or selective iron supplementation [131] in otherwise well-nourished pregnant women.

The general health of the women in my study as indicated by the SF-36 scores was comparable to the population survey [165]. The lower proportion of smoking among women

who participated in the follow up study compared with those who did not participate may indicate that the women in this follow-up study were more health conscious and had healthier lifestyle habits than the general population. This may contribute to the slightly better health status in some areas of the SF-36 assessment for the women in this study compared with the population data.

Although there is a lack of population data on the prevalence of IDA among child-bearing age women in Australia, the 3.6 % of IDA among women in this study is what would be expected in a well nourished population. The establishment of the recommended dietary intake for a specific nutrient in Australia is set to prevent the nutritional deficiency in 97% of a population for the nutrient in question. The actual rate of IDA among women in this study might be underestimated as symptoms associated with mild IDA such as fatigue and tiredness are usually attributed to work and stress, and tend to be overlooked by both health care professionals and patients. The rate of hospital admissions (16%) among women in this study was similar to Hemminki's study (14%) [132].

The percentage of women who had at least one subsequent pregnancy is also similar to Hemminki's study (approximately 50%). The percentage of normal virginal delivery (NVD) for subsequent pregnancies was higher in our study compared with the population statistics [167]. However, the population data included pregnancy outcomes of all births, both nulliparous and multiparous, while all women in this study had at least one previous birth. Therefore it is not surprising that more women had normal virginal delivery in subsequent pregnancies. The mean birth weights of 1st and 2nd subsequent pregnancies in this study were also higher than the population statistics as a result of differences in data collection.

This study included only live births in analyses while the population data included stillbirth with birth weight at least 400g or gestational age greater than 20 weeks.

One of the perceived benefits of routine iron supplementation in pregnancy is to replenish iron stores of the women and to prevent IDA in subsequent pregnancy. However, there were no differences between the iron and placebo groups in the rate of IDA in either 1st or 2nd subsequent pregnancy. However, there were only a small number of women who had more than one subsequent pregnancy in this study. Therefore, this result may not be generalised to the general population.

Although more women who smoked during pregnancy did not participate in the follow up phase, there were no differences in the percentage of drop out or percentage of smoker between the iron and placebo groups. In addition, those women not included in the follow up had a higher mean haemoglobin level at baseline and it is unlikely they were at higher risk of developing ID/IDA. Therefore, the differences in the baseline characteristics of women between those who participated in the follow up phase and those who did not are unlikely to have significantly affected the results.

6.6 Conclusion

Routine iron supplementation in pregnancy has no long term effect on the general health and reproductive outcomes of women in otherwise well nourished populations.

Chapter 7. General discussion

Routine iron supplementation in pregnancy has been advocated as a strategy to prevent ID and IDA in pregnancy over the last three decades, and it is a common practice in many industrialised countries. However, systematic reviews of iron supplementation in pregnancy as part of the Cochrane Collaboration [30] and the US Preventive Services Task Force [29] have both concluded that there is a lack of evidence on the effect of iron supplementation in pregnancy on clinical outcomes for the mother, fetus and the newborn, and that further research designed to assessed clinical outcomes in relation to iron supplementation in pregnancy is needed. In particular, the effect of iron supplementation in pregnancy on long-term growth and cognitive development of the children was highlighted as an area of priority for research by the US Preventive Services Task Force [134], whereas the Cochrane review commented on the need to determine whether routine iron supplementation in pregnancy causes harm in well nourished women in industrialised countries [30] . The Policy statement from the US Preventive Services Task Force was that there was insufficient evidence to recommend for or against routine iron supplementation in pregnancy [134]. Despite that, the practice of routine iron supplementation in pregnancy is still widespread as it is generally considered an effective and inexpensive strategy to prevent ID and IDA in pregnancy without harmful effects.

My thesis addressed the issues highlighted by the US Preventive Services Task Force and the Cochrane review. The study presented in the thesis investigated the effect of routine iron supplementation in pregnancy on IQ, behaviour, growth and iron status of children, and on the general health and subsequent pregnancy outcomes of women. Although there are numerous randomised controlled trials of iron supplementation in pregnancy, major

clinical outcomes other than low birth weight and preterm birth, are rarely assessed and long term follow up data are scarce. My study provides the first evidence on the developmental outcomes of the children in relation to maternal iron supplementation in pregnancy.

My results showed that improved maternal iron nutrition through iron supplementation in pregnancy did not affect the IQ of children with or without adjustment for predictors of IQ. There are many potential confounders that may influence IQ of children. The design of my study, which was a long term follow-up of a double blinded randomised controlled trial, ensured that any influences of confounders on the outcomes were minimised. Although mothers whose children did not participate in the follow-up reported a higher percentage of smoking during pregnancy, there were no differences between groups in the follow-up in terms of the proportion of mothers who smoked or other baseline characteristics of the families. The only difference between the groups was the intervention, which resulted in a significant difference in maternal iron status at the end of pregnancy. The differences in the frequency of ID and IDA at the end of pregnancy between the iron and placebo groups were comparable between all women randomised and women who participated in the follow-up. A post hoc sample size estimate indicated that my study has 95% power to detect a 5 IQ points difference between groups. These points highlighted that my study provides the first high quality evidence that routine iron supplementation in pregnancy in industrialised countries has no beneficial effect on the IQ of children.

My study also showed that children whose mothers received routine iron supplementation in pregnancy had a higher risk of abnormal behaviour compared with children whose mothers received placebo. This finding was unexpected and should be treated with some

caution as there were no other behavioural differences noted between the groups. Nevertheless, there has been increasing concern about the potential risks associated with routine iron supplementation in pregnancy. This includes increased oxidative stress [168, 169] and impaired maternal-placental blood flow as a result of iron-induced macrocytosis and increased haemoglobin concentration [170]. Oxidative stress from iron supplements in pregnancy has the potential to damage the conceptus and increase the risk of congenital defects, preterm delivery and low birth weight in the short term [42]. Abnormal high haemoglobin has also been linked with poor pregnancy outcomes in population studies [100, 171]. Whether routine iron supplementation in pregnancy in well-nourished women has any potential adverse effects on long term child development remains an issue that needs to be addressed. Further follow-up of the AMBIT children will help to determine whether the behavioural difficulties reported by the parents persist when the children are older or whether it was a chance finding.

Also there is concern regarding the potential long term risks of cancer and heart disease associated with oxidative tissue damage as a result of free radical production from excess iron in the body [172, 173]. Assessment of these risks requires longer-term follow-up of the women and this was beyond the scope of my study. Nevertheless, my study showed routine iron supplementation in pregnancy had no effect on the general health and well-being of women, or the percentage of women who had medical problems detected since the completion of the supplementation phase and the category of the problems between groups.

There is increasing recognition that the current recommendations by WHO for iron supplementation in pregnancy [174] and the current cut-off of haemoglobin for diagnosis of

anaemia in pregnancy may be higher than necessary. Iron supplements at doses between 60mg/day and 120mg/day are equivalent to approximately 3mg to 6mg of absorbed iron assuming the lowest absorption rate of 5%, which is much higher than the estimated extra requirement of iron in pregnancy of approximately 1mg/day [141]. The existing goal appears to be based on the maximal haemoglobin concentration that can be achieved with iron supplementation of well-nourished women [174]. It may be more appropriate to establish a range of optimal haemoglobin concentrations to be achieved during pregnancy based on functional outcomes, and to develop functional criteria for the diagnosis of anaemia in pregnancy. It is well recognised that severe IDA has many adverse health consequences and treatment with iron is necessary. However, prevention of the decline in haemoglobin during pregnancy with routine iron supplementation may not be desirable.

Currently in Australia, the draft revision of the RDIs stipulates an estimated average requirement (EAR) for iron in pregnancy at 23mg/day and an RDI of 27mg/day [175]. The EAR is set to meet the requirement of half the healthy individuals while the RDI is set to meet the requirement of 97-98% of healthy individuals in a life stage [175]. However, recommendations for RDI are often made from metabolic and biochemical studies without assessment of long term health outcomes. To the best of my knowledge, the AMBIT [89] and long term follow up of the AMBIT in my study is the first RCT to assess the relationship between iron intake, iron status of pregnant women and the long term health outcomes. The mean iron intake of women in the placebo group in the AMBIT was 14mg/day and 11% of them had IDA at the end of pregnancy using the current diagnostic criteria of WHO [89]. Furthermore, increasing the iron intake of pregnant women to meet the RDI with low dose iron supplements as in the AMBIT had no benefit on long term outcomes assessed in my study. These results suggest that the proposed EAR and RDI for iron in

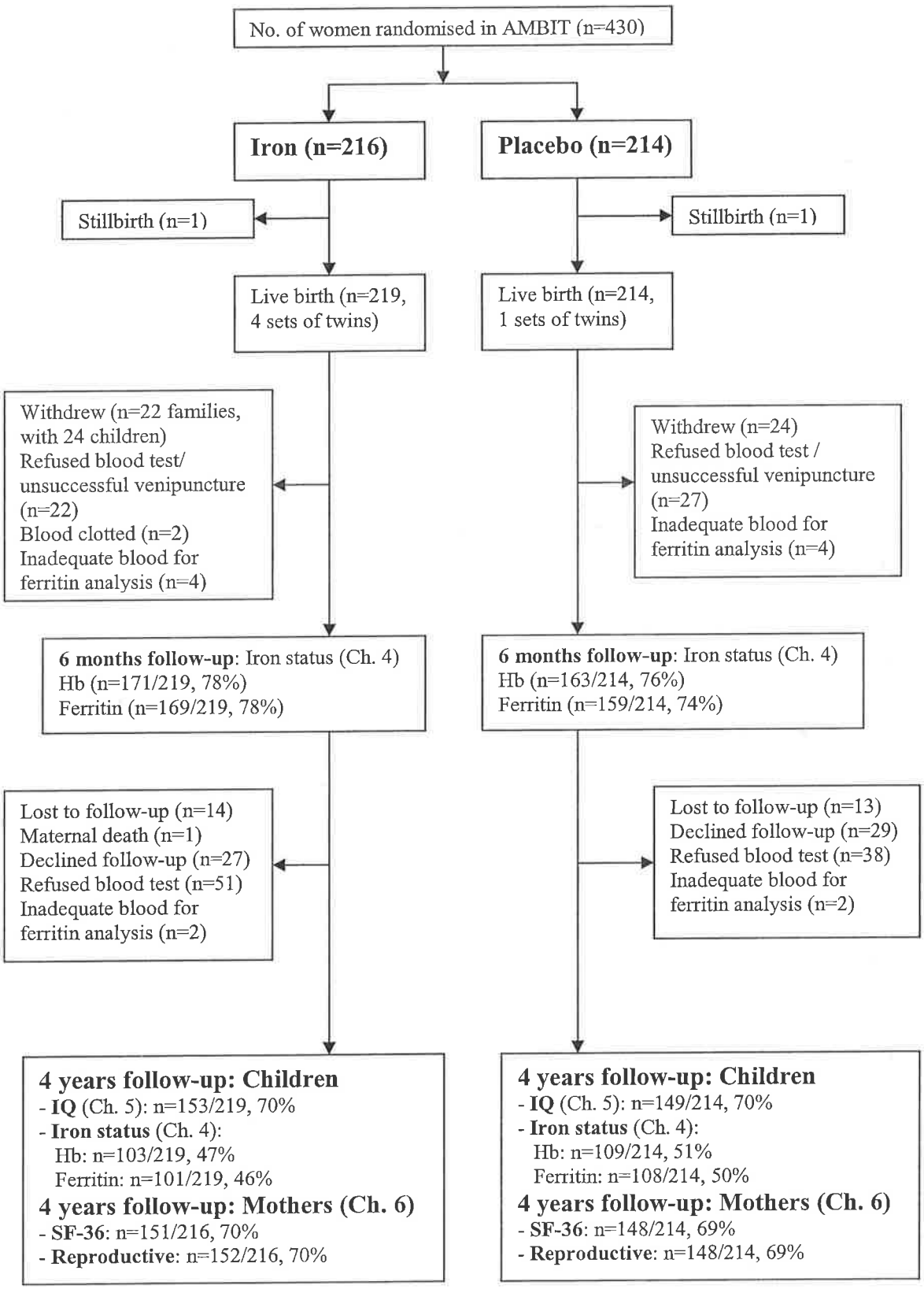
pregnancy may be higher than the actual requirement necessary to affect long term health outcomes.

The finding from my study is likely to be applicable to other industrialised countries as the dietary iron intake of pregnant women in my study is comparable with a nationally representative sample of pregnant women in Australia [176] as well as women in other industrialised countries [177]. The prevalence of IDA in pregnancy in my study population is also similar to pregnant women in other industrialised countries [110]. However, my results may not be generalised to other populations where iron deficiency and anaemia are more prevalent and more severe, or where under nutrition is common.

Conclusion

The existing evidence has failed to show any beneficial effect of routine iron supplementation in pregnancy on growth and development of children, or general health of mothers in industrialised countries while the potential adverse effects on the developing fetus cannot be excluded. Currently, iron overdose from accidental ingestion of iron tablets remains the most common cause of childhood poisoning [135]. From a public health perspective, the lack of benefits in clinical outcomes and the potential risk associated with routine iron supplementation in pregnancy suggest that the risk may outweigh benefits in well-nourished populations, and a re-evaluation of policy and practice in relation to routine iron supplementation in pregnancy is warranted.

Appendix 1. Participants flow chart



Participants flow diagram: follow-up of women and children

Appendix 2 Publication in support of thesis

Publication

Zhou SJ, Schilling MJ, Makrides M. Evaluation of an iron specific checklist for the assessment of dietary iron intake in pregnant and post-partum women. Nutrition (In Press).

Published abstracts

Zhou SJ, Makrides M, Gibson RA, Baghurt P. Effect of iron supplementation in pregnancy on IQ of children at 4 years of age. Asia Pac J Clin Nutr. 2004;13(Suppl):S39 (abstract).

Conference presentations

Zhou SJ, Makrides M, Gibson RA. Effect of iron supplementation in pregnancy on IQ of children at 4 years of age. The Second World Congress of Paediatrics Gastroentrology Hepatology and Nutrition. Paris, July 2004.

Zhou SJ, Makrides M, Gibson RA & Baghurt P. Effect of iron supplementation in pregnancy on IQ of children at 4 years of age. Brisbane, August 2004 (Best student oral presentation award)

Zhou SJ, Makrides M, Gibson RA & Crowther CA . Effect of iron supplementation in pregnancy on the health of mothers and the development of their children: a 4-year follow

up. The 9th Annual Congress of the Perinatal Society of Australia & NZ, Adelaide, March 2005. (Yong investigator award).

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The effects of iron supplementation during pregnancy on childhood growth and development: a long term follow up of the Adelaide Mothers' and Babies' Iron Trial

Information sheet

Why is this study being done?

You are invited to participate in a follow-up study of the Adelaide Mothers' and Babies' Iron Trial (AMBIT). You may recall that the aim of AMBIT was to determine if women would benefit from taking a low dose of iron during pregnancy. Our results showed that women in the iron group were less likely to be iron deficient or have iron deficiency anaemia during pregnancy and more likely to have better iron stores at 6 months after the birth of their babies, compared with women who did not receive iron. We now want to assess if supplementing women during pregnancy has any effect on the longer term growth and development of children.

What does this study involve?

If you agree to participate in this study, we will ask you and your child to attend one appointment only at the Women's and Children's Hospital for about 1-1.5 hours. The appointment will take place within 2-4 weeks of your child's 4th birthday. We will send you two short questionnaires about your general health and that of your child to fill out and bring them with you to the appointment. Each questionnaire takes about 5-10 minutes to complete.

At the appointment, your child's weight, height and head circumference will be measured. Your child will also have a development test called the Stanford-Binet. This involves showing picture cards, repeating sentences, copying and building certain patterns using blocks and beads, and some simple comprehension tasks. It takes approximately 30-60 minutes to complete. Your child will also have a blood test for iron status in which 1 ml of blood will be taken by an experienced nurse. The nurse will insert a fine needle into your child's arm to take the blood sample. Additional information on feeding history, home environment, parent's education and occupation will also be collected. At the end of the appointment, your group allocation in the AMBIT study will be revealed.

If you have become pregnant since participating in AMBIT, we would like to access your medical records to check outcomes of any subsequent pregnancy.

You will receive $15 of reimbursement for car parking or travel costs to the hospital.

Your rights

You are free to withdraw from the study at any time without any explanation of why you have chosen to do so and without prejudice to you and your child's future care or treatment at the hospital.

All information gathered would be treated with confidence and no information that could identify you will be released to any person not associated directly with the study. The results from the study may eventually be published in medical journals or at professional meetings, but you will not be identified in any way.

Any questions?

If at any time you have any queries or questions, please telephone Jo Zhou on 8161 7512. Alternatively you can contact Dr Maria Makrides on 8161 6067.

This study has been approved by the Research Ethics Committee at the WCH. Should you wish to discuss the study with someone not directly involved, in particular in relation to matters concerning policies, information about the conduct of the study or your rights as a participant, or should you wish to make a confidential complaint, you may contact the Executive Secretary of the Research Ethics Committee at the WCH, Ms Brenda Penny on 8161 6521.

WOMEN'S & CHILDREN'S HOSPITAL RESEARCH ETHICS COMMITTEE

CONSENT FORM

I _____

hereby consent to my and my child's involvement in the research project entitled:
The effects of iron supplementation during pregnancy on childhood growth and development: a long term follow up of AMBIT

1. The nature and purpose of the research project described on the attached Information Sheet has been explained to me. I understand it, and agree to taking part.

2. I understand that I and/or my child may not directly benefit by taking part in this study.

3. I acknowledge that the possible risks and/or side effects, discomforts and inconveniences, as outlined in the Information Sheet, have been explained to me.

4. I understand that while information gained in the study may be published, I and my child will not be identified and information will be confidential.

5. I understand that I and my child can withdraw from the study at any stage and that this will not affect medical care or any other aspects of my and my child's relationship with this hospital.

6. I understand that I will receive $15 to cover my transport costs to the hospital for taking part in this study as specified in the Information Sheet.

7. I have had the opportunity to discuss taking part in this research project with a family member or friend and/or have had the opportunity to have a family member or friend present whilst the research project was being explained by the researcher.

8. I am aware that I should retain a copy of the Consent Form, when completed, and the Information Sheet.

9. a) I consent to a specimen of blood being taken from my child, access to my and my child's medical records if required and to complete the health questionnaires in the above project.

b) I do / do not consent to the blood samples being used in any other research project, provided the project has the approval of the Women's & Children's Hospital Research Ethics Committee.

Signed: .....

Relationship to participant(s) .....

Full name of participant (s) .....

Dated:.....

I certify that I have explained the study to the mother and consider that she understands what is involved.

Signed: ..... Title: .....

Dated: .....

AMBIT FOLLOW-UP STUDY

CASE REPORT FORM

Mother's First Name:_____Surname_____

Mother's ID:_____UR:_____

Child's First Name:_____Surname:_____

Child's ID:_____UR:_____

Child's DOB: _ _ / _ _ / _ _ _ _ Child's Gender: M / F

Address: _____

_____

_____P/C_____

Phone: _____(H)

_____(W)

_____(Mobile)

CASE REPORT FORM CHECK LIST

- ❑ Consent form
- ❑ Appointment form for information collected at appt
- ❑ Strength and difficulties questionnaire (SDQ)
- ❑ Home screening questionnaire (HSQ)
- ❑ Mother's health questionnaire (SF-36)
- ❑ Pregnancy outcome questionnaire
- ❑ Stanford – Binet Intelligence Scale record booklet
- ❑ Stanford – Binet results summary form
- ❑ Iron status report

AMBIT follow up Mother's ID_ _ _ _ _ _ Child's ID_ _ _ _ _ _ _ _

INFORMATION COLLECTED AT APPOINTMENT

Date of apponitment: _ _ / _ _ / _ _ _ _

PARENTS INFORMATION

Mother's highest education level completed: _____ Occupation: _____
_____ Scores: _____

Father's highest education level completed: _____ Occupation: _____
_____ Scores: _____

FEEDING INFORMATION

1. Did you breastfed your child? ❑ Yes ❑ No
   If yes, length of BF: _____ weeks
2. Does your child drink cow's milk? ❑ Yes ❑ No
   If yes, on average how much cow's milk does your child drink daily? _____ ml/d

PRESCHOOL/DAYCARE ATTENDENCES

Does your child attend preschool/daycare? ❑ Yes ❑ No
If yes,
a) how many days does your child attend preschool per week? _____ day/week
how many days does your child attend daycare per week? _____ day/week

b) how often does your child miss preschool/daycare because not feeling well?
❑ Never ❑ Almost never ❑ Sometimes ❑ Often

HEALTH

1. Does your child have problem with hearing?
   ❑ Yes ❑ No ❑ Don't know
   If yes, please specify: _____
   _____
   _____
2. Does your child have problem with eyesight?
   ❑ Yes ❑ No ❑ Don't know
   If yes, please specify: _____
   _____
   _____
3. Does your child have any physical problem, which affect his/her normal activities?
   ❑ Yes ❑ No ❑ Don't know
   If yes, please specify: _____
   _____
   _____

ASSESSMENTS AT THE APPOINTMENT

1. Growth assessment completed? ❑ Yes ❑ No
   If yes, Wt:_____kg, Ht:_____cm, HC:_____cm.
   If no, why?_____

2. Stanford – Binet test completed? ❑ Yes ❑ No
   If no, why?_____
   _____

3. Blood sample taken? ❑ Yes ❑ No
   If yes, venipuncture / finger prick
   If no, why?_____

4. Pregnancy outcome questionnaire completed? ❑ Yes ❑ No
   If no, why?_____

FOLLOW-UP REQUIRED? ❑ Yes ❑ No

If yes, specify:_____

OTHER CONTACTS

Name:_____ Relationship to child:_____
Contact address:_____ Phone Numbers
_____ Home:_____
_____P/C_____ Work:_____
Mobile:_____

4th pregnancy: BWt:_____g, Length:_____cm, HC:_____cm, Apgar Score (5min):_____

Complication: ❑ convulsion ❑ neonatal death

❑ Others, specify:_____

7. Did you have anaemia during the pregnancy (pregnancies)?

1st pregnancy since AMBIT	❑ Yes	❑ No
2nd pregnancy since AMBIT	❑ Yes	❑ No
3rd pregnancy since AMBIT	❑ Yes	❑ No
4th pregnancy since AMBIT	❑ Yes	❑ No

8. Did you take iron tablets or multivitamin tablets contain iron during the pregnancy (pregnancies)?

1st pregnancy since AMBIT	❑ Yes	❑ No
2nd pregnancy since AMBIT	❑ Yes	❑ No
3rd pregnancy since AMBIT	❑ Yes	❑ No
4th pregnancy since AMBIT	❑ Yes	❑ No

Section 2: About your general health

1. Have you been diagnosed with anaemia since participating in AMBIT?

❑ Yes ❑ No

If yes, has iron treatment been prescribed?

❑ Yes ❑ No

2. Have you had any illnesses or health problems since participating in AMBIT that required active medical treatment

❑ Yes ❑ No

If yes, what was the illness? Please specify:

_____

_____

3. Have you been admitted to hospital at any time since participating in AMBIT which was not pregnancy related?

❑ Yes ❑ No

If yes, please answer the following questions

(a) which hospital(s) were you admitted? _____

(b) when were you admitted?_____

(c) What was the reason for the admission(s)? _____

4th pregnancy : BWt:_____g, Length:_____cm; HC:_____cm, Apgar Score (5min):_____

Complication: ❑ convulsion ❑ neonatal death

❑ Others, specify:_____

. Did you have anaemia during the pregnancy (pregnancies)?

1st pregnancy since AMBIT	❑ Yes	❑ No
2nd pregnancy since AMBIT	❑ Yes	❑ No
3rd pregnancy since AMBIT	❑ Yes	❑ No
4th pregnancy since AMBIT	❑ Yes	❑ No

. Did you take iron tablets or multivitamin tablets contain iron during the pregnancy (pregnancies)?

1st pregnancy since AMBIT	❑ Yes	❑ No
2nd pregnancy since AMBIT	❑ Yes	❑ No
3rd pregnancy since AMBIT	❑ Yes	❑ No
4th pregnancy since AMBIT	❑ Yes	❑ No

Section 2: About your general health

. Have you been diagnosed with anaemia since participating in AMBIT?

❑ Yes ❑ No

If yes, has iron treatment been prescribed?

❑ Yes ❑ No

. Have you had any illnesses or health problems since participating in AMBIT that required active medical treatment

❑ Yes ❑ No

If yes, what was the illness? Please specify:

_____

_____

. Have you been admitted to hospital at any time since participating in AMBIT which was not pregnancy related?

❑ Yes ❑ No

If yes, please answer the following questions

(a) which hospital(s) were you admitted? _____

(b) when were you admitted?_____

(c) What was the reason for the admission(s)? _____

AMBIT follow up Mother's ID_____ Child's ID_____

Strengths and Difficulties Questionnaire

For each item, please mark the box for Not True, Somewhat True or Certainly True. It would help us if you answered all items as best you can even if you are not absolutely certain or the item seems daft! Please give your answers on the basis of the child's behaviour over the last six months.

	Not True	Somewhat True	Certainly True
Considerate of other people's feelings	☐	☐	☐
Restless, overactive, cannot stay still for long	☐	☐	☐
Often complains of headaches, stomach-aches or sickness	☐	☐	☐
Shares readily with other children (treats, toys, pencils etc.)	☐	☐	☐
Often has temper tantrums or hot tempers	☐	☐	☐
Rather solitary, tends to play alone	☐	☐	☐
Generally obedient, usually does what adults request	☐	☐	☐
Many worries, often seems worried	☐	☐	☐
Helpful if someone is hurt, upset or feeling ill	☐	☐	☐
Constantly fidgeting or squirming	☐	☐	☐
Has at least one good friend	☐	☐	☐
Often fights with other children or bullies them	☐	☐	☐
Often unhappy, down-hearted or tearful	☐	☐	☐
Generally liked by other children	☐	☐	☐
Easily distracted, concentration wanders	☐	☐	☐
Nervous or clingy in new situations, easily loses confidence	☐	☐	☐
Kind to younger children	☐	☐	☐
Often argumentative with adults	☐	☐	☐
Picked on or bullied by other children	☐	☐	☐
Often volunteers to help others (parents, teachers, other children)	☐	☐	☐
Can stop and think things out before acting	☐	☐	☐
Can be spiteful to others	☐	☐	☐
Gets on better with adults than with other children	☐	☐	☐
Many fears, easily scared	☐	☐	☐
Sees tasks through to the end, good attention span	☐	☐	☐

Thank you very much for your help

AMBIT follow up Mother's ID_____ Child's ID_____

HOME SCREENING QUESTIONNAIRE
Age 3 – 6 Years

Please answer all of the following questions about how your child's time is spent and some of the activities of your family. On some questions, you may want to tick more than one box.

1. a) Do you get any magazines in the mail?
   ☐ YES ☐ NO

   b) If yes, what kind?
   ☐ home and family magazines
   ☐ news magazines
   ☐ children's magazines
   ☐ other

2. Does your child have a toy box or other special place where he/she keep his/her toys? ☐ YES ☐ NO

3. How many children's books does your family own?
   ☐ 0 to 2
   ☐ 3 to 9
   ☐ 10 or more

4. How many books do you have besides children's books?
   ☐ 0 to 9
   ☐ 10 to 20
   ☐ more than 20

   Where do you keep them?
   ☐ in boxes (packed)
   ☐ on a bookcase
   ☐ other (explain_____
   _____)

5. How often does someone take your child into a grocery store?
   ☐ hardly ever; I prefer to go alone
   ☐ at least once a month
   ☐ at least twice a month
   ☐ at least once a week

6. About how many times in the past week did you have to spank your child?_____

7. Do you have a T.V?
   ☐ YES ☐ NO
   If yes, about how many hours is the T.V on each day?_____

8. How often does someone get a chance to read stories to your child?
   ☐ hardly ever
   ☐ at least once a week
   ☐ at least 3 times a week
   ☐ at least 5 times a week

9. Do you ever sing to your child when he/she is nearby?
   ☐ YES ☐ NO

10. Does your child put away his/her toys by himself/herself most of the time?
    ☐ YES ☐ NO

11. Is your child allowed to walk or ride his tricycle by himself/herself to the house of a friend or relative?
    ☐ YES ☐ NO

12. What do you do with your child's art work?
    ☐ let him/her keep it
    ☐ put it away
    ☐ hang it somewhere in the house
    ☐ throw it away shortly after looking at it

13. In the space below write what you might say if your child said, "look at that big truck"
    _____
    _____

14. What do you usually do when a friend visiting you in your home and your child has nothing to do?
    ☐ suggest something for him/her to do
    ☐ offer him/her a toy
    ☐ give him/her a cookie or something to eat
    ☐ put him/her to bed for a nap
    ☐ play with him/her

Please turn over

15. How often does your child eat a meal at the table with <u>both</u> mother and father (or other adult male)?
- ☐ never
- ☐ at least once a month
- ☐ at least once a week
- ☐ at least twice a week
- ☐ at least 3 or 4 times a week
- ☐ at least once a day

16. How often does your child spend time playing or "working" with his/her father (or other adult male)?
- ☐ at least 4 times a week
- ☐ at least twice a week
- ☐ at least once a week
- ☐ at least once a month
- ☐ never

17. How often does someone get a chance to take your child out of the house for an outing (shopping, park, zoo, restaurant, museum, car trip, library etc.)?
- ☐ at least 6 times a year
- ☐ at least once a month
- ☐ at least twice a month
- ☐ at least once a week

18. Tick the things which you (or other adult or older child) are helping or have helped your child to learn:
- ☐ colours (like naming colours of things)
- ☐ alphabet
- ☐ numbers
- ☐ understanding of time (like morning-afternoon and now-later)
- ☐ shapes (like drawing circles or squares)
- ☐ reading new words or writing his/her name

19. Has your child learned any songs, prayers, or nursery rhymes?
☐ YES ☐ NO
If yes, where did he/her learn them?
- ☐ at day care or preschool
- ☐ from a sister or brother
- ☐ at church or Sunday school
- ☐ from mother or father
- ☐ from television

20. It is 30 minutes before dinner and your child is hungry. Most of the time you would:
- ☐ give him/her a snack
- ☐ have him/her wait for dinner

21. Which items do you sometimes let your child choose for himself/herself?
- ☐ part of what to have for breakfast or lunch
- ☐ favourite foods in the grocery store (fruit, cereal, cookies, etc.)
- ☐ the clothes he/she wants to put on
- ☐ non of the above

22. What would you do if your child got angry and hit you?
- ☐ hit him/her to show him/her it hurts
- ☐ send him/her to his/her room
- ☐ spank him/her
- ☐ talk to him/her
- ☐ ignore it

23. Do you have any pets?
☐ YES ☐ NO

24. Do you have any plants in your house?
☐ YES ☐ NO

25. Which of the following best describes your neighbourhood:
- ☐ it is not as clean as I would like it
- ☐ the houses are not well cared for
- ☐ it is well cared for
- ☐ it is well cared for and attractive

26. a) How many bedrooms does your house have?_____
b) How many people are living in your house?_____

27. Do you occasionally try new recipes that you find in the newspaper or in magazines?
☐ YES ☐ NO

28. Is anyone in the family presently taking a class in school at the college level?
☐ YES ☐ NO

Please turn over

29. Who buys the groceries for the family?

Mother	☐ sometimes	☐ often
Father	☐ sometimes	☐ often
Grandparent	☐ sometimes	☐ often
Older child	☐ sometimes	☐ often
Other	☐ sometimes	☐ often

30. Most of the decisions about how the family income is to be spent are made by
    - ☐ mother
    - ☐ father
    - ☐ grandparent
    - ☐ friend

31. How often do you and your child get a chance to play together (like pretend games, dolls, house, cars and trucks, or table games)?
    - ☐ hardly ever; too young
    - ☐ at least once a week
    - ☐ at least 3 – 4 times a week
    - ☐ everyday

32. Do you have any friends or relatives with children about the same age as your child?
    ☐ YES ☐ NO

33. When your child asks if he/she can do something you think he/she is too young to do, would you be more likely to say
    - ☐ no, I don't want you to
    - ☐ no
    - ☐ not now
    - ☐ no. You're too young now but when you're older you'll be able to do it

34. What would happen if your child spilled his/her milk?
    - ☐ he/she would be spanked
    - ☐ he/she would have to clean it up
    - ☐ someone else would clean it up
    - ☐ he/she would be sent to his/her room

Please complete the checklist on the reverse side

Please turn over

We are interested in finding out what kinds of toys children have in their homes. The items listed below are for children of different ages.

Please tick any of the following that you have in your home AND that your child is allowed to play with. Do not tick the ones that you do not have now or ones that are broken.

We do not expect a child to have all of these items.

1. ☐ Dolls with clothes or paper dolls
2. ☐ Stuffed animals, animal toys or animal books
3. ☐ dress-up clothes or costumes
4. ☐ tricycle, bicycle or scooter
5. ☐ stroller or walker
6. ☐ wagon
7. ☐ big wheel or child-size car
8. ☐ pull or push toy
9. ☐ mobile
10. ☐ child-size furniture
11. ☐ high chair
12. ☐ playpen
13. ☐ puzzles – at least three
14. ☐ alphabet toy, alphabet game or alphabet book
15. ☐ number toy, number game or number book
16. ☐ colouring book
17. ☐ dot-to-dot or colour-by-number book
18. ☐ scissors
19. ☐ pegboard
20. ☐ toy telephone
21. ☐ plastic snap-together beads
22. ☐ musica. or music box
23. ☐ children's books
24. ☐ ball
25. ☐ shape ball or box
26. ☐ crib gym or gym set
27. ☐ jump seat or door swing
28. ☐ squeeze toys
29. ☐ rattles
30. ☐ T.V.
31. ☐ busy box
32. ☐ gun
33. ☐ clay or play dough
34. ☐ real or toy musical instruments
35. ☐ sand box
36. ☐ homemade building toys
37. ☐ blocks
38. ☐ Tinker toys, Lego or Lincoln Logs
39. ☐ record player, tape or CD player
40. ☐ children's records, tapes, CDs
41. ☐ chalkboard or white board
42. ☐ swings
43. ☐ jungle gym
44. ☐ car, truck or train
45. ☐ measuring cups
46. ☐ pots and pans
47. ☐ toy dishes
48. ☐ doll carriage
49. ☐ plastic tools and workbench
50. ☐ crayons, paints or pencils

AMBIT follow up

Mother's ID_ _ _ _ _ _ _ _

General Health of the Mother (SF-36)

Date completed: _ _ /_ _ /_ _ _ _

INSTRUCTIONS: This questionnaire asks for your views about your health, how you feel and how well you are able to do your usual activities.

Answer every question by marking the answer as indicated. If you are unsure about how to answer a question, please give the best answer you can.

1. In general, would you say your health is: (circle one)

Excellent	1
Very good	2
Good	3
Fair	4
Poor	5

2. Compared to one year ago, how would you rate your health in general now? (circle one)

Much better now than one year ago	1
Somewhat better now than one year ago	2
About the same as one year ago	3
Somewhat worse now than one year ago	4
Much worse now than one year ago	5

3. The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

(circle one number on each line)

ACTIVITIES	Yes Limited A Lot	Yes Limited A Little	No, Not Limited At All
a. **Vigorous activities**, such as running, lifting heavy objects, participating in strenuous sports	1	2	3
b. **Moderate activities**, such as moving a table, pushing a vacuum cleaner, bowling or playing golf	1	2	3
c. Lifting or carrying groceries	1	2	3
d. Climbing **several** flights of stairs	1	2	3
e. Climbing **one** flight of stairs	1	2	3
f. Bending, kneeling or stooping	1	2	3
g. Walking **more than one kilometre**	1	2	3
h. Walking **half a kilometre**	1	2	3
i. Walking **100 metres**	1	2	3
j. Bathing or dressing yourself	1	2	3

4. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

(circle one number on each line)

	YES	NO
a. Cut down on the **amount of time** you spent on work or other activities	1	2
b. **Accomplished less** than you would like	1	2
c. Were limited in the **kind** of work or other activities	1	2
d. Had **difficulty** performing the work or other activities (for example, it took extra effort)	1	2

5. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious?)

(circle one number on each line)

	YES	NO
a. Cut down on the **amount of time** you spent on work or other activities	1	2
b. **Accomplished** less than you would like	1	2
c. Didn't do work or other activities as **carefully** as usual	1	2

6. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours, or groups?

(circle one)

Not at all	1
Slightly	2
Moderately	3
Quite a bit	4
Extremely	5

7. How much bodily pain have you had during the past 4 weeks? (circle one)

No bodily pain	1
Very mild	2
Mild	3
Moderate	4
Severe	5
Very severe	6

8. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework?)

(circle one)

Not at all	1
A little bit	2
Moderately	3
Quite a bit	4
Extremely	5

9. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks -

(circle one number on each line)

	All of the time	Most of the time	A good bit of the time	Some of the time	A Little of the time	None of the time
a. Did you feel full of life?	1	2	3	4	5	6
b. Have you been a very nervous person?	1	2	3	4	5	6
c. Have you felt so down in the dumps that nothing could cheer you up?	1	2	3	4	5	6
d. Have you felt calm and peaceful?	1	2	3	4	5	6
e. Did you have a lot of energy?	1	2	3	4	5	6
f. Have you felt down?	1	2	3	4	5	6
g. Did you feel worn out?	1	2	3	4	5	6
h. Have you been a happy person?	1	2	3	4	5	6
i. Did you feel tired?	1	2	3	4	5	6

10. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives, etc?)

(circle one)

All of the time	1
Most of the time	2
Some of the time	3
A little of the time	4
None of the time	5

Mother's ID_____

11. How **TRUE** or **FALSE** is <u>each</u> of the following statements for you?

(circle one number on each line)

	Definitely true	**Mostly true**	**Don't know**	**Mostly false**	**Definitely false**
a. I seem to get sick a little easier than other people	1	2	3	4	5
b. I am as healthy as anybody I know	1	2	3	4	5
c. I expect my health to get worse	1	2	3	4	5
d. My health is excellent	1	2	3	4	5