Iron deficiency in young Australian women: Role of iron knowledge, dietary intake and supplementation, and the effects on cognition

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A thesis submitted for the degree of PhD (Nutrition and Dietetics)

University of Newcastle, NSW, Australia

July 2014
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Alecia J Leonard
Acknowledgements

I would like to express my gratitude to the following people for their contribution to my thesis.

First and foremost I sincerely thank my three supervisors and mentors, Dr. Amanda Patterson, Dr. Kerry Chalmers and Professor Clare Collins. I am so fortunate to have been supervised by such highly regarded researchers. Without your continuing advice, support and guidance this thesis would not have been possible. Your passions, skills and commitment to research have been an inspiration to me. I am indebted to you all for the immeasurable part you have played in my experience as a researcher.

I thank my fellow Nutrition and Dietetics RHD students with whom I shared many research frustrations as well as countless fun times both in and out of the office. Great friendships evolved in HA06 that will continue into the future. A special mention goes to Tracy for sharing your statistical knowledge on many an occasion.

To my incredibly supportive husband (Steve), thank you for being there for me every day, making me laugh, supporting me during the stressful times and reminding me that everything will be even better than OK. Thanks to my Mum (Karen), Dad (Tony) and Sister (Lauren), you have provided me with endless and unconditional love and support and are only ever a phone call away. I would like to thank my good friends who bring so much to my life and have been integral of my PhD journey by providing me with much needed downtime along the way.

I was fortunate to receive an Australian Post-Graduate Award for this PhD and additional financial support from Meat and Livestock Australia and the School of Health Sciences at the University of Newcastle.

Finally, but so importantly, thank you to the young women who participated in my research studies. I appreciate your interest in research, your time and your commitment as participants.
Conflict of Interest

Alecia Leonard received a postgraduate scholarship top-up from Meat and Livestock Australia Pty Ltd. Meat and Livestock had no role in the: design on the studies; analysis of data; writing of this thesis or the manuscripts it contains; or decision to submit the manuscripts for publication.
Publications and presentations arising from this thesis

Manuscripts in peer-reviewed journals: Published


Conference abstracts: Published in conference proceedings or peer-reviewed journals


Glossary of common abbreviations

WHO  World Health Organization
RCT  Randomised controlled trial
DQES  Dietary Questionnaire of Epidemiological Studies
HMRI  Hunter Medical Research Institute
JBI  Joanna-Briggs Institute
JBI-MAStARI  Joanna Briggs Institute-Meta Analysis of Statistics Assessment and Review Instrument
NUTTAB  Nutrient Data Tables for use in Australia
ADP  Australian Digital Theses
HAPS  Hunter Area Pathology Service
Fe$^{3+}$  Ferric iron
Fe$^{2+}$  Ferrous iron
sTfR  Soluble transferrin receptor
sTfR-ferritin index  Soluble transferrin receptor/Log ferritin
Ft  Serum ferritin
Hb  Haemoglobin
AAG  Alpha-1-glycoprotein
CRP  C-reactive protein
DMTI  Divalent metal transporter
ALSWH  Australian Longitudinal Study of Women’s Health
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>AusDiab</td>
<td>Australian Diabetes, Obesity and Lifestyle Study</td>
</tr>
<tr>
<td>CCV</td>
<td>Cancer Council of Victoria</td>
</tr>
<tr>
<td>SF-36</td>
<td>Short-form 36 Health Survey</td>
</tr>
<tr>
<td>FFQ</td>
<td>Food Frequency Questionnaire</td>
</tr>
<tr>
<td>NKQ</td>
<td>Nutrition Knowledge Questionnaire</td>
</tr>
<tr>
<td>NSW</td>
<td>New South Wales</td>
</tr>
<tr>
<td>US</td>
<td>United States</td>
</tr>
<tr>
<td>UK</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>WMS-R</td>
<td>Wechsler Memory Scale revised</td>
</tr>
<tr>
<td>WAIS</td>
<td>Welscher Adult Intelligence Scale</td>
</tr>
<tr>
<td>WAIS-R</td>
<td>Revised Welscher Adult Intelligence Scale</td>
</tr>
<tr>
<td>CANTAB</td>
<td>Cambridge Neuropsychologcical Test Automated Battery</td>
</tr>
<tr>
<td>N/A</td>
<td>Not applicable</td>
</tr>
<tr>
<td>NRV’s</td>
<td>Nutrient Reference Values</td>
</tr>
<tr>
<td>RDI</td>
<td>Recommended Dietary Intake</td>
</tr>
<tr>
<td>EAR</td>
<td>Estimated Average Requirement</td>
</tr>
<tr>
<td>NNS</td>
<td>National nutrition survey</td>
</tr>
<tr>
<td>NHMRC</td>
<td>National Health and Medical Research Council</td>
</tr>
<tr>
<td>kg</td>
<td>Kilograms</td>
</tr>
<tr>
<td>m</td>
<td>Metres</td>
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<td>mg</td>
<td>Milligrams</td>
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<tr>
<td>Symbol</td>
<td>Term</td>
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</tr>
<tr>
<td>g</td>
<td>Grams</td>
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<td>µg</td>
<td>Micrograms</td>
</tr>
<tr>
<td>L</td>
<td>Litre</td>
</tr>
<tr>
<td>SMD</td>
<td>Standardised Mean Difference</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>TAFE</td>
<td>Technical Education College</td>
</tr>
<tr>
<td>ANOVA</td>
<td>One-way analysis of variance</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of mean</td>
</tr>
<tr>
<td>n</td>
<td>Numbers (sample)</td>
</tr>
<tr>
<td>OCP</td>
<td>Oral contraceptive pill</td>
</tr>
<tr>
<td>EIA</td>
<td>Enzyme immunoassay</td>
</tr>
<tr>
<td>IEMA</td>
<td>Immunoezymometric assay</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme linked immunosorbent assay</td>
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Abstract

Iron deficiency was labelled a major public health concern in the late 1980s due to its recognition as the most prevalent nutritional disorder in the world. According to the World Health Organization (2001), iron deficiency remains the most common nutritional deficiency worldwide. Within Australia, one in five young women are affected by iron deficiency. A strong link between iron deficiency anaemia and impaired cognitive function has been established in children. The effect of latent iron deficiency on cognition in young women has not been well investigated.

The aims of this research were to: 1) determine the level of nutrition knowledge of dietary iron in a subgroup of young women living in Newcastle, NSW and its relationship to their iron intake and iron status; 2) examine the suitability of a validated battery of tests (IntegNeuro) for assessing cognitive function in iron deficient and iron sufficient women; 3) determine an appropriate sample size for a future RCT on iron deficiency and cognition in young women; 4) determine an effective dose of elemental iron to treat iron deficiency in latent iron-deficient participants, while maintaining blinding to treatment.

A study on the effect of nutrition knowledge on dietary iron intake and iron status was conducted in young women. This involved the distribution of a Nutrition Knowledge Questionnaire and Food Frequency Questionnaire to females who were enrolled or interested in enrolling in the pilot RCT (described below). The results of this study showed a significant relationship between knowledge and total iron intake. However, better knowledge did not result in better iron status. Results also showed a positive relationship between the frequency of flesh food intake and iron status.

A pilot double-blinded, placebo-controlled intervention trial was conducted in iron-deficient and iron-sufficient young women (18-35 years). Cognitive function and haematological markers of iron status were measured at baseline and follow-up. Iron-deficient participants were randomised to receive placebo, 60mg or 80mg elemental iron daily for 16 weeks. A control group of iron-sufficient participants was allocated placebo capsules. Participants in the iron treatment groups had greater cognitive change scores.
compared to no-treatment groups. Change scores for Impulsivity and Attention were significantly greater in plasma ferritin improvers than in non-improvers ($p=.004$, and $p=.026$, respectively). IntegNeuro was easy to administer and acceptable to young women. Based on differences in Memory and Attention scores between iron-sufficient and iron-deficient participants, further research with a sample size of 26 or 84 iron-deficient participants per group is required for an adequately powered trial.

In conclusion, this thesis contributes to various areas of iron deficiency research in young women. Nutrition knowledge regarding iron in young women is a novel area of research. Some positive associations between knowledge and intake were found. Results revealed that a validated questionnaire with a greater focus on dietary enhancers and inhibitors of iron is required to further this area of research. Dietary intake of flesh foods was positively related to serum ferritin. There is a need to establish strategies for increasing iron intake and absorption in young women. Such strategies may include educating non-vegetarians about the benefits of increased flesh food consumption and vegetarians about dietary iron enhancers and inhibitors.

Few significant differences in cognition scores between iron-deficient and iron-sufficient young women at baseline, and after the 16 week intervention were expected in an underpowered pilot study. It is important to consider that the detection of differences in cognition was not the primary aim of this pilot study. Future studies in this area should be well powered, multi-centred randomised-controlled trials using a cognitive battery that has been validated for use in this population.

The pilot RCT revealed some important information relating to methodological advancements in this area of research. These include: 1) the IntegNeuro battery of cognitive tests is a useful method for use in young women, which is important due to the diverse range of cognitive tests used in previous research; 2) a 60mg dose of elemental iron is as effective in treating iron deficiency and causes fewer side effects than an 80mg dose; and 3) sTfR alone does not enhance the ability to detect iron deficiency in its early stages, although the sTfR-ferritin index is more useful.
Chapter 1. Introduction
1.1 Overview
This introductory chapter will provide an overview of the rates of iron deficiency, its possible causes, consequences and treatment. Detail on the metabolic process of iron deficiency and existing knowledge regarding each of the sections that make up the current chapter is included in the Literature review (presented in Chapter 2) and the Systematic review (presented in Chapter 3).

1.2 Iron deficiency: A nutritional crisis
In the late 1980s iron deficiency was labelled a major public health concern as it had become the most prevalent nutritional disorder in the world (DeMaeyer, Dallman et al. 1989, Stoltzfus 2001). According to the World Health Organization (WHO), iron deficiency remains the most common nutritional deficiency worldwide (World Health Organization 2001), affecting over 30% of the world’s population (World Health Organization 2012). As shown in Figure 1.1, between the years 1993 and 2005 iron deficiency anaemia was either a mild, moderate or severe public health problem in every country worldwide, with the exception of Chile (World Health Organization 2008).
Iron deficiency can affect all population groups, but is most commonly seen in children within their first two years of life and in women of reproductive age (Webb and Oski 1973, Scrimshaw 1984). Within Australia, 20% of women aged 25-50 years are diagnosed with iron deficiency anaemia (Ahmed, Coyne et al. 2008). The high rates of iron deficiency have led to it being an area of research interest (World Health Organization 2001). The causes of iron deficiency, its effect on health and well-being and its treatment options remain the primary aspects requiring further research.

The economic impact of iron deficiency includes the cost of therapeutic measures incurred by public and private health sectors, societal consequences of increased maternal mortality and long-term projected consequences of impaired mental development (World Health Organization 2001). A scarcity of information exists about the actual cost of programs for the control of iron deficiency and the benefit obtained by its correction (World Health Organization 2001). However, an estimation on the cost of
iron deficiency in Southern Asia (Bangladesh, Bhutan, India, Maldives, Nepal, Pakistan and Sri Lanka) was reported as 2.7 billion dollars annually (Horton and Ross 2007).

1.2.1 Factors contributing to iron deficiency in young women
The management of iron deficiency is a challenging area due to multiple possible contributing factors and causes. The most common factors that contribute to iron deficiency in young women include increased iron losses as a result of menstruation and childbirth, insufficient dietary iron intake and inadequate absorption (Miller 2013). Women can lose up to 137mg of iron per year from menstruation alone (Baynes and Cook 1996, Beard, Dawson et al. 1996). Other causes of iron loss are blood donation and basal losses (Miller 2013).

Due to menstruation and pregnancy, young women aged 19-35 years have higher iron requirements than any other population subgroup (National Health and Medical Research Council 1991). The Recommended Dietary Intake (RDI) is therefore set higher for women in these life stages, which are known to be associated with iron deficiency.

Also, insufficient dietary iron intake may be a result of high rates of vegetarianism in this population (Ball and Bartlett 1999). Limited knowledge about the most useful food sources of dietary iron may also be a contributing factor (De Vriendt, Matthys et al. 2009). However, the latter area is not well investigated. Iron absorption can be affected by a number of factors such as infection, and consumption of dietary iron enhancers such as ascorbic acid and inhibitors such as polyphenols and phytates (Hurrell and Egli 2010). Polyphenols occur in various plant foods such as vegetables, fruit, cereals and legumes, as well as beverages including tea, coffee and wine. Phytates also occur in plant based foods, predominately seeds (Hurrell and Egli 2010).

1.2.2 The consequences of iron deficiency
The consequences of iron deficiency anaemia include poor work capacity, and effects on immunity and thermoregulation (World Health Organization 2001). In addition, deficits in cognitive function (such as memory, attention, and reaction time) have been shown in iron-deficient infants and children (Lozoff, Beard et al. 2006). However, the effect of iron deficiency in adult populations is less clear. Although the exact mechanism by which
Iron deficiency affects the brain is not completely understood, possibilities include abnormalities in neurotransmitter metabolism, decreased myelin formation, and alterations in brain energy metabolism (Tucker, Sandstead et al. 1984, Beard and Connor 2003). The consequence of latent iron deficiency, which is more common than iron deficiency anaemia, on the health and wellbeing of young women has not been well investigated.

1.3 Iron deficiency research in women of childbearing age

There is limited understanding of the effects of latent iron deficiency, which is much more common than iron deficiency anaemia in young women. The lack of definitive findings limits understanding of the effects of iron deficiency or strategies for addressing it, therefore, there is little in the way of recommendations within the public health arena. The following section highlights areas of iron deficiency research in young women that require further research.

1.3.1 The impact of knowledge on dietary iron intake, and dietary iron intake on iron status

Assessing young women’s level of knowledge relating to dietary iron will help researchers and clinicians to understand one factor potentially contributing to iron deficiency in this population. Nutrition knowledge is difficult to measure and is known for being a challenging area of research (Worsley 2002). To date, no study has focused on assessing knowledge of dietary iron and its impact on dietary iron intake. It is currently unclear what young women know about dietary iron in regard to how much iron they need, or which foods are high in iron, and importantly, what foods they should increase if their iron is low. If there is an association between nutrition knowledge of dietary iron and dietary iron intake in young women, it is important that this is identified to enable targeted education programs to be developed. Detail on the effect of nutrition knowledge and dietary iron intake on iron status is included in Chapter 2, Section 2.9 and Chapter 4.

Evidence of the contribution of dietary iron intake on the incidence of iron deficiency remains unclear. Research into dietary iron intake is limited by inconsistency in
assessment tools used to measure iron intake (Ball and Bartlett 1999, Ball, Mishra et al. 2004, Zhou, Schilling et al. 2005, Pynaert, Delanghe et al. 2007). It is also limited by a lack of useful data on the bioavailability of dietary intake with regard to iron enhancing and inhibiting factors (Collings, Harvey et al. 2013).

1.3.2 The effect of iron deficiency on cognitive function

Iron deficiency in infants and children has been well investigated with regards to its effect on cognitive function, including memory, attention and reaction time (Lozoff, Brittenham et al. 1982, Lozoff, Brittenham et al. 1982, Lozoff and Brittenham 1986, Laessle, Platte et al. 1996, Lozoff, Jimenez et al. 2000, Lozoff, Beard et al. 2006, Lozoff, Jimenez et al. 2006). Research has shown strong associations between iron deficiency and cognitive deficits in children up to two years of age (Lozoff, Beard et al. 2006). Research on the effect of iron deficiency on cognitive function in young women is scarce and is limited by substantial variation in methods used to measure iron status and cognitive function (Elwood and Hughes 1970, Groner, Holtzman et al. 1986, Beard 2003, Murray-Kolb and Beard 2007), which makes the detection of an effect difficult (Further discussion in Section 2.6.7).

1.3.2.1 The usefulness of tools to assess cognition in young women

Few studies that have measured the effect of iron deficiency on cognition in young women use comparable cognitive measures (Groner, Holtzman et al. 1986, Patterson 1999, Khedr, Hamed et al. 2008). There is no gold standard for tools to measure cognitive function in this population. To determine whether cognitive functioning is affected by iron deficiency in young women, the use of a reliable assessment tool is required. This will enable a benchmark for other researchers to compare, and to maximise the reliability of the results.

1.3.3 Appropriate iron dosage for use in a blinded trial

Blackening of stools, constipation, nausea, and bloating are common side effects of iron supplementation (Zhu, Kaneshiro et al. 2010). As a result of such side effects, iron-deficient women commonly do not take iron supplements consistently or for the recommended time frame (Rimon, Kagansky et al. 2005, Zhu, Kaneshiro et al. 2010).
Recommended doses of elemental iron supplements vary in guidelines from 20-120mg per day (Makrides, Crowther et al. 2003, Rimon, Kagansky et al. 2005, Therapeutic Guidelines Ltd 2006, Australian Medicines Handbook Pty Ltd 2010, Mozaffari-Khosravi, Noori-Shakam et al. 2010). Currently, there is no gold standard on the most effective dose for sufficiently improving iron status in young women with latent iron deficiency. The most commonly recommended iron supplement is dried ferrous sulphate, which is recommended in the United States (Stoltzfus and Dreyfuss), United Kingdom (Goddard, James et al. 2011) and Australia (Australian Medicines Handbook Pty Ltd 2010). Clinical practice guidelines in Australia recommend a daily dose of 100-210mg elemental iron for treatment of iron deficiency anaemia in adults (National Prescribing Service 2010). Treatment of latent iron deficiency and the impact of using lower dose iron treatment on iron status and side-effects are not articulated within current iron treatment guidelines. The most appropriate dose of iron supplementation in iron-deficient young women is further discussed in Chapter 2, section 2.10 and Chapter 7.

1.3.4 Markers used to measure iron status

A variety of markers are used to assess iron status and diagnose iron deficiency, but there is no definitive method, or universally accepted reference ranges for commonly used tests (Olivares, Walter et al. 2000). It is imperative that sensitive, specific and reliable biochemical markers are identified and used to evaluate iron status in its early stages. Detail on the markers used to assess iron status is included in Chapter 2, section 2.4 and Chapter 8.

1.3.5 Strategies to recruit and retain women of childbearing age in health research studies

Young women are underrepresented in nutrition research, despite the importance of diet, lifestyle and physiological transitions experienced at this life stage (Griffin, O’Connor et al. 2013). The ability to successfully recruit and retain participants in health research studies largely determines the impact of the research (Ashery and McAuliffe 1992). Difficulties exist in recruiting young women and keeping them involved in research studies (Griffin, O’Connor et al. 2013). Minimal research into factors affecting their recruitment and engagement in nutrition research has been conducted (Hure,
Smith et al. 2008). In order to optimise study quality within nutrition research, there is a need to evaluate data related to recruitment and retention in young women, and present practical advice related to recruiting and retaining young women in health research. Practical considerations relating to the recruitment and retention of young women into nutrition research studies are presented in Chapter 9.

1.4 Research aims and hypotheses

The aims of the research reported in this thesis were: 1. to determine the level of nutrition knowledge of dietary iron in a group of young women living in Newcastle, NSW, and its effect on their iron intake and iron status; 2. to examine the suitability of a validated battery of tests for assessing cognitive function in iron-deficient and iron-sufficient women; 3. using pilot data, determine an appropriate sample size for a RCT on iron deficiency and cognition in young women; and 4. to determine an effective iron dose to improve the iron status of participants with latent iron deficiency, while maintaining blinding to treatment.

More specific aims of this research were to:

6. Systematically review published and unpublished work in the area of iron deficiency, cognition, mental health and fatigue.

7. Conduct analysis of baseline data to assess:
   • the level of nutrition knowledge of dietary iron in young women
   • the association between nutrition knowledge and iron intake in young women
   • the association between dietary iron intake and iron status in young women

8. Conduct a pilot RCT to determine:
   • the suitability of the IntegNeuro battery of tests for assessing cognitive function in iron-deficient and iron-sufficient women
   • an appropriate sample size for an adequately powered RCT examining the effects of iron treatment on cognition in iron-deficient women
   • an efficacious iron dose to improve iron status of iron-deficient young women, while maintaining blinding to treatment
• any differences in cognitive function using IntegNeuro in iron-deficient and iron-sufficient young women
• any change in cognitive function using the IntegNeuro battery at follow-up, after iron-deficient participants complete a 16 week iron treatment intervention

1.5 Thesis structure and study design

1.5.1 Overview

This thesis begins with a comprehensive review of the background literature supporting this work (Chapter 2), followed by a study on the effect of nutrition knowledge on iron intake and iron intake on iron status (Chapter 4). These two chapters will be followed by two chapters presenting the results of a pilot randomised controlled trial of iron supplementation among iron-deficient young women and the effect on cognition. The three following chapters will present the methodological issues arising from the thesis and the learned outcomes from these. The final chapter will discuss findings from the research and provide concluding remarks.

This thesis is based on six manuscripts. Figure 1.2 shows the process by which these manuscripts came about.
Figure 1.2. Flow diagram of the six manuscripts that form this thesis

Note: JBI-MASARI= Joanna Briggs Institute-Meta Analysis of Statistics Assessment and Review Instrument; Ft= serum ferritin; sTfR= soluble transferrin receptor to serum ferritin ratio; AAG= alpha-1-glycoprotein; criteria for iron deficiency= Ferritin <20µg/L, Haemoglobin ≥120g/L and iron sufficiency (Ferritin ≥20µg/L, Haemoglobin ≥120g/L; IntegNeuro= a self-administered (touch screen) battery for assessing cognitive function and including the domains of Memory, Response Speed, Impulsivity, Attention, Information Processing, Executive Function, Emotion Identification; DQES= Dietary Questionnaire of Epidemiological Studies.
1.5.2 Systematic review – Chapter 3
A comprehensive systematic review of the effects of latent iron deficiency on cognition, mental health and fatigue was conducted. This review aimed to provide a clear justification of the need for more investigation in this area.

1.5.3 Relationships between knowledge of dietary iron and iron status - Chapter 4
In addition to measuring cognition, the cross-sectional analysis of data collected during baseline assessments for the RCT was designed to measure the effect of nutrition knowledge of iron on dietary iron intake, and to also examine the effects of dietary iron intake on iron status. Two questionnaires were distributed to the RCT pilot (described in Section 1.4.4) sample of young women aged 18-35 years in Newcastle, NSW. The questionnaires (knowledge of dietary iron and a food frequency questionnaire) were distributed simultaneously. Data obtained from the questionnaires provided a quantitative assessment of nutrition knowledge of iron and dietary iron intake.

1.5.4 Pilot randomised controlled trial – Chapters 5 & 6
A pilot study on the effects of latent iron deficiency on cognitive functioning was conducted. This study is described in Chapters 5 (baseline analyses only) and Chapter 6 (intervention participants only). The design was a double-blinded, randomised controlled trial of iron supplementation in young women. This study provided a basis for establishing an adequately powered RCT, and aimed to determine: 1) the suitability of the IntegNeuro testing battery; 2) an appropriate sample size by measuring cognitive change scores for those whose iron status improved with treatment; and 3) an appropriate iron dose to treat latent iron deficiency whilst maintain blinding to treatment allocation.

The pilot RCT on the effects of latent iron deficiency on cognitive functioning in young women included three stages:

Baseline testing
The baseline testing of the pilot RCT involved assessment of iron status and cognitive function in women aged between 18 and 35 years, at the University of Newcastle. Those
who provided initial blood samples but were not interested or able to participate in the assessment of cognitive functioning exited the study after their initial blood test.

**Intervention**

The intervention phase of the pilot RCT included those participants who completed an initial assessment of iron status and cognitive function, and who were found to have latent iron deficiency, as well as a control group of iron sufficient participants. These participants were invited by phone/email to participate in a 16 week supplementation intervention (60mg iron, 80mg iron, or placebo).

**Follow-up testing**

The follow-up phase involved another assessment of iron status at Hunter Area Pathology Service and repeat cognition test using IntegNeuro. Figure 1.3 shows the recruitment process and RCT flow chart.
Figure 1.3. Flow chart describing the study design of a pilot double-blinded, placebo-controlled randomised controlled trial of the effects of iron supplementation (60 or 80mg iron or placebo for 16 weeks) on the cognitive function of iron-deficient (Ferritin <20ug/L, Haemoglobin ≥120g/L) and iron-sufficient (Ferritin ≥20ug/L, Haemoglobin ≥120g/L) women (18-35 years).

Note: Ferritin = serum ferritin; sTfR-ferritin index = soluble transferrin receptor to serum ferritin ratio; A1GP = alpha-1-glycoprotein; IntegNeuro = a self-administered (touch screen) battery for assessing cognitive function and including the domains of Memory, Response Speed, Impulsivity, Attention, Information Processing, Executive Function, Emotion Identification. Elemental iron in the form of ferrous sulfate.

1.5.5 Comparison of two doses of elemental iron in the treatment of latent iron deficiency – Chapter 7

Analysis of RCT pilot data (Chapter 6) was conducted to determine the efficacy of two different doses of iron supplementation in improving iron status whilst maintaining blinding to treatment groups. Thirty two women were included in the intervention. Young women found to be iron deficient at baseline were randomly assigned to one of two different doses (60mg or 80mg) of elemental iron as ferrous sulfate or placebo for 16 weeks. A control group of iron sufficient participants were also blinded to their iron status and given placebo. Participants were contacted on a four weekly basis to report any potential side-effects of treatment, using a specifically designed questionnaire (Appendix 10). Immediately following the 16 week intervention, participants were asked
to guess which treatment protocol they thought they had been allocated to. Participants had repeat blood tests after 16 weeks and were not informed of their treatment or iron status until trial completion.

1.5.6 The use of soluble transferrin receptor as a marker in early stage iron deficiency – Chapter 8

Haematological data from the pilot RCT presented in Chapter 5 were examined to determine the usefulness of sTfR in the assessment of early stage iron deficiency. In addition, a search of peer-reviewed literature from earliest record to June 2013 was conducted. Studies using sTfR as a marker of early stage iron deficiency were examined with a focus on the various assays and reference ranges used. Reference ranges and sTfR values were tabulated and compared with results from the pilot RCT.

1.5.7 Recruitment and retention of young women – Chapter 9

Chapter 9 presents a study addressing the practical considerations of recruitment and retention of young women for nutrition research. Within this chapter recommendations are made for optimising these aspects in future research. Recruitment and retention strategies targeted towards young women (18-35 years) for the RCT pilot were critiqued against a crossover validation study and a cross-sectional survey that were conducted at the University of Newcastle, Australia between 2010 and 2013.
Chapter 2. Background Literature
2.1 Overview

This chapter will review published literature on the function of iron in the body, the stages of development of iron deficiency, and its prevalence in Australia and internationally. The consequences of iron deficiency will be examined, particularly with regards to cognitive functioning, as well as its determinants and treatment options.

2.2 Iron in the body

Researchers began publishing their work on the uptake, regulation and function of iron in the early 1950’s. There have been few contributions to this work since the late 1980’s. The first study to investigate dietary and storage factors in iron deficiency was in 1957 (Dawson and Desforges 1957).

2.2.1 Function of iron

Iron is found in the body in two molecular states, Ferrous (2⁺) and Ferric (3⁺) iron. The most stable state is Ferrous (2⁺) iron. The ability of iron metabolism to access these two states underlies its participation in several essential metabolic roles including the activation of molecular oxygen, nitrogen and hydrogen. Iron is also involved in DNA synthesis and respiratory enzyme production (Moore and Dubach 1956, Aisen and Listowsky 1980). Iron is also required for various physiological processes such as the binding of oxygen to haemoglobin, and myoglobin, cytochromes, and cellular respiration (Moore and Dubach 1956, Aisen and Listowsky 1980).

2.2.2 Iron distribution and homeostasis

In normal conditions the iron content of the body is 3-4 grams (Franchini 2006). Over half of the iron in the human body is present in circulating haemoglobin (2.5g), the remainder is in iron-containing proteins (0.4g), for example haem proteins, bound to iron transport mechanisms (5mg) (Camaschella, De Gobbi et al. 2000). Iron is contained in its storage forms as ferritin, the main storage form, and haemosiderin (0.5-1g) (Moore and Dubach 1956, Camaschella, De Gobbi et al. 2000). Both compounds are capable of being mobilised for haemoglobin synthesis when iron is needed (Moore and Dubach 1956).
In the human body, iron is absorbed in its ferrous form (Fe\(^{2+}\)). A divalent metal transporter (DMT1) is responsible for bringing iron into the absorptive enterocytes in the small intestine, where it is then stored as ferritin (Moore and Dubach 1956, Andrews 2010). Iron uptake by enterocytes and homeostasis is tightly regulated by a negative feedback loop, due to the body’s limited capacity to excrete iron (Casanovas, Banerji et al. 2014). The amount of iron in the enterocyte regulates the amount exported to other cells in the body (Frazer and Anderson 2003). A diagram of the iron uptake and transport system is shown in Figure 2.1.

Iron is consumed in food sources as either haem (flesh foods) or non-haem (plant-based, as well as flesh foods). Non-haem iron is converted from Fe\(^{3+}\) to Fe\(^{2+}\) which is transported to mucosal ferritin storage or to the blood cells via DMT1, ferroportin1 and plasma transferrin. Haem iron is transported directly to either mucosal ferritin storage or to blood cells via a haem transporter, ferroportin1 and plasma transferrin. Mucosal ferritin stores iron, and some is lost via shedding of epithelial cells (Schuster 2011). Hepcidin is a peptide hormone produced by the liver which senses the iron blood level and regulates iron reabsorption (Casanovas, Banerji et al. 2014).

Iron absorption into cells has greater influence on regulating iron homeostasis than iron excretion (Finch 1994). Disturbances in the feedback loop lead to iron-related abnormalities such as deficiency, overload and inflammation (Casanovas, Banerji et al. 2014).

**Figure 2.1 Absorption of iron in an enterocyte (Schuster 2011)**
Iron deficiency is the most common disturbance in iron homeostasis (Finch 1994). The first diagnosis of iron deficiency was not made until the 1930's, as there was no routine haematological measurement of iron markers prior to this (Chanarin 2000). In iron deficient states, enterocytes up-regulate the production of DMT1, therefore increasing the absorption of dietary iron (Malope, MacPhail et al. 2001).

### 2.3 Stages of iron deficiency

The progression from normal iron balance to iron deficiency anaemia is characterised by three phases (Dallman 1986). These stages are iron depletion, latent iron deficiency and iron deficiency anaemia (Cook and Lynch 1986, Dallman 1986). In the context of research trials, the stages of iron deficiency are referred to in past (Skikne, Flowers et al. 1990) and recent literature (World Health Organization 2004, Goddard, James et al. 2011).

#### 2.3.1 Iron depletion

Iron depletion is the state in which storage iron is absent but the tissues that need iron are able to maintain normal physiological functions (Dallman 1986, Carley 2003, World Health Organization 2004). Generally this stage is asymptomatic, creates no effect on erythropoiesis and is not detected by haemoglobin and haematocrit testing (Carley 2003).

#### 2.3.2 Latent iron deficiency

Latent, early stage or non-anaemic iron deficiency, refers to a decrease in transport iron and is characterised as a substantial reduction in storage iron and haemoglobin synthesis as well as an increased iron-binding capacity and a decrease in serum iron (Carley 2003).

#### 2.3.3 Iron deficiency anaemia

The third stage in this process is iron deficiency anaemia, which occurs when iron stores are insufficient to maintain haemoglobin production and haemoglobin falls (Dallman 1986, Scholl and Hediger 1994). This advanced stage is characterised by low haemoglobin and haematocrit levels. At this stage iron stores have already been significantly depleted (Carley 2003). Detail on the biochemical markers and reference ranges are presented in Section 2.5.
2.4 Haemochromatosis

Haemochromatosis affects approximately 1 in every 200-400 individuals worldwide (Merryweather-Clarke, Pointon et al. 1997). It is a congenital condition that is characterised by progressive iron overload in tissues and is the most common disorder of iron overload (Franchini 2006). The first diagnosis of haemochromatosis was made in 1889 (Pietrangelo 2003). The genetic form of iron overload has an autosomal recessive inheritance associated with mutations of the HFE gene on chromosome 6 (Franchini 2006). During iron overload, enterocytes would normally down-regulate the production of DMT1, reducing the absorption of dietary iron (Olivares, Walter et al. 2000). However, this does not occur in haemochromatosis and therefore excessive iron is absorbed from the gastrointestinal tract. Iron overload can lead to irreversible organ damage, predominately to the liver, if therapeutic phlebotomy is not timely (Siah, Ombiga et al. 2006).

2.5 Measuring iron status

Haematological markers have been used by health professionals and researchers to define iron deficiency for decades. However, consensus on the most reliable markers to identify iron deficiency is yet to be reached. In 2004, the World Health Organization (WHO) recommended the best approach to assessing iron status was to measure ferritin, haemoglobin and soluble transferrin receptor (sTfR) (World Health Organization 2004). The WHO uses the following haematological criteria for latent iron deficiency: Ft <20ug/L, Hb >120g/L (World Health Organization 2001). Moderate iron deficiency occurs when Hb remains >120g/L, Ft is <15ug/L and sTfR is raised (based on varying reference ranges) (Leonard, Patterson et al. 2013). Iron deficiency anaemia can be classified as Hb <120g/L, Ft <20ug/L, and reduced mean red cell volume (World Health Organization 2001). Latent iron deficiency is three times as common as iron deficiency anaemia (World Health Organization 2001).

Haas and Brownlie (2001) and Punnonen et al. (1997) both reported on the difficulty in diagnosing latent iron deficiency, as opposed to iron deficiency anaemia due to wide reference ranges for iron markers (Punnonen, Irijala et al. 1997, Haas and Brownlie 2001). In addition to soluble transferrin receptor and haemoglobin, Punnonen et al. (1997) and
Haas (2001) also report the benefit of serum iron, total iron binding capacity (TIBC), transferrin saturation and mean red cell volume (MCV) in the assessment of iron status (Punnonen, Irjala et al. 1997, Haas and Brownlie 2001). Haas (2001) reports that each of these tests has merit, however if considered individually, they are unable to reliably diagnose latent iron deficiency (Haas and Brownlie 2001).

2.5.1 Ferritin

Ferritin (Ft) is known as the most useful haematological measure of iron deficiency, and has been used for the past 25 years (Cook 2005, Zhu, Kaneshiro et al. 2010). Ferritin is a standardised marker of iron depletion and reflects residual iron stores (Punnonen, Irjala et al. 1997, Haas and Brownlie 2001). A limitation of ferritin is its vulnerability to elevate independently of iron deficiency in individuals with acute inflammation (Cook 2005). This means that ferritin is an unreliable measure on its own.

2.5.2 Haemoglobin

Haemoglobin (Hb) measures deficits in functional iron and is a universally available marker used in the diagnosis of iron deficiency anaemia (Cook, Lipschitz et al. 1974, Haas and Brownlie 2001). An individual with normal iron stores must lose a significant amount of body iron before Hb falls below reference ranges for anaemia (Cook 2005). The reference range for Hb varies throughout literature, however is commonly <120g/L (World Health Organization 2001). An important consideration when screening for iron deficiency is the low specificity of Hb, due to the many clinical causes of low Hb besides iron deficiency, such as anaemia of inflammation (Cook 2005). As a result of the low specificity of Hb, more definitive measures such as Ft and soluble transferrin receptor (sTfR) are required to accurately define iron deficiency anaemia (Cook, Lipschitz et al. 1974).

2.5.3 Alpha-1 Glycoprotein

Many haematological tests, especially Ft, are affected markedly by the body’s acute phase response and should therefore be conducted in conjunction with an acute phase protein marker (Thomas and Thomas 2002, World Health Organization 2004). Two of the main acute phase proteins are C-reactive protein (CRP) and Alpha-1-glycoprotein
(A1GP). A1GP is slower to rise, but remains at a high concentration longer than C-reactive protein, so may be a better indicator of chronic sub-clinical infection than CRP, and may better reflect changes in the concentration of ferritin during infections. CRP responds quickly to inflammation (within 5 hours), but subsides quickly. Using both CRP and A1GP together is the most accurate way to estimate the prevalence of iron deficiency (Thurnham, McCabe et al.).

2.5.4 Soluble transferrin receptor

In most cases, soluble transferrin receptor concentration is not elevated in response to inflammation (World Health Organization 2001). Therefore, if Ft is within normal ranges or high, and sTfR is high, the reliability of the Ft result could be questioned, as the person may actually be iron deficient.

Soluble transferrin receptor (sTfR) has been shown to be a more accurate marker of latent iron deficiency than serum ferritin alone (Thomas and Thomas 2002). sTfR is a soluble form of the cellular iron membrane receptor, and its concentration is inversely related to intracellular iron (Malope, MacPhail et al. 2001), as the cells will up-regulate the membrane receptors in order to bind more iron and increase intracellular iron concentrations. Like haemoglobin, sTfR reflects the functional iron compartment (tissue iron supply) (Punnonen, Irjala et al. 1997, Malope, MacPhail et al. 2001). This test is not an acute phase reactant which enhances its reliability (Olivares, Walter et al. 2000). Circulating levels of sTfR have been identified as a sensitive measure in the diagnosis of iron deficiency anaemia and latent iron deficiency since the late 1900s (Haas and Brownlie 2001, Malope, MacPhail et al. 2001). Levels of circulating sTfR increase with increased cellular iron needs (Punnonen, Irjala et al. 1997). In a study of baseline haematological data from the pilot RCT implemented for this PhD, sTfR was not found to be as useful a marker of latent iron deficiency as first envisaged. This study on the usefulness of sTfR as a marker of latent iron-deficiency is further discussed in Chapter 7.
2.5.5 sTfR/LogFt-Index

The combination of storage and functional iron markers is represented by the sTfR/LogFt-Index (Makrides, Crowther et al. 2003), which aims to increase the accuracy of iron deficiency diagnosis. There is no gold standard reference range for sTfR or the sTfR/LogFt-Index, it is dependent upon the assay and laboratory used.

2.6 Prevalence of iron deficiency

Iron deficiency affects up to two thirds of young women in developing countries (Scrimshaw 1991, Stoltzfus). It is not only widespread in developing countries, iron deficiency is also prominent in developed countries including the U.S, Japan and Europe, where 10 to 20 per cent of women are affected (Scrimshaw 1991, World Health Organization 2001). Iron deficiency affects approximately 10 to 20 per cent of preschool children in developed countries and up to 80 per cent in developing countries (World Health Organization 2012). Men can also be affected by iron deficiency, but at much lower rates (World Health Organization 2001).

2.6.1 Prevalence of iron deficiency in Australian women

There is limited reliable data on the prevalence of iron deficiency among young Australian women, as few studies have examined this in representative samples of women since the 1980’s. In 2007, a study was conducted to investigate biochemical markers of nutrition status in women (Fayet, Samman et al. 2007). The study included 308 University educated females with a mean age of 22.6 years. Blood samples were obtained and eating behaviours were assessed using the Three-Factor Eating Questionnaire (Fayet, Samman et al. 2007). Data from this study revealed that 32% of the sample had low iron levels (Fayet, Samman et al. 2007). Rangan et al. (1998) reported that 26.6% of their young sample of 255 female University students aged 15-30 years were iron deficient (Rangan, Blight et al. 1998). While the study was among an older cohort of women, the Australian Diabetes, Obesity and Lifestyle Study (AusDiab) (2008) found that 20% of their sample of 1634 women aged 25-50 years were iron deficient (Ahmed, Coyne et al. 2008).
In these studies there was inconsistency in the criteria used for determining iron status, with Fayet et al. using Hb <120g/L and Ft<15 µg/L; Rangan et al. using Hb 120-160g/L, Ft <12 µg/L; and the AusDiab study using Hb <120g/L, Ft <12µg/L for iron depletion, and between 12 µg/L and <20 µg/L for marginal iron deficiency. Also, Rangan et al. and Fayet et al. restricted their recruitment to female University students aged 15-30 years, whereas the AusDiab study recruited a large, community based sample of a greater age range. A limitation of the AusDiab study and the study by Fayet et al. was that they did not measure markers of inflammation, possibly leading to an overestimation of serum ferritin and underestimation of iron deficiency (Ahmed, Coyne et al. 2008). Rangan et al. measured sTfR which is not influenced by inflammation (Rangan, Blight et al. 1998). Australian prevalence data from these three studies are reported in Table 2.1.

**Table 2.1 Recent data on prevalence of iron deficiency among Australia females**

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Sample population</th>
<th>Prevalence of iron deficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Rangan, Ho et al. 1997)</td>
<td>1997</td>
<td>255 female university students aged 15-30 years (NSW, Aus)</td>
<td>27</td>
</tr>
<tr>
<td>Australian Diabetes, Obesity and Lifestyle study (Ausdiab) (Ahmed, Coyne et al. 2008)</td>
<td>2008</td>
<td>1634 females aged 25-50 years (QLD, Aus)</td>
<td>20</td>
</tr>
<tr>
<td>(Fayet, Samman et al. 2007)</td>
<td>2007</td>
<td>308 female University students 18-30 years (NSW, Aus)</td>
<td>32</td>
</tr>
</tbody>
</table>

### 2.7 Non-dietary determinants of iron deficiency

The underlying cause of iron deficiency is an imbalance between iron requirements and iron absorption (World Health Organization 2001). Imbalance may be due to blood loss, caused by menstruation, childbirth or blood donation, or basal losses. These mechanisms of iron loss and loss per annum are reported in Table 2.2.

**Table 2.2 Annual blood and iron losses in reproductive aged females Lifestyle/Physiology**

<table>
<thead>
<tr>
<th>Mechanisms of iron loss</th>
<th>Blood lost per Annum</th>
<th>Iron loss per Annum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal losses (Baynes and Bothwell 1990)</td>
<td>N/A</td>
<td>~365mg</td>
</tr>
<tr>
<td>Gastrointestinal lesions (Zhu, Kaneshiro et al. 2010)</td>
<td>N/A</td>
<td>~365mg</td>
</tr>
<tr>
<td>Menstruation (normal) (Price, Forsyth et al. 1964)</td>
<td>430ml (33ml/month)</td>
<td>~137mg</td>
</tr>
<tr>
<td>Menstruation (menorrhagia) (Janssen, Scholten et al. 1998)</td>
<td>960-1,140ml (80-120ml/month)</td>
<td>~500mg</td>
</tr>
<tr>
<td>Pregnancy (Beard and Tobin 2000)</td>
<td>N/A</td>
<td>1,000mg</td>
</tr>
<tr>
<td>Blood donation (Finch, Cook et al. 1977, Newman 2006)</td>
<td>~2000ml (400-500ml/donation)</td>
<td>~204-300mg</td>
</tr>
</tbody>
</table>
2.7.1 Basal and gastrointestinal iron loss

Basal iron loss is unavoidable and occurs via the sloughing of endothelial and epithelial cells. It can equate to approximately 1mg/day (Baynes and Bothwell 1990, Zhu, Kaneshiro et al. 2010). Basal losses are related to body size and iron status, they increase in states of iron deficiency and decrease in states of iron overload (Hallberg and Rossander-Hulten 1991). Gastrointestinal lesions can account for 1-2mg iron loss daily (Zhu, Kaneshiro et al. 2010). Bleeding lesions occur in approximately 50% of people, with peptic ulcer disease being the most common cause (Powell and McNair 2008).

2.7.2 Menstruation

Menstruation is the most significant factor that increases a female’s risk of iron deficiency (Denic and Agarwal 2007). Young women commonly have insufficient iron stores to counter the substantial iron losses that occur through normal menstrual bleeding (Galan, Yoon et al. 1998). Volume of menstrual blood loss among females can vary significantly (Price, Forsyth et al. 1964). It is estimated that an average of 33ml of menstrual blood may be lost per month. Iron loss as a result of menstruation can be between 1.5mg to 2.1mg each day of menstruation (Beard, Dawson et al. 1996). Menorrhagia is excessive menstrual blood loss of more than 80ml per month (Kadir, Economides et al. 1998). Approximately 5% of women aged 30 to 49 years consult their General Practitioner about menorrhagia and 12% of all gynaecological referrals are for this complaint (Kadir, Economides et al. 1998).

2.7.3 Pregnancy and childbirth

Pregnant females are also at an increased risk of iron deficiency (Beard and Tobin 2000). Increased red blood cell mass and expansion of plasma volume occur during pregnancy. Approximately 1000mg of iron is lost over the course of the pregnancy, due to basal losses, increased maternal red cell mass, foetal needs and amniotic fluid (Beard, Dawson et al. 1996). Substantial blood loss that occurs during labour may also contribute to the risk of iron deficiency (Beard and Tobin 2000). Scholl et al. (1994) found that iron deficiency was associated with poorer pregnancy and birth outcomes, including low birth weight and preterm delivery (Scholl and Hediger 1994). In this study of 826 females
aged 12-29 years, the odds of preterm delivery more than doubled and the odds of low birth weight infants tripled, in iron-deficient participants (Scholl and Hediger 1994).

### 2.7.4 Blood donation

Latent iron deficiency is prevalent in blood donors, especially in premenopausal females and frequent donors (Salvin, Pasricha et al. 2014). Blood donors are 2.5 times more likely to have low iron stores (Fogelholm, Alopaeus et al. 1993). Individuals in Australia and the United States are able to provide up to four donations per year (Newman 2006, Salvin, Pasricha et al. 2014). Standard whole blood donations in Australia are 470mL which result in the loss of 200 to 250 mg of iron (Finch, Cook et al. 1977, Cook, Flowers et al. 2003).

### 2.8 Dietary determinants of iron deficiency

To maintain a normal iron balance, iron losses should be replaced by an equivalent amount of iron derived from dietary sources (Beard, Dawson et al. 1996).

#### 2.8.1 Nutrient Reference Values for iron

In 2006, the Australian National Health and Medical Research Council released Nutrient Reference Values (NRV’s) that were based on the 1995 National Nutrition Survey (National Health and Medical Research Council 1991). Young women have the highest iron requirements of all population groups, due to the effects of menstruation and childbirth. Hence the Recommended Dietary Intake (RDI) for iron is set at 18mg/day for women aged 19-30 years in Australia (National Health and Medical Research Council 1991). The RDA in the US is similarly high (18mg/day) (National Research Council 2001), and the RDI in the UK is 14.8mg/day (Scientific Advisory Committee on Nutrition 2010), while the male equivalent is set at 8mg/day (National Health and Medical Research Council 1991). This recommendation reflects the very high variability in iron requirements in this population because of the large range in menstrual loss experienced by women (National Health and Medical Research Council 1991). The RDIs are set to cover 97.5% of the population’s requirements, and while only a small proportion of women experience extremely high menstrual blood loss, the RDI is set at 18mg/d to cover the majority of women’s requirements, and is therefore higher than actually
needed for many women. An upper limit was set at 18% iron absorption with the aim of accounting for typical iron absorption rates from an omnivorous diet. Iron absorption rates for vegetarian diets are lower. The estimated average requirement for iron is 8mg/day which is the daily intake estimated to meet the requirements of half the healthy females in this particular life stage (National Health and Medical Research Council 1991).

2.8.2 Iron intakes of young women

Women with latent iron deficiency or concerns about their iron status are commonly advised by General Practitioners to increase their intake of dietary iron rather than commencing iron supplementation (Heath, Skeaff et al. 2001). Intensive dietary programs have been shown to improve iron status in young women in a limited number of studies (Heath, Skeaff et al. 2001, Patterson, Brown et al. 2001), though whether advice from GPs to do so is efficacious is unknown.

Data from the Australian Longitudinal Study of Women’s Health has shown that many young women do not consume foods in accordance with the national food selection guide (Australian Guide to Healthy Eating), and that this is associated with inadequate nutrient intakes, including iron (Blumfield, Hure et al. 2011). In 1999 the CSIRO reported that 66% of Australian females aged between 19 and 44 years consumed less than 12mg of iron per day (the RDI at the time) (Baghurst 1999). In France, Galan et al. (1998) determined that 93% of menstruating females, aged 35-60 years, consumed lower than the RDI for iron (≥16mg per day) as assessed using six 24 hour food recalls collected during a 12 month period (Galan, Yoon et al. 1998). Comparisons of the 1983 and 1995 National Nutrition Surveys in Australia show notable increases in iron intakes for young women (Ball, Mishra et al. 2004). However, the 1995 National Nutrition Survey revealed that mean dietary iron intake for women aged 19-24 years was 11.9mg/day and 12mg/day for women aged 24-44 years, which is well below the RDI of 18mg/day (Ball and Bartlett 1999). Fayet et al. (2007) revealed that 9.4% of the Australian females of childbearing age in their study did not eat red meat at all (Fayet, Samman et al. 2007). Reasons for the gap between the RDI for iron and actual iron intake have been hypothesised as a lack of affordability of flesh foods and poor access to fresh foods (Ball, Mishra et al. 2004). Cereal sources of iron are much more affordable than flesh foods,
and make a considerable contribution to total iron intakes for all population sub-groups in Australia (Australian Bureau of Statistics 1998). Data from the 1995 National Nutrition Survey revealed that approximately 55% of iron intake was provided by cereal products and meat products, with cereal-based products and vegetable products contributing an additional 20%. Cereal foods made a larger contribution to the dietary intakes of children than that of adolescents and adults, the reverse applied to meat sources of iron (Australian Bureau of Statistics 1998).

Vegetarians are at increased risk of iron deficiency due to little or no intake of haem iron (Ball and Bartlett 1999). Several studies have reported that iron deficiency is more common in vegetarians and in those who eat meat less than twice a week (Heath, Skeaff et al. 2001, Gibson 2004). Heath et al. (2001) reported low intake of flesh foods in their sample of young women (Heath, Skeaff et al. 2001). Significantly lower ferritin levels in vegetarians compared with omnivores have been reported despite adequate intakes of total iron (McEndree, Kies et al. 1983, Hunt 2003, Ball, Mishra et al. 2004).

### 2.8.3 Bioavailability of dietary iron

The National Health and Medical Research Council (NHMRC) state that adult menstruating women need to absorb 1.5mg of iron per day to achieve iron balance, although this is dependent upon individual variation in quantities of menstrual blood iron loss (National Health and Medical Research Council 1991, Birgegard, Gascon et al. 2006). The absorption and bioavailability of iron in the body is more important for iron status than total iron intake (National Health and Medical Research Council 1991). Iron bioavailability is estimated to be 14-18% for mixed diets and 5-12% for vegetarian diets, in individuals with low iron stores (Hurrell and Egli 2010). The bioavailability of dietary iron is influenced by the type of iron consumed. There are two types of dietary iron: haem, which comes from haemoglobin and myoglobin in animal food sources; and non-haem, which comes predominantly from plant food sources (Hurrell and Egli 2010). Haem iron contributes approximately 10-15% of total iron for individuals who eat meat, and it is estimated that 15-35% of this iron is absorbed (Hurrell and Egli 2010).

Non-haem iron is not absorbed as efficiently due to its dependence on the balance between absorption enhancers and inhibitors, approximately 2-15% is absorbed from the
gastrointestinal tract (Monsen, Hallberg et al. 1978, National Health and Medical Research Council 1991, Hurrell and Egli 2010). Ascorbic acid, such as found in citrus, is known to increase absorption of non-haem iron when consumed at the same time, by converting ferric iron ($\text{Fe}^{3+}$) to ferrous iron ($\text{Fe}^{2+}$) (Monsen, Hallberg et al. 1978, Hacisevkd 2009). Other acids, such as vinegar, can have a similar effect in increasing iron absorption from non-haem sources. The absorption of non-haem iron is also increased by consuming meat, poultry or fish in the same meal (Collings, Harvey et al. 2013). The enhancing effect of meat, poultry and fish on non-haem iron foods (also known as the meat, poultry, fish factor) especially cereal-and legume-based meals has been well reported, however the nature of this effect is unclear (Hurrell, Reddy et al. 2006). In 1976, a two-fold increase in the absorption of non-haem iron was reported when beef, pork, chicken and fish were consumed with the same meal (Cook and Monsen 1976).

Certain dietary components inhibit the absorption of iron, these include tannins (found in coffee and tea) phytates (found in cereals, legumes and nuts), and calcium (Monsen, Hallberg et al. 1978). These foods are best not avoided, because they are beneficial for other reasons, for example: phytates are predominantly found in high fibre foods; tannins in tea and coffee act as antioxidants; and calcium is an essential mineral for whom intake is often marginal. It is therefore important to manage these competing interests by consuming these foods separately from iron-rich foods to assist in the prevention of iron deficiency (Monsen, Hallberg et al. 1978, National Health and Medical Research Council 1991). Phytates and tannins inhibit the absorption of non-haem iron only, whereas, calcium has been shown to inhibit both haem and non-haem iron (Hallberg, Brune et al. 1991).

### 2.9 Nutrition knowledge of iron

Low dietary iron intakes in young women may be a result of poor knowledge and awareness about nutrition, specifically in regard to iron (Kabir, Shahjalal et al.). Evidence on the impact of nutrition knowledge on health is scarce, which may be due to poor conceptualisation of nutrition knowledge, perceived lack of relevance, poor measurement, poor matching of knowledge and outcome variables or insufficient statistical power to indicate a significant result (Worsley 2002).
To date, the only study to examine nutrition knowledge on dietary iron intake was conducted in 2011 in South America in 1,301 children aged 6-14 years from 17 schools (Garcia-Casal, Landaeta-Jimenez et al. 2011). Garcia-Casal et al. (2011) educated teachers about the importance of sound nutrition with an emphasis on iron deficiency prevention and assessed its effect on prevention of iron deficiency among the sample population. The study conducted haematological assessments at baseline and follow-up. The nutrition education intervention was designed with consideration of students’ baseline level of nutrition knowledge, which was assessed at the beginning of the study by means of a written test. A ‘learning by doing’ approach was employed in the program which included workshops, participative talks, game activities, a cooking course and a recipe competition. Results indicated serum ferritin improved from 12.00±10.17µg/L before, to 24.33±18.34µg/L after the intervention, indicating that educational initiatives have an impact on improving nutritional health in children (Garcia-Casal, Landaeta-Jimenez et al.).

There are also few studies that have examined the influence of nutrition knowledge on other elements of dietary intake, although the evidence that does exist suggests a positive relationship. Wardle et al. (2000) examined food choice behaviours in 19,278 male and female University students aged 17-30 years from 23 countries (Wardle, Haase et al. 2004). They determined that higher nutrition knowledge scores were significantly associated with ‘healthy eating’ and participants who had an increased level of knowledge were 25 times more likely to consume adequate amounts of fruit and vegetables (Wardle, Parmenter et al. 2000, Worsley 2002). Harnack et al. investigated the presence of a relationship between nutrition knowledge for cancer prevention and dietary behaviour for cancer prevention in a large sample of 10,286 US adults (Harnack, Block et al. 1997). These authors reported that, after adjustment for confounding co-variates, knowledge and belief constructs were predictive of dietary behaviour (Harnack, Block et al. 1997). A Belgian study conducted in 2009 used a nutrition knowledge questionnaire originally developed by Parmeter et al. (1999) in a population of 803 Belgian women aged 18-39 years (Parmenter and Wardle 1999, De Vriendt, Matthys et al. 2009). Authors found that improved nutrition knowledge was related to increased fruit and vegetable intake (De Vriendt, Matthys et al. 2009).
2.10 Consequences of iron deficiency

Iron deficiency anaemia is known to be detrimental to work performance, immunity, and thermoregulation (World Health Organization 2001). Impaired mental health and cognitive function, and increased fatigue have also been reported as being effects of iron deficiency (Elwood and Hughes 1970, Haas and Brownlie 2001, Murray-Kolb and Beard 2007).

2.10.1 Work performance

In 1977 iron deficiency was shown to significantly impair physical work performance due to reduced total work time, ability to reach maximal work load, heart rate in response to work and increased post-exercise blood lactate (Gardner, Edgerton et al. 1977). A systematic review of more recent studies in this area described work capacity as measured by aerobic capacity, endurance, efficiency, voluntary activity and work load (Haas and Brownlie 2001). This review revealed that iron deficiency as defined by subnormal iron status, particularly resulting in anaemia, reduces maximal oxygen uptake (Haas and Brownlie 2001). The reduced oxygen uptake leads to an inability to sustain moderate to heavy physical labour (Haas and Brownlie 2001).

2.10.2 Immunity

The effect of iron deficiency on normal development of the immune system has been extensively studied and functional immune defects have been consistently shown (Ekiz, Agaoglu et al. 2005). Iron is necessary for immune cell proliferation, and iron deficiency is associated with defective host mechanisms (Bhaskaram and Reddy 1975). The clinical importance of a relationship between iron deficiency and immune function is not well defined due to unclear aetiology (Lynch, Sazawal et al. 2001). However, increased susceptibility to infections, assessed by levels of mature T-lymphocytes, in iron-deficient patients has been observed (Ekiz, Agaoglu et al. 2005).

2.10.3 Thermoregulation

Poor thermoregulation in iron-deficient humans has been documented (Martinez-Torres, Cubeddu et al. 1984, Beard, Borel et al. 1990). Beard et al. (1990) revealed significantly lower body temperature (36.0±2 cf. 36.2±1°C) in iron-deficient anaemic
females aged between 18 and 44 years compared with latent iron-deficient and control females (Beard, Borel et al. 1990). Beard also reported significant improvements in the ability to maintain body temperature after supplementation in previously iron-deficient participants (Beard, Borel et al. 1990).

2.10.4 Fatigue

Evidence of a relationship between iron deficiency and increased levels of fatigue has been reported in several studies (Elwood and Hughes 1970, Haas and Brownlie 2001, Patterson, Brown et al. 2001, Brutsaert, Hernandez-Cordero et al. 2003, Verdon, Burnand et al. 2003, Mansson, Johansson et al. 2005, Vaucher, Druais et al. 2012). Iron deficiency leads to a greater energy cost to perform the same task as an iron-sufficient individual, rendering the iron-deficient individual more fatigued (Haas and Brownlie 2001). Brutsaert et al. (2003) report that iron deficiency decreases skeletal muscle capacity for aerobic muscle metabolism, increasing susceptibility to fatigue (Brutsaert, Hernandez-Cordero et al. 2003). Reports of a relationship between anaemic iron deficiency and fatigue appear stronger than those in latent iron deficiency (Galan, Yoon et al. 1998). Verdon et al. (2003) conducted a double blind placebo controlled trial to determine the subjective response to iron therapy in 144 latent iron-deficient females aged 18-55 years (Verdon, Burnand et al. 2003). This study found that iron supplementation reduced fatigue measured by a 10 point visual analogue scale (Verdon, Burnand et al. 2003). Similarly, Vaucher (2012) revealed that iron supplementation in 198 females aged 18-53 years, with latent iron deficiency improved previous feelings of fatigue (Vaucher, Druais et al. 2012). Patterson et al. (2001) examined the effects of iron deficiency and its treatment by iron supplementation or a high iron diet on fatigue in 66 women aged 18-50 years. This study found both treatment options reduced fatigue in women of childbearing age, however improvements in fatigue scores were not proportional to changes in iron status (Patterson, Brown et al. 2001). Rangan et al. (1998) measured the effect of iron deficiency on levels of fatigue and found no association (Rangan, Blight et al. 1998). Authors of this study attribute this finding to small sample size of iron-deficient anaemic participants (n=11), stating that with this sample, it was not possible to associate severe iron deficiency with fatigue. Studies have used various assessment tools to measure fatigue.
2.10.5 Mental health

Iron deficiency has been related to impaired mental health in a limited number of studies (Greig, Patterson et al. 2013), but the mechanism by which this relationship occurs is unclear (Rangan, Blight et al. 1998). Validated mental health assessment tools most commonly used are the General Health Questionnaire (Goldberg, Gater et al. 1997) and the Short-form 36 Health Survey (SF-36) (Patterson, Brown et al. 2001). Rangan et al. (1998) reported that anaemic participants scored significantly higher (poorer health) on the General Health Questionnaire than latent, iron-deficient participants (Rangan, Blight et al. 1998). Three studies have shown poorer mental health scores in latent iron-deficient young women at baseline compared with controls. In all three studies mental health scores improved after iron supplementation (Ballin, Berar et al. 1992, Patterson, Brown et al. 2001, Mansson, Johansson et al. 2005). Of these studies one used SF-36 General Health Short-form Survey, the other two studies used unvalidated assessment tools (Ballin, Berar et al. 1992, Mansson, Johansson et al. 2005). Elwood et al. (1970) also revealed poorer mental health scores at baseline as measured by an unvalidated self-appraisal questionnaire, however this study found no improvement after iron treatment (Elwood and Hughes 1970).

2.10.6 General health and wellness

Several non-specific symptoms have been related to iron deficiency, including dizziness, weakness, breathlessness, and irritability, however mechanisms for these effects have not been well defined (Beutler 1959, Elwood and Hughes 1970, Ballin, Berar et al. 1992). Two studies have revealed a relationship between iron deficiency and impaired general health and wellbeing. Patterson et al. (2000) examined associations between self-reported diagnosed ‘low iron’, general health and well-being, vitality and tiredness in women using data from the Australian Longitudinal Study of Women’s
Health (Patterson, Brown et al. 2000). This study used the physical component summary score, mental component summary scores and vitality score from the SF-36 General Health Short-form Survey and showed significantly lower mean physical health, mental health and vitality scores in young women (18-23 years) who reported (ever) having ‘low iron’ at baseline than women with no history of iron deficiency (Patterson, Brown et al. 2000). In addition, this study revealed that young women who reported recent iron deficiency (in the last 2 years) had significantly lower physical health, mental health and vitality scores between baseline and follow-up than other respondents (Patterson, Brown et al. 2000).

Patterson et al. (2001) conducted a randomised controlled trial to examine the effect of iron deficiency on general health and wellbeing among their sample of young women using the SF-36 General Health and Well-being scale. Results from this research showed significant reductions in mental health and vitality scores in the iron-deficient population when compared with controls at baseline. Both mental health and vitality improved with iron treatment (Patterson, Brown et al. 2001).

### 2.10.7 Cognitive functioning

An important consequence of iron deficiency is its effect on cognitive behaviours such as learning, memory, attention and reaction time (Fretham, Carlson et al., Beard and Connor 2003). The exact mechanism by which iron deficiency affects the brain is not well understood, however, potential factors include abnormalities in neurotransmitter metabolism, decreased, myelin formation, and alterations in brain energy metabolism (Tucker, Sandstead et al. 1984, Beard 2003). Within the brain there is a plasma pool of transferrin receptor cells that are responsible for the acquisition of iron. In addition, there is a mechanism for the dispersal of iron (transferrin) and for cell-specific iron storage (ferritin) (Beard 2003). The blood brain barrier regulates the rate of iron uptake and is affected by iron status (Beard 2003). This regulatory process increases iron uptake when iron status is low and decreases it when status is high (Beard 2003). Loss of iron from the brain can occur with dietary iron deficiency and can increase with iron supplementation (Beard 2003). Cognitive effects were once thought to arise only if iron deficiency anaemia was present, however, its impact at earlier stages of iron deficiency is of interest because
the abnormalities have been shown to last long after the resolution of anaemia (Fretham, Carlson et al., Beard and Connor 2003).

2.10.7.1.1 Children and adolescents

Since initial studies in 1978, most research on the effects of iron deficiency on cognitive function have focused on infants and young children (Oski, Honig et al. 1983, Bruner, Joffe et al. 1996). Studies have shown that iron deficiency can cause impaired neurological development and function in infants and children through effects on neurotransmitter function (Oski, Honig et al. 1983, Galan, Yoon et al. 1998, Grantham-McGregor and Ani 2001, Lozoff, Haas et al. 2001). Such changes have been shown to specifically affect concentration, attention and short-term memory in childhood (Lozoff, Brittenham et al. 1982, Lozoff, Brittenham et al. 1982, Walter, Kovalskys et al. 1983, Pollitt, Hathirat et al. 1989, Laessle, Platte et al. 1996). Lozoff et al. (2006) conducted a review of longitudinal research into the effects of iron deficiency on cognition from infancy through to adolescence (Lozoff, Beard et al. 2006). They determined adolescents who had iron deficiency anaemia during infancy continued to perform less well at spatial memory and selective attention tasks than peers who had good iron status in infancy (Lozoff, Beard et al. 2006). Lozoff et al. concluded by stating that direct effects of iron deficiency on the developing brain may result in long term adverse impacts on development.

Ballin et al. (1992) showed improvement in the ability to concentrate in iron-deficient adolescents after iron levels normalised (Ballin, Berar et al. 1992), and Bruner et al. (1996) showed improvements in attention scores after iron treatment in adolescents with non-anaemic iron deficiency (Bruner, Joffe et al. 1996), indicating that some cognitive effects may be reversible with iron treatment.

2.10.7.1.2 Adults

A systematic review of the literature (presented in Chapter 3) found that few studies have investigated the effects of iron deficiency on cognitive function in adults (Greig, Patterson et al. 2013). Of the studies that have been conducted, Murray-Kolb et al. (2007) compared quintiles of iron status at baseline, and reported significantly better performance at an Attention task by those females in the upper quintile compared to the
lowest quintile. They also found that females in the upper quintile completed tasks faster than participants in the lowest quintile (Murray-Kolb and Beard 2007). There was no difference in Attention score between iron-sufficient controls and latent iron-deficient participants (Murray-Kolb and Beard 2007). Three other studies revealed poorer cognition in iron-deficient young women at baseline, when compared with their iron sufficient counterparts (Ballin, Berar et al. 1992, Patterson, Brown et al. 2001, Khedr, Hamed et al. 2008). After iron treatment, studies have shown improvement in some cognitive tasks (attention, learning memory, arithmetic) (Ballin, Berar et al. 1992, Patterson, Brown et al. 2001, Murray-Kolb and Beard 2007, Khedr, Hamed et al. 2008). However, other studies in adults have shown no difference in such tasks between iron-deficient and iron-sufficient participants at baseline (Beard, Hendricks et al. 2005, Manssson, Johansson et al. 2005) or in iron-deficient participants following iron treatment (Elwood and Hughes 1970, Mansson, Johansson et al. 2005). Blanton (2013) recently examined the haematologic and cognitive responses to increases in dietary iron in 43 females with a mean age of 21.1 years (Blanton 2013). Blanton determined that participants who showed increases in ferritin had significantly higher improvements in planning speed, spatial working memory strategy and memory (Blanton 2013).

2.10.7.1.3 Cognitive assessment tools

Studies examining the relationship between iron deficiency and cognitive function have used a range of individual cognitive tests and test batteries (Table 2.3) (Greig, Patterson et al. 2013). Groner et al. (1986) used The Welscher Adult Intelligence Scale (WAIS) and showed no significant difference in cognitive results between iron-sufficient and iron-deficient participants at baseline(Groner, Holtzman et al. 1986). Tasks that make up the WAIS, including Digit Span, Digit Symbol, Maze Test, Block Design and Arithmetic, have also been used individually (Greig, Patterson et al. 2013). Patterson et al. (2000) used the revised version of the WAIS (WAIS-R) (Patterson 1999, Kaufman and Lichtenberger 2006). The WAIS-R consists of six verbal subtests and five performance subtests and is recommended for use in a clinical, educational or research settings (Kaufman and Lichtenberger 2006). Using the WAIS-R, Patterson et al. found significant differences on four subtests between iron-sufficient and iron-deficient participants at baseline (Block Design, Digit Span, Digit Symbol and Arithmetic). Blanton (2013) used the Cambridge
Neuropsychological Test Automated Battery (CANTAB) which contains 25 computerized neuropsychological tests (Blanton 2013, Cambridge Cognition 2013). Blanton found that body iron had significant beneficial effects on spatial working memory and planning speed (Blanton 2013). Murray-Kolb et al. used the Cognitive Abilities Test (CAT; 22) and found a significant difference in cognitive results between iron-sufficient participants and those with iron-deficiency anaemia (Murray-Kolb and Beard 2007).

<table>
<thead>
<tr>
<th>Source</th>
<th>Cognitive/mental health/fatigue assessment tool</th>
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<tr>
<td>Blanton (2013)</td>
<td>Cambridge Neuropsychological Test Automated Battery (CANTAB)</td>
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<tr>
<td>Beard et al. (2005)</td>
<td>Raven’s Coloured Progressive Matrices test</td>
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<tr>
<td>Murray-kolb et al. (2007)</td>
<td>Cognitive abilities test (CAT;22)</td>
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<tr>
<td>Bruner et al. (1996)</td>
<td>Brief Test of Attention (BTA), Symbol Digit Modalities Test (SDMT), Visual Search Attention Test (VSAT), Hopkins Verbal Learning Test (HVLT)</td>
</tr>
<tr>
<td>Ballin et al. (1992)</td>
<td>Self-report (Medical questionnaire)</td>
</tr>
<tr>
<td>Elwood et al. (1970)</td>
<td>Serial Sevens, E-test, Maze test, Card Sorter</td>
</tr>
<tr>
<td>Groner et al. (1986)</td>
<td>Wechsler Adult Intelligence Scale (WAIS), Wechsler Intelligence Scale Children (WISC), Consonant Trigrams, Rey Auditory Verbal Learning Test</td>
</tr>
<tr>
<td>Patterson et al. (2000)</td>
<td>WAIS-R</td>
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<tr>
<td>Mansson et al. (2005)</td>
<td>Self-report (Quality of Life questionnaire)</td>
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<tr>
<td>Khedr et al. (2008)</td>
<td>Wechsler Memory Revised Scale (WMS-R), Wechsler Adult Intelligence Scale-Revised (WAIS-R)</td>
</tr>
<tr>
<td>Fordy et al. (1994)</td>
<td>General Health Questionnaire (GHQ), Simple and Complex Reaction Time, Memory, Digit Symbol Substitution, Continuous Attention Test</td>
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<tr>
<td>Kretsch et al. (1998)</td>
<td>Bakan Vigilance Task, Word Recall Task, Two Finger Tapping Task, Ericksen Effect</td>
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Table modified from Greig et al. (2003) (Chapter 3) (Greig, Patterson et al. 2013).

2.11 Management of iron deficiency

Recommendations for the treatment of iron deficiency anaemia include elemental iron supplementation and increased dietary iron intake (Gibson, Heath et al. 2002, Mora 2002). Patterson et al. (1998) described the importance of investigation into the treatment of latent iron deficiency, which is an area of research that is currently limited (Patterson 1999). It is imperative that iron deficiency is effectively managed to prevent progression to anaemia.

In many cases, young women are not prescribed supplementation for latent iron deficiency, and instead are recommended to increase their dietary iron intake (Galloway and McGuire 1994). However, dietary iron intake in this population remains much lower than the RDI (Ball, Mishra et al. 2004). Ball et al. (2004) analysed data from the Australian
Longitudinal Study of Women’s Health (ALSWH) and found that fewer than one third of women met the RDI for iron (Ball, Mishra et al. 2004). They suggest that knowledge and awareness about nutrition and poor access to fresh food, may act as barriers to compliance with dietary guidelines (Ball, Mishra et al. 2004).

Clinical practice guidelines for the management of iron deficiency have been developed in the United States (Stoltzfus and Dreyfuss), the United Kingdom (Goddard, James et al. 2011) and in Australia (Therapeutic Guidelines Ltd 2006, Australian Medicines Handbook Pty Ltd 2010). These all recommend the use of dried ferrous sulfate which contains approximately 33% elemental iron. Ferrous sulphate is the most commonly used iron supplement; it has high bioavailability and is cost effective (Gillespie, Kevany et al. 1991). Clinical practice guidelines recommend a daily dose of 100-210mg elemental iron for a minimum of two to four months in the treatment of iron deficiency anaemia in adults (National Prescribing Service 2010). Treatment of latent iron deficiency and the impact of using lower dose iron treatment on iron status are not articulated within current iron treatment guidelines.

Ideally, supplementation should achieve maximal absorption with minimal side-effects (Hallberg, Ryttinge.L et al. 1966). Oral iron therapy has been associated with a high incidence of gastrointestinal side-effects such as nausea, constipation and darkening of stools which lead to discontinuation of treatment in 21% of patients (Australian Medicines Handbook Pty Ltd 2010, Bayraktar and Bayraktar 2010, Zhu, Kaneshiro et al. 2010). Such side-effects can compromise blinding and adherence to treatment protocols within trials. Few studies have examined the effect of different doses of iron therapy on iron status (Makrides, Crowther et al. 2003, Rimon, Kagansky et al. 2005, Mozaffari-Khosravi, Noori-Shadkam et al. 2010). Of the studies that have examined this, findings indicate that lower doses of elemental iron (20-60mg per day) have fewer side-effects (Macdougall 1999, National Prescribing Service 2010).
2.12 Summary

This chapter provides a comprehensive review of iron deficiency, its causes and consequences. It is apparent that iron deficiency remains the most common nutritional disorder worldwide, and most commonly affects young women. There are limited recent Australian data on the prevalence of iron deficiency in young women.

Currently, haematological testing of iron status utilises a variety of markers, assays and reference ranges because no formalised procedures have been published. The most recently developed method is sTfR which has been shown to most accurately define iron deficiency anaemia. There is a need for further research on the usefulness of sTfR as a marker of latent iron deficiency as this has rarely been reported in literature.

Dietary iron intake in young women is significantly lower than the RDI for iron and has been for decades. This review identifies the need for research into the cause of the inadequate dietary intake of iron. One possibility is poor nutrition knowledge. Nutrition knowledge of topics such as Food Guide Pyramid, childhood nutrition, food sources and functions of nutrients has been positively associated with weight loss (Klohe-Lehman, Freeland-Graves et al. 2006). However, the effect of nutrition knowledge on dietary iron intake is unknown and should be investigated.

This review describes the consequences of iron deficiency, which include impaired thermoregulation, immunity and cognitive function. Studies have shown that alterations in brain energy metabolism, decreased myelin formation, and abnormalities in neurotransmitter metabolism are responsible for the correlation between iron deficiency and cognitive deficits, but most of the work has been in infants and young children. Other commonly reported effects of iron deficiency are fatigue and increased levels of depression and anxiety. A high quality systematic review of literature on the effects of iron deficiency on cognition, mental health and fatigue in young women is required. There is a limited amount of research on the effect of iron deficiency on cognitive function in young women. However, the research that does exist is confounded by the use of multiple and various cognitive tests. A randomised controlled trial using an easy
to administer cognitive test battery is required to examine the effects of latent iron deficiency on cognition in young women.

Finally, recommendations for the management of iron deficiency include increasing dietary iron intake and using elemental iron supplementation. The most appropriate dosage of elemental iron to treat latent iron deficiency without causing side-effects is currently unknown. Therefore, research comparing dosages is required to determine an ideal iron supplement dosage to adequately improve iron status without negative side-effects.
Chapter 3. Systematic Review

This article was published in 2013.


The work presented in the manuscript was presented at The Annual Scientific Meeting Nutrition Society of Australia and New Zealand in November 2011. Queenstown, New Zealand (Oral presentation).

The work presented in the manuscript was completed in collaboration with the co-authors (Appendix 14).
3.1 Overview

Previous research has shown that iron deficiency negatively impacts cognitive function in children, probably by altering brain energy metabolism and neurotransmitter function. However, whether iron deficiency has detrimental effects on cognition, mental health and fatigue in women of childbearing age is unclear. The primary aim of this systematic review was to determine whether iron deficiency in women of childbearing age affects cognition, mental health and fatigue, and whether change in iron status results in improvements in cognition, mental health and fatigue. Paper 1 commences verbatim from Section 3.2. The methods are presented in Section 3.4, results in Section 3.5, and discussion in Section 3.6.

3.2 Abstract

It is known that iron deficiency negatively impacts on cognitive function in children by altering brain energy metabolism and neurotransmitter function. Whether iron deficiency has detrimental effects on cognition, mental health and fatigue in women of childbearing age is unclear. Our aim was to systematically review the literature to determine whether iron deficiency in women of childbearing age affects cognition, mental health and fatigue, and whether change in iron status results in improvements in cognition, mental health and fatigue. Studies using iron supplement interventions were reviewed to examine the effect of iron deficiency in women of childbearing age (13-45 years) on cognition, mental health and fatigue. English-language articles from the earliest record to 2011 were sourced. Quality of retrieved articles was assessed and iron pathology, cognitive, mental health and fatigue data were extracted. Means and standard deviations from cognitive test data were included in meta-analyses of combined effects. Of 1348 studies identified, 10 were included in the review. Three studies showed poorer cognition and mental health scores and increased fatigue with iron deficiency at baseline. Seven studies reported improvement in cognitive test scores with iron treatment. Results from three of these studies were included in meta-analyses of the effect of iron supplement intervention on cognition. The results of the meta-analyses showed a significant improvement in Arithmetic scores after treatment (p < .01), but no effect on digit symbol, digit span or block design. While improvement in
cognition after iron treatment was seen in 7 of 10 studies, the evidence base is limited by poor study quality and heterogeneity across studies. Additional high quality studies using consistent measures are warranted.

3.3 Introduction

Iron deficiency is the most prevalent nutritional deficiency worldwide (World Health Organization 2012). Internationally, rates of iron deficiency are highest for infants and young children during their first two years of life and women of childbearing age (World Health Organization 1992). Women of childbearing age are at particular risk of iron deficiency due to the increased demand for iron during pregnancy, as well as the iron losses resulting from menstruation and during childbirth (Lee, Dobson et al. 2005). Other possible causes of iron deficiency include diets that are low in iron and high in iron absorption inhibitors such as phytates and polyphenols (Samman 2007).

Iron deficiency is characterised by a reduction in stored iron, which is most commonly measured by the marker, serum ferritin (World Health Organization 2001). Functional iron is often measured by haemoglobin (Cook 2005). For the adult female population, normal serum ferritin is usually defined as $>20\mu g/L$ and normal haemoglobin as $>120g/L$ (World Health Organization 2001, World Health Organization 2004). For the purposes of this review, participants with normal serum ferritin and haemoglobin levels were considered as iron sufficient. Non-anaemic iron deficiency was classified as serum ferritin $\leq 20\mu g/L$, haemoglobin $>120g/L$ in conjunction with two other markers indicative of iron deficiency (serum iron $<10\mu mol/L$, total iron binding capacity $>68\mu mol/L$, serum transferrin saturation $<15\%$) (World Health Organization 2001). Iron deficiency anaemia is the most severe form of iron deficiency and results in haemoglobin $\leq 120g/L$, in addition to satisfying the markers for iron deficiency (Falkingham, Abdelhamid et al. 2010).

Up to two thirds of women of childbearing age in developing countries suffer from iron deficiency (Scrimshaw 1991). Iron deficiency in women of childbearing age is not merely a phenomenon of developing nations, with rates of 10 to 20% found in the USA, Japan and Europe (Scrimshaw 1984, World Health Organization 1992). In Australia, prevalence
estimates for women of childbearing age are limited, but recent data from the Queensland cohort of the Ausdiab study indicate that rates are high, with one in five women aged 25-50 years having either mild (9.7% with serum ferritin 12-20ug/L) or moderate iron deficiency (10.6% with serum ferritin <12ug/L) (Ahmed, Coyne et al. 2008). Another Australian study has shown high rates of iron deficiency with 32% of a convenience sample of women of childbearing age from the University of Sydney (mean age 22 years) having a serum ferritin of <15ug/L, corresponding with depleted iron stores (Fayet, Samman et al. 2007). Note that each of these studies used a different cut-off for serum ferritin. A New Zealand study assessed the dietary iron intakes and biochemical iron status of a nationally representative sample of women aged 15-49 years. Results indicated that the prevalence of iron deficiency anaemia and non-anaemic iron deficiency ranged from 1.4-5.5%, and for iron deficiency without anaemia from 0.7-12.6% (Ferguson, Morison et al. 2001).

3.4 Objectives

The aim of this paper was to review the literature on the effects of iron deficiency in women of childbearing age on cognitive functioning, mental health and fatigue, published from earliest record to 2011.

This review considered two main questions:

1. What is known about the effects of iron deficiency in women of childbearing age on cognitive functioning, mental health and fatigue?

2. Is change in iron status related to improvements in cognitive performance or mental health and fatigue?

The review provides a summary of the literature, including the measures used to assess iron status, cognition, mental health and fatigue. For intervention studies included in the review, iron dosages and consequent changes in iron status are also reported, summarised by meta-analysis where appropriate. The review also provides recommendations for future research and practice in the area.
3.5 Methods

3.5.1 Protocol and registration

The protocol for this review was peer reviewed by the Joanna-Briggs Institute (JBI), and is registered in the JBI library of systematic review protocols (http://www.joannabriggs.edu.au/Search.aspx).

3.5.2 Eligibility criteria

Types of participants

Studies that include female human participants aged between 13 and 45 years were included.

Types of intervention

Studies that met the above criteria and included assessment of cognition and any form of iron treatment, for any time period, were considered.

Types of studies

This review considered, but was not limited to, randomised controlled trials (RCTs). In the absence of RCTs, other research designs such as non-randomised controlled trials and before and after studies were considered, to enable inclusion of the current best evidence regarding the effects of iron deficiency in women of childbearing age on cognition, mental health and fatigue.

3.5.3 Terms

For the purposes of this review, cognitive functioning refers to the mental process by which we acquire and use knowledge and generally relates to concentration, attention and memory (Falkingham, Abdelhamid et al. 2010). Cognitive functioning domains include verbal memory, working memory, sustained attention, information processing speed and impulsivity. Both validated and un-validated measures of cognitive functioning were considered. Measures of mental health taken from general health perception measures were considered. We accepted assessment of fatigue by a range of methods, including self-report as described by Piper et al. 1989 (Piper 1989).
3.5.4 Types of outcome measures

Studies that included the following outcome measures were considered:

Assessment of iron status (iron deficiency, iron deficiency anaemia or iron sufficiency) using standardised laboratory methods, for example, serum ferritin, haemoglobin, serum transferrin receptor, serum iron and markers of inflammation.

Iron treatment intervention (distribution of iron supplementation), for any specified time period, to participants who have previously had iron status and cognition, mental health or fatigue measured.

Follow-up testing of iron status post supplement period in intervention studies.

Measures of Cognitive function

Measures of mental health and fatigue included the assessment of any aspect of mental health or fatigue when iron status and cognition were also being measured.

3.5.5 Keywords used in search

[Women of childbearing age, young women, females, iron deficiency] AND cognition, iron status AND cognitive functioning, iron status AND attention, iron status AND memory, iron status AND concentration, iron status AND mental health, iron status AND fatigue.

3.5.6 Search strategy

The search was conducted in September 2010 and updated in December 2011. The search strategy aimed to find both published and unpublished studies written in the English language from earliest record to 2011. A three-step search strategy was used. Databases searched were PRE-MEDLINE® and MEDLINE (Ovid), CINAHL, Scopus, Embase, and PsycINFO. Following an initial search, analysis of the text contained in the title, abstract, index and reference list of retrieved articles was conducted. A second search using all identified keywords and index terms was then undertaken across all included databases. Thirdly, the reference list of all identified reports and articles was searched for additional
studies. The search for unpublished studies was conducted using Mednar, the online unpublished Australian Digital Theses (ADP) Program.

3.5.7 Study selection
Studies were screened for eligibility and articles were retrieved if information in the title, abstract and descriptor headings met eligibility criteria. Eligibility was independently assessed by two reviewers. Once retrieved, background and methods were examined. Investigators were contacted if articles contained insufficient information to meet inclusion criteria. If no reply was received, studies were excluded. Studies that met criteria then underwent critical appraisal to examine the quality of the processes used in the study, assessing for bias and strength of methodological techniques. Critical appraisal was achieved using the Joanna-Briggs Institute critical appraisal tool (JBI-MAStARI).

3.5.8 Data extraction process
For studies that met inclusion criteria, the following data were extracted: study details, iron status assessment, and measures of cognitive function, mental health and fatigue.

3.5.8.1 Assessing risk of bias in individual studies
The use of the JBI-MAStARI tool enabled a comprehensive assessment of bias within individual studies at both the study and outcome levels. The Joanna-Briggs Institute provides researchers with an assessment and review instrument (JBI-MAStARI). This tool is designed to manage, appraise, extract and analyse quantitative data as part of a systematic review of evidence. JBI-MAStARI is a web-based database and incorporates a critical appraisal scale, data extraction forms, and a data analysis function (built with JBI-CReMS). This information was used when examining the quality of the data synthesized. Studies that included unexplained bias were classified as lower quality, with less emphasis placed on data and outcomes of lower quality studies. Publication bias was not measureable due to the limited number of studies in the analysis. Given that many of the studies have negative findings, publication bias is less likely (Dwan, Altman et al. 2008). Having limited studies included in the review reduces the type II error, or the ability to distinguish chance from asymmetry (Sterne, Sutton et al.).
3.5.9 Analysis

Meta-analyses were conducted using STATA11 (Sterne 2008) to estimate the combined effect of iron status on cognitive function across studies. Standard deviations and standard error of means were calculated from available data. The mean difference was then calculated if it was not provided by the authors, enabling a complete data set in preparation for analysis. This was calculated by dividing the difference in mean outcome scores between groups by the standard deviation of outcome among participants. Standardised Mean Difference (SMD) was included as a summary statistic in the meta-analyses. The assumption of SMD is that studies included random samples and that the population distribution is normal. Differences between studies were measured using the $I^2$ statistic. A guide to the interpretation of the $I^2$ statistic is as follows: 0% to 40% might not be important, 30%-60% may represent moderate heterogeneity, and 50%-90% may represent substantial heterogeneity, 75%-100% considerable heterogeneity (Cochrane Statistical Methods Group 2008). The choice of model used was based upon heterogeneity determined from the $I^2$ statistic. Both fixed and random effects models were examined in the meta-analysis to enable comparison of each model.

3.6 Results

3.6.1 Study characteristics

The flow diagram in Figure 3.1 shows the studies screened, assessed for eligibility, and included in the review, with reasons listed for those excluded. Ten studies satisfied eligibility criteria and were included in the review, the characteristics of these studies are summarised in Table 3.1. Of the included studies, there were seven RCTs, two non-randomised controlled trials, and one pre-post intervention study. The pre-post intervention study measured cognition after an iron therapy intervention.
Figure 3.1 Flow diagram of number of studies screened, assessed for eligibility, and included in the review with reasons for exclusion
<table>
<thead>
<tr>
<th>Source</th>
<th>Design</th>
<th>N, population</th>
<th>Dose &amp; type of oral iron</th>
<th>Iron Supplementation Duration</th>
<th>Follow-up (Months)</th>
<th>Study Arms</th>
<th>Retention %†</th>
<th>Intention-to-Treat Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beard et al (2005)</td>
<td>RCT</td>
<td>95, mothers, 18-30 yrs</td>
<td>125mg FeSO4 tablets</td>
<td>10 wk</td>
<td>2.5</td>
<td>IIT+CT+MV+Fe+FeSO4</td>
<td>85</td>
<td>No</td>
</tr>
<tr>
<td>Murray-kolb et a (2007)</td>
<td>RCT</td>
<td>152, females, 18-35 yrs</td>
<td>160mg FeSO4 (60mg Fe) tablets</td>
<td>16 wk</td>
<td>4</td>
<td>IIT+CT+GPA/PA +Fe/Plac.</td>
<td>74</td>
<td>No</td>
</tr>
<tr>
<td>Bruner et al (1996)</td>
<td>RCT</td>
<td>81, adolescent girls, 13-18 yrs</td>
<td>1300mg FeSO4 (260mg Fe) tablets</td>
<td>8 wk</td>
<td>2</td>
<td>IIT+CT+Fe/Plac.</td>
<td>90</td>
<td>Yes</td>
</tr>
<tr>
<td>Ballin et al (1992)</td>
<td>RCT</td>
<td>59, adolescent girls, 16-17 yrs</td>
<td>10mL iron polystyrene sulfonate (105mg elemental iron) tablets</td>
<td>8 wk</td>
<td>2</td>
<td>IIT+CT+PA+HQ+Fe/Plac.</td>
<td>27</td>
<td>No</td>
</tr>
<tr>
<td>Elwood et al (1970)</td>
<td>RCT</td>
<td>47, females, ≥20 yrs</td>
<td>150mg FeCO3 tablets</td>
<td>8 wk</td>
<td>2</td>
<td>IIT+CT+Fe/Plac.+HQ</td>
<td>87</td>
<td>No</td>
</tr>
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<td>Groner et al (1986)</td>
<td>RCT</td>
<td>38, pregnant females, 14-24 yrs</td>
<td>90mg C4H2FeO4 (60mg Fe) capsules</td>
<td>4 wk</td>
<td>1</td>
<td>IIT+CT+Fe+MV</td>
<td>75</td>
<td>No</td>
</tr>
<tr>
<td>Patterson et al (1999)</td>
<td>RCT</td>
<td>76 females 18-35 yrs</td>
<td>350mg FeSO4 (105mg Fe)</td>
<td>12 wk</td>
<td>3</td>
<td>IIT+CT+Fe/Plac.+HQ +DT</td>
<td>74</td>
<td>No</td>
</tr>
<tr>
<td>Khedr, et al (2008)</td>
<td>Non-RCT</td>
<td>53, adults, 16-28 yrs</td>
<td>600mg C4H2FeO4 (195mg Fe) tablets</td>
<td>12 wk</td>
<td>3</td>
<td>IIT+CT+Fe</td>
<td>100</td>
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</tr>
<tr>
<td>Mansson et al (2005)</td>
<td>Non-RCT</td>
<td>75375, students, 16-19 yrs</td>
<td>100mg FeSO4 tablets</td>
<td>12 wk</td>
<td>3</td>
<td>IIT+CSR+HQ+Fe</td>
<td>128</td>
<td>No</td>
</tr>
<tr>
<td>Kretsch et al (1996)</td>
<td>Pre-post intervention</td>
<td>24, obese dieting females, 25-42 yrs</td>
<td>55mg C4H2FeO4 (18mg Fe) tablets</td>
<td>20 wk</td>
<td>N/A</td>
<td>++CT</td>
<td>58</td>
<td>No</td>
</tr>
</tbody>
</table>

Abbreviations: †Retention rates reported post intervention if no follow-up or at latest point of follow-up; FeSO4, ferrous sulfate therapy; IIT, iron testing; CT, cognition testing; MV, multivitamin; Fe, iron therapy; Plac., placebo therapy; GPA, grade point average assessed; PA, physical assessment; HQ, health questionnaire; DT, Diet therapy; CSR, cognition self-report; N/A, not applicable
3.6.2 Study aims

The predominant focus of the seven RCTs was to investigate the effect of iron status and iron treatment on cognitive functioning in female participants. Both of the two non-randomised controlled trials assessed the effects of iron status and iron treatment on cognitive functioning in iron-deficient participants (Mansson, Johansson et al. 2005, Khedr, Hamed et al. 2008). One of the two non-randomised controlled trials also assessed changes in symptoms of mental health and fatigue in iron-deficient participants after treatment (Mansson, Johansson et al. 2005). The pre-post intervention study measured change in iron status after an iron therapy intervention during a weight loss trial and the consequent effect on cognitive functioning (Kretsch, Fong et al. 1998).

3.6.3 Sample demographic information

Sample sizes ranged from 24 to 716. Sample populations were from geographically defined areas, and included pregnant women, mothers, University and secondary school students, healthy women, General Practitioner referred iron-deficient women, iron-deficient women from Haematology Outpatient Clinics, and obese dieting women.

3.6.4 Iron status at baseline

All 10 included studies assessed iron status at baseline. Iron status data from 9 of the 10 studies that conducted an intervention are summarised in Table 3.2. One intervention study did not provide sufficient data for inclusion in the table (Kretsch, Fong et al. 1998). As shown in Table 3.2, there is substantial variation in the methods used for testing iron status.

3.6.5 The markers used to assess iron status

The markers used to assess iron status varied between studies. The most commonly used markers of iron status were serum ferritin, haemoglobin, and serum iron. However, more than nine other markers were used to assess iron status across the included studies (Table 3.2). Where a marker was used by more than one study, the reference range criteria were comparable. For example, the reference range for normal serum ferritin was defined as >15-20µg/L and normal haemoglobin as >120g/L throughout studies.
3.6.6 Iron supplement interventions

All 10 included studies conducted an iron supplement intervention (Elwood and Hughes 1970, Groner, Holtzman et al. 1986, Ballin, Berar et al. 1992, Bruner, Joffe et al. 1996, Kretsch, Fong et al. 1998, Patterson 1999, Beard, Hendricks et al. 2005, Mansson, Johansson et al. 2005, Murray-Kolb and Beard 2007, Khedr, Hamed et al. 2008). As shown in Table 3.1, the iron supplements used varied across studies, and included ferrous sulphate, iron polystyrene sulfonate (liquid iron), ferrous carbonate, and ferrous fumerate. Dosage and duration of the intervention also varied between studies. Dosage of elemental iron supplementation ranged from 18mg to 260mg per day. The shortest iron supplementation intervention was four weeks (Groner, Holtzman et al. 1986), and the longest intervention was 20 weeks (Kretsch, Fong et al. 1998).
Table 3.2. Iron status measures and results

<table>
<thead>
<tr>
<th>Source</th>
<th>Iron status results</th>
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<tr>
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<tr>
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<td>120</td>
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<tr>
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IDA, Fe deficiency anaemia; CN, control; PI, placebo; BL, baseline; MCV, mean corpuscular volume; TSAT, transferrin saturation; Ft, ferritin; ID, Fe deficient; CNPL, control placebo; CNFE, control Fe supplementation; IDPL, Fe deficient placebo; IDFE, Fe deficient Fe supplementation; IDAPL, Fe deficiency anaemia placebo; IDAFe, Fe deficiency anaemia Fe supplementation; EP, end point; Hct, haematocrit; RDW, red blood cell distribution width; sTFR, soluble transferrin receptor; RCD, red cell distribution width; sFe, serum Fe, TIBC, total Fe binding capacity; sTFR, soluble transferrin receptor; Fe SAT, Fe saturation
Iron status after iron supplementation intervention

All studies that conducted iron supplement interventions measured iron status during follow up testing. As shown in Table 3.2, iron status improved with treatment in all except two studies (Groner, Holtzman et al. 1986, Kretsch, Fong et al. 1998). However, not all of the studies reporting improved iron status after iron supplement intervention included a control group (Groner, Holtzman et al. 1986, Patterson 1999). Haemoglobin improved in six of the seven studies reporting the measure, with an average improvement of 11g/L after iron treatment. Six studies measured serum ferritin levels, with improvements after treatment reported in 5 of the 6 studies (Groner, Holtzman et al. 1986). The average improvement in serum ferritin levels was 26µg/L.

The largest improvements were seen in the studies with the two longest interventions (10 and 16 weeks) (Beard, Hendricks et al. 2005, Murray-Kolb and Beard 2007). The study reporting a decrease in serum ferritin and haemoglobin after treatment (Groner, Holtzman et al. 1986) was one of the shortest interventions (eight weeks). The study with the longest intervention (20 weeks) was conducted on dieting women, with caloric restriction imposed during the iron intervention (Kretsch, Fong et al. 1998). This study found an improvement in haemoglobin of 6g/L in 43% of their 14 participants, and a decrease of 6g/L in 57 % of participants (Kretsch, Fong et al. 1998).

Three of the 10 studies that included an intervention reported assessment of participant compliance (Groner, Holtzman et al. 1986, Bruner, Joffe et al. 1996, Mansson, Johansson et al. 2005). Mansson reported a 24% compliance rate over a three month intervention period (Mansson, Johansson et al. 2005). Groner reported an 88% compliance rate over a one month intervention (Groner, Holtzman et al. 1986). One study did not report the result of their assessment of compliance.

3.6.7 The effect of iron deficiency on cognitive functioning

Of the eight studies that included both iron-deficient and iron-sufficient participants at baseline (Groner, Holtzman et al. 1986, Ballin, Berar et al. 1992, Kretsch, Fong et al. 1998, Patterson 1999, Beard, Hendricks et al. 2005, Mansson, Johansson et al. 2005, Murray-Kolb and Beard 2007, Khedr, Hamed et al. 2008), four reported higher cognitive scores
for iron-sufficient than iron-deficient participants at baseline and improved scores after iron treatment:

**Ballin et al.** showed that self-reported ability to concentrate was lower in iron-deficient participants at baseline compared with iron-sufficient controls, and that the iron-deficient participants reported a significant improvement in ability to concentrate after iron treatment (Ballin, Berar et al. 1992).

**Murray-Kolb et al.** showed that at baseline, iron-sufficient participants performed better on cognitive tasks and completed them faster than iron-deficient participants. After iron treatment, learning, attention and memory scores all improved, and the time taken to complete tasks decreased. As the severity of iron deficiency increased, cognition decreased and time taken to complete tasks increased (Murray-Kolb and Beard 2007).

**Khedr et al.** showed that at baseline, iron-deficient participants performed poorer on cognitive tasks, including intelligence and memory (Wechsler Memory Scale revised (WMS-R), Wechsler Adult Intelligence Scale- Revised (WAIS-R), which significantly improved with iron treatment (Khedr, Hamed et al. 2008).

**Patterson et al.** included iron-deficient and iron-sufficient participants and found that there were significant differences on four tests overall between iron-deficient and iron-sufficient participants at baseline (Block Design, Digit Span, Digit Symbol, and Arithmetic). Following treatment there was no improvement for the iron-deficient participants on Digit Span. There was a learning effect for Digit Symbol, as iron-deficient participants and controls both improved after treatment. There was improvement for iron-deficient participants on Arithmetic and Block Design (Patterson 1999).

Three of the eight studies reported no difference in cognition between iron-deficient participants compared with iron-sufficient controls at baseline. These studies did show improvement in cognitive function in previously iron-deficient participants after iron treatment:

**Kretsch et al.** recruited participants based on BMI and not iron status for a weight loss intervention. They showed decreasing haemoglobin occurred with dieting, and that this
correlated with decreased sustained attention, as measured by the Bakan Sustained Attention task (Kretsch, Fong et al. 1998).

**Groner et al.** found a significant improvement in Arithmetic scores in iron-deficient participants after treatment. On comparison of the change between baseline and follow-up scores, the experimental group showed significantly greater improvement than controls on tests of short term memory and attention. (Groner, Holtzman et al. 1986).

**Beard et al.** included iron-deficient participants and iron-sufficient controls and found no difference in cognitive tasks at baseline. Iron treatment resulted in significant improvement in previously iron-deficient participants on intelligence and short-term memory scores (Beard, Hendricks et al. 2005).

One study showed no difference in cognitive function between iron-deficient and iron-sufficient groups either at baseline or at follow-up, after iron treatment:

**Mansson et al.** found no significant difference between the iron levels of iron-deficient and iron-sufficient participants reporting inability to concentrate at baseline and no difference after treatment which the study attributed to small sample size (n = 375) (Mansson, Johansson et al. 2005).

Two studies recruited only iron-deficient participants (Elwood and Hughes 1970, Bruner, Joffe et al. 1996). One of these studies reported an improvement in cognitive tasks with iron treatment:

**Bruner et al.** only included iron-deficient participants and showed that verbal learning and memory improved with iron treatment (Bruner, Joffe et al. 1996).

And one study showed no difference in cognitive function after iron treatment:

**Elwood et al.** showed no improvement in cognitive scores with treatment in iron-deficient participants. The authors suggested that this may have been due to participants iron status not being low enough for an effect to be shown (Elwood and Hughes 1970).
3.6.8 Meta-analysis of the effects of iron supplement intervention on cognition

Results from three of the RCTs that met the inclusion criteria were pooled in meta-analyses. The three studies included in the analyses were the only studies that provided sufficient data to do so (Groner, Holtzman et al. 1986, Patterson 1999, Khedr, Hamed et al. 2008).

Heterogeneity between studies was tested using the I² statistic. The I² result was zero for each cognition test included in the analysis, indicating that there was no significant variation between the studies. There was no difference between the results of fixed or random effects models. A fixed effects model was used as it was considered more reliable than the random effects model due to so few studies being included in the analysis.

A range of tests was used to measure cognition, with few of the included studies using the same tests. Digit Symbol, Digit Span, Arithmetic and Block Design (assessing attention, working memory, and visuo-spatial ability) were the only cognitive tests used in more than one study, and therefore were the ones included in the meta-analyses. The studies in which each of these tests was used are shown in Table 3.3. Digit forward and digit backward are combined to form digit span. Total scores for digit span were included in the meta-analysis.

<table>
<thead>
<tr>
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<th>Khedr et al.</th>
<th>Groner et al.</th>
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**Table 3.3. Cognitive tests used in the studies included in the meta-analysis**

*Digit Symbol*

Digit Symbol was used in three of the included studies as a measure of the cognitive construct, attention. Two studies (Groner, Holtzman et al. 1986, Khedr, Hamed et al. 2008)
2008) specified using the (WAIS-R); the other (Beard, Hendricks et al. 2005) did not specify which version of the test was used. The meta-analysis of Digit Symbol included pre- and post-iron treatment intervention scores in iron-deficient participants (Groner, Holtzman et al. 1986, Beard, Hendricks et al. 2005, Khedr, Hamed et al. 2008) (Figure 3.2). There was no significant difference between combined Digit Symbol test scores before and after treatment, SMD 0.29, 95% CI -0.07, 0.65, n=186, p=0.114, I²=0.0%.

**Figure 3.2. Digit Symbol scores at baseline and after iron treatment intervention**

**Digit Span and Arithmetic**

Digit Span and Arithmetic were used in three of the included studies as a measure of working memory. Working memory provides temporary storage and manipulation of information required for complex cognitive tasks such as comprehension, learning and reasoning (Baddeley 1992). Two of these three studies reported using the (WMS-R) battery (Groner, Holtzman et al. 1986, Khedr, Hamed et al. 2008), while the third did not specify. Total scores for Digit Span (i.e., Digits Forward and Digits Backward) were included in the meta-analysis (as one study did not provide separate results for Digits Forward and Digits Backward). The meta-analysis of Digit Span included scores pre- and post-iron treatment in iron-deficient participants (Groner, Holtzman et al. 1986, Beard, Hendricks et al. 2005, Khedr, Hamed et al. 2008) (Figure 3.3). Combined change
scores for Digit Span were: SMD -0.11, 95% CI -0.47, 0.25, n=186, p=0.564, I²=0.0%. The analysis showed no significant difference in Digit Span scores before and after treatment.

The meta-analysis of Arithmetic scores significantly improved after iron treatment (Figure 3.4). Combined change scores for Arithmetic were: SMD 0.84, 95% CI 0.47, 1.22, n=186, p=0.01, I²=0.0%.
Block Design

Block Design was used by two of the included studies (Patterson 1999, Khedr, Hamed et al. 2008), and each used the WAIS-R as a measure of visuo-spatial ability. The meta-analysis of Block Design scores included pre- and post-iron treatment interventions in iron-deficient participants (Figure 3.5). There was no significant difference in Block Design scores after treatment. Combined change scores for Block Design were: SMD 0.35, 95% CI -0.07, 0.76, n=186, p=0.103, I^2=0.0%.

![Block Design scores before and after iron treatment intervention](image)

Figure 3.5. Block Design scores before and after iron treatment intervention

3.6.9 The effect of iron deficiency on Mental Health and Fatigue

In total, 4 of the 10 included studies measured mental health (Elwood and Hughes 1970, Ballin, Berar et al. 1992, Patterson, Brown et al. 2001, Mansson, Johansson et al. 2005) and three studies measured fatigue (Elwood and Hughes 1970, Patterson, Brown et al. 2001, Mansson, Johansson et al. 2005). Mental Health was measured using the General Health Questionnaire and the Perceived Stress Scale. Fatigue was measured using the General Health Questionnaire, the Piper Fatigue Scale, and the Edinburgh Postnatal Depression Scale.
Mental Health

Three of the five studies that measured mental health reported lower scores for iron-deficient participants compared with controls, which improved with treatment (Ballin, Berar et al. 1992, Patterson, Brown et al. 2001, Mansson, Johansson et al. 2005). Of the three studies, only one used validated tools to assess mental health: the General Health Questionnaire and the SF-36 (Patterson, Brown et al. 2001). The other two studies that found significant results used un-validated assessment tools (Ballin, Berar et al. 1992, Mansson, Johansson et al. 2005). The study that reported no improvement, used an un-validated, self-appraisal questionnaire designed by Ingham et al. 1965 (Elwood and Hughes 1970). This study only recruited anaemic participants (Elwood and Hughes 1970).

Fatigue

Of the three studies that measured fatigue, one reported a higher prevalence of self-reported fatigue in iron-deficient participants at baseline, which significantly reduced with treatment (Mansson, Johansson et al. 2005). This study used a standardised questionnaire consisting of 30 questions about different symptoms related to quality of life. One study, which recruited only anaemic women, found no evidence of a benefit of iron therapy on fatigue, measured by self-report (Elwood and Hughes 1970). The third study that considered fatigue used the Piper Fatigue Scale (PFS) and found higher PFS scores in iron-deficient participants at baseline and reported significant improvement in PFS scores after iron treatment (Patterson, Brown et al. 2001).

3.6.10 Overall study quality

Study quality was assessed using the Joanna Briggs Institute critical appraisal tool. Quality was low in four studies (Elwood and Hughes 1970, Ballin, Berar et al. 1992, Kretsch, Fong et al. 1998, Mansson, Johansson et al. 2005), moderate in two (Groner, Holtzman et al. 1986, Khedr, Hamed et al. 2008) and high in four (Bruner, Joffe et al. 1996, Patterson 1999, Beard, Hendricks et al. 2005, Murray-Kolb and Beard 2007) (Table 3.4). The reasons for a low quality rating were: not having follow-up testing; no report of handling participant withdrawals; no discussion of participant blinding; and no description of treatment intervention. Studies assessed as being of high quality were
RCTs, with comparable study groups, defined research questions and outcome measures, and they described the intervention adequately and used appropriate statistical analysis.

Table 3.4. Study quality

<table>
<thead>
<tr>
<th>Author</th>
<th>Design</th>
<th>Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beard J et al (2005)</td>
<td>RCT</td>
<td>+</td>
</tr>
<tr>
<td>Murray-Kolb et al (2007)</td>
<td>RCT</td>
<td>+</td>
</tr>
<tr>
<td>Bruner et al (1996)</td>
<td>RCT</td>
<td>+</td>
</tr>
<tr>
<td>Patterson AJ (2000)</td>
<td>RCT</td>
<td>+</td>
</tr>
<tr>
<td>Groner et al (1986)</td>
<td>RCT</td>
<td>0</td>
</tr>
<tr>
<td>Ballin et al (1992)</td>
<td>RCT</td>
<td>-</td>
</tr>
<tr>
<td>Elwood et al (1970)</td>
<td>RCT</td>
<td>-</td>
</tr>
<tr>
<td>Mansson J et al (2005)</td>
<td>Non-RCT</td>
<td>-</td>
</tr>
<tr>
<td>Kretsch et al (1998)</td>
<td>cohort</td>
<td>-</td>
</tr>
</tbody>
</table>

Symbols: + high quality, 0 moderate quality, - low quality

3.7 Discussion

3.7.1 Variation in the measurement and diagnosis of iron deficiency

The World Health Organisation (WHO) recommends that the approach to assessing iron status should be to measure serum ferritin (SF) and soluble transferrin receptor (sTFR) (World Health Organization 2004). Haemoglobin concentration is also recommended to provide information about the severity of iron deficiency (World Health Organization 2004). This systematic review of the literature on the effects of iron deficiency in women of childbearing age on cognition, mental health and fatigue has shown there is significant variation between studies in the methods used to evaluate iron status (Baynes and Cook 1996). The most common measures reported in the included studies were haemoglobin and serum ferritin. These measures were often accompanied by soluble transferrin receptor, serum iron, mean corpuscular volume, haematocrit, transferrin saturation, and total iron binding capacity.
3.7.2 Relationship between iron status and cognitive function, mental health and fatigue

Most research on the effects of iron deficiency on cognition has been conducted in children and infant populations (Lozoff and Brittenham 1986). This review demonstrates that relatively few studies have examined the relationship between iron deficiency and cognition in women of childbearing age. While there does appear to be a relationship between iron status and cognition in this population, it is difficult to specify which domains of cognitive function, or whether cognition is adversely impacted on, due to the small number of studies using comparable cognitive measures.

Eight of the 10 studies in this review compared iron-deficient participants to iron-sufficient controls at baseline (Groner, Holtzman et al. 1986, Ballin, Berar et al. 1992, Kretsch, Fong et al. 1998, Beard, Hendricks et al. 2005, Mansson, Johansson et al. 2005, Khedr, Hamed et al. 2008). Four studies found that iron-deficient participants had poorer results at baseline compared with controls, but this improved following iron supplementation (Ballin, Berar et al. 1992, Patterson 1999, Murray-Kolb and Beard 2007, Khedr, Hamed et al. 2008). The studies found differences in results for tests of attention, working memory and reaction time (Ballin, Berar et al. 1992, Patterson 1999, Murray-Kolb and Beard 2007, Khedr, Hamed et al. 2008). Not all studies that included an iron-sufficient control group found a relationship between iron status and cognition at baseline (Groner, Holtzman et al. 1986, Kretsch, Fong et al. 1998, Beard, Hendricks et al. 2005).

Eight studies included in this review reported improvement in cognitive function with iron treatment in iron-deficient women (Groner, Holtzman et al. 1986, Ballin, Berar et al. 1992, Bruner, Joffe et al. 1996, Kretsch, Fong et al. 1998, Patterson 1999, Beard, Hendricks et al. 2005, Murray-Kolb and Beard 2007, Khedr, Hamed et al. 2008). Improvements were shown in digit symbol, arithmetic and digit span scores. The largest impact was on Arithmetic (assumed to assess working memory), which was tested in three studies and included in the meta-analysis (Groner, Holtzman et al. 1986, Patterson 1999, Khedr, Hamed et al. 2008). Two of the studies that were not included in the meta-analysis, showed no improvements in cognitive tests (Elwood and Hughes 1970, Mansson,
Johansson et al. 2005). Of the studies showing no effect of iron treatment on cognition, one study relied on self-report of cognitive symptoms (Mansson, Johansson et al. 2005). The other study that showed no effect had a short duration intervention of 4 weeks and hence is not likely to have been long enough to improve performance on cognitive measures (Elwood and Hughes 1970).

These results indicate a substantial amount of variation across included studies. Performance on cognitive tests at baseline was not consistently poorer in iron-deficient participants compared with iron-sufficient controls. Similarly, not all studies reported improvement in test scores after treatment. It is difficult to quantify the clinical importance due the limited number of studies using the same cognitive tests. Factors that may confound the relationship between iron status and cognition in women of childbearing age include level of education, parity, dietary intake, sleep patterns, menstruation, and waist-to-hip-ratio (Laessle, Platte et al. 1996, Maki, Rich et al. 2002, Lassek and Gaulin 2008). However, none of the studies included in this review reported on potential confounding factors. Inconsistency in cognition testing methods and iron status markers used in the studies included in this review hinders the comparison of results. Future research needs consistency across markers used to assess iron status and the tests used to assess cognitive function to further characterise any relationship between iron deficiency and cognition in women of childbearing age.

There was only adequate literature to support a meta-analysis for cognition, the literature on fatigue and mental health was so limited and heterogeneous that only a narrative review of these areas was feasible. Results for mental health and fatigue assessments varied, with some studies finding iron deficiency was related to poorer mental health scores and higher levels of fatigue at baseline (Ballin, Berar et al. 1992, Patterson, Brown et al. 2001, Mansson, Johansson et al. 2005), and one study finding no difference (Elwood and Hughes 1970). Three studies found improved mental health after treatment (Ballin, Berar et al. 1992, Patterson, Brown et al. 2001, Mansson, Johansson et al. 2005) and two showed reduced fatigue scores after iron treatment (Patterson, Brown et al. 2001, Mansson, Johansson et al. 2005). One study, that measured both mental health and fatigue, showed no improvement for either following iron treatment (Elwood and
Hughes 1970). Study quality appeared to account for the different results between studies. The study that reported no improvement relied on self-report to measure mental health and fatigue, whereas the studies that reported an effect used validated assessment tools. Another factor that may affect results is the method of recruitment used (eg. volunteers vs. random sampling). Those volunteering for a study on iron deficiency and fatigue are particularly likely to self-select based on their own perceived fatigue levels. Fatigue and poor mental health or vitality may be the result of numerous causes and be completely unrelated to iron status, and this would impact on the results of any trial where iron supplementation is the only treatment. Hence comprehensive assessment of potential confounders is recommended.

Two recently conducted RCTs measuring the effects of iron deficiency on fatigue in young women were not eligible to be included in the review due to no measure of cognitive function. One study, conducted in 2012, found fatigue levels decreased by 47.7% in the iron treatment group and 28.8% in the placebo group (Vaucher, Druais et al. 2012). The other was conducted in 2011 and examined the effect of intravenous iron in the treatment of fatigue in premenopausal women. This study reported that fatigue decreased during their iron intervention in 82% of participants compared with 47% of controls (Krayenbuehl, Battegay et al. 2011).

### 3.7.3 Effect of iron treatment on iron status in women of childbearing age

It is generally recognised by medical practitioners that three months supplementation is required to improve iron status significantly (Patterson 1999). Various forms and doses of iron treatment were used in the included studies. The duration of iron treatment interventions in the studies included in the current review ranged from four weeks to 20 weeks. All but one of the included studies found iron treatment successfully improved markers of iron status. The greatest improvements in iron status were seen for interventions of at least 10 weeks duration.

### 3.7.4 Limitations

This review was affected by a number of limitations that need to be acknowledged. Firstly, a large range of measures were used to assess iron status across the included
studies, and there was a lack of consistency both in the type of tests and the reference ranges chosen. Most studies did not consider factors that may be related to, and thus be confounded with, iron status and cognition, such as socioeconomic status, pregnancy and dietary intake patterns. The use of a range of tests to measure cognition, mental health and fatigue made it difficult to compare studies. (Ballin, Berar et al. 1992, Bruner, Joffe et al. 1996, Mansson, Johansson et al. 2005). The form of iron supplementation varied, as did the dose of iron used. In fact, the dosage range was quite extreme and varied from 18mg to 260mg, making it difficult to compare the impact of supplementation on iron status. The length of supplementation also varied, and short duration interventions may have had insufficient time for alterations in iron status to occur, especially with respect to brain iron. There was limited assessment of compliance with iron supplementation. Only three studies assessed compliance, and only two of these reported their findings (Groner, Holtzman et al. 1986, Bruner, Joffe et al. 1996).

Limited studies on the effects of iron deficiency on cognitive function in women of childbearing age were available to inform the review. The small numbers of studies informing the meta-analysis means its utility for clarifying the results is limited and results need to be interpreted with caution (Cochrane Statistical Methods Group 2008).

3.7.5 Implications for practice

The results of this review indicate that short term (<8 weeks) iron treatment had the lowest impact on iron status. Health practitioners should therefore prescribe iron supplements to women of childbearing age with low iron stores for longer than eight weeks and ideally for at least three months. This is the consensus standard treatment for iron deficiency in Australia, to ensure sufficient time for iron stores (as measured by serum ferritin and soluble transferrin receptor) to be replenished (Patterson 1999).

3.7.6 Implications for research

This review highlights the variation in methodology used for testing cognition, mental health and fatigue in iron-deficient women of childbearing age. This variation makes it difficult to adequately compare results, and therefore indicates that high quality RCTs with similar study design and methodology are needed to enable a more conclusive determination of an effect. A standardised approach to measuring cognition, mental
health and fatigue, including the use of validated assessment tools, will enable benchmarking.

3.8 Conclusion

Relatively few published studies have examined the relationship between iron deficiency in women of childbearing age and cognition, mental health and fatigue. In iron-deficient participants, small improvements in fatigue and moderate improvements in mental health scores with supplemental iron treatment were seen. The majority of included studies showed some evidence of improvement in cognitive function after iron supplementation. However, few studies used the same measures of cognitive functioning making comparison of results difficult. Meta-analysis of four cognitive tasks (Digit Symbol, Digit Span, Arithmetic, Block Design) revealed significant improvement following iron treatment in only one task, Arithmetic, which is a measure of working memory.

Many of the included studies are limited by short treatment interventions and poor assessment of compliance. Studies also varied in their focus on different aspects of cognition (e.g. memory or intelligence) and in the tests they use to measure these constructs. Further high quality randomised controlled trials of a similar design and using similar evaluation methods to determine iron status and cognitive functioning will assist in clarifying the relationship between iron status and cognition in women of childbearing age.

3.9 Acknowledgements

Funding

This research was supported by a grant from Meat and Livestock Australia (AP, KC, and CC). Alecia J Greig receives a Australian postgraduate award and top-up scholarship from Meat and Livestock Australia. Clare E Collins is supported by a National Health and Medical Research Council Australian Career Development Fellowship.
3.10 Author contributions

The authors thank Dr. Patrick McElduff of the University of Newcastle, Australia, for his advice and guidance with the statistical analyses; Debbie Booth, medical librarian at the University of Newcastle for conducting the database searches and Hannah Lucas, Accredited Practising Dietitian for assistance with identifying studies for inclusion. AG is first author, conducted statistical analysis, extracted data from the studies and prepared the manuscript. AP, KC and CC designed the review and participated in the data extraction and quality assessments, and drafting and review of the manuscript. All authors have contributed to the systematic review and approved the final version of the manuscript.

3.11 Conflicts of interest

The authors state that there are no conflicts of interest.
Chapter 4. The effect of nutrition knowledge and dietary iron intake on iron status in young women

This paper was published in 2014.


The work presented in the manuscript was presented at the Annual Scientific Meeting Nutrition Society of Australia and New Zealand in December 2013. Brisbane, Australia (2 Poster presentations).

The work presented in the manuscript was completed in collaboration with the co-authors (Appendix 15).
4.1 Overview

Limited studies exist on the nutrition knowledge on dietary intake; a recently published systematic review identified a need for well-designed studies in this area (Spronk, Kullen et al. 2014). This chapter assessed young women’s knowledge of dietary iron and its relationship with iron intake and iron status, a novel contribution to the evidence base in this area. The aims were to assess the level of nutrition knowledge of dietary iron in a sample of young women and its effect on dietary iron intake and to assess the effect of dietary iron intake on iron status in the sample of young women. Paper 6 commences verbatim from Section 4.2. Methods are presented in Section 4.4, results are presented in Section 4.5 and discussion is presented in Section 4.6.

4.2 Abstract

Background: Previous research on the relationships between general nutrition knowledge and dietary intake, and dietary iron intake and iron status has produced inconsistent results. Currently, no study has focused on knowledge of dietary iron and its effect on dietary iron intake. Objectives: This study aimed to determine whether nutrition knowledge of iron is related to dietary iron intake in young women, and subsequently whether greater knowledge and intake translates into better iron status. Methods: A cross-sectional assessment of nutrition knowledge of iron, dietary iron intake and iron status was conducted in women aged 18–35 years living in Newcastle, NSW, Australia. Iron status was assessed by serum ferritin, haemoglobin, soluble transferrin receptor and alpha-1-glycoprotein. Results: One hundred and seven women (27.8 ± 4.7 years) completed the nutrition knowledge questionnaire and FFQ. Of these, 74 (70%) also had biomarkers of iron status measured. Mean iron intake was 11.2 ± 3.8 mg/day. There was no association between nutrition knowledge score and whether the women met the RDI for iron (F (1, 102) = .40, P = .53). A positive correlation was shown between nutrition knowledge score and iron intake (mg/day) (r = 0.25, P = .01). Serum ferritin was positively associated with the frequency of flesh food intake (r = .27 P = .02). Vegetarians (including partial vegetarians) had significantly lower serum ferritin levels than non-vegetarians (F (1, 71) = 7.44, P = .01). Conclusions: Significant positive correlations found between higher flesh food intake and biomarkers of iron status
suggest that educating non-vegetarians about the benefits of increased flesh food consumption and vegetarians about dietary iron enhancers and inhibitors may have potential for addressing the high rates of iron deficiency among young women.

4.3 Introduction

Iron deficiency affects approximately one in five young Australian females (Ahmed, Coyne et al. 2008) and has been negatively associated with several aspects of health and wellbeing, including decreased work capacity and impaired neurological function (Cook and Lynch 1986, Patterson, Brown et al. 2001, Murray-Kolb and Beard 2007). Young women are at particular risk of iron deficiency due to losses from menstruation and childbirth (World Health Organization 2001). Iron deficiency is characterised by a reduction in stored iron, which is most commonly measured by the marker, serum ferritin (World Health Organization 2001). For the adult female population, normal serum ferritin is defined by the World Health Organization as ≥15µg/L and normal haemoglobin as ≥120g/L (World Health Organization 2001, Pasricha, Flecknoe-Brown et al. 2010). Assessment of soluble transferrin receptor-ferritin index (sTfR-ferritin index) in addition to ferritin and haemoglobin is useful in determining early stage iron deficiency (Leonard, Patterson et al. 2013).

Determining the cause of iron deficiency is a complex and challenging process for health professionals as numerous factors contribute (Pasricha, Flecknoe-Brown et al. 2010). The most common causes are iron losses, and an imbalance between iron requirements and absorption from dietary iron intake (World Health Organization 2001). Menstruation is the most significant factor that increases a female’s risk of iron deficiency (Denic and Agarwal 2007), with females of reproductive age commonly having insufficient iron stores to cover losses that occur through normal menstrual bleeding (Galan, Yoon et al. 1998). Volume of menstrual blood loss varies (Price, Forsyth et al. 1964) from approximately 4 to 10ml of blood per day, equating to approximately 2.5mg to 10mg/day iron loss (Price, Forsyth et al. 1964, Baynes and Bothwell 1990, Beard, Dawson et al. 1996).

Iron status of young women is influenced by their dietary iron intake (Beard, Dawson et al. 1996). Young women have the highest iron requirements of all population groups,
including females at other life stages, hence the recommended dietary intake (RDI) for iron is set at 18mg/day for women aged 19-50 years in Australia (National Health and Medical Research Council 1991) and the US (National Research Council 2001), and 14.8mg/day in the UK (Scientific Advisory Committee on Nutrition 2010). The estimated average requirement for iron is 8mg/day which is the daily intake estimated to meet the requirements of half the healthy females in this particular life stage (National Health and Medical Research Council 1991).

According to the Australian Department of Health and Ageing, females do not consume iron in sufficient quantities (National Health and Medical Research Council 1991). In 1999, the Commonwealth Scientific and Industrial Research Organisation (CSIRO) determined that 66% of Australian females aged between 19 and 44 years consume less than 12mg/day of iron; 6mg/day less than the recommended daily intake of 18mg/day set by the National Health and Medical Research Council (National Health and Medical Research Council 1991, Baghurst 1999). Reasons for the gap between the RDI for iron and actual iron intake have been hypothesised as lack of affordability, poor access to fresh foods, and lack of knowledge and awareness about nutrition (Ball, Mishra et al. 2004). Assessing young women’s level of knowledge relating to dietary iron will help researchers and clinicians to understand a potential factor contributing to iron deficiency in this population.

The absorption of dietary iron is a complex process which has been well investigated (Collings, Harvey et al. 2013). Absorption and availability of iron in the body is more important for iron status than total iron intake (National Health and Medical Research Council 1991). Dietary iron consists of elemental iron, and either haem or non-haem iron (Zhu, Kaneshiro et al. 2010). Between 25 and 35% of haem iron is absorbed through the intestinal wall, whereas only 2-15% of non-haem iron is absorbed (Monsen, Hallberg et al. 1978, National Health and Medical Research Council 1991). Ascorbic acid is known to enhance the absorption of non-haem iron, largely due to its ability to reduce ferric to ferrous iron (Monsen, Hallberg et al. 1978, Hurrell and Egli 2010). Meat, fish and poultry have an enhancing effect on iron absorption from plant-based meals; the effect of 30g of meat, fish or poultry is equivalent to 25mg of ascorbic acid (Hurrell and Egli 2010). Some
nutrients and dietary compounds inhibit the absorption of iron and consideration should be given to their simultaneous consumption with iron-rich foods (Monsen, Hallberg et al. 1978, National Health and Medical Research Council 1991, Hurrell and Egli 2010). These include polyphenols (found in coffee and tea), phytates (found in legumes and nuts), and calcium (found in dairy) (Monsen, Hallberg et al. 1978, Hurrell and Egli 2010). The biochemical mechanism for the inhibitory effect of polyphenols and phytates on the absorption of non-haem iron remains unclear (Hurrell, Juillerat et al. 1992, Hurrell and Egli 2010). Calcium inhibits the absorption of haem and non-haem iron, which occurs during the initial uptake of iron in to the enterocyte (Hurrell and Egli 2010).

Nutrition knowledge is difficult to measure and is known for being a challenging area of research (Worsley 2002). To our knowledge, no published study has focused on assessing knowledge of dietary iron and its impact on dietary iron intake. This study aims to determine whether nutrition knowledge of iron is related to dietary iron intake in young women and to determine whether a greater level of knowledge and intake, results in better iron status.

4.4 Methods

4.4.1 Design and participants

This cross-sectional study was conducted at the University of Newcastle, Australia from 2012 to 2013. Participants in this study were being recruited for a randomised controlled trial on the effects of iron deficiency and cognitive function. Eligibility criteria were that participants were female and aged between 18-35 years, were not diagnosed as iron deficient within the past 12 months and not currently taking iron supplements. Recruitment was conducted via flyers at the University of Newcastle (Callaghan campus), Technical Education (TAFE) College, accessing the volunteer register at Hunter Medical Research Institute and word-of-mouth. Informed consent was obtained from all participants.

The study was approved by the Human Research Ethics Committee of The University of Newcastle, Australia.
4.4.2 Materials

Nutrition Knowledge Questionnaire (NKQ)

A four part, 30-item questionnaire to assess participants’ nutrition knowledge of dietary iron was developed specifically for the current study (Appendix 7). Part A included four socio-demographic questions. Part B asked eight questions about nutrition knowledge of dietary iron, for example “What is the main function of iron?” “Which of the following are features of haem iron (as opposed to non-haem iron)?” and “Is the recommended daily intake of iron higher for men or women?” Part C asked 12 questions on iron status, cooking and dietary practices, including questions such as “Are you vegetarian? “ or “Are you partially vegetarian?” and “Have you ever been told by your doctor that you have low iron levels?” Part D asked six questions on health and wellbeing.

As shown in Table 4.1, each correct response to questions in Part B of the nutrition knowledge questionnaire was allocated one point, with a maximum possible score of 19. The sum of participants’ correct responses to this part of the questionnaire determined their total nutrition knowledge of iron score. One question in Part B, “Do you think your level of nutrition knowledge of iron is low, moderate or high?” was not included in participants’ total score.
Table 4.1: Questions, correct answers and maximum possible scores for the Food Knowledge and Iron Physiology subscales from Part B of the Nutrition Knowledge Questionnaire

<table>
<thead>
<tr>
<th>Subscale</th>
<th>Questions from Part B of the NKQ</th>
<th>Correct Answer(s)</th>
<th>Maximum Possible Score</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Food Knowledge</strong></td>
<td>Which of these foods are good sources of iron?</td>
<td>Chickpeas, lamb, chicken*</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Which of these vegetarian foods are good sources of</td>
<td>Baked beans, lentils, spinach, eggs*</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>iron?</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Which of the following statements is true/false</td>
<td>(a) Red meat has more than double</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>regarding iron in the diet?</td>
<td>the amount of iron of chicken or</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>fish (gram for gram)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) The absorption of iron from</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>meat foods is increased by Vitamin</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C consumed in the same meal</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c) Tea and coffee inhibit iron</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>absorption</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(d) Calcium enhances iron</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>absorption</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Iron Physiology</strong></td>
<td>What is the main function of iron in the body?</td>
<td>Forms part of haemoglobin</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Which of the following are features of haem iron (as</td>
<td>Iron is attached to a protein,</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>opposed to non-haem iron)?</td>
<td>found in animal foods, more easily</td>
<td></td>
</tr>
<tr>
<td></td>
<td>For adults aged 19-30 years, is the recommended</td>
<td>absorbed by humans*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>daily intake of iron higher for men or women?</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Why do you believe this is the case?</td>
<td>Menstrual blood loss, losses</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>during childbirth, to build iron</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>stores during pregnancy*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total possible score</td>
<td></td>
<td>19</td>
</tr>
</tbody>
</table>

Note: *Participants were asked to tick as many options as applicable

Dietary Iron Intake

The Food Frequency Questionnaire (FFQ) used in the study was the Dietary Questionnaire for Epidemiological Studies Version 2 (DQESv2), which was designed by the Cancer Council of Victoria and validated in pre-menopausal Australian women. Correlation coefficients for the relationship between weighed food record and FFQ ranged from 0.28 for vitamin A to 0.78 for carbohydrate. The correlation coefficient for iron was 0.51 (Hodge, Patterson et al. 2000). The FFQ consists of 74-items and is an easy to use, semi-quantitative, self-administered questionnaire about usual dietary intake (Hodge, Patterson et al. 2000, Xinying, Noakes et al. 2004). The first 10 questions refer to daily consumption of fruit, vegetables and dairy, types and amounts of breads, spreads, sugar, cheese and eggs. The questionnaire then includes four questions on portion size, and together these 14 questions are used to adjust frequencies allowing for quantification of food in grams. The main section of the FFQ assesses daily equivalent frequencies of
whole foods consumed (never, less than once per month, 1 to 3 times per month, 2 times per week, 3 to 4 times per week, 5 to 6 times per week, 1 time per day, 2 times per day, 3 or more times per day).

**Scoring**

Responses from Part B of the NKQ were divided into two subscales: Food Knowledge and Iron Physiology. Questions contributing to each subscale are shown in Table 4.1. Data on vegetarians and partial vegetarians from Part C of the NKQ were combined to form the variable ‘vegetarians (including partial vegetarians)’. Responses to the FFQ were processed by the Cancer Council of Victoria (CCV) using scanning technology, and food amounts in grams were computed. Micro-and macro-nutrient intakes (µg/day, mg/day or g/day) were computed using dietary analysis software based on NUTTAB 95 nutrient composition data (Lewis, Milligan et al. 1995, Hodge, Patterson et al. 2000). Haem and non-haem iron components are not available in the NUTTAB95 database. The influence of haem and non-haem iron intake on iron status was considered in the current study by the researchers identifying and analysing the daily equivalent frequencies and grams of individual haem iron containing foods (beef, chicken, lamb, veal, sausages, fish, bacon, ham salami) and non-haem iron containing foods (spinach, eggs, legumes, cereals) from the CCV dataset provided, with biomarkers of iron status. Non-haem and haem iron containing foods were identified if their iron content was >1mg/100g.

**4.4.3 Procedure**

The NKQ and FFQ were distributed to participants on campus at the University of Newcastle, or via mail-out.

**Haematological testing**

Serum ferritin, haemoglobin, soluble transferrin receptor (sTfR) and alpha-1-glycoprotein were used as biomarkers of iron status. Blood tests were performed by Hunter Area Pathology Service, accredited by the National Association of Testing Authorities Australia, using standard techniques. Non-anaemic, iron deficient participants were defined as having serum ferritin <20µg/L with haemoglobin and alpha-1 glycoprotein within normal reference ranges (haemoglobin 115-165g/L, alpha-1
glycoprotein 0.51-1017g/L). sTfR-ferritin index was used to reflect functional and storage iron. This marker increases in response to lower ferritin and is not influenced by inflammatory markers. Improved iron status should result in lower sTfR-ferritin index levels. The reference range for serum ferritin was higher than that recommended by the World Health Organization (<15 µg/L) so that it was inclusive of those with early stage iron deficiency.

### 4.4.4 Statistical analysis

STATA-IC 11 statistical analysis software (StataCorp 2013) was used with an alpha level of 0.05. Pairwise deletion was used to handle missing data. Spearman’s rank correlation was used to examine the association between total nutrition knowledge of iron score and dietary iron intake, and between each of the two subscales (Food Knowledge and Iron Physiology) of the NKQ and dietary iron intake. Spearman’s correlation was used to examine the relationship between biomarkers of iron status (serum ferritin, sTfR-ferritin index, and haemoglobin) and dietary intake of iron, and other minerals, vitamins and compounds available from the FFQ output, that may increase (vitamin C) or inhibit (fibre, calcium) iron absorption. Spearman’s correlation analysis was also used to examine the relationships between biomarkers of iron status (serum ferritin, sTfR-index and haemoglobin) and the portion of steak consumed, as well as daily equivalent frequencies of iron containing foods per day (beef, chicken, veal, pork, fish, spinach, legumes, eggs, cereals). One-way analysis of variance (ANOVA) was used to examine the effect of knowledge score on meeting the RDI for iron, the effect of vegetarian status on dietary iron intake and serum ferritin level and frequency of non-haem iron containing foods per day.

### 4.5 Results

#### 4.5.1 Sample information

One hundred and seven participants completed the FFQ and the NKQ. Of these, serum ferritin was measured in 74 participants; haemoglobin was measured in 73 participants, sTfR-index in 63 participants, and alpha-1-glycoprotein in 60 participants. A total of 54 participants had a result for all haematological markers measured (serum ferritin, sTfR-
index, haemoglobin and alpha-1-glycoprotein). Of these, 23 participants (42%) had non-anaemic iron deficiency. The mean age of respondents who completed both questionnaires and had all haematological markers of iron status measured was 27.8±4.7 years. Forty per cent of participants had a University degree, (41% of these were in the field of Health), and 41% of participants worked as a professional (eg. scientist, doctor, registered nurse, allied health professional, teacher, or artist).

4.5.2 Nutrition knowledge of dietary iron

For the 107 participants who completed the NKQ, the average total score for nutrition knowledge regarding iron was 11.2±3.1, out of a maximum possible score of 19. Total knowledge score was low (0-6) in 34.6%, moderate (7-13) in 49.5% and high (14-19) in 15.9% of participants. Self-perceived nutrition knowledge of iron was low in 18.7% (n=20) of participants, moderate in 65.4% (n=70), and high in 15.9% (n=17) of participants.

4.5.3 Relationship between nutrition knowledge of dietary iron and dietary iron intake

Of the 107 participants who completed the FFQ, mean daily intake of iron (calculated from responses to the FFQ) was 11.2±3.8mg. Only 3.7% of women met the iron RDI of 18mg/day; however, 80% of participants met the iron EAR of 8mg/day. There was no significant difference in total knowledge score between participants who met versus did not meet the RDI for iron (10.8±3.0 vs. 11.1±3.4, F (1, 102) = .40, p=.53). A statistically significant positive correlation between total nutrition knowledge score and dietary iron intake was observed, although the correlation coefficient indicates the strength of this relationship was only moderate (r=0.25, p=.01). The correlation between total knowledge score and intake of haem iron containing foods was not significant (r=0.12, p=.23).

Responses to Part B of the NKQ were divided into two subscales, Food Knowledge and Iron Physiology. Spearman’s correlation analysis revealed a significant positive association between the Food Knowledge subscale and dietary iron intake (r=.30, p<.01). Three participants had iron intakes greater than three standard deviations above the mean (>11.2mg) and so analyses of Food Knowledge and iron intake were performed with (r=.30, p<.01) and without (r=.30, p<.01) these participants included, with no differences in the outcome. With the outliers (defined above) removed, there was a
positive association between the Iron Physiology subscale and dietary iron intake \((r=.20, p=.049)\). This correlation was non-significant when the outliers were included \((r=.15, p=.130)\).

### 4.5.4 Relationship between dietary intake and iron status

As shown in Table 4.2, there was no significant correlation between total dietary intake of iron and any of the biomarkers of iron status (serum ferritin, sTfR-ferritin index, and haemoglobin). No associations were found between iron status and the dietary intake of minerals (other than iron), vitamins or other compounds that may enhance or inhibit iron absorption.

**Table 4.2 Correlation coefficients for the relationship between dietary intake of iron, calcium, fibre, vitamin C and zinc per day and haematological markers of iron status**

<table>
<thead>
<tr>
<th>Dietary Intake per Day</th>
<th>Serum Ferritin (\mu)g/L ((n=74))</th>
<th>sTfR-Ferritin Index ((n=60))</th>
<th>Haemoglobin g/L ((n=73))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron (mg)</td>
<td>Mean (and SD) 11.2±3.8</td>
<td>.01 (.92)</td>
<td>.05 (.68)</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>880.9±268.1</td>
<td>-.04 (.72)</td>
<td>.11 (.39)</td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>19.8±6.7</td>
<td>-.19 (.10)</td>
<td>.16 (.22)</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>100.9±43.9</td>
<td>-.13 (.26)</td>
<td>-.01 (.96)</td>
</tr>
<tr>
<td></td>
<td>Spearman’s rho (and p value)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Spearman’s correlation was used to analyse the relationship between daily equivalent frequencies of iron containing food intake and biomarkers of iron status. As shown in Table 4.3, there was a positive correlation between serum ferritin and the frequency of fresh meat consumption (beef, veal, chicken, lamb, pork, fish) \((r=.31, p=.01)\) and all meat consumption (also including sausages and frankfurters, bacon, ham and salami) \((r=.27, p=.02)\). There was a positive association between haemoglobin and the portion of steak usually consumed \((r=.25, p=.03)\). Serum ferritin was negatively associated with the frequency of spinach \((r=-.24, p=.04)\) and egg intake \((r=-.28, p=.01)\). In addition, sTfR-Index was negatively correlated with the frequency of fresh meat consumption \((r=-.36, p<.01)\), and all meat consumption \((r=-.34, p=.01)\).
Of the 107 participants who completed both the NKQ and FFQ, nine participants (8%) reported being vegetarian and 22 (20%) reported being partially vegetarian. The remaining 76 participants consumed non-vegetarian diets. Vegetarians (including partial vegetarians) had a higher total knowledge score than non-vegetarians (12.1±3.7, 10.6 ±3.2, \( F (1, 101) =4.2, p=.04 \)). Vegetarians (including partial vegetarians) consumed spinach (4.7±2.2, 3.3 ±1.7, \( F (1, 101) = 10.94, p<.01 \)), soybeans (3.4±1.9, 1.7 ±1.2, \( F (1, 101) =31.15, p<.01 \)) and other beans (4.2±1.6, 4.1 ±1.5, \( F (1, 101) = 22.55, p<.01 \)) significantly more frequently than non-vegetarians. There were no significant differences between these groups in the frequency of consumption of other non-haem iron containing foods. There was also no significant difference in mean iron intake between vegetarians (including partial vegetarians) and non-vegetarians (10.54mg/day±3.31, 11.5mg/day±4.0, \( F (1, 104) =1.4, p=.23 \)). However, of the 23 vegetarian (including partial vegetarian) and

---

**Table 4.3** Spearman’s correlation coefficients (*p*value) for the relationship between haematological markers of iron status and daily equivalent frequencies of iron containing foods

<table>
<thead>
<tr>
<th>Dietary intake frequency</th>
<th>Serum Ferritin (n=74)</th>
<th>sTfR-Ferritin index (n=60)</th>
<th>Haemoglobin (n=73)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Haem iron foods</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef</td>
<td>.18 (.13)</td>
<td>-.15 (.26)</td>
<td>.22 (.06)</td>
</tr>
<tr>
<td>Veal</td>
<td>.15 (.20)</td>
<td>-.12 (.37)</td>
<td>.09 (.46)</td>
</tr>
<tr>
<td>Chicken</td>
<td>.23 (.04) *</td>
<td>-.19 (.14)</td>
<td>.15 (.21)</td>
</tr>
<tr>
<td>Lamb</td>
<td>.16 (.17)</td>
<td>-.18 (.18)</td>
<td>.02 (.88)</td>
</tr>
<tr>
<td>Pork</td>
<td>.18 (.12)</td>
<td>-.30 (.02)*</td>
<td>.11 (.37)</td>
</tr>
<tr>
<td>Fish</td>
<td>-.14 (.24)</td>
<td>-.29 (.03) *</td>
<td>-.08 (.48)</td>
</tr>
<tr>
<td>Sausages and frankfurters</td>
<td>.27 (.02)*</td>
<td>-.33 (.01)*</td>
<td>.18 (.12)</td>
</tr>
<tr>
<td><strong>Fresh meat</strong></td>
<td>.31 (.01) *</td>
<td>-.36 (&lt;.01) *</td>
<td>.09 (.42)</td>
</tr>
<tr>
<td><strong>Processed meat</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacon</td>
<td>.21 (.07)</td>
<td>-.34 (.01) *</td>
<td>.13 (.28)</td>
</tr>
<tr>
<td>Ham</td>
<td>.06 (.63)</td>
<td>-.08 (.53)</td>
<td>.10 (.40)</td>
</tr>
<tr>
<td>Salami</td>
<td>.14 (.24)</td>
<td>-.12 (.34)</td>
<td>.16 (.17)</td>
</tr>
<tr>
<td><strong>All meat</strong></td>
<td>.27 (.02) *</td>
<td>-.34 (.01) *</td>
<td>.11 (.33)</td>
</tr>
<tr>
<td><strong>Selected Non-haem iron foods</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spinach</td>
<td>-.24 (.04)*</td>
<td>.25 (.05)</td>
<td>-.07 (.54)</td>
</tr>
<tr>
<td>Baked beans</td>
<td>.06 (.57)</td>
<td>.06 (.62)</td>
<td>-.02 (.85)</td>
</tr>
<tr>
<td>Soybeans</td>
<td>-.08 (.48)</td>
<td>.10 (.44)</td>
<td>-.10 (.39)</td>
</tr>
<tr>
<td>Other beans</td>
<td>-.13 (.26)</td>
<td>.02 (.83)</td>
<td>.18 (.16)</td>
</tr>
<tr>
<td>Eggs</td>
<td>-.28 (.01)*</td>
<td>.21 (.10)</td>
<td>.11 (.36)</td>
</tr>
<tr>
<td>Iron fortified breakfast cereals</td>
<td>-.06 (.63)</td>
<td>-.05 (.71)</td>
<td>-.06 (.61)</td>
</tr>
<tr>
<td>Rice</td>
<td>-.15 (.21)</td>
<td>.09 (.48)</td>
<td>-.00 (.99)</td>
</tr>
<tr>
<td>Pasta</td>
<td>.10 (.37)</td>
<td>-.06 (.64)</td>
<td>.15 (.20)</td>
</tr>
<tr>
<td>Bread</td>
<td>-.02 (.85)</td>
<td>.10 (.42)</td>
<td>.17 (.15)</td>
</tr>
</tbody>
</table>

Note: * p<.05. Fish: fried, grilled and tinned combined. Fresh meat: beef, veal, chicken, lamb, pork, fish. All meat: beef, veal, chicken, lamb, pork, fish, sausages, bacon, ham, salami. Other beans: chickpeas, lentils. Iron fortified breakfast cereals: weetbix, cornflakes.
48 non-vegetarian participants who had ferritin measured, vegetarians (including partial vegetarians) had significantly lower serum ferritin levels than non-vegetarians (26.3µg/L±18.7, 42.3µg/L ±25.6, F (1, 71) =7.4, p=.01).

4.5.5 Relationship between nutrition knowledge of dietary iron and iron status

Using Spearman’s correlation coefficient we found a statistically significant positive relationship between total knowledge score and sTfR-Index (r=.32, p=.01), though in the opposite direction to that expected (i.e., better knowledge was associated with poorer iron status). There was no relationship between total knowledge score and serum ferritin (r=-0.16, p=.18), or between total knowledge score and haemoglobin (r=.03, p=.78). There was no relationship between the Food Knowledge subscale and serum ferritin (r=-0.10, p=.39), sTfR-Index (r=.24, p=.07) or haemoglobin (r=.00, p=.98). The relationship between the Iron Physiology subscale and sTfR-Index was close to significance (r=.25, p=.06). There was no relationship between the Iron Physiology subscale and serum ferritin (r=-.13, p=.26) or haemoglobin (r=.12, p=.31).

4.6 Discussion

The current study examined associations between nutrition knowledge and dietary iron intake, and between dietary iron intake and iron status in young women. To our knowledge this is the first study to examine associations between nutrition knowledge of iron and dietary iron intake. Examination of potential factors that may influence the iron intake of a population at risk of iron deficiency has been identified as an area requiring research (World Health Organization 2001).

Overall, women with a higher total nutrition knowledge score had higher dietary iron intake than those with lower knowledge scores. This was due to a relatively strong relationship between the Food Knowledge subscale and iron intake, and a relationship between the Iron Physiology subscale and dietary iron intake. Positive associations between food knowledge and iron intake found in this study demonstrate that nutrition knowledge of dietary iron is an important component of nutrition interventions aimed at optimising dietary iron intake and iron status in young women. However, further to
these positive associations, other results suggest that the relationship between knowledge of dietary iron and iron intake remains complex. There was no difference in knowledge score between women who consumed the RDI and women who consumed lower than the RDI. Despite higher knowledge scores in vegetarians than non-vegetarians, there was no significant difference in mean iron intake between vegetarians and non-vegetarians.

Most Australian women fail to consume foods in accordance with the national food selection guide (Australian Guide to Healthy Eating), and this has been reported to be associated with inadequate nutrient intakes (Blumfield, Hure et al. 2011). Responses from the FFQ demonstrated that fewer than 4% of women met the iron RDI of 18mg/day. Average total dietary iron consumed was 11.2mg±3.8mg per day, which is lower than has previously been reported in the literature. For example, the average iron intake in young Australian women was reported as 12.6±3.9mg per day (Rangan, Binns et al. 1997) and in a sample of women living in France, iron intake was reported as 12.3±3.4mg per day (Galan, Yoon et al. 1998). Data from the 1995 National Nutrition Survey found mean iron intakes for women were below recommended levels, with fewer than 25% meeting the RDI for iron, which at the time was 12mg/day (Jamison 1995, Ball and Bartlett 1999).

Similarly, in 1999 the CSIRO reported that 66% of Australian females aged between 19 and 44 years consumed less than 12mg of iron per day (Baghurst 1999). In regard to the EAR (8mg/day), 80% of participants met this target. The EAR is the daily nutrient level estimated to meet the requirements of half the healthy female population (National Health and Medical Research Council 1991), therefore, it is a more achievable dietary target than the RDI, which is designed to meet the physiological requirements of 98% of women.

The second aim of this study was to determine whether greater knowledge and intake translates into better iron status. Although participants with higher nutrition knowledge scores had higher dietary iron intake, there were no associations found between total dietary iron intake and biomarkers of iron status. Furthermore, while most relationships between knowledge scores and iron status were not significant, better total knowledge scores were actually related to poorer iron status as measured by sTfR-Index. The
relationship between sTfR-Index and knowledge may be a result of vegetarians (including partial vegetarians) having significantly higher total knowledge scores than non-vegetarians. There was no significant difference in the mean total iron intake of vegetarians (including partial vegetarians) compared with non-vegetarians; however, those identifying as vegetarians had significantly lower serum ferritin concentrations. Low ferritin levels in vegetarians have previously been reported by other researchers (Ball and Bartlett 1999, Hunt 2003).

Analysis of associations between biomarkers of iron status and frequency of consumption of haem and non-haem iron containing foods from the FFQ demonstrated significant relationships. Frequency of consumption of haem iron containing foods (beef, veal, chicken, lamb, pork, fish, sausages and frankfurters, bacon, ham, salami) was related to higher ferritin and lower sTfR-ferritin index, whereas frequency of consumption of spinach and eggs was related to lower serum ferritin levels. A relationship between haem iron intake and iron status has been reported in the literature several times (Ball and Bartlett 1999, Collings, Harvey et al. 2013), and while the association between haem containing foods and iron status found in the present study is probably partly the result of higher haem iron intakes, this was not calculated. Instead we examined frequency of consumption of haem containing foods, which has rarely been reported, and we expect that the relationship is the result not only of higher haem consumption, but also more frequent consumption of the component of flesh foods that significantly increases non-haem iron absorption from meals (Collings, Harvey et al. 2013). This is supported by the comparison of gram intakes of haem containing foods with iron status measures, which found the associations to be less strong than with frequency of consumption. While both spinach and eggs are reasonable sources of non-haem iron, both also contain significant concentrations of potent iron absorption inhibitors (oxalates in spinach and phosvitin in eggs). It is therefore likely that the presence of these iron inhibitors may have contributed to the findings of a negative correlation between these foods and iron status biomarkers. Vegetarians may have a heightened interest in nutrition, resulting in their higher knowledge scores, than non-vegetarians. Therefore, it is not surprising that these women consumed spinach, soybeans and other beans significantly more frequently than non-vegetarians with the
knowledge that they are iron rich foods. This may help to explain the poorer iron status among the vegetarian women, despite them having similar total iron intakes. Certainly, a 2003 review by Hunt supports the idea that vegetarians, especially females, tend to have lower iron stores despite high intakes of non-haem iron (Hunt 2003). This review further discusses the evidence around iron absorption inhibitors found in significant quantities in traditionally vegetarian food options (e.g., phytic acid in legumes, vegetables and whole-grains, polyphenols and tannins in tea and vegetables, calcium in dairy, and soy and egg protein). The complicated nature of these inhibitory and enhancing relationships suggests that especially vegetarian women could benefit from improved nutrition knowledge about dietary iron and iron absorption enhancers and inhibitors. This study supports the concept that better knowledge about dietary iron can translate into better total dietary iron intakes. While we could not demonstrate a positive relationship between knowledge and iron status, there is some suggestion that if young women can be educated about increasing frequency of meat consumption, this could translate into improved iron status.

The cross sectional design used in the present study does not allow us to infer causality. Further research using an intervention that aims to improve dietary iron knowledge in women and measures iron status over time is therefore warranted. There are several other limitations in the current study that also need to be considered. Participants were recruited from only one region of NSW, which may have reduced the external validity of the sample of young Australian women. Women were largely university educated or university students, with a large proportion from health backgrounds, so they may have had higher levels of knowledge than the general population. The psychometric properties of the NKQ have not yet been determined. Assessment of the reliability and construct validity of the NKQ has not yet been undertaken and therefore the present results need to be interpreted with caution. Further research is required to establish the validity of the NKQ and whether it is indeed a good measure of iron knowledge. Self-report bias is a limitation of questionnaire based studies, as misreporting can be common (Westerterp and Goris 2002). A further limitation of the NKQ is that it does not assess in detail the knowledge about the iron enhancing and inhibiting factors that are crucial to the vegetarian sub-group, and this should be addressed in further research. Finally, the
assessment of iron intake using the FFQ provided a measure of total intake but does not consider haem and non-haem iron separately. However, the FFQ does provide grams of foods consumed in addition to nutrient data and frequencies, and the correlation coefficient for the relationship between seven day weighed food records and the FFQ was reported to be 0.51 for iron in the validation paper (Hodge, Patterson et al. 2000).

4.7 Conclusion
This study highlights a complex relationship between women’s knowledge about iron containing foods and iron status. The results indicate that knowledge about food sources of iron and iron physiology are related to higher dietary iron intake. However this association did not translate into better iron status. Hence, further research is required to examine the complex relationship between knowledge and iron status. Educating young non-vegetarian women about the benefits of increased frequency of flesh food consumption may have potential for addressing the high rates of iron deficiency among this group. In addition, more detailed assessment of knowledge of iron enhancing and inhibiting factors should be conducted as this is particularly important for vegetarian women.
Chapter 5. A study of the effects of latent iron deficiency on measures of cognition: A pilot randomised controlled trial of iron supplementation in young women - Baseline analysis
5.1 Overview

Previous studies that have examined the effect of iron deficiency on cognition have used a number of different cognitive assessment methods, leading to difficulty comparing results, as shown in the systematic review (Chapter 3). The current chapter aims to determine whether any differences in cognitive function in iron-deficient and iron-sufficient young women are detected using IntegNeuro. Following this chapter, Chapter 6 will present further detail on the relationship between iron deficiency and cognition by presenting follow-up results from the pilot RCT.

5.2 Introduction

Cognition encompasses various functions including memory, attention and concentration (Falkingham, Abdelhamid et al. 2010) and can be measured using standardised test batteries or by individual tests designed to measure a specific cognitive ability (e.g., digit span as a test of short-term memory). There is good evidence of an association between iron deficiency and impaired cognitive function in infants and children (Webb and Oski 1973, Lozoff, Brittenham et al. 1982, Oski, Honig et al. 1983, Walter, Kovalskys et al. 1983, Pollitt, Hathirat et al. 1989). However, the effect of latent iron deficiency on cognitive function in young women has not been well investigated. In Australia, one in five young women is affected by iron deficiency (Ahmed, Coyne et al. 2008); and consequently, research into its effect on cognitive function in this population is warranted.

The exact mechanism by which iron deficiency affects the brain is not well understood. Possibilities include abnormalities in neurotransmitter metabolism, decreased myelin formation, and alterations in brain energy metabolism (Tucker, Sandstead et al. 1984, Beard 2003). Neurocognitive assessment remains the primary way to examine both brain development and degeneration of the brain (Clark, Paul et al. 2006). It is both safe and portable and can characterise cognitive deficits. Timed neurocognitive tests have greater sensitivity than untimed tests (1996). As shown in two systematic reviews, many different test batteries are used, including the Wechsler Memory Scale-Revised (WMS-
IntegNeuro™ (Brain Resource Company, Ltd) is a battery of cognitive tests that consists of automated stimulus presentation and response recording, using a touch-screen and voice recording software (Paul, Lawrence et al. 2005). This computerised battery incorporates 12 tasks that take approximately 50 minutes to complete. The tests included in the IntegNeuro battery are designed to assess the following domains: Memory, Attention, Response Speed, Emotion Identification, Information Processing, Impulsivity and Executive Function (Clark, Paul et al. 2006). Global standardisation of tasks enables validation in a wide variety of settings and populations, including young women (Brain Resource Company 2011).

This study aimed to determine any differences in cognitive function using IntegNeuro in iron-deficient and iron-sufficient young women.

5.3 Methods

This Chapter presents baseline comparative data from a pilot randomised-controlled trial that was conducted at the University of Newcastle, Callaghan Campus, NSW, Australia between April 2010 and April 2013.

5.3.1 Participants and recruitment

Flyers were distributed at the University of Newcastle, Callaghan campus and Technical Education (TAFE) College (Appendix 2). Recruitment was also conducted by accessing the volunteer register at Hunter Medical Research Institute and by word-of-mouth. All interested individuals were screened for eligibility against the inclusion criteria (Appendix 3), which were: female, 18-35 years; BMI (kg/m²) 18-30; English as primary language; not iron deficient within the last 12 months; not currently taking iron supplementation (those on a standard multivitamin were eligible to participate); no
chronic medical condition; not taking medication that could potentially interfere with results (anti-inflammatory medications, antacids, histamine receptor antagonists, proton pump inhibitor); ability to provide blood samples for biomarkers of iron status; not pregnant, or planning a pregnancy within the following 4 months; available to participate in the intervention for 4 months. Those eligible to participate were provided with an information statement (Appendix 4) and written informed consent was obtained prior to the commencement of the study (Appendix 5).

5.3.2 Cognitive testing

Cognitive function was measured at baseline and follow-up using the IntegNeuro Battery of cognitive tests developed by the Brain Resource Company (Brain Resource Company 2011). The IntegNeuro battery assesses the following cognitive domains, with the individual tasks within each domain in parentheses: Memory (recall, recognition, digit span, span of visual memory); Response speed (motor tapping); Impulsivity (reaction time); Attention (sustained attention); Information Processing (switching of attention, choice reaction time, verbal interference); Executive Function (maze) and Emotion Identification (emotion recognition). A detailed description of these tests is presented in Appendix 12.

The IntegNeuro battery was self-administered using a computer touchscreen to present the tasks and record responses. A headset was used to deliver auditory instructions and tasks.

A standardised score out of ten (sten) was calculated for each of the cognitive domains. Sten scores divide the score scale into ten units (Brain Resource Company 2011, Psychometric Success 2013). Each unit has a bandwidth of half a standard deviation, except the highest Sten score (10) which extends upwards from two standard deviations above the mean, and the lowest Sten score (1), which extends downwards from two standard deviations below the mean (Brain Resource Company 2011, Psychometric Success 2013). Higher Sten scores indicate better cognitive functioning. The IntegNeuro scoring system is described in Appendix 13.
5.3.3 Haematological testing

The haematological markers used in this study were serum ferritin, haemoglobin, soluble transferrin receptor (sTfR) and alpha-1-glycoprotein (an inflammatory marker). Hunter Area Pathology Service collected blood samples. Latent iron deficiency was defined as having ferritin <20µg/L (Ahmed, Coyne et al. 2008) and all other markers within reference ranges (haemoglobin 115-165g/L (Suominen, Punnonen et al. 1998, Ahmed, Coyne et al. 2008, Leonard, Patterson et al. 2013), alpha-1 glycoprotein 0.51-1.17g/L (Larsson A 2008)). sTfR is expected to be raised above the assay reference range in the presence of iron deficiency. In this study however, all participants had sTfR within normal ranges, therefore it was not useful for defining iron deficiency (further explanation on this is presented in Chapter 8). Anaemic participants (Ferritin <20µg/L, haemoglobin <120g/L) were excluded from analysis and recommended to see a General Practitioner about their results.

5.4 Statistical analysis

Ferritin categories were determined by dividing results into quartiles (<10µg/L, 10-19µg/L, 20-29µg/L, ≥30µg/L) for analysis of iron-sufficient vs. iron-deficient participants. Chi-square tests of independence were performed to examine the relationship between demographic variables and iron status (using the above ferritin categories).

Kruskal-Wallis analysis was used to examine differences in cognitive scores (domain and individual measures that contribute to domain) between iron-deficient and iron-sufficient participants based on ferritin categories. Kruskal-Wallis analysis was also used to determine differences in cognitive scores between iron-deficient and iron-sufficient participants based on cut-offs used in previous studies (<12 vs. ≥12 (World Health Organization 1989), <15 vs. ≥15 (World Health Organization 2011), <20 vs. ≥20 (Ahmed, Coyne et al. 2008), <30 vs. ≥30 (Mast 1998)).

Factor analysis (principal component with varimax rotation) was conducted to determine the dimensions emerging from the cognitive test results. Kruskal-Wallis analysis was then performed to examine differences between iron-deficient and iron-
sufficient participants, and between participants in the different ferritin categories, on the resulting factors (dimensions).

5.5 Results

Baseline testing was completed by 112 participants. Of these, 83 were iron-sufficient and 28 had latent iron deficiency. One participant was anaemic.

The mean age was 27.2 ± 5.2 years and 26.6 ± 4.3 years for iron-sufficient and iron-deficient participants respectively. No participants had alpha-1 glycoprotein results outside of the normal reference range. Forty six per cent of participants had a University degree and 33% per cent of participants worked as a professional (eg. scientist, doctor, registered nurse, allied health professional, teacher, and artist).

No relationship was shown between iron status (using ferritin categories) and the following demographic variables: occupation, $X^2 (27, N = 70) = 30.62, p=.287$; education, $X^2 (12, N = 70) = 5.26, p=.949$; having heavy periods, $X^2 (3, N = 71) = 6.66, p=.084$; severe tiredness, $X^2 (3, N = 71) = 4.81, p=.186$; intense anxiety, $X^2 (3, N = 71) = 2.79, p=.425$; feelings of depression, $X^2 (3, N = 71) = 0.31, p=.959$; or making a regular blood donation, $X^2 (3, N = 71) = 2.59, p=.460$. Receiving a diagnosis of depression from a General Practitioner (either: less than 3 months ago; 3-12 months ago; more than 12 months ago) was reported in 24% of participants. No relationship was found between iron status and having a diagnosis of depression ($X^2 (3, N = 70) = 3.05, p=.384$). There was also no relationship between iron status and the regularity of menstruation in the last 3 months (none, one, two, three, four, five or more) ($X^2 (12, N = 71) = 5.05, p=.956$). The use of hormonal contraceptives was reported in 51% of participants. There was no relationship between this and iron status ($X^2 (3, N = 71) = 2.106, p=.551$).

5.5.1 Relationship between iron status and cognitive function

The first analysis presented is the effect of iron status on the cognitive domain scores. Following this, the relationship between iron status and individual measures that contribute to the overall cognitive domain scores are presented.
5.5.1.1 Analysis of relationship between cognitive domain scores and ferritin

As shown in Table 5.1, there were no significant differences in cognitive domain scores between participants as a function of ferritin category (<10 µg/L, 10-19 µg/L, 20-29 µg/L or ≥30 µg/L). As shown in Table 5.2, analysis of differences in cognitive domain scores between iron-deficient and iron-sufficient participants was conducted based on cut-offs used in previous research (<12 vs. ≥12, <15 vs. ≥15, <20 vs. ≥20, <30 vs. ≥30). The results of this analysis showed a significant difference in Response Speed between iron-sufficient and iron-deficient participants based on ferritin <30 µg/L and ≥30 µg/L. Based on this ferritin cut-off, iron-sufficient participants performed significantly better (i.e., faster responses) than iron-deficient participants. There were no other significant differences in cognitive domain scores between iron-deficient and iron-sufficient participants based on the different classifications of iron deficiency.

Table 5.1. Differences in cognitive domain scores between ferritin categories at baseline

<table>
<thead>
<tr>
<th>Cognitive Sten score</th>
<th>Ft&lt;10µg/L (n=15)</th>
<th>Ft 10-19 µg/L (n=15)</th>
<th>Ft 20-29 µg/L (n=28)</th>
<th>Ft≥30µg/L (n=55)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Memory</td>
<td>4.77±1.93</td>
<td>5.87±1.70</td>
<td>5.50±2.18</td>
<td>5.17±1.98</td>
<td>.380</td>
</tr>
<tr>
<td>Response speed</td>
<td>5.53±1.68</td>
<td>5.57±1.57</td>
<td>4.98±1.79</td>
<td>5.90±1.70</td>
<td>.102</td>
</tr>
<tr>
<td>Impulsivity</td>
<td>6.10±2.34</td>
<td>6.73±2.37</td>
<td>6.46±2.57</td>
<td>6.35±2.35</td>
<td>.917</td>
</tr>
<tr>
<td>Attention</td>
<td>5.67±2.78</td>
<td>5.29±3.12</td>
<td>5.96±3.12</td>
<td>6.03±2.66</td>
<td>.868</td>
</tr>
<tr>
<td>Information processing</td>
<td>6.87±1.86</td>
<td>6.67±1.70</td>
<td>6.61±1.84</td>
<td>6.26±1.88</td>
<td>.612</td>
</tr>
<tr>
<td>Executive function</td>
<td>6.90±2.11</td>
<td>7.00±2.14</td>
<td>6.64±1.76</td>
<td>7.05±2.05</td>
<td>.656</td>
</tr>
<tr>
<td>Emotion identification</td>
<td>5.33±2.27</td>
<td>5.73±2.42</td>
<td>5.32±2.51</td>
<td>5.45±2.37</td>
<td>.958</td>
</tr>
</tbody>
</table>

Note: K-Wallis used in analysis
Table 5.2. Differences in cognitive domain scores between iron-deficient and iron-sufficient participants at baseline (based on ferritin cut offs used in published literature)

<table>
<thead>
<tr>
<th>Cognitive change Sten score</th>
<th>Ft&lt;12µg/L (n=16)</th>
<th>Ft≥12µg/L (n=98)</th>
<th>pvalue</th>
<th>Ft&lt;15µg/L (n=21)</th>
<th>Ft≥15µg/L (n=93)</th>
<th>pvalue</th>
<th>Ft&lt;20µg/L (n=30)</th>
<th>Ft≥20µg/L (n=84)</th>
<th>pvalue</th>
<th>F&lt;30µg/L (n=58)</th>
<th>F≥30µg/L (n=56)</th>
<th>pvalue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Memory</td>
<td>4.75±1.86</td>
<td>5.38±2.00</td>
<td>.255</td>
<td>4.79±1.88</td>
<td>5.41±2.01</td>
<td>.178</td>
<td>5.32±1.87</td>
<td>5.28±2.04</td>
<td>.812</td>
<td>5.41±2.01</td>
<td>5.17±1.98</td>
<td>.495</td>
</tr>
<tr>
<td>Response speed</td>
<td>5.34±1.80</td>
<td>5.62±1.72</td>
<td>.340</td>
<td>5.52±1.65</td>
<td>5.59±1.75</td>
<td>.562</td>
<td>5.55±1.60</td>
<td>5.59±1.78</td>
<td>.658</td>
<td>5.28±1.70</td>
<td>5.90±1.70</td>
<td>.026*</td>
</tr>
<tr>
<td>Impulsivity</td>
<td>6.28±2.37</td>
<td>6.4±2.39</td>
<td>.862</td>
<td>6.10±2.36</td>
<td>6.46±2.39</td>
<td>.566</td>
<td>6.42±2.33</td>
<td>6.39±2.41</td>
<td>.979</td>
<td>6.44±2.43</td>
<td>6.35±2.35</td>
<td>.836</td>
</tr>
<tr>
<td>Attention</td>
<td>5.47±2.80</td>
<td>5.94±2.72</td>
<td>.563</td>
<td>5.30±2.79</td>
<td>5.99±2.70</td>
<td>.353</td>
<td>5.48±2.90</td>
<td>6.01±2.66</td>
<td>.439</td>
<td>5.71±2.79</td>
<td>6.03±2.66</td>
<td>.575</td>
</tr>
<tr>
<td>Information processing</td>
<td>6.88±1.79</td>
<td>6.42±1.84</td>
<td>.343</td>
<td>6.67±1.80</td>
<td>6.44±1.85</td>
<td>.661</td>
<td>6.77±1.75</td>
<td>6.38±1.86</td>
<td>.358</td>
<td>6.69±1.78</td>
<td>6.26±1.88</td>
<td>.206</td>
</tr>
<tr>
<td>Executive function</td>
<td>7.00±2.08</td>
<td>6.91±1.98</td>
<td>.983</td>
<td>6.59±2.38</td>
<td>7.00±1.89</td>
<td>.504</td>
<td>6.95±2.09</td>
<td>6.92±1.96</td>
<td>.712</td>
<td>6.80±1.93</td>
<td>7.05±2.05</td>
<td>.554</td>
</tr>
<tr>
<td>Emotion identification</td>
<td>5.19±2.27</td>
<td>5.48±2.40</td>
<td>.600</td>
<td>5.45±2.32</td>
<td>5.44±2.39</td>
<td>.993</td>
<td>5.53±2.31</td>
<td>5.41±2.40</td>
<td>.842</td>
<td>5.43±2.39</td>
<td>5.45±2.37</td>
<td>.986</td>
</tr>
</tbody>
</table>

Note: KWallis analysis used for the comparisons
5.5.1.2 Analysis of the relationship between individual cognitive measures that contribute to the domain scores and ferritin

A significant difference between ferritin categories was shown in the percentage of correctly identified sad faces \( (p=0.009) \) (Emotion Identification task). Post-hoc analysis using Bonferroni’s method revealed that participants with ferritin 20-29µg/L had significantly higher percent correct than those with ferritin ≥30µg/L \( (80.80±14.62, 69.32±17.97, p=0.035) \). A significant difference between ferritin categories was also shown in verbal fluency scores \( (p=0.014) \). Post-hoc analysis revealed that participants with ferritin 10-19µg/L had marginally lower scores than those with ferritin ≥30µg/L \( (11.51±5.05, 14.31±3.37, p=0.059) \). Participants with ferritin of 10-19µg/L correctly recalled a significantly higher number of words in 30 seconds than those with ferritin ≥30µg/L \( (36.5±3.06, 33.5±4.10, p=0.049) \). There were no other significant differences in individual measures of cognition that contribute to domain scores and ferritin categories. The differences shown may have been due to Type I error.

5.5.1.3 Factor analysis

The standardised scores on each of the cognitive tests were analysed in a factor analysis. Kaiser’s criterion suggesting that factors with an eigenvalue greater than 1.0 are retained, was applied, as was varimax rotation, leaving seven factors. Kaiser’s criterion was reinforced by observation of the scree plot. Factor loadings less than 0.5 were discounted. The resulting factors (Memory; Executive function; Verbal short term memory; Speed; Inhibition; Visual short term memory and Intrusions) and loadings are shown in Table 5.3.
Table 5.3 Factor loadings based on a principle components analysis with varimax rotation for the 34 items from the IntegNeuro battery of cognitive tests (n=112)
<table>
<thead>
<tr>
<th>Variable</th>
<th>Factor1 Memory</th>
<th>Factor2 Executive function</th>
<th>Factor3 Verbal short-term memory</th>
<th>Factor4 Speed</th>
<th>Factor5 Inhibition</th>
<th>Factor6 Visual short-term memory</th>
<th>Factor7 Intrusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of words from (auditory) list correctly recalled in 30 seconds- 1st attempt</td>
<td>0.6315</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of words from (auditory) list correctly recalled in 30 seconds- 2nd attempt</td>
<td>0.7575</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of words from (auditory) list correctly recalled in 30 seconds- 3rd attempt</td>
<td>0.7410</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of words from (auditory) list correctly recalled in 30 seconds- 4th attempt</td>
<td>0.7529</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total memory recall</td>
<td>0.9327</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.5459</td>
</tr>
<tr>
<td>Memory intrusions trials 1-4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of words from the original list correctly recalled in 30 seconds- attempt immediately follows the distracter list trial</td>
<td>0.7056</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of words from the original list correctly recalled in 30 seconds- following a half hour delay</td>
<td>0.7151</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Memory intrusions trial 6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.5692</td>
</tr>
<tr>
<td>Memory intrusions trial 7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.6316</td>
</tr>
<tr>
<td>Digit span forwards (recall span)</td>
<td>0.7749</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digit span trials correct forwards</td>
<td>0.7825</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Digit span Backward (recall span)</td>
<td>0.8035</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Digit span trials correct backwards</td>
<td>0.8028</td>
<td></td>
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<tr>
<td>Span of visual memory</td>
<td>0.7698</td>
<td></td>
<td></td>
<td>0.8007</td>
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</tr>
<tr>
<td>Switching of attention errors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.6819</td>
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<tr>
<td>Switching of attention average connection time</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>0.7211</td>
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<tr>
<td>Switching of attention completion time</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.5804</td>
</tr>
<tr>
<td>Maze test completed trials</td>
<td>0.8590</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variable</td>
<td>Factor1 Memory</td>
<td>Factor2 Executive function</td>
<td>Factor3 Verbal short-term memory</td>
<td>Factor4 Speed</td>
<td>Factor5 Inhibition</td>
<td>Factor6 Visual short-term memory</td>
<td>Factor7 Intrusions</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
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<td>-----------------------------</td>
<td>----------------------------------</td>
<td>---------------</td>
<td>-------------------</td>
<td>----------------------------------</td>
<td>-------------------</td>
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<tr>
<td>Maze test completion time</td>
<td>0.8673</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Maze test path learning time</td>
<td>0.8840</td>
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<tr>
<td>Maze test total errors</td>
<td>0.7429</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Maze test number of overruns</td>
<td>0.7229</td>
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<tr>
<td>Go/No-Go reaction time variability</td>
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<td>0.6971</td>
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<tr>
<td>Go/No-Go false alarms</td>
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<td></td>
<td>0.8436</td>
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<tr>
<td>Go/No-Go false misses</td>
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<td>0.8713</td>
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<td></td>
</tr>
<tr>
<td>Go/No-Go total errors</td>
<td></td>
<td></td>
<td>0.9712</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Emotion recognition</td>
<td></td>
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<tr>
<td>Fear reaction time</td>
<td>0.7815</td>
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<tr>
<td>Fear reaction time</td>
<td>0.7138</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Anger reaction time</td>
<td>0.7236</td>
<td></td>
<td></td>
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<tr>
<td>Disgust reaction time</td>
<td>0.8008</td>
<td></td>
<td></td>
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<tr>
<td>Happy reaction time</td>
<td>0.5168</td>
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<td></td>
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<tr>
<td>Neutral reaction time</td>
<td>0.6509</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Between-groups analysis**

There were no significant differences between iron-sufficient vs. iron-deficient participants on any of the seven factor scores (Long-term Memory $p = .155$, Executive function $p = .973$, Verbal short-term memory $p = .641$, Speed $p = .246$, Inhibition $p = .973$, Visual short-term memory $p = .406$, Intrusions $p = .602$).

There were also no between-group differences on any of the seven factors for the ferritin categories: F1 Memory $p = .207$, F2 Executive function $p = .971$, F3 Verbal short term memory $p = .823$, F4 Speed $p = .747$, F5 Inhibition $p = .961$, F6 Visual short term memory $p = .515$, F7 Intrusions $p = .185$. 
5.6 Discussion

This chapter presents the baseline haematological and cognitive results of the pilot RCT. This study aimed to determine any differences in cognitive function using IntegNeuro in iron-deficient and iron-sufficient young women.

IntegNeuro was simple to administer and well accepted by the sample of young women. However, the ability of the battery to detect differences in cognition as a function of iron status in this population was unable to be confirmed by the present results. Few statistically significant relationships between iron status and cognitive function based on composite domain scores and the individual measures that contribute to the domain scores were shown. Based on the ferritin cut-off <30µg/L and ≥30µg/L, iron-deficient participants had significantly lower Response Speed scores than iron-sufficient participants. This was the only significant difference shown between cognitive domain scores using several ferritin cut-offs.

Some studies have shown no association between iron status and cognitive function at baseline but have reported improvement in cognitive function in previously iron-deficient participants after iron treatment (Groner, Holtzman et al. 1986, Kretsch, Fong et al. 1998, Beard, Hendricks et al. 2005). However, other studies have shown higher cognitive scores for iron-sufficient compared with iron-deficient participants at baseline (Patterson 1999, Murray-Kolb and Beard 2007, Khedr, Hamed et al. 2008). Differences reported in these studies include, intelligence, memory (Murray-Kolb and Beard 2007, Khedr, Hamed et al. 2008), and arithmetic ability (Patterson 1999). Incomparable cognitive testing methods between the present study and previous studies make it difficult to determine the reason for the differences in results. Khedr et al., used the Wechsler Memory Scale – Revised. Patterson used the The Welscher Adult Intelligence Scale - Revised and Murray-Kolb and Beard used Detterman’s Cognitive Abilities Test. The age and gender of participants in these three studies were comparable with the current pilot RCT, however, Khedr et al. only included participants with iron deficiency anaemia and Patterson and Murray-Kolb and Beard both included anaemic participants in addition to participants with latent iron deficiency, which was not the case for the
current study. It is possible that iron deficiency does not significantly affect cognitive performance until anaemia is present. This was evident in the study by Murray-Kolb and Beard who only found differences when they compared the 1st and 5th quintiles (iron-sufficient vs. iron-deficient anaemic participants).

Factor analysis was conducted to determine the dimensions of IntegNeuro derived from the current sample of participants. The seven dimensions of IntegNeuro determined by the factor analysis (Memory; Executive function; Verbal short term memory; Speed; Inhibition; Visual short term memory and Intrusions) were disparate to the seven cognitive domains produced by The Brain Resource Company (Memory, Response Speed, Impulsivity, Attention, Information processing, Executive Function, Emotion Identification) (Gordon, Barnett et al. 2008). The Brain Resource Company classified the seven cognitive domains using WebNeuro data (a shorter, web version of IntegNeuro) from 1000 healthy individuals between the ages 6 and 91 years (53.5% female). They also used principle component analysis, however with oblimin rotation instead of varimax (Gordon, Barnett et al. 2008, Mathersul, Palmer et al. 2009). The reason for the differences in cognitive dimensions between our factor analysis and that of the Brain Resource Company (BRC) may be a result of the WebNeuro testing battery taking a shorter amount of time to complete, BRC testing a larger sample size, or participants having a wider age range and including both genders.

This pilot study has several limiting factors that may have reduced the likelihood of the baseline data showing significant associations. Due to the nature of a pilot, the study included a relatively small sample of iron-deficient participants. It is possible that a larger sample of iron-deficient participants would show different results. There was no control of dietary intake prior to or during testing. There was also no control of menstrual cycle, exercise habits, the use of stimulants, sleep patterns or stress prior to testing. Another limitation is that, although participants were asked to have their blood samples collected within the following 24-48 hours after cognition testing, there was no strict control for the time of the day for cognitive or blood testing. Although the haematological tests used in the study are reasonably stable irrespective of the day and
time of day they were measured, the duration of time between cognitive testing and blood sample collection varied between participants. This was due to logistical barriers associated with participants’ availability and the Hunter Area Pathology availability.

Overall, this chapter shows little evidence of differences in cognitive function between iron-sufficient and iron-deficient participants at baseline. Future studies need to be conducted to determine if differences are shown with a larger sample of iron-deficient participants, and with better controls around testing conditions. Chapter 6 examines follow-up data from the pilot RCT to determine whether iron-deficient participants improve on cognitive tasks following a 16 week iron treatment intervention.

This paper was published in 2014.


The work presented in the manuscript was presented at the 16th International Congress of Dietetics in September 2012. Sydney, Australia (Poster presentation).

The work presented in the manuscript was completed in collaboration with the co-authors (Appendix 16).
6.1 Overview
The effect of iron deficiency on cognition in young women has not been well reported in literature. In the studies that do exist, methods to assess cognition and iron status are heterogeneous making results difficult to compare (as presented in Chapter 3). The aims of this chapter were to determine: the suitability of the IntegNeuro battery for assessing cognitive function in iron-deficient and iron-sufficient women; and the effect of iron treatment on cognitive function; an appropriate sample size for an adequately powered RCT examining the effects of iron treatment on cognition in iron-deficient women. Paper 5 commences verbatim from Section 6.2. Methods for the collection of cognitive and haematological data are presented in Section 6.4, results are presented in Section 6.5 and discussion is presented in Section 6.6.

6.2 Abstract
Rates of iron deficiency are high amongst healthy young women. Cognitive impairment occurs secondary to iron deficiency in infants and children, but evaluation of the impact on cognition among young women is inconsistent. The aim was to determine the suitability of the IntegNeuro test battery for assessing cognitive function in iron-deficient and iron-sufficient young women. A pilot double-blinded, placebo-controlled intervention trial was conducted in iron-deficient (serum ferritin ≤ 20 µg/L and haemoglobin > 120 g/L) and iron-sufficient young women (18–35 years). Cognitive function and haematological markers of iron status were measured at baseline and follow-up. Iron-deficient participants \((n = 24)\) were randomised to receive placebo, 60 mg or 80 mg elemental iron daily supplements for 16 weeks. A control group of iron-sufficient participants \((n = 8)\) was allocated to placebo. Change scores for Impulsivity and Attention were significantly greater in plasma ferritin improvers than in non-improvers \((p = 0.004, p = 0.026)\). IntegNeuro was easy to administer and acceptable to young women. Based on the differences in Memory and Attention scores between iron-deficient participants on iron treatment and those on placebo, it was decided that between 26 and 84 participants would be required in each iron treatment group for an adequately powered extension of this pilot RCT.
6.3 Introduction

Iron deficiency is the most prevalent nutritional deficiency worldwide (World Health Organization 1992). No population group is unaffected, but rates are highest for young women, infants and children in their first two years of life (World Health Organization 2001). Young women are at particular risk of iron deficiency due to increased iron requirements secondary to menstruation and pregnancy. Up to two thirds of young women in developing countries suffer from iron deficiency (Scrimshaw 1991). However, it is not only a phenomenon of developing nations, with prevalence rates of between 10 and 20 per cent found in the U.S. and Europe (World Health Organization 2001).

Latent iron deficiency is characterised by individuals having serum ferritin \( \leq \) 20µg/l and haemoglobin >120g/l (World Health Organization 2001). Iron-deficiency anaemia is the most severe form of iron deficiency and is characterised by having haemoglobin \( \leq \) 120g/l, in addition to having low serum ferritin (Falkingham, Abdelhamid et al. 2010).

There is now good evidence of an association between iron deficiency and impaired cognitive function in infants and children (Webb and Oski 1973, Lozoff, Brittenham et al. 1982, Oski, Honig et al. 1983, Walter, Kovalskys et al. 1983, Pollitt, Hathirat et al. 1989). Cognition is important for quality of life and encompasses various functions including memory, attention and concentration (Falkingham, Abdelhamid et al. 2010). The exact mechanism by which iron deficiency affects the brain is not well understood. Possibilities include abnormalities in neurotransmitter metabolism, decreased myelin formation, and alterations in brain energy metabolism (Tucker, Sandstead et al. 1984, Beard 2003).

Studies in infants and children have shown that iron deficiency without anaemia can cause changes in brain development and function (Grantham-McGregor and Ani 2001). These changes have been shown to specifically affect concentration, attention and short-term memory (Lozoff, Brittenham et al. 1982, Lozoff, Brittenham et al. 1982, Walter, Kovalskys et al. 1983, Pollitt, Hathirat et al. 1989). A review of longitudinal studies found that adolescents who had experienced iron-deficiency anaemia during infancy
continued to perform less well in spatial memory and selective attention tasks compared to peers who had adequate iron status in infancy (Lozoff, Beard et al. 2006).

Due to the high rates of iron deficiency in young women, it is important to conduct well designed randomised controlled trials to investigate whether iron deficiency is associated with deficits in cognitive function in this population. Of the studies that have been conducted in this population, some have found poorer cognitive performance in iron-deficient participants compared with iron-sufficient participants (Patterson 1999, Murray-Kolb and Beard 2007, Khedr, Hamed et al. 2008), whereas others have found no difference (Groner, Holtzman et al. 1986, Kretsch, Fong et al. 1998, Beard 2003). Following iron supplementation, the majority of studies have reported an improvement in cognitive function in young women who were iron-deficient at baseline (Groner, Holtzman et al. 1986, Ballin, Berar et al. 1992, Bruner, Joffe et al. 1996, Kretsch, Fong et al. 1998, Patterson 1999, Beard 2003, Murray-Kolb and Beard 2007, Khedr, Hamed et al. 2008). However, some studies have found no difference (Elwood and Hughes 1970). Two systematic reviews have examined this topic and concluded that there is some evidence of an effect but findings are confounded by extremely varied methodologies, particularly with regard to measures of cognitive function and definitions of iron deficiency (Falkingham, Abdelhamid et al. 2010, Greig, Patterson et al. 2013).

The most recent work in this area has been conducted by Murray-Kolb and Beard (2007) (Murray-Kolb and Beard 2007) and Blanton (2013) (Blanton 2013). Both conducted prospective randomised controlled intervention trials in young women (18-35 years and 18-30 years, respectively) of varied iron status. Murray-Kolb and Beard used iron supplements and Blanton used high bioavailable iron meals as the intervention. Murray-Kolb and Beard found that at baseline, iron-sufficient women performed better on cognitive tasks and completed them faster than women with iron deficiency anaemia (haemoglobin<120g/L). They also showed that participants with latent iron deficiency (haemoglobin 105-119g/L and ≥2 abnormal iron status values) performed intermediary between the two extremes of iron status which compared the 1st vs 5th quintiles. There were no significant differences between latent iron-deficient and iron-sufficient participants. After treatment with iron supplements, a significant improvement in serum
ferritin was associated with improvements in cognitive performance, while improvement in haemoglobin, defined as percentage change greater than 4.4%, was related to improved speed in completing the cognitive tasks (Murray-Kolb and Beard 2007). Similarly, in a trial comparing beef and non-beef lunches three times weekly for 16 weeks, Blanton (Blanton 2013) reported that participants whose ferritin increased had significantly greater improvements in planning, speed, spatial working memory and strategy than those whose ferritin did not increase.

In addition to using different interventions, the studies also differed with regard to the cognitive tests used. Murray-Kolb and Beard (Murray-Kolb and Beard 2007) used Detterman’s Cognitive Abilities Test whereas Blanton (Blanton 2013) used the Cambridge Neuropsychological Test Automated Battery. Both batteries claim to measure similar domains, including verbal memory, spatial memory and visual information processing. However, they include different individual cognitive tests and are therefore not directly comparable. The two systematic reviews mentioned above found that some studies have used individual tests such as Digit Span or Block Design (Greig, Patterson et al. 2013), whereas others have used composite test batteries such as the Wechsler Memory Scale-Revised (WMS-R), Wechsler Adult Intelligence Scale-Revised (WAIS-R), and Hopkins Verbal Learning Test (Falkingham, Abdelhamid et al. 2010, Greig, Patterson et al. 2013).

In order to achieve some homogeneity in the area of iron deficiency and cognition in adults, research groups need to use the same tools in studies of similar design. The preferred tool needs to have good reliability and validity and standardised administration procedures. IntegNeuro is a battery of cognitive tests that has good reliability and validity. Paul et al. (2005) reported on the validity of the IntegNeuro battery in assessing seven cognitive domains (Memory, Response Speed, Impulsivity, Attention, Information Processing, Executive Function, Emotion Identification) in a sample of 50 healthy adults (25 women and 25 men, age 22-80 years) (Paul, Lawrence et al. 2005). This study assessed validity, conducting correlation analyses between IntegNeuro and paper based tests, and examined the influence of age, education and sex on test results. They found strong relationships between IntegNeuro tests and standard
measures of cognitive function (Paul, Lawrence et al. 2005). Clark et al. (2006) examined the effects of age, gender and education on cognitive function using the IntegNeuro battery and reported its sensitivity regarding the assessment of cognition (Clark, Paul et al. 2006).

The current research examines the effect of latent iron deficiency on cognitive function in young women. The aim was to determine the suitability of the IntegNeuro battery for assessing cognitive function in iron-deficient and iron-sufficient women in a double-blind placebo-controlled trial.

6.4 Methods

This study was conducted at the University of Newcastle, Callaghan Campus in NSW, Australia, between April 2010 and April 2013. Ethics approval was provided by the Human Research Ethics Committee at University of Newcastle (H-2010-1079) (Appendix 1). Women aged 18-35 years were recruited via flyers (Appendix 2), promotion in lectures, through the Hunter Medical Research Institute volunteer register and by word-of-mouth. All interested individuals were screened for eligibility against inclusion criteria (Appendix 3). These were: female, 18-35 years; BMI 18-30 kg/m²; English as primary language; not iron deficient within the last 12 months; not currently taking iron supplementation (those on a standard multivitamin were eligible to participate); no chronic medical condition; not taking medication that could potentially interfere with results (anti-inflammatory medications, antacids, histamine receptor antagonists, proton pump inhibitor); ability to provide blood samples for biomarkers of iron status; not pregnant, or planning a pregnancy within the following 4 months; available to participate in the intervention for 4 months. Those eligible were provided with an information statement (Appendix 4) and informed consent was obtained prior to the commencement of the study (Appendix 5).

The study included baseline cognitive and haematological testing, a 16 week intervention with two separate doses of elemental iron (60mg or 80mg) in the form of ferrous sulfate or placebo, an iron-sufficient control group allocated to placebo, and follow-up testing. Figure 6.1 shows the recruitment process and study flow chart.
### Study Recruitment and Randomisation Flow chart

This describes the participant flow through a pilot double-blinded, placebo-controlled randomised control trial of the effect of iron supplementation (60mg or 80mg iron or placebo for 16 weeks) on the cognitive function of iron-deficient (ferritin <20µg/L, haemoglobin ≥120g/L) and iron-sufficient (ferritin ≥20µg/L, haemoglobin ≥120g/L) women (18-35 years).

**IntegNeuro** = a self-administered (touch screen) battery for assessing cognitive function including the domains of Memory, Response Speed, Impulsivity, Attention, Information Processing, Executive Function, Emotion Identification.

### 6.4.1 Haematological testing

Serum ferritin, haemoglobin, soluble transferrin receptor (sTfR) and alpha-1-glycoprotein (an inflammatory marker) were measured at baseline and follow-up. Blood samples were collected by Hunter Area Pathology Service. Latent iron deficiency was defined as having ferritin <20µg/L and all other markers within reference ranges (haemoglobin 115-165g/L (Ahmed, Coyne et al. 2008), sTfR 0.9-2.30mg/L (Suominen, Punnonen et al. 1998, Leonard, Patterson et al. 2013), alpha-1 glycoprotein 0.51-1.17g/L (Larsson A 2008)). sTfR is expected to be raised in the presence of iron deficiency. However, all participants in the current study had normal sTfR. Ferritin reflects storage iron and sTfR reflects functional iron, therefore, sTfR-Ferritin Index (sTfR-Index) has been proposed as a measure that may increase the accuracy of iron deficiency diagnosis (Malope, MacPhail et al. 2001). Anaemic participants (Ferritin <20µg/L, haemoglobin <120g/L) were excluded from the remainder of the trial and data analyses. Participants
were classified as ferritin improvers or non-improvers according to the criteria used by Blanton (2013) (Blanton 2013) and Murray-Kolb and Beard (2007) (Murray-Kolb and Beard 2007). This criterion considers whether participants had a change in ferritin greater or less than the known biological day-to-day variation (27%) (Borel, Smith et al. 1991, Cooper and Zlotkin 1996, Stupnicki, Malczewska et al. 2003). Participants’ percentage ferritin change during the trial was calculated. Participants with percentage changes above 27% were deemed ferritin improvers and those with percentage changes of ≤27% were considered ferritin non-improvers. Participants were also classified as haemoglobin improvers or non-improvers based on whether they had a change in haemoglobin greater or less than the known biological day-to-day variation (4.4%) (Murray-Kolb and Beard 2007, Blanton 2013).

6.4.2 Cognitive testing

Cognitive function was measured at baseline and follow-up using the IntegNeuro Battery of Cognitive Tests developed by the Brain Resource Company (Brain Resource Company 2011). The IntegNeuro battery includes the following cognitive domains, with the individual tasks within each domain in parentheses: Memory (recall, recognition, digit span, span of visual memory); Response speed (motor tapping); Impulsivity (reaction time); Attention (sustained attention); Information Processing (switching of attention, choice reaction time, verbal interference); Executive Function (maze) and Emotion Identification (emotion recognition). A detailed description of these tests is presented in Appendix 12.

The IntegNeuro battery was self-administered using a touchscreen to present the tasks and record responses. A headset was used to deliver auditory instructions and tasks. The tests yield a standardised score out of ten (sten) for each of the cognitive domains. Sten scores divide the score scale into ten units. Each unit has a bandwidth of half a standard deviation, except the highest Sten score (10) which extends upwards from two standard deviations above the mean, and the lowest Sten score (1), which extends downwards from two standard deviations below the mean (Brain Resource Company 2011, Psychometric Success 2013). Higher Sten scores indicate better cognitive function. The IntegNeuro scoring system is described in Appendix 13.
Practice trials were included before scored trials for relevant cognitive tasks. Cognition testing was conducted in a private, sound-proof testing room. The experimenter was present in the adjoining room but there was no communication with participants during testing. Participants completed the IntegNeuro battery in one session of approximately 50 minutes.

Participants with latent iron deficiency were randomised to one of two doses of ferrous sulfate (60mg or 80mg), or placebo. The first eight iron-sufficient (ferritin ≥20ug/L) participants who were screened were invited into the intervention as a control group. A single blinding approach was used with the control group who all received placebo capsules, whereby researchers knew they were iron-sufficient, but participants remained blinded to both their iron status and their capsule formulation. Subsequent iron-sufficient participants exited the study following baseline testing. Every four weeks participants were contacted and asked to report possible side effects associated with the treatment. The study piloted the two doses of iron treatment (60mg and 80mg) to determine an efficacious dose to which participants could remain blinded. Those results form the basis of another paper (Leonard, Chalmers et al. 2014).

There was no specific time of the day that participants were required to take their capsules. Participants were provided with a tips sheet (Appendix 8) which included information about taking their capsules two hours apart from any other medications (except the oral contraceptive pill) as a precaution. The tips sheet also included strategies for remembering to take the capsules, such as placing them next to their toothbrush or in their handbag using the small extra capsule container provided.

**6.4.3 Treatment blinding**

Compounding chemists were contracted to provide the iron and placebo supplements. To ensure blinding, the active and placebo supplements were identical in appearance, were packaged in identical containers and tasted the same. Participants were provided with 112 capsules (1 capsule per day for 16 weeks). The researchers and participants remained blinded to the treatment protocol and the randomisation code was held by the compounding chemists only to be broken once the final results were collected. Researchers remained blinded to treatment allocations until all participants had
completed the study. A University of Newcastle researcher, external to the study, unblinded participants to their allocated treatment group and forwarded them a letter explaining their treatment group and their iron status at the follow-up blood assessment (Appendix 11). At the end of the intervention participants were asked to guess which treatment they were taking.

6.4.4 Required Sample Size for An Adequately Powered RCT

The sample size calculation was estimated using the difference in cognitive change score results between iron-deficient participants on iron treatment and those on placebo in the current trial. More specifically, data used in this calculation were: .05 as the type I error probability; power of 80%; the difference in means from baseline to follow-up for Memory and Attention sten scores for iron-deficient participants taking iron treatment and those taking placebo; and within group standard deviation for each of these cognitive domains.

6.4.5 Statistical Analysis

STATA-IC 11 (StataCorp 2013) was used for performing statistical analyses. An alpha level of .05 was used for statistical significance. Due to the small sample size and low power to detect significant differences, we also report differences at an alpha level of <.10 as marginally significant.

Kruskal Wallis tests were used to examine differences between treatment (60mg and 80mg) and no-treatment (placebo and control) groups in iron status, cognitive domain scores, and the individual measures that contribute to the cognitive domain scores, at baseline and follow-up. Kruskal Wallis tests were also used to analyse the effect of treatment (60mg and 80mg combined vs. placebo and control combined) on change scores (baseline to follow-up) for each cognitive domain and for each of the measures that contribute to the domain scores. Two additional sets of analyses were performed on cognitive changes scores: one with ferritin improvers versus non-improvers as the independent variable; the other with haemoglobin improvers versus non-improvers as the independent variable.
6.5 Results

6.5.1 Participants

Eligibility screening was completed by 134 young women, of whom 128 were eligible and 95 completed the trial. Of the 24 participants who were iron-deficient at baseline, 19 completed the trial (placebo, n=6; 60mg iron, n=7; 80mg iron n=6). Six iron-sufficient participants in the control group, completed. Reasons given for withdrawal were: an unrelated illness (n=3) or being too busy (n=3). One participant gave no reason. Of the 25 participants who completed the trial, the majority was Australian (90%), the mean age was 25.7±4.1 years and 12 participants reported taking the oral contraceptive pill (OCP). There was no difference in age or OCP use across the allocated groups. Participants complied adequately with the intervention. The average number of capsules remaining of the 112 provided to participants was 12±12 (9.6%).

6.5.2 Iron Status

There were no significant differences in ferritin, haemoglobin, sTfR-Index or A1GP between the three iron-deficient groups (60mg, 80mg and placebo) at baseline. As expected, iron-sufficient controls had significantly higher ferritin and lower sTfR-Index than iron-deficient groups. Following the intervention, a significant difference in ferritin change score was revealed between treatment groups (60mg and 80mg) and no treatment groups (iron-sufficient controls and iron-deficient placebo). There was no significant difference in either haemoglobin or sTfR-Index change scores between iron treatment (60mg and 80mg) and no treatment groups (iron-sufficient controls and iron-deficient placebo). A1GP results were within the reference range for all participants pre- and post-intervention. Full details regarding iron status of participants pre- and post-intervention are presented elsewhere (Leonard, Chalmers et al. 2014).

6.5.3 Cognitive scores

Median cognitive domain scores (and interquartile range, IQR) for each group at baseline and follow-up are presented in Table 6.1. Medians are reported due to non-normal distributions, primarily due to small sample size.
6.5.3.1 Baseline Comparison of Iron-deficient Groups (combined) vs Iron-sufficient Group

Analyses comparing iron-sufficient (controls) and iron-deficient participants (60mg, 80mg and placebo groups combined) at baseline revealed no significant between-group differences on any of the cognitive domains [Memory ($p=.523$); Response Speed ($p=.652$); Impulsivity ($p=.655$); Attention ($p=.263$); Information Processing ($p=.353$); Executive Function ($p=.543$), or Emotion Identification ($p=.178$)].

6.5.3.2 Baseline Comparison of Iron-deficient Groups

Analysis of cognitive domain scores at baseline for the three iron-deficient groups (60mg iron, 80mg iron, placebo) revealed a significant difference in the Executive Function domain score ($p=.012$). Post hoc analysis indicated that the 80mg iron group had lower Executive Function scores than the 60mg iron group ($p=.006$). There was no significant difference in Executive Function scores between placebo and 60mg ($p=.411$) or placebo and 80mg groups ($p=.182$). There was a marginally significant difference in Memory domain scores between the three iron-deficient groups ($p<.053$). Post hoc analysis showed the 80mg iron group had marginally lower Memory scores than the 60mg group ($p=.098$). There was no difference in Memory score between placebo and 60mg ($p=1.000$) or placebo and 80mg groups ($p=.128$).

There were no significant differences between the three iron-deficient groups at baseline for Response speed ($p=.390$); Impulsivity ($p=.702$); Attention ($p=.537$); Information Processing ($p=.272$), or Emotion Identification ($p=.767$).

Changes in cognitive performance from baseline to follow-up are presented in Table 6.1. We first present the analysis of the effect of iron treatment (60mg and 80mg combined) versus no treatment (placebo and control combined) on scores for each cognitive domain. Following this, scores on each of the individual measures that contribute to the domain scores are compared between treatment and no treatment groups. Finally, domain scores and the individual measures that contribute to the domain scores were analysed for ferritin improvers versus non-improvers, and haemoglobin improvers versus non-improvers. The results of the statistical analyses are presented in Table 6.2.
Table 6.1 Median (and interquartile range) for each cognitive domain score from a pilot double-blinded, placebo-controlled randomised control trial of the effects of iron supplementation (60 or 80mg iron or placebo for 16 weeks) on the cognitive function of iron-deficient (Ferritin <20ug/L, Haemoglobin ≥120g/L) and iron-sufficient (ferritin ≥20ug/L, haemoglobin ≥120g/L) women (18-35 years), at baseline and follow-up by group (60 or 80mg iron, iron-deficient placebo or iron-sufficient control).

<table>
<thead>
<tr>
<th>Cognitive domain</th>
<th>60mg Iron (n=7)</th>
<th>80mg Iron (n=6)</th>
<th>Placebo (n=6)</th>
<th>Control (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Follow-up</td>
<td>Baseline</td>
<td>Follow-up</td>
</tr>
<tr>
<td>Memory</td>
<td>6.00 (6.0-7.0)</td>
<td>6.50 (6.5-8.0)</td>
<td>4.25 (3.5-5.0)</td>
<td>4.25 (4.0-5.0)</td>
</tr>
<tr>
<td>Response Speed</td>
<td>4.50 (3.0-6.0)</td>
<td>6.50 (2.0-7.5)</td>
<td>6.00 (5.0-6.5)</td>
<td>4.00 (3.5-4.5)</td>
</tr>
<tr>
<td>Impulsivity</td>
<td>6.50 (5.0-7.5)</td>
<td>8.50 (4.0-9.0)</td>
<td>4.75 (4.0-8.0)</td>
<td>6.25 (4.5-9.0)</td>
</tr>
<tr>
<td>Attention</td>
<td>4.50 (2.5-6.5)</td>
<td>5.00 (3.0-7.0)</td>
<td>4.25 (1.0-8.0)</td>
<td>7.50 (3.5-9.5)</td>
</tr>
<tr>
<td>Information</td>
<td>6.00 (4.5-9.0)</td>
<td>8.00 (6.0-9.0)</td>
<td>4.50 (4.5-6.0)</td>
<td>6.50 (4.5-8.0)</td>
</tr>
<tr>
<td>Executive Function</td>
<td>8.00 (7.5-9.0)</td>
<td>7.50 (6.5-8.0)</td>
<td>4.50 (3.0-7.0)</td>
<td>7.00 (6.5-7.5)</td>
</tr>
<tr>
<td>Emotion Identification</td>
<td>6.50 (3.0-7.5)</td>
<td>4.00 (1.5-8.0)</td>
<td>6.00 (5.0-8.0)</td>
<td>5.75 (4.5-7.0)</td>
</tr>
</tbody>
</table>

Note: Scores are Sten scores (0-10), higher scores indicate better performance. 60mg and 80mg iron groups took ferrous sulfate. Control: iron-sufficient participants taking placebo. Placebo: iron-deficient participants taking placebo. Statistical analyses on differences in cognitive domain scores between baseline and follow-up for each group were not conducted due to small sample sizes.
6.5.3.3 Cognitive Change Scores

While statistically significant differences were not necessarily expected due to the small sample size, analysis of cognitive domain change scores (follow-up minus baseline) revealed that Impulsivity improved significantly more in the treatment (60mg iron and 80mg iron combined) than no treatment groups (placebo and controls combined) \((p=.047)\). There were no other statistically significant differences in cognitive domain change scores between treatment and no treatment groups at the \(p<.05\) level. At the \(p<.10\) level, Attention change scores were larger for treatment than no treatment groups \((p=.085)\).

Analysis of change scores for the individual measures that contribute to the domain scores found that the iron treatment groups had significantly higher recognition memory change scores than the placebo groups \((p=.029)\). There were no other statistically significant differences in cognitive change scores between the iron treatment and the no-treatment groups at the \(p<.05\) level. At the \(p<.1\) level, the iron treatment groups showed a greater improvement in reaction time on a sustained attention task (correctly pressing the same letter twice in a row) than the no-treatment groups \((p=.064)\). For the Go/No-go task, reduction in total errors \((p=.053)\) and omission errors \((p=.083)\) was also greater for treatment than no treatment groups. In contrast, the results of an emotion recognition task showed that the placebo groups had a greater change score for correctly identifying fear faces than the iron treatment groups \((p=.056)\), and a greater improvement in reaction time scores for that task than the iron treatment groups \((p=.050)\).
Table 6.2 Cognitive domain change scores for iron treatment and no treatment groups

<table>
<thead>
<tr>
<th>Cognitive change Sten score</th>
<th>Iron treatment groups (n=13) (Mean ±SD)</th>
<th>No treatment groups (n=12) (Mean ±SD)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Memory</td>
<td>0.67±0.78</td>
<td>0.08±1.76</td>
<td>0.210</td>
</tr>
<tr>
<td>Response speed</td>
<td>-0.27±2.21</td>
<td>-0.46±2.15</td>
<td>0.512</td>
</tr>
<tr>
<td>Impulsivity</td>
<td>0.62±1.83</td>
<td>-0.88±2.42</td>
<td>0.047*</td>
</tr>
<tr>
<td>Attention</td>
<td>1.31±2.80</td>
<td>-0.54±2.36</td>
<td>0.085</td>
</tr>
<tr>
<td>Information processing</td>
<td>1.00±1.86</td>
<td>-0.21±2.48</td>
<td>0.107</td>
</tr>
<tr>
<td>Executive function</td>
<td>0.62±2.48</td>
<td>0.58±1.43</td>
<td>0.805</td>
</tr>
<tr>
<td>Emotion identification</td>
<td>-0.50±2.16</td>
<td>0.96±1.67</td>
<td>0.105</td>
</tr>
</tbody>
</table>

Note: *Sig p<.05. Iron treatment groups=60mg and 80mg iron, no treatment groups= iron-deficient placebo and iron-sufficient controls.

6.5.3.4 Analysis of cognitive scores in ferritin improvers and non-improvers

Cognitive domain change scores for Impulsivity and Attention were significantly greater for ferritin improvers than non-improvers (p=.004, p=.026, for Impulsivity and Attention, respectively). Change scores for Emotion Identification were significantly smaller for ferritin improvers than non-improvers (p=.022) (Table 6.3).

Analysis of individual measures that contribute to the domain scores found that ferritin improvers had a greater improvement in recognition memory compared with non-improvers (p=.003). Change scores for a sustained attention task were also significantly greater for ferritin improvers than non-improvers (p=.048). For the Go/No-go task, ferritin improvers had a greater reduction in total errors (p=.005) and omission errors (p=.009) than non-improvers. Ferritin non-improvers had a greater improvement in reaction time to identify fear and sad faces than the ferritin improvers (p=.023, p=.023).

There were no other statistically significant differences in cognitive change scores between ferritin improvers and non-improvers at the p<.05 level. At the p<0.1 level, on the Go/No-go task, ferritin improvers had a greater improvement in reaction time variability (p=.081), and omission errors (p=.067) than non-improvers. Ferritin improvers also had greater improvement than non-improvers on digit span forwards (p=.069), and a greater reduction in total errors on a sustained attention task (p=.068).

Table 6.3 Cognitive domain change scores for ferritin improvers and non-improvers
### 6.5.3.5 Analysis of cognitive scores in haemoglobin improvers and non-improvers

Analysis of cognitive domain change scores showed no significant differences between haemoglobin improvers and haemoglobin non-improvers (Table 6.4).

Analyses of individual measures that contribute to the domain scores found that haemoglobin improvers had significantly greater improvement on digit span forwards ($p=0.034$) and greater improvement in accuracy on a switching of attention task ($p=0.022$) than non-improvers. There were no other statistically significant differences in cognitive change scores between haemoglobin improvers and non-improvers at the $p<0.05$ level. At the $p<0.1$ level, haemoglobin improvers had a greater reduction in errors on a verbal interference task, ($p=0.064$) compared with non-improvers. Haemoglobin improvers also had a larger reduction in reaction time to identify happy faces than non-improvers ($p=0.067$).

#### Table 6.4 Cognitive domain change scores for haemoglobin improvers and non-improvers

<table>
<thead>
<tr>
<th>Cognitive change Sten score</th>
<th>Haemoglobin improvers (n=10)</th>
<th>Haemoglobin non-improvers (n=15)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Memory</td>
<td>0.61±1.50</td>
<td>0.23±1.31</td>
<td>0.741</td>
</tr>
<tr>
<td>Response speed</td>
<td>0.30±1.75</td>
<td>-0.07±2.46</td>
<td>0.521</td>
</tr>
<tr>
<td>Impulsivity</td>
<td>0.05±2.23</td>
<td>-0.20±2.29</td>
<td>0.739</td>
</tr>
<tr>
<td>Attention</td>
<td>0.75±2.47</td>
<td>0.20±2.92</td>
<td>0.802</td>
</tr>
<tr>
<td>Information processing</td>
<td>1.35±2.56</td>
<td>-0.20±1.79</td>
<td>0.112</td>
</tr>
<tr>
<td>Executive function</td>
<td>0.65±2.12</td>
<td>0.57±1.99</td>
<td>0.538</td>
</tr>
<tr>
<td>Emotion identification</td>
<td>0.50±1.43</td>
<td>0.00±2.39</td>
<td>0.780</td>
</tr>
</tbody>
</table>

Note: Haemoglobin improvers are those whose haemoglobin increase by more than the known biological day-to-day variation of 4.4%, non-improvers either had no change or a decrease in haemoglobin.
6.5.4 Effect of guessing treatment allocation on cognitive change

Within the treatment group, there were no significant differences in cognitive change scores between participants who correctly guessed that they were taking iron and those who did not (Memory $p=0.130$, Response Speed $p=0.946$, Impulsivity $p=1.00$, Attention $p=0.462$, Information Processing $p=1.00$, Executive Function $p=0.382$, Emotion Identification $p=0.893$). Within the no-treatment group, participants who incorrectly guessed they were taking iron had a significantly lower Attention change score than those who correctly guessed they were taking placebo ($0.50 \pm 1.87$, $-2.0 \pm 2.34$, $p=0.026$). There were no other significant differences in cognitive change scores between participants who correctly guessed that they were taking placebo and those who did not (Memory $p=0.456$, Response Speed $p=0.286$, Impulsivity $p=0.683$, Information Processing $p=0.935$, Executive Function $p=0.459$, Emotion Identification $p=0.621$).

6.5.5 Required sample size for an adequately powered RCT

Power calculations were conducted to determine sample size for a future adequately powered RCT. Based on the difference in Memory scores between iron-deficient participants on iron treatment (60mg and 80mg) and those on placebo, 26 participants would be required in each iron treatment group for an adequately powered RCT. Based on scores in the Attention domain, a sample size of 84 iron deficient participants would be required in each treatment group for an adequately powered RCT.
6.6 Discussion

This is the first study to report on the use of the IntegNeuro battery of cognitive tests for assessing cognition in iron-deficient young women. IntegNeuro is a validated tool that is suitable for people aged 6-96 years (Clark, Paul et al. 2006). It is available internationally, with versions in English, Spanish, Dutch and Hebrew (Brain Resource Company 2011). The battery can be self-administered using a standard personal computer and touchscreen monitor. Scoring of test results is done centrally by the Brain Resource Company (San Francisco, CA) and standardised against the International Brain database which contains data from more than 20,000 people (Brain Resource Company 2011). IntegNeuro therefore offers the potential for researchers around the world to use a highly standardised and controlled method for cognitive research generally, but would allow some homogeneity in testing methods in the area of iron deficiency and cognition, which is currently lacking.

6.6.1 Suitability of IntegNeuro for iron deficiency research

The primary aim of this pilot study was to examine the suitability of the IntegNeuro battery of tests for assessing Cognitive Function in iron-deficient and iron-sufficient young women. The IntegNeuro was simple to administer and well accepted by the population of young women included in the study. Three participants reported that the response time of the touchscreen was slow, but no other problems were reported. The process of uploading data to the Brain Resource Company for scoring and subsequent return of standardised data occurred without incident. Logistically, the IntegNeuro was found suitable for use in research in iron-deficient and iron-sufficient young women.

The suitability of the IntegNeuro battery to provide useful cognitive data in iron-deficient young women and trials of its treatment are less clear from this small pilot study. There were no differences detected in cognition scores for any domains for iron-deficient versus iron-sufficient young women at baseline. However, there were some statistically significant differences in cognitive domain change scores for ferritin improvers (irrespective of treatment group) compared with non-improvers, including the Attention and Impulsivity domains.
6.6.2 Required sample size for an adequately powered RCT

The secondary aim of this pilot study was to determine an appropriate sample size for an adequately powered RCT. Previous research has shown a relationship between iron deficiency and performance on Memory and Attention tasks (Groner, Holtzman et al. 1986, Ballin, Berar et al. 1992, Patterson 1999, Murray-Kolb and Beard 2007), therefore these cognitive domains were used in the sample size calculations. The sample size used in this pilot study was insufficient to detect a statistically significant difference in cognitive function between groups at baseline for Memory and Attention and this should be considered when interpreting the results. More importantly, the results of the power analyses provided guidance regarding sample size for a future RCT.

6.6.3 Effect of iron deficiency on cognition at baseline and after iron treatment

This study found no statistically significant differences for any of the cognitive domains for iron-deficient versus iron-sufficient women at baseline. Participants in iron treatment groups had significantly higher change scores for the Impulsivity domain, and an individual task for Memory compared with the no-treatment groups.

While there has been limited research to determine the effect of iron deficiency on cognitive function in young women (Greig, Patterson et al. 2013), of the studies that do exist, there is a great deal of heterogeneity in cognitive testing methods and specific sample populations (pregnant, overweight, receiving medical treatment) making comparisons difficult (Tucker, Sandstead et al. 1984, World Health Organization 1992). A recently conducted systematic review (Greig, Patterson et al. 2013) reports on eight studies that included both iron-deficient and iron-sufficient participants at baseline (Groner, Holtzman et al. 1986, Ballin, Berar et al. 1992, Kretsch, Fong et al. 1998, Patterson 1999, Beard, Hendricks et al. 2005, Mansson, Johansson et al. 2005, Murray-Kolb and Beard 2007, Khedr, Hamed et al. 2008). Of these, four reported higher cognitive scores for iron-sufficient than iron-deficient participants at baseline, as well as improved scores after iron treatment (Ballin, Berar et al. 1992, Patterson 1999, Murray-Kolb and Beard 2007, Khedr, Hamed et al. 2008). Three studies reported no difference in cognition between iron-deficient participants compared with iron-sufficient controls at baseline.
These studies did show improvement in cognitive function in previously iron-deficient participants after iron treatment. One study showed no difference in levels of concentration between iron-deficient and iron-sufficient groups either at baseline or following iron treatment, which the authors attributed to small sample size (n = 375) (Mansson, Johansson et al. 2005).

Two of the studies included in the systematic review recruited only iron-deficient participants (Elwood and Hughes 1970, Bruner, Joffe et al. 1996). One of these studies reported an improvement in performance on cognitive tasks following iron treatment (Bruner, Joffe et al. 1996), and the other study showed no difference in cognitive function after iron treatment (Elwood and Hughes 1970). The latter study was limited by the use of haemoglobin as the only marker of iron status, with no real screening to determine cause of anaemia (Elwood and Hughes 1970). Differences in the effect of iron deficiency on cognitive function reported between studies may be due to the different cognitive tools used, as some used individual tests (such as digit span, digit symbol (Groner, Holtzman et al. 1986) and maze test (Elwood and Hughes 1970), sustained attention (Bruner, Joffe et al. 1996)) and others used composite test batteries (such as the Cambridge Neuropsychological Test Automated Battery (Blanton 2013), the Cognitive Abilities Test (Murray-Kolb and Beard 2007)). Variations in sample size between the studies do not appear to be a contributor to the differences in results reported. The sample size in studies were varied (e.g., n=152 (Murray-Kolb and Beard 2007), n=53 (Khedr, Hamed et al. 2008), n=222 (Ballin, Berar et al. 1992), n=76 (Patterson 1999), n=24 (Kretsch, Fong et al. 1998), n=95 (Beard, Hendricks et al. 2005), n=38 (Groner, Holtzman et al. 1986), n=375 , n=38 (Groner, Holtzman et al. 1986), n=375 (Mansson, Johansson et al. 2005)).

Blanton (2013) has very recently reported improvements in the individual tasks of planning speed and spatial working memory strategy in previously iron-deficient women (n=54) (Blanton 2013). Prior to this, Murray-Kolb and Beard (2007) performed a large (n=149) well controlled study that showed improvements in the cognitive domains of attention, memory and learning in previously iron-deficient and iron-deficient
anaemic young women after iron treatment (Murray-Kolb and Beard 2007). Both Blanton and Murray-Kolb and Beard’s studies differed from the previous studies in the way in which they were analysed. Rather than examining the data by treatment group, or ‘intention to treat’ they instead used analyses that compared those participants who had an improvement in iron status (ferritin and/or haemoglobin) with those who showed no improvement, irrespective of treatment. While this is not usual practice, it can be justified by the large variations in response to iron treatment for iron deficiency, which may be due to individual variations in iron losses and in gastrointestinal iron absorption.

In fact, our serum ferritin data were not consistent with what might be expected across 60mg, 80mg and placebo treatment groups (Leonard, Chalmers et al. 2014). For example, some improvements were seen in the placebo group (not associated with inflammation as measured by alpha-1-glycoprotein) and some decreases were seen in the treatment groups (Leonard, Chalmers et al. 2014). Therefore it was decided to analyse the data in a similar manner to Blanton and Murray-Kolb and Beard and this resulted in the detection of some significant changes in domain and individual cognitive scores for ferritin improvers and individual scores in haemoglobin improvers.

Most participants in the iron treatment group correctly guessed their treatment allocation (Leonard, Chalmers et al. 2014). However, there was no statistical difference in participants’ ability to guess their treatment allocation between the treatment and placebo groups. As reported above, participants who guessed that they were taking iron, when they were in fact taking placebo, actually performed worse on the Attention task than those who correctly guessed that they were taking placebo.

Limitations of the current study include that participants were primarily university educated, which is not necessarily representative of reproductive aged females in the community. Another limitation is that strict instructions regarding the time of the day to take capsules were not provided because it was felt that an additional degree of burden regarding time restrictions may have reduced compliance. Further, this study did not control for the dietary intake prior to or during testing, menstrual cycle, exercise habits, the use of stimulants, sleep patterns or stress prior to testing. The time of the day for cognitive or blood testing were also not controlled, to accommodate for the busy
schedules of the volunteers. Participants were asked to have their blood samples collected within the following 24-48 hours after cognition testing. However, due to logistical barriers associated with participants’ availability and the Hunter Area Pathology availability, the duration of time between cognitive testing and blood sample collection varied between participants. Regarding compliance, efforts were made to remind participants to take capsules, some chose not to have text message reminders. However strengths were that the double blinded intervention and the use of validated assessment of cognitive function adhere to the Consolidated Standards of Reporting Trials statement (Schulz, Altman et al. 2011).

6.7 Conclusions

IntegNeuro is an easy to administer tool for the assessment of cognition in young women. Some cognitive change scores were significantly higher for ferritin improvers (irrespective of treatment group) than non-improvers, and for women who had latent iron deficiency at baseline and were treated with iron supplements. Further research, using a larger sample of approximately 26-84 iron-deficient participants in each group, is required to determine the effectiveness of IntegNeuro in assessing the relationship between iron deficiency and cognitive function in this population.
Chapter 7.  Comparison of Two Doses of Elemental Iron in the Treatment of Latent Iron Deficiency: Efficacy, Side Effects and Blinding Capabilities

This paper was published in 2014.


The work presented in the manuscript was presented at completed in collaboration with the co-authors (Appendix 17).
7.1 Overview

There are currently no national recommendations on the most appropriate dose of elemental iron for the treatment of non-anaemic iron deficiency. In addition, common side-effects that are associated with iron therapy (including nausea, vomiting and blackening of stools) have been shown to be significantly more common in individuals taking higher doses of iron treatment. Using haematological data from the pilot RCT (presented in Chapters 5 & 6), the aim of this chapter was to determine a suitable dose of ferrous sulfate for the treatment of non-anaemic iron deficiency, at which participants could remain blinded. Paper two commences verbatim from Section 7.2. Methods for the collection of haematological data for the pilot RCT are presented in Section 7.2, baseline and follow-up results are presented in Section 7.3 and discussion is presented in Section 7.4.

7.2 Abstract

Adherence to iron supplementation can be compromised due to side effects, and these limit blinding in studies of iron deficiency. No studies have reported an efficacious iron dose that allows participants to remain blinded. This pilot study aimed to determine a ferrous sulfate dose that improves iron stores, while minimising side effects and enabling blinding. A double-blinded RCT was conducted in 32 women (18–35 years): 24 with latent iron deficiency (serum ferritin < 20 µg/L) and 8 iron sufficient controls. Participants with latent iron deficiency were randomised to 60 mg or 80 mg elemental iron or to placebo, for 16 weeks. The iron sufficient control group took placebo. Treatment groups (60 mg $n = 7$ and 80 mg $n = 6$) had significantly higher ferritin change scores than placebo groups (iron deficient $n = 5$ and iron sufficient $n = 6$), $F(1, 23) = 8.46, p \leq 0.01$. Of the 24 who completed the trial, 10 participants (77%) on iron reported side effects, compared with 5 (45%) on placebo, but there were no differences in side effects ($p = 0.29$), or compliance ($p = 0.60$) between iron groups. Nine (69%) participants on iron, and 11 (56%) on placebo correctly guessed their treatment allocation. Both iron doses were equally effective in normalising ferritin levels. Although reported side-effects were similar for both groups, a majority of participants correctly guessed their treatment group.
7.3 Introduction

Young women are at high risk of iron deficiency secondary to menstruation and childbirth (World Health Organization 2001). The nutritional disorder affects one in five young women in Australia (Fayet, Flood et al. 2010) and is associated with poorer general health and wellbeing and high levels of fatigue (Patterson, Brown et al. 2000, Patterson, Brown et al. 2001). It is imperative that iron deficiency is effectively managed to prevent progression to anaemia. Increased dietary iron intake, iron fortification and iron supplementation are used to improve iron status (Mora 2002). Clinical practice guidelines for the management of iron deficiency have been developed in the United States (Stoltzfus and Dreyfuss), the United Kingdom (Goddard, James et al. 2011) and in Australia (Therapeutic Guidelines Ltd 2006, Australian Medicines Handbook Pty Ltd 2010). These all recommend the use of dried ferrous sulfate which contains approximately 33% elemental iron. Clinical practice guidelines recommend a daily dose of 80–105 mg of elemental iron for treatment of iron deficiency anaemia in adults (National Prescribing Service 2010). A systematic review conducted in 2011 assessed the effects of intermittent oral iron supplementation on anaemia in menstruating women, compared with no intervention, a placebo or daily supplementation (Fernandez-Gaxiola and De-Regil 2011). This study found weekly supplementation with 60 to 120 mg elemental iron was effective in improving haematological markers (Fernandez-Gaxiola and De-Regil 2011). Treatment of latent iron deficiency and the impact of using lower dose iron treatment on iron status are not articulated within current literature and iron treatment guidelines.

Ideally, supplementation should achieve maximal absorption with minimal side effects (Hallberg, Ryttinge.L et al. 1966). Oral iron has been associated with gastrointestinal side effects such as nausea, constipation and darkening of stools which can decrease compliance (Australian Medicines Handbook Pty Ltd 2010, Zhu, Kaneshiro et al. 2010). Such side effects can compromise blinding within trials. Lower dose iron supplements have fewer side effects (Macdougall 1999, National Prescribing Service 2010) yet the effect of varying the dosage of iron on iron status (Makrides, Crowther et al. 2003, Rimon, Kagansky et al. 2005, Mozaffari-Khosravi, Noori-Shadkam et al. 2010) has rarely been
studied, with no studies conducted in non-pregnant young women. Whether lower doses are absorbed as efficiently as higher doses in non-pregnant young women remains unknown (Rockey 2006). Therefore, the current study aims to determine the efficacy of two different doses of iron supplementation in improving iron status whilst maintaining blinding to treatment groups.

7.4 Methods

Testing was conducted at the University of Newcastle, Callaghan Campus in NSW, Australia between April 2010 and April 2013. Women aged 18–35 years were recruited via flyers and promotion in lectures within the University. Recruitment also included flyers at the Technical Education (TAFE) College, accessing the volunteer register at Hunter Medical Research Institute and word-of-mouth. All interested individuals were screened for eligibility against inclusion criteria using an author designed questionnaire (refer to supplementary material). The inclusion criteria were: female, 18–35 years; BMI 18–30 kg/m²; English as primary language; not iron deficient within the last 12 months; not currently taking iron supplementation (those who had been on a standard multivitamin, containing minimal or no iron, were eligible to participate and asked to cease the supplement); no chronic medical condition; not taking medication that could potentially interfere with results; ability to provide blood samples for biomarkers of iron status; not having donated blood within the last three months and will not donate blood during the trial; not pregnant, or planning a pregnancy within the following 4 months; available to participate in intervention for 4 months. Those eligible were provided with an information statement and informed consent was obtained prior to the commencement of the study.

7.4.1 Participants

Thirty two women were included in the intervention. As shown in Figure 7.1, eight participants were included in the iron-sufficient control group and were provided placebo capsules, and 24 iron-deficient participants were randomised to either placebo, or treatment (60 mg or 80 mg iron).
7.4.2 Haematological testing

Serum ferritin, haemoglobin and soluble transferrin receptor (sTfR) were used as biomarkers of iron status, and alpha-1-glycoprotein (A1GP) was used as a marker of inflammation. A1GP is slower to rise, but remains at a high concentration longer than C-reactive protein (CRP), so is a better indicator of chronic sub-clinical infection than CRP, and may better reflect changes in the concentration of ferritin during infections (World Health Organization 2001). Blood tests were performed by Hunter Area Pathology Service, accredited by the National Association of Testing Authorities Australia, using standard techniques. The timing of the blood testing was not restricted in order to optimise recruitment and compliance (Leonard, Hutchesson et al. 2014). Results of the blood tests were sent directly to the research team at the University, and participants remained blinded to blood test results until the completion of the trial. Iron deficiency
was defined as having ferritin < 20 µg/L (Ahmed, Coyne et al. 2008) and all other markers within reference ranges (haemoglobin 115–165 g/L (Ahmed, Coyne et al. 2008), soluble transferrin receptor 0.9–2.30 mg/L (Suominen, Punnonen et al. 1999, Leonard, Patterson et al. 2013), A1GP 0.51–1.17 g/L (Larsson A 2008)). sTfR reflects the number of iron receptors expressed on cell membranes and is raised once tissue iron starts to become limited (Koulaouzidis, Said et al. 2009). It should theoretically represent a definitive marker of latent iron deficiency (Olivares, Walter et al. 2000). Participants with haemoglobin results below the reference range were excluded from the intervention and were immediately referred to their General Practitioner. At completion of the trial, all women were given copies of their blood test results for communication with their General Practitioner.

7.4.3 Pilot testing of supplementation

Group allocation and progression through the trial is summarised in Figure 7.1. Young women found to be iron deficient at baseline were randomly assigned to one of two different doses (60 mg or 80 mg) of ferrous sulfate or placebo for 16 weeks. Ferrous sulfate was used as it is the most common type of elemental iron used to treat iron deficiency; two different doses were used to determine the most effective dose in improving iron status with the fewest side-effects. The doses 60 mg and 80 mg have been associated with fewer side-effects than the doses recommended in the National Guidelines (Macdougall 1999, National Prescribing Service 2010). The duration of supplementation was chosen based upon the correction of iron deficiency anaemia taking between 2 and 4 months (National Prescribing Service 2010). The first eight iron-sufficient participants were invited into the intervention as a control group. A single blinding approach was used with the control group who were all provided with placebo capsules, which contained Lactose. Subsequent iron-sufficient participants exited the study following baseline testing. Participants were not informed of their treatment or iron status until trial completion. All participants were contacted on a four weekly basis to report any potential side-effects, using a specifically designed questionnaire. It was explained to participants that any remaining capsules would be counted following the intervention to increase compliance. In addition, participants were provided with a “Tips and
Reminders” sheet for taking capsules. This included the following information: Take one capsule per day; Leave your container of capsules next to your tooth brush; Keep one or two capsules in the small container provided and leave this in your handbag in case you forget to take it in the morning and then remember part way through the day; Use the calendar to cross off each day once you have taken your capsule. This will help you to keep a track of how regularly you are remembering to take them; Take the capsule two hours apart from any other regular medication (except the oral contraceptive pill which can be taken at the same time); Do not take two capsules on the same day to compensate for missing your capsule the previous day—Take only one capsule per day; Return any left-over capsules in your container when you return for your follow up testing; Please take note of your compliance with the treatment regimen and any side effects you may have experienced and report this to the research team when you are contacted by phone every four weeks. Immediately following the 16 week intervention participants were asked to guess which treatment protocol they thought they had been allocated to. Participants had repeat blood tests after 16 weeks.

7.4.4 Capsules and randomisation

Compounding chemists were contracted to provide the iron and placebo supplements and used Random Allocation Software to allocate treatments to participant Identification Numbers (Saghaei. M 2004). The active and placebo supplements were identical in appearance and were packaged in identical containers. The researchers and participants remained blinded to the treatment protocol and the randomisation code was held by a third party researcher only to be broken once the final results were collected. The study protocol was approved by the University of Newcastle Human Research Ethics Committee.

7.4.5 Statistical analysis

STATA-IC 11 statistical analysis software was used with an alpha level of 0.05 set for statistical significance. Kruskal-Wallis rank test was used to analyse the effect of group on iron markers at baseline and follow-up and the difference in ferritin change score between oral contraceptive pill users and non-users on iron treatment. One-way analysis
of variance (ANOVA) was used to examine the difference in iron marker change scores between treatment (60 mg iron and 80 mg iron) and no treatment groups (control and placebo). Fischer’s exact test was used to examine the frequency of reported side effects and the frequency of correct treatment guesses between treatment and no treatment groups.

7.5 Results

7.5.1 Participants

Twenty-four (75%) participants (mean age ± SD 25.6 ± 4.1 years) completed the intervention (60 mg iron n = 7, 80 mg iron n = 6, placebo n = 5, control n = 6). Reasons given for withdrawing from the study were unrelated illness (n = 3) or being too busy (n = 3). Two participants gave no reason. Participant demographics are shown in Table 7.1. Participants were primarily Australian, had a mean BMI of 21.2 kg/m² and 48% used an oral contraceptive pill (OCP).

<table>
<thead>
<tr>
<th>Table 7.1. Participant demographics (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
</tr>
<tr>
<td>Origin</td>
</tr>
<tr>
<td>Australia</td>
</tr>
<tr>
<td>Asia</td>
</tr>
<tr>
<td>Canada</td>
</tr>
<tr>
<td>United Kingdom</td>
</tr>
<tr>
<td>OCP use (total)</td>
</tr>
</tbody>
</table>

Note: BMI: Body mass index, Age and BMI data is provided as mean ± SD

7.5.2 Iron status

Ferritin, haemoglobin, and sTfR levels at baseline and follow-up, together with change scores for each group (60 mg iron, 80 mg iron, iron-deficient placebo, iron-sufficient control) are presented in Table 7.2. The A1GP was normal in all participants and was unchanged following the intervention.

<table>
<thead>
<tr>
<th>Table 7.2. Mean (±SEM) haematological markers of iron status at baseline, follow-up and change scores by treatment group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron marker</td>
</tr>
<tr>
<td>Ferritin (µg/L)</td>
</tr>
<tr>
<td>Baseline</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>Haemoglobin (g/L)</td>
</tr>
<tr>
<td>Baseline</td>
</tr>
<tr>
<td>Follow-up</td>
</tr>
<tr>
<td>Change</td>
</tr>
<tr>
<td>Change score</td>
</tr>
</tbody>
</table>
| Note: 60 mg and 80 mg iron: ferrous sulfate, sTfR-Index: soluble transferrin receptor-ferritin index. Normal ranges for haematological markers: ferritin > 20 µg/L; haemoglobin 115–165 g/L; soluble transferrin receptor 0.9–2.30 mg/L.

7.5.2.1 Baseline

Kruskal-Wallis analyses performed on iron status markers for the three iron deficient groups (60 mg, 80 mg and placebo) confirmed there were no significant between group differences in ferritin ($p = 0.38$), haemoglobin ($p = 0.34$) or sTfR-Index ($p = 0.82$) at baseline. As shown in Table 7.3, analyses comparing iron-sufficient (controls) and iron-deficient participants (60 mg, 80 mg and placebo groups combined) revealed that controls had significantly higher ferritin ($p < 0.01$) and lower sTfR-Index ($p < 0.01$) than the combined iron-deficient groups at baseline, but no difference in haemoglobin ($p = 0.30$).

Table 7.3. Comparison of haematological markers at baseline, follow-up and change scores

<table>
<thead>
<tr>
<th>Comparisons (p value)</th>
<th>Ferritin (µg/L)</th>
<th>Haemoglobin (g/L)</th>
<th>sTfR-Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls vs. Iron-deficient</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>&lt;0.01</td>
<td>0.30</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Follow-up</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo vs. Controls, 60 mg, 80 mg</td>
<td>&lt;0.01</td>
<td>1.0</td>
<td>0.11</td>
</tr>
<tr>
<td>Change score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron treatment vs. Placebo</td>
<td>&lt;0.01</td>
<td>0.45</td>
<td>0.07</td>
</tr>
</tbody>
</table>

7.5.2.2 Follow-Up

Analysis of iron status at follow-up revealed a significant difference between the placebo group vs. the 60 mg, 80 mg and iron-sufficient controls combined in ferritin ($p \leq 0.01$), but no difference in haemoglobin ($p = 1.0$) or sTfR-Index ($p = 0.11$) (as shown in Table 7.3). Post hoc analysis showed the placebo group had significantly lower ferritin at follow-up than the 60 mg iron group, 80 mg iron group and controls ($p = 0.02, p = 0.02, p = 0.04$). There was no significant difference in ferritin between controls and 60 mg iron.
(p = 0.57), controls and 80 mg iron (p = 0.87), or 60 mg and 80 mg groups (p = 0.89) at follow-up.

### 7.5.2.3 Change Scores

Change scores between baseline and follow-up for the iron treatment (60 mg and 80 mg combined) and placebo (iron-deficient placebo and iron-sufficient controls combined) groups were compared using one-way ANOVA. As shown in Table 7.3, the analyses revealed that the increase in ferritin levels was significantly greater following iron treatment compared with placebo, $F(1, 23) = 8.46, p \leq 0.01$. There were no differences in haemoglobin change, $F(1, 22) = 0.60, p = 0.45$, or sTfR-Index change, $F(1, 15) = 3.95, p = 0.07$, between iron treatment and placebo groups.

Based on our criteria for iron deficiency (ferritin < 20 µg/L, haemoglobin 115–165 g/L, soluble transferrin receptor 0.9–2.30 mg/L, A1GP 0.51–1.17 g/L), at follow-up six (75%) iron-deficient participants on 60 mg iron became iron sufficient and one remained iron deficient (13%). Four iron-deficient (57%) participants on 80 mg iron became iron sufficient and two remained iron deficient (28%). Two iron-deficient (25%) participants on placebo become iron sufficient and four (50%) remained iron deficient. All except one (who became iron deficient) of the iron-sufficient controls (80%) remained iron sufficient (Table 7.4). Of the participants on iron treatment, there was no difference in ferritin change score between oral contraceptive pill users and non-users ($p = 0.94$).

#### Table 7.4. Iron status outcome, compliance, side effects and treatment guesses by treatment group

<table>
<thead>
<tr>
<th>Participant group</th>
<th>IS</th>
<th>ID</th>
<th>DNF</th>
<th>Outcome</th>
<th>Compliance (%) *</th>
<th>Side effects</th>
<th>Treatment guess</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 mg iron (n = 7)</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>Nil</td>
<td>85.7 ± 17.7</td>
<td>5</td>
<td>1 1 0 0 6 1 0</td>
</tr>
<tr>
<td>80 mg iron (n = 6)</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>Nausea</td>
<td>93.3 ± 10.6</td>
<td>2</td>
<td>1 4 1 2 3 2 1</td>
</tr>
<tr>
<td>ID placebo (n = 5)</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>Dark stools</td>
<td>92.3 ± 5.3</td>
<td>4</td>
<td>1 0 0 2 2 1 2</td>
</tr>
<tr>
<td>IS controls (n = 6)</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>Constipation</td>
<td>88.7 ± 9.1</td>
<td>5</td>
<td>1 1 0 0 1 5 0</td>
</tr>
</tbody>
</table>

Note: 60 mg and 80 mg iron: ferrous sulfate; ID: iron-deficient; IS: iron-sufficient; DNF: Did not finish;
* Compliance is based on the percentage of capsules participants returned at the end of the 16 weeks trial period out of a maximum of 112
7.5.3 Side effects and compliance

Reported side effects included nausea, darkening of stools and constipation. While these were more commonly reported by participants in the 80 mg elemental iron group, particularly dark stools (Table 7.4), a Fischer’s exact test indicated there was no statistically significant differences in the frequency of reported side effects between the 60 mg and 80 mg groups ($p = 0.29$), the placebo and controls ($p = 0.55$), or between the treatment and placebo groups ($p = 0.42$). Kruskal-Wallis analyses performed on compliance scores (% of capsules taken) showed no statistically significant difference between the 60 mg and 80 mg groups ($p = 0.22$), between the placebo and controls ($p = 0.25$), or between the treatment and placebo groups ($p = 0.60$).

7.5.4 Participants’ treatment guesses

Of the 13 participants taking iron supplements who completed the trial, 9 (69%) correctly guessed they were taking iron supplements. A Fischer’s exact test showed no difference in the number of correct treatment guesses between the 60 mg and 80 mg groups ($p = 0.27$). Of the 11 participants taking placebo capsules who completed the trial, 6 participants (56%) correctly guessed that they were taking placebo capsules. There was also no significant difference in the number of correct treatment guesses between the placebo and control groups ($p = 0.08$).

7.6 Discussion

7.6.1 Change in iron status

Limited knowledge exists on the efficacy of different doses of iron supplementation on iron status in non-pregnant young women with latent iron deficiency. Our study aimed to determine a ferrous sulfate dose that improves iron stores in women with latent iron deficiency, while minimising side effects. At follow-up, iron-deficient participants who were randomised to ferrous sulfate (60 mg or 80 mg) had significant improvements in their ferritin from baseline levels. Following this improvement, there was no difference in ferritin when compared to controls at follow-up. Iron-deficient participants randomised to placebo had significantly lower ferritin than iron treatment groups and controls at follow-up. This shows that 16 weeks of
elemental iron is effective in normalising iron levels in most participants in this population of young women, and that without such iron treatment iron stores remain depleted. This pilot study had 68% power to detect a difference in ferritin change score between treatment or no treatment groups. The analysis showed a significantly higher ferritin change in treatment compared to no treatment groups. The power in this study will be used to inform the sample size in future studies as data on treatment of latent iron deficiency in non-pregnant women is limited.

Ferritin increased in iron-deficient participants on placebo to a much lesser degree than those on iron treatment. Altering dietary iron was not part of the intervention and participants were not given any advice about changing their diet in order to keep their current intakes stable during the trial. It is possible that participants altered their dietary iron intake, which may explain some of the change in iron status and is a limitation that must be acknowledged. However, participants remained blinded to their iron status until the completion of the trial, so it is just as likely that iron-sufficient controls changed their dietary iron intake as the iron-deficient participants. Results also demonstrated that a daily 60 mg dose was as effective as an 80 mg dose in treating latent iron deficiency. Seventy five per cent of participants on 60 mg iron dose became iron sufficient at the end of the trial as compared with 57% of participants in the 80 mg iron group. However, there was no significant difference in iron status at follow-up between the 60 mg vs. 80 mg iron groups.

A systematic review of the literature has actually shown that weekly dosing at 60–120 mg is adequate for treating iron deficiency in menstruating women (Fernandez-Gaxiola and De-Regil 2011), however national guidelines recommend 80 or 105 mg daily, which is also recommended by General Practitioners in Australia (National Prescribing Service 2010). We have shown that 60 mg daily is efficacious in young women with latent iron deficiency.

### 7.6.2 Compliance

The incidence of reported side effects was not statistically significantly different between placebo or treatment groups in this trial. The gastrointestinal effects of iron supplementation appear to be highly individual. Clear dose related side effects have
been reported in previous studies using low (15 mg) and high doses (222 mg) (Rimon, Kagansky et al. 2005, Australian Medicines Handbook Pty Ltd 2010), whereas others have found no difference in side effects between placebo and treatment groups, even when daily doses of 260 mg were used (Bruner, Joffe et al. 1996). In the current study, there was no statistically significant difference in the compliance between groups. Galloway et al. (1994) reviewed literature on participants’ compliance with iron supplement regimes in research studies and reported that compliance decreases when dose increases, however, as in the current study, Galloway found little evidence of side effects causing low compliance (Galloway and McGuire 1994).

7.6.3 Side effects and treatment guess

This study also aimed to examine the effect of potential side effects of the two different doses of iron supplementation on awareness of blinding to treatment groups. To assist with blinding, capsules were used in the study rather than tablets. This is due to ferrous sulfate being slightly green in colour and having a distinctly metallic taste. Therefore, to produce tablets for a blinded trial would involve finding inactive compounds to mimic or hide both the colour and taste of ferrous sulfate. Seventy seven per cent of participants in the treatment groups could guess that they were on iron, which is much higher than the 48% of 191 correctly guessing they were taking iron reported by Makrides et al. (Makrides, Crowther et al. 2003), though this study was in pregnant women who were obviously undergoing significant bodily changes making any additional effects of iron treatment difficult to identify. In the current study, the incidence of reported side effects was not different between treatment groups and placebo. This suggests that factors other than side effects play a role in the identification of their treatment, such as perhaps feeling more energetic. Although there was no formal assessment of fatigue and vitality in the current study, Patterson et al. (2001) showed improved vitality and decreased fatigue after treatment of iron deficiency in young women (Patterson, Brown et al. 2001).

7.6.4 Limitations

Several limitations of this study must be acknowledged. These include the small sample size, and low power, which are likely to have affected the reliability of results. Some participants may have self-selected for this study given that they thought they were iron
deficient, however, we made it clear that participants with iron deficiency within the 12 months prior to their enrolment in the study were not eligible. Also, physical activity and dietary intake were not assessed. These factors may have influenced individuals iron status at follow-up (Beard and Tobin 2000). Despite possible influence of day-to-day variation (Borel, Smith et al. 1991), menstruation (Kim, Yetley et al. 1993) and seasonal variation (Maes, Bosmans et al. 1997) on hematological results, the timing of the blood testing was not controlled to prevent unnecessary increased participant burden.

7.7 Conclusions

Results of this study revealed that a 60 mg iron dose can normalize iron status in non-pregnant young women with latent iron deficiency. No differences were found in the incidence of reported side effects or the level of compliance between treatment groups and placebo. Further double-blinded trials should examine the effectiveness of iron doses lower than 60 mg for improving iron status in young women, and to determine if awareness of treatment allocation is reduced.
Chapter 8. Is soluble transferrin receptor a useful marker in early stage iron deficiency?

This paper was published in 2013.


The work presented in the manuscript was completed in collaboration with the co-authors (Appendix 18).
8.1 Overview

Soluble transferrin receptor (sTfR) is one of the most recently developed markers used to assess iron status. sTfR does not increase during an acute phase response as ferritin does, therefore, it has been shown to enhance the diagnosis of iron deficiency anaemia. However, its usefulness in the diagnosis of early stage iron deficiency is not as clear. This chapter presents a review of the usefulness of sTfR in the assessment of non-anaemic iron deficiency. The review included a search of the use of sTfR in peer-reviewed literature and documentation of the different assays and reference ranges used to assess sTfR. This chapter also reports unexpected baseline sTfR results from the pilot RCT. Paper 3 commences verbatim from Section 8.2. Methods are presented in Section 8.4, results of the literature search and baseline haematological results from the pilot RCT (Section 8.5) and discussion (Section 8.6).

8.2 Abstract

Background and Aims: Soluble transferrin receptor (sTfR) is a recent test used to assess iron status and diagnose iron deficiency. Unlike Ferritin, it does not change during acute phase responses. The aim was to 1) review literature on sTfR in the assessment of early stage iron deficiency and 2) report baseline sTfR from a recent randomised controlled trial.

Methods: A search from earliest record to June 2013 located peer-reviewed studies using sTfR as a marker of early stage iron deficiency. Reference ranges and sTfR values were tabulated and compared with results from a current trial conducted at the University of Newcastle, Australia.

Results: Of eight studies on early stage iron deficiency (iron storage depletion) that measured sTfR, seven different assays were used. Baseline results from the current trial demonstrated a significant difference in mean sTfR level between iron deficient and iron replete participants, (0.99 * 0.20, 1.26 * 0.36, p<0.01). However no participants (n=119) had sTfR levels outside the reference range (0.9-2.3mg/L).

Conclusions: While sTfR levels were higher in early stage iron deficiency, defined by low Ferritin, the reference range was not useful in identifying early stage iron deficiency.
Multiple assays using varying reference ranges make between study comparisons difficult.

### 8.3 Introduction

Iron deficiency is the most common nutritional disorder worldwide (Suominen, Punnonen et al. 1998). It is imperative that specific and reliable biochemical markers of early stage iron deficiency are identified (Olivares, Walter et al. 2000). Currently, there are no universally accepted procedures for measuring iron status, with a variety of markers, assays and reference ranges used. Uncertainty about the most reliable assessment techniques could mean that in a clinical setting iron deficiency is under diagnosed (Olivares, Walter et al. 2000).

Serum ferritin and serum iron are the most commonly used markers of iron status (Thomas and Thomas 2002). Ferritin is routinely used as a measure of iron stores (Choi 2005). The level of ferritin that defines early stage iron deficiency has always been controversial. Haemoglobin is also usually included as it is a measure of functional iron and if low, indicates iron deficiency anaemia (Suominen, Punnonen et al. 1998). Other markers include mean corpuscular volume, transferrin saturation, and total iron binding capacity, but these are usually used in conjunction with haemoglobin and/or ferritin.

Soluble Transferrin Receptor (sTfR) reflects the number of iron receptors expressed on cell membranes and is raised once tissue iron starts to become limited (Koulaouzidis, Said et al. 2009). It should theoretically represent a definitive marker of iron deficiency (Olivares, Walter et al. 2000). However, the severity of iron deficiency may be important when considering its usefulness (Choi 2005). Assays and reference ranges used to assess sTfR are extremely variable, with no current internationally recognised reference range, meaning its usefulness as a diagnostic marker of early stage iron deficiency has been questioned (Choi 2005, Koulaouzidis, Said et al. 2009). Because ferritin reflects storage iron and sTfR reflects functional iron, the sTfR-Ft Index has been proposed as a measure that may increase the accuracy of iron deficiency diagnosis (Malope, MacPhail et al. 2001).
The current paper reviews the usefulness of sTfR in the assessment of early stage iron deficiency as reported in the peer-reviewed literature, documenting the various assays and reference ranges used. We also report baseline sTfR results from a double-blinded, placebo-controlled RCT on early stage iron deficiency and cognition in women.

8.4 Methods

8.4.1 Literature review

A title and abstract search of PRE-MEDLINE* and MEDLINE (Ovid) was conducted from earliest record to June 2013, using specific terms (early stage iron deficiency, soluble transferrin receptor, reference ranges, accuracy, utility) to locate studies using sTfR to assess iron status. The assays used for assessment and the respective diagnostic values were tabulated.

8.4.2 Haematological assessment for the iron and cognition study

The Iron and Cognition Study was conducted at the University of Newcastle, Australia and included 119 females aged 18-35 years. Iron-deficient participants were invited into the intervention, which is ongoing. The iron status measures and corresponding reference ranges for normal iron status were: Ferritin (20.0-150.0µg/L); Haemoglobin (<120g/L); sTfR (0.9-2.30mg/L, Beckman Coulter Assay); and Alpha-1-acid glycoprotein (0.51-1.17g/L). sTfR-Index was calculated as sTfR/LogFerritin. To examine the performance of the sTfR marker, we defined participants as iron deficient (Ferritin<20µg/L with normal Alpha-1-acid glycoprotein and normal Haemoglobin results) or iron replete (Ferritin≥20µg/L) and compared between group differences using one-way analysis of variance and Kruskal-Wallis in StataIC-11 (version 11, College Station, TX).

8.5 Results

8.5.1 Literature review

Eight studies that investigated the use of sTfR to define early stage iron deficiency were reviewed. Table 8.1 summarises these studies.
In seven of the eight studies, authors reported absolute sTfR results (Suominen, Punnonen et al. 1998, Olivares, Walter et al. 2000, Malope, MacPhail et al. 2001, Thomas and Thomas 2002, Lopez, Carracedo et al. 2006) or percentage of results outside the reference range (Punnonen, Irjala et al. 1998, Choi 2005). These studies all showed that sTfR did not increase above the reference range in most participants with early stage iron deficiency. Two of these studies reported raised sTfR in 10% (n=10) and 21% (n=224) of their participants with early stage iron deficiency (Punnonen, Irjala et al. 1998, Choi 2005). One study compared three different assays used to measure sTfR (Roche Diagnostics, Dade Behring, Nichols institute EIA) and found strong correlations (Dade Behring, Nichols: $r^2=0.851$, Roche, Dade Behring: $r^2=0.934$, Roche, Nichols: $r^2=0.876$) (Thomas and Thomas 2002). One study reported that sTfR remained within normal range for all participants with early stage iron deficiency (Skikne, Flowers et al. 1990).


### Table 8.1. Studies Examining Soluble Transferrin Receptor as a Marker of Early Stage Iron Deficiency

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Participants</th>
<th>Commercial assay used</th>
<th>Reference range reported (mg/L)</th>
<th>Mean (±sd) sTfR values reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lopez et al. (2006)</td>
<td>251 healthy children 1-10 years (Gender not specified)</td>
<td>Enzymeimmunoassay</td>
<td>&lt;2.5</td>
<td>1.93±0.41 2.28±0.5</td>
</tr>
<tr>
<td>(Lopez, Carracedo et al. 2006)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choi et al. (2005)</td>
<td>224 adolescents 17-19 years (Male/Female)</td>
<td>Monoclonal antibody sandwich immunoezymometric assay (IEMA)</td>
<td>&lt;3.24</td>
<td>1.17±1.06 21%&gt;3.24</td>
</tr>
<tr>
<td>(Choi 2005)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thomas et al. (2002)</td>
<td>227 Hospitalised adults varying conditions 49-79 years (Male) 356 32-76 years (Female)</td>
<td>Beckman Coulter immunoassay monoclonal sandwich assay</td>
<td>0.4-1.8</td>
<td>Not reported 1.3±0.8</td>
</tr>
<tr>
<td>(Thomas and Thomas 2002)</td>
<td></td>
<td></td>
<td>0.7-4.2</td>
<td>Not reported 3.4±0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.9-4.4</td>
<td>Not reported 3.4±2.1</td>
</tr>
</tbody>
</table>
### Baseline results for the iron and cognition study

Of the 119 participants at baseline, a complete set of iron status measures were available for 89. At baseline, 28 of 89 women had low ferritin and one was anaemic (haemoglobin <120g/L). No participants had a sTfR result outside of the normal range of 0.9-2.3mg/L using the Beckman Coulter methodology. Iron status results for the iron deficient and iron replete groups are presented in Table 8.2. Mean sTfR was significantly higher for the iron deficient than for the iron replete group (F(1,87) = 20.62, p < .001). Mean sTfR-Index was also significantly higher for the iron deficient than iron replete women (F(1,87) = 59.29, p < .001).

<table>
<thead>
<tr>
<th>Marker</th>
<th>Ft &lt;20µg/L (n=28)</th>
<th>Ft≥20µg/L (n=61)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (115-165µg/L)</td>
<td>129.5± 8.78</td>
<td>132.54± 7.59</td>
</tr>
<tr>
<td>Ft (20-150µg/L)</td>
<td>11.58± 4.93</td>
<td>44.82± 20.28*</td>
</tr>
<tr>
<td>sTfR (0.9-2.30mg/L)</td>
<td>1.26 ± 0.36</td>
<td>0.99 ± 0.20*</td>
</tr>
<tr>
<td>sTfR-ferritin Index</td>
<td>1.32 ± 0.68</td>
<td>0.61 ± 0.16*</td>
</tr>
<tr>
<td>AAG (0.51-1.17g/L)</td>
<td>0.67 ± 0.19</td>
<td>0.69 ± 0.19</td>
</tr>
</tbody>
</table>

Abbreviations: Hb: Haemoglobin, Ft: Ferritin, sTfR: Soluble transferrin receptor; AAG: Alpha-1-glycoprotein. * Indicates that the level is significantly different in participants with Ft levels ≥20µg/L than in those with Ft <20µg/L.
8.6 Discussion

sTfR is designed to definitively assess functional iron status, that is not affected by inflammation. It suffers from a lack of standardisation of the method and significant variation in the references ranges used. Ferritin measures iron stores and although an acute phase reactant, it has been used extensively, and has well-defined reference ranges, readily identifiable by both researchers and clinicians. If measured concomitantly with a marker of inflammation (e.g., C-reactive protein or AAG), low ferritin can reliably identify early stage iron deficiency, if haemoglobin remains normal. Whether the addition of sTfR to these assays adds to the identification of early stage iron deficiency has been questioned (Skikne, Flowers et al. 1990, Punnonen, Irlaja et al. 1998, Suominen, Punnonen et al. 1998, Olivares, Walter et al. 2000, Malope, MacPhail et al. 2001, Thomas and Thomas 2002, Choi 2005, Lopez, Carracedo et al. 2006), and our recent experience with baseline trial results would suggest that it doesn’t. (Olivares, Walter et al. 2000, Malope, MacPhail et al. 2001). However, its usefulness in the diagnosis of early stage iron deficiency has been questioned.

While the sTfR/ferritin index has been reported as more useful in identifying early stage iron deficiency (Punnonen, Irlaja et al. 1998, Olivares, Walter et al. 2000, Malope, MacPhail et al. 2001, Thomas and Thomas 2002, Lopez, Carracedo et al. 2006), it suffers from the same lack of standardisation of reference ranges as it relies on the sTfR assay. Thus the sTfR/Ferritin index is not easily compared across studies and researchers and clinicians are unsure what their results mean relative to others.

8.6.1 Usefulness of sTfR in iron deficiency

The utility of sTfR in the diagnosis of iron deficiency anaemia has been assessed in two systematic reviews (Koulaouzidis, Said et al. 2009, Infusino, Braga et al. 2012). Koulaouzidis et al. (2009) found the use of sTfR improves the clinical diagnosis of iron deficiency anaemia (Koulaouzidis, Said et al. 2009). Infusino et al. (2012) reported the probability of a patient with iron deficiency anaemia having a positive sTfR result was approximately 23 fold higher than for a patient with anaemia of chronic disease (Infusino, Braga et al. 2012). Infusino et al. state the lack of standardisation of the sTfR measurement is a limitation when comparing results and no definitive conclusion can
be made about the diagnostic power of this marker (Infusino, Braga et al. 2012). The usefulness of sTfR in the diagnosis of early stage iron deficiency was not a consideration of either of these reviews. sTfR has received much less attention in the diagnosis of iron deficiency anaemia. The current report reviewed eight studies that assessed the utility of sTfR in the diagnosis of early stage iron deficiency. All studies report that sTfR is not reliable in diagnosis of early stage iron deficiency and adds little value to serum ferritin results alone (Skikne, Flowers et al. 1990, Punnonen, Irla et al. 1998, Suominen, Punnonen et al. 1998, Olivares, Walter et al. 2000, Malope, MacPhail et al. 2001, Thomas and Thomas 2002, Choi 2005, Lopez, Carracedo et al. 2006).

Two studies found no significant difference in sTfR levels between normal iron status and those with early stage iron deficiency (Punnonen, Irlala et al. 1998, Choi 2005). Studies reporting significant differences found most participants remained within sTfR reference ranges (Skikne, Flowers et al. 1990, Olivares, Walter et al. 2000, Malope, MacPhail et al. 2001, Thomas and Thomas 2002, Lopez, Carracedo et al. 2006). The Iron and Cognition Study found iron-deficient participants had significantly higher sTfR and sTfR-ferritin index values than iron replete participants, yet none of the values were outside the normal reference ranges. Even the one iron-deficient anaemic participant who had to be excluded from the intervention had a normal sTfR reading.

Olivares et al. (2000), report the sensitivity of sTfR <13.5mg/L as 23.6% and sTfR:Ft, 68% (Olivares, Walter et al. 2000). Choi et al. (2005), used a cut off of <3.24mg/L which had a sensitivity of 21.9% and specificity of 26.7% in non-anaemic iron deficiency and much higher for iron-deficient anaemic participants (70.8% and 90.6% respectively). With a reduction in the upper reference range to 2.8mg/L, Choi et al. found it only increased the sensitivity of sTfR to 29.3% (Choi 2005). Thomas et al. (2002), reported a close correlation between three sTfR assays and found that in early stage iron-deficient participants, sTfR results were within the reference range for each assay (Thomas and Thomas 2002). The higher sensitivity of sTfR-Ferritin index supports findings from five studies which consider this index a useful marker of latent iron deficiency (Punnonen, Irlala et al. 1998, Olivares, Walter et al. 2000, Malope, MacPhail et al. 2001, Thomas and Thomas 2002, Lopez, Carracedo et al. 2006). Suominen et al. (1998), found iron deficiency without
anaemia in 40% of the 43 apparently healthy women in their study, highlighting the importance of early detection.

8.7 Conclusion

Despite being commonly referred to as an important marker in the diagnosis of iron deficiency, sTfR alone appears not to be a sensitive diagnostic marker for early stage iron deficiency. The sTfR-ferritin index appears to be a useful marker along with serum ferritin and haemoglobin to enable early detection. Appropriate indications for the use of sTfR should be agreed upon by international consensus and comparable sTfR assays and reference ranges recommended.
Chapter 9. Recruitment and retention of young women into nutrition research studies: practical considerations


The work presented in the manuscript was completed in collaboration with the co-authors (Appendix 19).
9.1 Overview

Difficulty in attracting young women to participate in the pilot RCT lead to a collaboration to examine recruitment and retention data from three nutrition studies being conducted at the University of Newcastle. This chapter presents recruitment methods from these the three studies and practical advice on recruitment and retention of young women into nutrition research. The studies included were the pilot RCT (presented in Chapters 5 & 6), a cross-over validation study and a cross-sectional study, both on weight loss in young women. Paper 4 commences verbatim from Section 9.2. Methods and study descriptions are presented in Section 9.4. Results, including a comparison of the effectiveness of recruitment strategies, reasons for ineligibility and retention strategy effectiveness are presented in Section 9.5 and recommendations for researchers within the discussion are in Section 9.6.

9.2 Abstract

Background

Successful recruitment and retention of participants into research studies is critical for optimising internal and external validity. Research into diet and lifestyle of young women is important due to the physiological transitions experienced at this life stage. This paper aims to evaluate data related to recruitment and retention across three research studies with young women, and present practical advice related to recruiting and retaining young women in order to optimise study quality within nutrition research.

Methods

Recruitment and retention strategies used in three nutrition studies that targeted young women (18 to 35 years) were critiqued. A randomised controlled trial (RCT), a crossover validation study and a cross-sectional survey were conducted at the University of Newcastle, Australia between 2010 and 2013. Successful recruitment was defined as maximum recruitment relative to time. Retention was assessed as maximum participants remaining enrolled at study completion.
Results

Recruitment approaches included notice boards, web and social network sites (Facebook and Twitter), with social media most successful in recruitment. The online survey had the highest recruitment in the shortest time-frame (751 participants in one month). Email, phone and text message were used in study one (RCT) and study two (crossover validation) and assisted in low attrition rates, with 93% and 75.7% completing the RCT and crossover validation study respectively. Of those who did not complete the RCT, reported reasons were: being too busy; and having an unrelated illness.

Conclusion

Recruiting young women into nutrition research is challenging. Use of social media enhances recruitment, while Email, phone and text message contact improves retention within interventions. Further research comparing strategies to optimise recruitment and retention in young women, including flexible testing times, reminders and incentives is warranted.

9.3 Introduction

Attracting young women to participate in nutrition, health and medical research is essential in developing translatable diet, health and lifestyle education programs relevant to their life stage (Stoy, Mastorianni et al. 1999). However, difficulties exist in recruiting young women and keeping them involved in research studies (Griffin, O’Connor et al. 2013). Very little research into factors affecting their recruitment and engagement in nutrition research has been conducted (Hure, Smith et al. 2008). From the limited research conducted to date, some common challenges have been highlighted, such as making initial contact, arranging mutually suitable times for data collection, maintaining contact for study duration and reducing attrition (Faden, Day et al. 2004, Griffin, O’Connor et al. 2013). These challenges appear to be less prevalent in the recruitment and retention of young men (Stoy, Mastorianni et al. 1999). Two common recruitment goals are to obtain a sample sufficient to represent the target population and to recruit adequate numbers, with power to test the primary hypothesis (Hulley, Cimmings et al. 2001). Difficulty recruiting participants can disrupt the study timeline
and consequently strain resources and ability of researchers to complete the study as planned within budget and time limits (Ashery and McAuliffe 1992). Strategies traditionally used to recruit young women have included random digit dialing, media and community advertising campaigns or university-based recruitment (Morton, Cahill et al. 2006). Challenges of making initial contact with young women and then maintaining it appear to be associated with age-normative transitional events (Faden, Day et al. 2004), including commencement of university studies, moving residence, commencing new employment, travelling overseas, and time constraints related to work, study, co-habitation, family and peers. Strategies commonly used to facilitate retention include the use of phone calls, sending out reminder text messages, Email reminders or mail (Faden, Day et al. 2004, Griffin, O'Connor et al. 2013). New methods to recruit young women are being explored and include the use of social media such as Facebook and Twitter (Eagan 2012). There is growing evidence on the use of social media for study recruitment (Lohse 2013). Many social interactions with young women occur via the Internet, therefore social networking sites are being utilised more frequently to recruit participants (Fenner, Garland et al. 2012).

The Australian Longitudinal Study of Women’s Health (ALSWH) is now recruiting a new young cohort of females aged 18 to 23 years. Recruitment for this new young cohort is being conducted using the ALSWH website, which includes a link to their Facebook page and Twitter account, and offers incentives to participants (Women’s Health Australia 2013). The use of social media in recruitment is somewhat limited in its ability to reach individuals who have little or no access to the Internet, however, having Internet access is very common. The Australian Communications and Media Authority reported that as of June 2013, 10.8 million Australians accessed the Internet once per day, a 7% increase from June 2008 (Australian Broadcasting Corporation and Media Authority 2013). Also in 2012, a US report confirmed that Internet usage was not restricted to higher socio-economic status with 75% of lower socio-economic individuals using the Internet and 66% of adult Internet users accessing social media sites (Lohse 2013). In this paper, we examine recruitment and retention across three recent studies in young women. Our aim is to address the practical considerations surrounding recruitment and retention at
this life stage and to make recommendations for optimising these aspects within future research.

9.4 Methods

Comparative analysis of the processes used to recruit and retain young women in three research studies conducted at The University of Newcastle, New South Wales, Australia was conducted. Methodological details for each study, participant characteristics and retention rate are outlined in Table 9.1. Effectiveness of recruitment and retention was compared based on the numbers of women who contacted researchers and expressed initial interest in participation; numbers screened for eligibility; numbers eligible/ineligible for study inclusion and reason for ineligibility; numbers commencing the study; numbers completing the study; and reasons for withdrawing.

9.4.1 Study descriptions

Study one was a double-blind, placebo-controlled, randomized trial. The aim was to examine the efficacy of iron supplementation in iron-deficient women and its effect on cognitive function. Pre and post assessments involved completing a 50 minute computer-based cognition test, and having a blood sample taken on campus. Iron-deficient participants and a proportion of iron-sufficient participants continued to the intervention phase where they were given placebo, 60 mg or 80 mg elemental iron for 16 weeks. The target sample was 120 women. Participants received personal feedback regarding their blood test results. Study two used a randomised crossover design to compare the accuracy and acceptability of a web-based food diary completed via computer, to a food diary accessed via a Smartphone, to a paper-based food diary. Young women (18 to 30 years of age) completed all three food diary modes, over three separate seven-day periods with the order of completion assigned randomly (days 2 to 8, days 16 to 22 and days 30 to 36). On day 1, participants’ resting energy expenditure was measured via indirect calorimetry, as well as their height and body composition. From day 2 to day 8, their physical activity levels were monitored via accelerometry. On days 1, 9, 15, 23, 29 and 37, their weight was measured, and on days 9, 23 and 37, they completed a survey regarding the acceptability of the food diary completed. Participants attended assessment sessions on six occasions over 37 days, during which measures
were taken for demographics, weight, height, and body composition. The length of sessions varied from ten minutes to one hour, depending on which assessments were being measured. The target sample was 40 women. Study three was a brief (approximately 15 minutes) online cross-sectional survey to identify expectations of an eHealth weight management intervention (for example, mode of delivery, content, features). The aims were also to identify barriers to weight loss overall, as well as specific barriers to participating and engaging in an eHealth weight loss intervention. The target sample was at least 100 women.
Table 9.1. Details of three studies that recruited young women, conducted at the University of Newcastle, Australia

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants and eligibility criteria</th>
<th>Recruitment</th>
<th>Retention strategies</th>
</tr>
</thead>
</table>
| Study one (Iron and Cognition RCT) | Women aged 18-35 years  
BMI 18.5 to 30kg/m²  
English as first language  
Not diagnosed with iron deficiency within the last 12 months  
Not currently taking iron supplementation  
No chronic medical condition  
Not taking medication that could potentially interfere with results  
Not donated blood within three months prior to screening  
Able to provide blood samples  
Not pregnant, or planning to become pregnant within the following four months  
Available for the following four months. | Recruitment location and time period:  
University of Newcastle, Australia from August 2010 to March 2013  
Recruitment methods:  
Flyers distributed across the campus (notice boards, cafeterias and outside lecture theatres).  
In lectures, using PowerPoint slide  
Word of mouth around campus  
Provision of course credit in two University courses (Psychology and Nursing)  
Hunter Medical Research Institute (HMRI) research volunteer register  
Community flyers (gyms, Technical College campuses and by word-of-mouth).  
Emails sent around University mailing lists for each faculty  
Advertisement on the University of Newcastle Facebook page. | Text message reminders for taking capsules  
Recommendation to leave container of capsules next to their toothbrush  
Recommendation to use the calendar provided to cross days off after the capsule was taken  
A small container was provided for handbags if remembered later in the day  
This information was included in a tips sheet for participants  
Refer to information sheet provided if common symptoms were present |
| Study two (Crossover Food diary Validation Study) | Women aged 18 to 30 years  
Healthy weight or overweight (BMI 21 to 30kg/m²)  
Access to a computer and a Smartphone with Internet access  
Self-reported moderate level of internet and Smartphone skills  
Weight stability over previous three months, and willingness to remain weight stable over the 37-day study period  
Not currently or planning to become pregnant or currently breastfeeding  
Not taking medications that affect weight or appetite  
No diagnosed metabolic disorders  
Non-smokers | Recruitment location and time period:  
University of Newcastle, Australia from January to May 2012.  
Recruitment methods:  
Advertisements posted on staff and student bulletin boards, University website and social networking sites (that is Facebook and Twitter). | Email and text message reminders about each data collection session  
Thirty dollar reimbursement gift voucher to cover travel and parking costs |
<table>
<thead>
<tr>
<th>Study</th>
<th>Participants and eligibility criteria</th>
<th>Recruitment</th>
<th>Retention strategies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study three (online weight management survey)</td>
<td>Australian women aged 18 to 30 years</td>
<td>Recruitment location and time period: University of Newcastle in August 2012 Recruitment methods: Advertisements on University website Social networking sites Staff and student email lists A link to the online survey was provided as part of the advertisement/email. Virtual snowballing was used to increase the size, and representative nature of the sample, whereby survey respondents were asked to pass on details of the study to others within the target group via email (that is email the survey link to their friends) and/or social networking (for example share the survey link with their Facebook friends) Prize draws were used to attract young women, including shopping centre, beauty therapy and cinema vouchers.</td>
<td>N/A</td>
</tr>
</tbody>
</table>
9.5 Results

9.5.1 Comparing the effectiveness of recruitment

In study one a total of 155 participants expressed interest in participating from June 2010 to March 2013. Of these, 128 participants met eligibility criteria. The recruitment methods used during the first 12 months were flyers on campus notice boards at the University of Newcastle and the Newcastle Technical and Further Education (TAFE) College, Emails to University staff, promotion in lectures and word-of-mouth. Seventy-three participants (57% of eligible participants) were recruited within the initial 12 months. From 2012 to 2013 recruitment was extended to the wider community and included flyers in gyms and day care centres, word-of-mouth, and Facebook messages. Advertising the study in the Research Awareness Exercise Program for two University courses (psychology and nursing) was also included. Participation in a research awareness program enabled students to receive course credit for their participation, with 15 participants (12% of total) recruited via this method. Another approach undertaken from 2012 to 2013 was to extend community-based recruitment and utilise the Hunter Medical Research Institute (HMRI) volunteer register. Questionnaires were sent to 250 females who had placed themselves on the register, resulting in recruitment of 10 participants (8% of total). Repeating community-based methods and refreshing University campus flyers attracted the final 30 participants from 2012 to March 2013. In study two, 91 individuals expressed interest, of whom 22 participated (24%). For this study, flyers were displayed on University campus noticeboards and Emailed to staff and students. The University’s media unit also advertised it on the University website news, with a link to the story placed on the University’s Facebook site, and details released via Twitter. In study three, the recruitment methods used generated the required study sample (> 100) one month, the shortest time frame of the three included studies. The study was advertised via the University’s media unit on the University website news, and promoted on the University’s social media sites (Facebook and Twitter). Invitations to participate were also distributed to staff and students across various University Email lists. Participants were encouraged to share the survey link at
the end of the study with their friends via social networks or Email. Of the 798 individuals who expressed interest, 751 (94%) were assessed as eligible, and 570 (71.4%) completed the full survey (Table 9.2).

Table 9.2. Length of recruitment and participant flow in each of the three included studies

<table>
<thead>
<tr>
<th>Contact and screening</th>
<th>Study one (RCT) n (%)</th>
<th>Study two (crossover validation) n (%)</th>
<th>Study three (cross-sectional online Survey) n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of recruitment (months)</td>
<td>36</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Expressed interest in participating (n)</td>
<td>155</td>
<td>91</td>
<td>798</td>
</tr>
<tr>
<td>Number completing eligibility screening</td>
<td>134 (86)</td>
<td>83 (91)</td>
<td>780 (97)</td>
</tr>
<tr>
<td>Number of screened participants who were eligible</td>
<td>128 (95)</td>
<td>22 (26)</td>
<td>751 (96)</td>
</tr>
<tr>
<td>Number of eligible participants who completed the study</td>
<td>95 (74)</td>
<td>22 (100)</td>
<td>570 (75)</td>
</tr>
</tbody>
</table>

9.5.2 Reasons for ineligibility

In study one, of the 134 participants who completed eligibility screening, six participants were not eligible. Of these, one was unable to attend the assessment session at the University, two were male and three had donated blood within three months prior to screening. Study two screened 83 women, of whom 61 were ineligible. Of these, 22 did not meet body mass index (BMI) criteria, 18 were unable to make the assessment sessions at the University, six did not meet the definition for weight stability in the previous three months, five did not have a Smartphone, five were unwilling to remain weight stable during the study period, two were smokers, two had chronic health issues and one had no access to the Internet. Study three screened 780 women, of these 29 were deemed ineligible. Four were male, 13 did not meet age criteria and 12 did not live in Australia. As shown in Table 9.2, 18 of the 798 did not complete the eligibility screening questions.

9.5.3 Retention strategy effectiveness

Retention strategies used by study one and study two are summarised in Table 9.1, and include text message and Email reminders. Of the 128 eligible for study one, 95 (74%) provided complete data sets at baseline testing, 32 were subsequently enrolled in the RCT of whom 26 (81%) completed the 16 week intervention and follow-up testing. Of
the six participants who commenced but did not complete the intervention, reasons
given for withdrawing included unrelated illness (n = 4) or being too busy (n = 2).
Methods used to assist with retention and adherence in study one included optional text
message reminders, a paper-based calendar, a small capsule container for carrying
supplements in a handbag, and four-weekly symptom check phone calls. These methods
are detailed in Table 9.1. Only 10/32 participants opted to have text message reminders
sent to them. All ten of these participants reported that they liked receiving the
reminders. All except one participant in study one remained contactable by phone or
Email for the entirety of the study. Of the 124 capsules provided to participants, the
average number of capsules remaining and returned to researchers was 12 ± 12 (9.6%).
Data on the number of capsules missed indicated that receiving a text message reminder
made no difference to the number of capsules missed. All 22 women in study two had
completed the study within a six-month recruitment period (January to June 2012) but
only 18 completed more than 85% of recording days for each diary and provided enough
data to be included in the study analysis.

9.6 Discussion

This paper examined data related to recruitment and retention of young women across
three different research study designs in order to address the practical issues related to
their participation and representation within research. The results showed that the use
of social media, text messages and face-to-face contact were beneficial in the recruitment
of young women into nutrition research studies. These results are similar to those of a
2009 Cochrane Review by Mapstone et al. (Mapstone, Elbourne et al. 2009) that identified
15 trials evaluating the effectiveness of strategies to improve recruitment in research.
They reported that trials using monetary incentives, and treatment information on the
consent form demonstrated benefit (Mapstone, Elbourne et al. 2009). Furimsky et al.
(2008) examined the challenges of recruiting and retaining youth with mental illness
within RCTs and found incentives and flexibility in scheduling useful (Furimsky,
Cheung et al. 2008). Neither of these studies examined the influence of gender on
recruitment. Adamson et al. (2007) scrutinized recruitment strategies used in the
Australian Longitudinal Study on Women’s Health (ALSWH). Authors reported on the importance of piloting recruitment strategies and keeping records of recruitment successful processes (Adamson, Young et al. 2007). They also recommended ensuring contact could be made easily by participants and in 1996 when this study was recruiting this was by providing a free call number to participants and obtaining as many inward phone lines as possible. At this time, when there was limited access to the Internet, phone contact was one of the easiest methods of accessing participants as it provided an instant response. Each of the three studies examined in this paper placed recruitment information on social networks on-line and found such strategies particularly useful when recruiting this demographic. A recent study by Fenner et al. also found modern information and communication technologies useful in assisting in engaging young women in health research (Fenner, Garland et al. 2012). Findings demonstrating the utility of recruiting young participants using social media sites, such as Facebook and Twitter are not surprising when you consider that three-quarters of adult Internet users under the age of 25 users have profiles on social media sites (Fenner, Garland et al. 2012). The large number of people connected to the Internet means a large potential pool of participants (Hoonakker and Carayon 2009). Study one had social media added to recruitment methods after an initial 12 months of slow recruitment. Study two used social media from the beginning of recruitment and recruited 22 participants within four months, which was slightly slower than study one, which recruited 31 participants within the same timeframe. Study three recruited 751 participants in one month. It is important to note that both study one and two (crossover validation) had reasonably stringent eligibility criteria and had higher levels of participant burden than study three, therefore making recruitment more difficult. Reasons for ineligibility were recorded for each study, which gave insight about the barriers to successful enrolment into a study, even after individuals have expressed interest in research studies. A barrier is not being able to attend the face-to-face assessment sessions. Study one also included a mail-out of study information to volunteers who had placed themselves on a search register, in its recruitment methods. Less than 10% of participants were recruited using this method. This recruitment rate was considerably lower than the 40% response rate reported by
Potential reasons for the low number of participants recruited via this method in study one may be the time burden associated with having to attend the University for blood and cognition tests. Traditional methods such as flyers, PowerPoint slides in lectures, staff and student Email lists were used by the studies. These methods were effective in recruiting the majority of the participants in study one, however, resulted in slow recruitment, and required regular refreshing of flyers on the noticeboards. Direct comparison of recruitment between the three studies included was not an aim due to differences in the degree of burden associated with participation, and in the difference influence of incentives across the studies. The blood test required for study one was the most significant participant burden of the three studies. Study one also included taking capsules every day for a 16 week period. Study two involved burden associated with completing lengthy food records and having six face-to-face assessments. High participant burden is more likely to be acceptable if the personal benefit of study results is also high. For example, the numbers of iron-deficient females volunteering for the RCT early on in recruitment was significantly greater than the previously reported prevalence of iron deficiency in this age group, suggesting that these participants potentially had personal health benefits as a factor motivating participation.

Previous research has shown that when recruiting for an RCT, the use of incentives such as feedback, course credit, money or ‘lottery’ is useful (Griffin, O’Connor et al. 2013). Various incentives were offered in all studies. Study one offered individual feedback on blood test results and study two offered feedback on participants’ diets. Study one was advertised to students in psychology and nursing courses that offer course credit for research participation. Both psychology and nursing are large courses at the University of Newcastle (950 and 1,500 students, respectively) with high percentages of females. The ‘value’ of the credit is limited to five (nursing) or ten (psychology) marks out of 100 and it seems this may not be adequate incentive for large numbers of students to participate. In addition, students may have other re-search studies competing for their interest. Studies two and three offered vouchers as incentives for participants. The chance to win ten shopping vouchers valued at 150 dollars is likely to have assisted in the recruitment of 400 participants (70%) within the first day of online recruitment into
study three. The addition of a monetary or voucher based incentive may have improved recruitment for study one. The lower burden of the cross-sectional design, not involving blood testing, capsules or extensive food diaries (Patel, Doku et al. 2003) and the short one off online survey with an incentive of prize draws is very likely to have made study three more appealing to participants. It is possible to recruit a lot of young women quite quickly if incentive prize draws are offered and combined with the convenience of online participation that is one-off. The National Statement on Ethical Conduct in Human Research in Australia approve the reimbursement of participants for costs associated with participation, however, state that it is unacceptable to provide payment to participants that is disproportionate to the time involved or encourages them to take risks (National Health and Medical Research Council, Australian Research Council et al. 2007). Our findings suggest that text reminders, phone calls and face-to-face contact may improve retention of young women in nutrition research, however further research is required to compare different incentives and contact methods to enable more definitive conclusions. The recruitment of young males into research may also benefit from using the strategies identified and further research in young males is required.

9.6.1 Recommendations for researchers

A number of key recommendations emerge from our examination of recruitment and retention methods across three studies:

1. Social networking sites should be utilised to distribute or advertise the study.
2. Consider participants’ motivation for participating, such as health benefits or incentives.
3. Use appropriate reimbursement or incentives such as monetary reward relative to the time demand involved, vouchers and course credit that are targeted to the population group.
4. Be flexible regarding testing days and times and provide individual feedback of results where appropriate.
5. Speak with participants in person or on phone calls as soon as possible to build researcher-participant rapport.

6. Use Email, phone calls, text messages and face-to-face contact as much as possible to maintain communication with participants.

9.7 Conclusion

This paper aimed to address the practical considerations surrounding recruitment and retention of young women in research studies. Recruiting young women for intervention trials is challenging. However, strategies such as using technologies (for example, social networking, Email, text messages) already used by young women can facilitate both recruitment and retention. The appropriate use of incentives and Email or phone reminders are important and should be planned from the start in order
Chapter 10. Final discussion and recommendations for research and practice
10.1 Overview

This chapter outlines the key findings of the research undertaken for this thesis and discusses findings in the context of existing literature. The strengths and limitations of the research are presented in Section 10.2, followed by recommendations for practice and future research (Section 10.3) and finally some concluding remarks (Section 10.4).

10.2 Introduction

This thesis aimed to contribute to the evidence base informing the effect of latent iron deficiency on cognitive function in young women. A pilot RCT was conducted to determine the suitability of the IntegNeuro cognitive testing battery for use in young iron-deficient women. The research also assessed differences in cognitive function using IntegNeuro in iron-deficient and iron-sufficient young women and improvement in cognition following an iron treatment intervention. A cross-sectional study was conducted to examine the relationship between nutrition knowledge of iron on dietary iron intake and iron status in young women. Data from the pilot RCT were used to address two additional aims. Firstly, to determine an efficacious dose of ferrous sulfate to treat latent iron deficiency whilst enabling participants to remain blinded to their treatment and secondly, to determine an appropriate sample size for an adequately powered RCT. Two methodological issues arose from the pilot RCT which are reported in published manuscripts. These included unexpected soluble transferrin receptor (sTfR) results and difficulty recruiting young women into the study. These issues are reported in Chapters 8 and 9 of the thesis.

10.2.1 The relationship between nutrition knowledge and dietary iron intake on iron status in young women

An aim of this thesis was to assess the level of nutrition knowledge of dietary iron in a sample of young women and its effect on dietary iron intake. Another aim was to assess the relationship between dietary iron intake on iron status in the sample of young women. These aims were addressed in the study presented in Chapter 4.

The study described in Chapter 4 assessed young women’s knowledge of dietary iron and its relationship with iron intake and iron status, this is a novel contribution to the evidence base in this area. The Nutrition Knowledge Questionnaire (NKQ) was
specifically designed for this study. Being newly developed, the psychometric properties of the questionnaire and its validity and reliability have not been determined at this time point. However, the questionnaire was piloted before being used in the present research and was suitable in the study sample.

The Cancer Council Dietary Questionnaire of Epidemiological Studies (DQES) FFQ was used to assess dietary iron intake. The FFQ had been previously validated in young women (Hodge, Patterson et al. 2000). A limiting factor associated with using this tool to assess dietary iron intake is that this questionnaire does not consider haem and non-haem iron separately, or include many iron enhancing or inhibiting foods.

Results from the two questionnaires showed that there was a positive relationship between total nutrition knowledge score, as measured by the NKQ, and dietary iron intake (mg/day). When the questions used to derive the total NKQ score were divided into two subscales, Food Knowledge and Iron Physiology, significant but weak positive relationships between each of the subscales and dietary iron intake were shown. Another finding of this study was that vegetarians knew more about good dietary sources of iron, and consumed more of these foods than non-vegetarians. However, the lower iron content of vegetable based non-haem iron containing foods, as well as the interaction between dietary iron enhancers and inhibitors, is likely to have contributed the iron status of the vegetarians being lower than the non-vegetarians. These results highlight the importance of vegetarians having a good level of knowledge not only about good vegetarian sources of iron, but also about having knowledge and understanding in relation to how dietary iron enhancing and inhibiting factors impact on iron status. These results also indicate that having more detailed questions on dietary iron enhancing and inhibiting foods in the NKQ would be beneficial for future use.

Overall, iron intake results supported the notion that most Australian women fail to consume foods as part of a dietary pattern that is in accordance with the Australian Guide to Healthy Eating (Blumfield, Hure et al. 2011). Iron status was affected by the type of dietary iron consumed rather than total iron intake. As demonstrated by intakes of haem iron containing foods being associated with higher ferritin and lower sTfR-ferritin index; and non-haem iron containing food intakes being related to lower ferritin
levels. These results tie in with vegetarian participants having lower iron status than non-vegetarians, and indicate that the type of iron (haem vs. non-haem), rather than the total amount of iron consumed is more informative when examining the relationship between iron intake and iron status. In addition, frequency of consumption of flesh foods were related to higher ferritin levels. We would expect that the relationship is not only the result of higher haem iron consumption, but also more frequent consumption of the undefined meat, fish and poultry factors found in flesh foods that significantly increases non-haem iron absorption from meals (Hunt 2003).

The timeliness of this study has been highlighted by the recent publication of a systematic review on the effects of nutrition knowledge on dietary intake identifying a need for well-designed studies in this area (Spronk, Kullen et al. 2014).

10.2.2 Systematic review on the effect of iron deficiency on cognition, mental health and fatigue in young women

A systematic review of the literature on the effect of iron deficiency on cognition, mental health and fatigue was presented in Chapter 3. The review only included studies that measured cognition and had a treatment intervention. The protocol for this review was peer-reviewed by the Joanna-Briggs Institute (JBI) and was registered in the JBI library of systematic review protocols. The review included a comprehensive literature search conducted across six databases. Critical appraisal of retrieved studies was conducted using the JBI critical appraisal tool (JBI-MAStARI).

Among the ten studies that were included in the review, many different assessment tools were used to measure cognition, mental health and fatigue, including tools that were not standardised or validated. Three of the studies reported poorer cognition and mental health, and increased fatigue in iron-deficient participants at baseline. Seven studies reported improvement in performance on cognitive tests following iron treatment. Differences in the cognitive tools used led to difficulty in the comparison of effects between studies. A small meta-analysis of combined effects, containing three of the ten studies included in the review was conducted. The three studies included used the same cognitive tests (Digit Symbol, Digit Span, Block Design and Arithmetic). The meta-
analysis revealed significant improvement in the Arithmetic domain after iron treatment, but no effect on Digit Symbol, Digit Span or Block Design domains.

The small numbers of studies informing the meta-analysis means its utility for definitively confirming or refuting the results is limited and results need to be interpreted with caution (Cochrane Statistical Methods Group 2008). Despite improvement in overall cognition for seven of the ten studies included in the review, the evidence base is limited by poor study quality and heterogeneity of testing methods. As a result, this study concluded that additional high quality studies using consistent testing methods are warranted.

10.2.3 Pilot randomised controlled trial

The pilot RCT was designed to address issues in research on the effects of iron deficiency on cognition that emerged from the systematic review of the literature (reported in Chapter 3). The systematic review found inconsistency in the measurement tools used to assess cognition. Therefore the suitability of a relatively new cognitive assessment battery, IntegNeuro, for assessing cognitive function in iron-deficient and iron-sufficient women was examined. IntegNeuro was chosen because it has been previously validated for use in young women. Results of the RCT revealed that the IntegNeuro battery of cognitive tests was simple to administer and there were no barriers to using this touchscreen test to assess cognition in young women.

Another aim of the pilot RCT was to determine whether any differences in cognitive function between iron-deficient and iron-sufficient young women could be detected using the using IntegNeuro test battery. Results from the baseline analysis of the RCT were presented in Chapter 5. There was no relationship been serum ferritin and individual cognitive results that contributed to the domain scores or the overall domain scores at baseline.

The paucity of significant results from this cross-sectional analysis is likely to be due to the small number of iron-deficient participants (N=24) in this pilot study. In addition, although cognitive testing conditions were controlled by using a private, sound-proof testing room, other factors that may impact cognition, such as dietary intake prior to or
during testing, menstrual cycle, exercise habits, the use of stimulants, sleep patterns or stress prior to testing were not controlled. Due to the need to accommodate for the busy schedules of the volunteers, the time of the day for cognitive or blood testing were also not controlled.

Contradictory findings have been reported in previously published research in this area. Some studies have shown no difference in cognitive function between iron-deficient and iron-sufficient participants at baseline (Beard, Hendricks et al. 2005, Mansson, Johansson et al. 2005) and others show differences in memory and attention (Kretsch, Fong et al. 1998, Murray-Kolb and Beard 2007). As reported in Chapter 2, comparisons between different studies are difficult due to the use of many different individual cognitive tests and test batteries. The reason for the differing results between studies on the effect of iron deficiency on cognitive function therefore remains unclear.

The pilot RCT also aimed to assess change in cognitive function (using the IntegNeuro battery) of iron-deficient participants after a 16 week iron treatment intervention. These results were presented in Chapter 6. There were no significant differences between the iron-deficient placebo group and the iron treatment groups (60mg elemental iron, 80mg elemental iron) and iron-sufficient controls taking placebo in performance on the cognitive tests at follow-up. Analysis of cognitive domain change scores showed that the Impulsivity change score was significantly better in iron treatment groups than placebo groups. When haematological results were analysed as ferritin and haemoglobin improvers vs. non-improvers, ferritin improvers had a greater improvement in recognition memory compared with non-improvers. In addition, change scores for a sustained attention task were also significantly greater for ferritin improvers than non-improvers. For the Go/No-go task, ferritin improvers had a greater reduction in total errors and omission errors than non-improvers. Haemoglobin improvers had significantly greater improvement on digit span forwards and greater improvement in accuracy on a switching of attention task than non-improvers.

Results from previous studies on the effect of iron deficiency on cognition have been mixed. Some show no differences in cognitive results between iron-deficient and iron-sufficient participants (Elwood and Hughes 1970, Bruner, Joffe et al. 1996, Mansson,

Significant findings were shown with regard to ferritin and haemoglobin improvers and cognition however, it was not expected that the current study would have the power to determine such differences due to it being a pilot study. Rather we hoped to examine the performance of the IntegNeuro among iron-deficient and iron-sufficient women in order to determine an appropriate sample size for an adequately powered RCT in the future. Previous research supports a relationship between iron deficiency and Memory and Attention, therefore these cognitive domains were used in the sample size calculation (Groner, Holtzman et al. 1986, Ballin, Berar et al. 1992, Patterson 1999, Murray-Kolb and Beard 2007). Required sample sizes for a future adequately powered RCT were produced from two power calculations. The calculations were based on the difference in Memory and Attention scores between iron-deficient participants on iron treatment (60mg and 80mg) and those on placebo. Twenty six participants would be required in each iron treatment group for an adequately powered RCT based on Memory scores. A sample of 84 iron-deficient participants would be required in each treatment group for an adequately powered RCT based on scores in the Attention domain.

10.2.4 The efficacy of elemental iron dosage in the treatment of iron deficiency in young women

The pilot RCT enabled the assessment of an efficacious dose of ferrous sulfate at which participants could feasibly remain blinded (the results for the efficacious dose of ferrous sulphate for the treatment of latent iron deficiency were presented in Chapter 7). While high dose iron supplementation (100mg/day) is recommended for the treatment of iron deficiency anaemia (National Prescribing Service 2010), there are currently no national recommendations on the most appropriate dose of elemental iron for the treatment of latent iron deficiency. In addition, common side-effects reported to be associated with iron therapy, including nausea, gastrointestinal disturbance and blackening of stools, have been shown to be significantly more common in individuals taking higher doses of
iron treatment (>80 mg/day) (Rimon, Kagansky et al. 2005). Therefore, while the ability to blind to treatment is essential for research purposes, it is also important to determine a suitable dose for the treatment of latent iron deficiency while limiting side effects.

Two doses of elemental iron (60mg and 80mg) in the form of ferrous sulfate were used in the 16 week intervention. Capsules were used in the study due to the likelihood that the colour and taste of iron tablets would be obvious to participants. Despite these efforts, most participants in the iron treatment group correctly guessed their treatment allocation. There was no difference in participants' ability to correctly guess their treatment allocation between iron treatment and placebo groups, so factors other than the physical characteristics of the capsules were at play.

There was no detectable difference in the reporting of side-effects or the rate of compliance between the 60mg and 80mg iron treatment groups, or between the two iron treatment groups and the placebo groups. Other studies have shown distinctly lower compliance rates with doses higher than 80mg (Rimon, Kagansky et al. 2005, Australian Medicines Handbook Pty Ltd 2010). Ferrous sulfate was effective in improving the ferritin levels of all participants who were iron-deficient at baseline. Following this improvement, there was no difference in ferritin between iron-deficient participants and iron-sufficient controls at follow-up. The lower dose treatment (60mg) was as effective in treating latent iron deficiency as the 80mg dose. This is a similar finding to that of other studies in other population groups, that have investigated the effect of level of ferrous sulfate dosage and shown that lower doses are as effective as higher doses (Makrides, Crowther et al. 2003, Rimon, Kagansky et al. 2005, Mozaffari-Khosravi, Noori-Shadkam et al. 2010).

### 10.2.5 The usefulness of soluble transferrin receptor in the diagnosis of latent iron deficiency

Soluble transferrin receptor (sTfR) is a recently developed marker used to assess iron status. Unlike Ferritin, STfR does not change during acute phase responses. It has been shown to enhance the diagnosis of iron deficiency anaemia (Lee, Oh et al. 2002). However, its usefulness in the diagnosis of early stage iron deficiency is not as clear. We had unexpected sTfR results at baseline in the RCT, whereby no participants had values
outside of the normal range. A review of the literature and an examination of the usefulness of sTfR in the assessment of latent iron deficiency was therefore performed and was presented in Chapter 8. The review included a search of the use of sTfR in peer-reviewed literature and documentation of the different assays and reference ranges used to assess sTfR. This chapter also reported the unexpected baseline sTfR results from the pilot RCT.

The literature search found eight studies that measured sTfR to diagnose latent iron deficiency. Among these studies, seven different assays were used. Multiple assays using varied reference ranges make between study comparisons difficult. Baseline results from the pilot RCT demonstrated a significant difference in mean sTfR level between iron-deficient and iron-sufficient participants. However, there were no participants with sTfR levels outside the reference range of the assay (0.9-2.3mg/L) used in this pilot RCT.

These results showed that although sTfR levels were higher in participants with latent iron deficiency than in iron-sufficient participants (as defined by low Ferritin), the reference range for sTfR was not useful in identifying the iron deficiency. Therefore, despite being commonly referred to as an important marker in the diagnosis of iron deficiency, sTfR alone appears not to be a sensitive diagnostic marker for early stage iron deficiency.

The sTfR-ferritin index appears to be a more useful marker in addition to ferritin and haemoglobin to enable early detection of iron deficiency. For iron researchers and health practitioners assessing iron status in young women, appropriate indications for the use of sTfR should be agreed upon by international consensus. Homogeneity on the assays used to assess sTfR and reference ranges recommended are essential for further assessment of its usefulness.

10.2.6 Recruitment and retention of young women in health research

Difficulty in attracting young women to participate in the pilot RCT implemented for this research led to collaboration to examine recruitment and retention data from three
nutrition studies being conducted at the University of Newcastle. These were analysed together and published to provide practical advice to nutrition researchers recruiting young women (the results of the research on recruitment and retention of young women in nutrition research were presented in Chapter 9). The studies included in this analysis were the pilot RCT reported in Chapters 5 and 6, a cross-over validation study and a cross-sectional study, both on weight loss in young women. No direct comparison between the studies was conducted, due to differences in the degree of burden associated with participation in the studies. Also there were different incentives offered to participants making it difficult to compare the studies directly.

Three quarters of adult internet users under the age of 25 have profiles on social media sites (Correa, Hinsley et al. 2010). Social networks online were used by all three studies to attract participants which was one of the most useful recruitment strategies used. In addition to the use of social media, text-messages and face-to-face contact were also beneficial in the recruitment of young women in to nutrition research. The use of modern information and technology was reported to be useful in engaging young women in health research (Fenner, Garland et al. 2012). The large number of adult internet users means there is a large pool of potential participants (Hoonakker and Carayon 2009).

Study one was the pilot RCT which had the longest recruitment period and only added social media as a recruitment method after 12 months of slow recruitment. Studies two and three used social media from the beginning of their recruitment process. Mail-out was also used by study one, however only 8% of participants were recruited using this method, substantially less than the 40% response rate from mail-out (Hoonakker and Carayon 2009). Study one required participants to attend cognition testing at the University, which may have accounted for the low response to mail-out recruitment. Study one also used more traditional methods such as flyers, power point slides in lectures and staff and student email lists. These methods were useful in recruiting the majority of the participants in study one, however over a lengthy period. Previous studies have shown that using incentives is useful in recruiting for RCTs (Griffin, O'Connor et al. 2013). To optimise the retention of young women in research, text
reminders, phone calls and face-to-face contact are useful. These methods were useful in enabling high retention rates in study one.

This research aimed to address the practical considerations surrounding recruitment and retention of young women in research studies. A number of key recommendations emerged from this study that may optimize retention and study quality, statistical power and research outcomes: social networking sites should be utilised to distribute or advertise the study; consider participants’ motivation for participating, such as health benefits or incentives; use appropriate reimbursement or incentives such as monetary reward relative to the time demand involved, vouchers and course credit that are targeted to the population group; be flexible regarding testing days and times and provide individual feedback of results where appropriate; speak with participants in person or on the phone as soon as possible to build researcher-participant rapport; use email, phone calls, text messages and face-to-face contact as much as possible to maintain communication with participants.

10.3 Implications of the body of research

10.3.1 For practice

The implications of this body of research for practice are addressed to health practitioners who assess and prescribe treatment for iron deficiency, health educators and for iron-deficient young women.

For health practitioners, this body of research suggests that sTfR does not enhance the diagnosis of latent iron deficiency. Using Ferritin and Haemoglobin as markers in the assessment of iron deficiency are essential, but due to the vulnerability of Ferritin to increase in the presence of inflammation, it is necessary to consider the addition of an inflammatory marker, such as AAG or CRP.

For health practitioners prescribing treatment for latent iron deficiency, a lower dose of iron supplementation is likely to be just as effective as a higher dose and should be considered when side-effects are a concern or problem for patients. Research needs to be conducted in women who experience side-effects from iron supplements to determine whether lower doses reduce side-effects while still being efficacious in terms of iron
status. Reducing the side-effects commonly associated with iron treatment is likely to enhance compliance with taking capsules, and in turn, may lead to better iron status outcomes long term.

Results from the study on nutrition knowledge and dietary iron intake showed that higher nutrition knowledge of dietary of iron translated in to higher dietary iron intake, however this did not translate into better iron status. This is likely to be a result of vegetarians having superior knowledge about dietary iron, with no difference found in total iron intakes based on consumption of adequate amounts of vegetarian food sources of iron, despite the lower iron content, and lower iron status. The iron status of vegetarians is largely affected by dietary iron inhibitors, therefore, vegetarian diets should be consumed with dietary iron enhancing foods such as those high in vitamin C. For iron-deficient non-vegetarians, increasing flesh foods such as beef, lamb, chicken and pork appears beneficial in increasing Ferritin.

10.3.2 Future research

Further research is required in the area of nutrition knowledge and its effect on dietary iron intake. Additional questions, which assess knowledge of dietary iron enhancers and inhibitors in more detail, should be added to the nutrition knowledge questionnaire developed for this thesis. The questionnaire should be validated for use in a broad sample of young women so that it can then be used as a tool to assess the viability of a nutrition education intervention for the improvement of iron status.

To strengthen the evidence in the area of latent iron deficiency and its effects on cognition, more methodologically consistent research is required. This should include a well-designed, blinded, randomised controlled trial using the IntegNeuro battery. IntegNeuro should be used in an adequately powered trial as it is simple to use, validated in young women and provides good normative data.

Further recommendations for such a trial that have arisen from this thesis include:

1. Use a multi-centred approach to recruitment
2. Use social media to enhance recruitment in young women and provide adequate incentives
3. Do not include sTfR as a marker of latent iron deficiency, as it is expensive, difficult to access and is not useful for identifying latent iron-deficient individuals
4. Use a 60mg dose of elemental iron which is efficacious, as it was found sufficient to treat latent iron deficiency
5. Trial a 40mg dose to determine its suitability for enabling participants to remain blinded to treatment

In addition, limitations of the pilot RCT that should be considered in more detail for a larger trial are: assessment of dietary intake prior to cognition testing; the limiting of stimulants; measurement of physical activity; assessment of sleep patterns; oral contraceptive use and stage of menstrual cycle prior to testing. Also, more stringent control of the timing of cognitive and blood testing is recommended.

10.4 Concluding remarks

The research reported in this thesis contributes to a number of areas of iron deficiency research in young women. Nutrition knowledge regarding iron in young women is a novel area of research that led to some positive associations between knowledge and intake, and overall revealed that a validated questionnaire with more focus on dietary enhancers and inhibitors of iron is needed. Higher frequency of intake of flesh foods was related to better iron status despite no detectable relationship with total iron intake. There is a need to establish strategies for increasing iron intake and absorption in young women. Such strategies may include focusing on a whole dietary approach to consuming the most bioavailable food sources of iron and decreasing inhibitors of iron absorption, while incorporating enhancers appropriately.

We found few significant differences in cognitive function between iron-deficient and iron-sufficient young women at baseline, and after the 16 week intervention for the RCT, but this was expected in an under-powered pilot study. This study aimed to assess the viability of IntegNeuro as a tool for the assessment of cognition in iron-deficient young women and to determine an adequate sample size for a future trial. With this information, and the knowledge that a 60mg elemental dose of iron is efficacious in the treatment of latent iron deficiency, planning can commence for a multi-centred, double-
blinded, randomised-controlled trial to assess the effects of iron treatment on the cognitive, general health and well-being, mental health and fatigue of iron-deficient young women.
References


National Health and Medical Research Council, Australian Research Council and Australian Vice-Chancellors' Committee (2007). National Statement on Ethical Conduct in Human Research (Updated May 2013). Canberra, Australia, Commonwealth of Australia


StataCorp (2013). Stata Statistical Software. College Station, TX, StataCorp LP. Release 13.


Appendices

Appendix 1: Ethics approval
HUMAN RESEARCH ETHICS COMMITTEE

Notification of Expedited Approval

To Chief Investigator or Project Supervisor:          Doctor Kerry Chalmers
CC Co-investigators / Research Students:          Doctor Amanda Patterson
                                                  Professor Clare Collins
                                                  Ms Alecia Greig

Re Protocol:                                      The effects of non-anaemic iron deficiency on cognitive
                                                  functioning: a blinded randomised controlled trial of iron
                                                  supplementation in women of childbearing age.

Date:                                            17-Jun-2010
Reference No:                                    H-2010-1079
Date of Initial Approval:                        17-Jun-2010

Thank you for your Response to Conditional Approval submission to the Human Research Ethics Committee (HREC) seeking approval in relation to the above protocol.

Your submission was considered under Expedited review by the Chair/Deputy Chair.

I am pleased to advise that the decision on your submission is Approved effective 17-Jun-2010.

In approving this protocol, the Human Research Ethics Committee (HREC) is of the opinion that the project complies with the provisions contained in the National Statement on Ethical Conduct in Human Research, 2007, and the requirements within this University relating to human research.

Approval will remain valid subject to the submission, and satisfactory assessment, of annual progress reports. If the approval of an External HREC has been "noted" the approval period is as determined by that HREC.

The full Committee will be asked to ratify this decision at its next scheduled meeting. A formal Certificate of Approval will be available upon request. Your approval number is H-2010-1079.

If the research requires the use of an Information Statement, ensure this number is inserted at the relevant point in the Complaints paragraph prior to distribution to potential participants You may then proceed with the research.
Conditions of Approval

This approval has been granted subject to you complying with the requirements for Monitoring of Progress, Reporting of Adverse Events, and Variations to the Approved Protocol as detailed below

PLEASE NOTE:

In the case where the HREC has "noted" the approval of an External HREC, progress reports and reports of adverse events are to be submitted to the External HREC only. In the case of Variations to the approved protocol, or a Renewal of approval, you will apply to the External HREC for approval in the first instance and then Register that approval with the University’s HREC.

Monitoring of Progress

Other than above, the University is obliged to monitor the progress of research projects involving human participants to ensure that they are conducted according to the protocol as approved by the HREC. A progress report is required on an annual basis. Continuation of your HREC approval for this project is conditional upon receipt, and satisfactory assessment, of annual progress reports. You will be advised when a report is due.

Reporting of Adverse Events

It is the responsibility of the person first named on this Approval Advice to report adverse events.

Adverse events, however minor, must be recorded by the investigator as observed by the investigator or as volunteered by a participant in the research. Full details are to be documented, whether or not the investigator, or his/her deputies, consider the event to be related to the research substance or procedure.
Serious or unforeseen adverse events that occur during the research or within six (6) months of completion of the research, must be reported by the person first named on the Approval Advice to the (HREC) by way of the Adverse Event Report form within 72 hours of the occurrence of the event or the investigator receiving advice of the event.

Serious adverse events are defined as: Causing death, life threatening or serious disability. Causing or prolonging hospitalisation. Overdoses, cancers, congenital abnormalities, tissue damage, whether or not they are judged to be caused by the investigational agent or procedure. Causing psycho-social and/or financial harm. This covers everything from perceived invasion of privacy, breach of confidentiality, or the diminution of social reputation, to the creation of psychological fears and trauma. Any other event which might affect the continued ethical acceptability of the project.

Reports of adverse events must include: Participant's study identification number; date of birth; date of entry into the study; treatment arm (if applicable); date of event; details of event; the investigator's opinion as to whether the event is related to the research procedures; and action taken in response to the event.

Adverse events which do not fall within the definition of serious or unexpected, including those reported from other sites involved in the research, are to be reported in detail at the time of the annual progress report to the HREC.

Variations to approved protocol

If you wish to change, or deviate from, the approved protocol, you will need to submit an Application for Variation to Approved Human Research. Variations may include, but are not limited to, changes or additions to investigators, study design, study population, number of participants, methods of recruitment, or participant information/consent documentation. Variations must be approved by the (HREC) before they are implemented except when Registering an approval of a variation from an external
HREC which has been designated the lead HREC, in which case you may proceed as soon as you receive an acknowledgement of your Registration.

Linkage of ethics approval to a new Grant

HREC approvals cannot be assigned to a new grant or award (ie those that were not identified on the application for ethics approval) without confirmation of the approval from the Human Research Ethics Officer on behalf of the HREC.

Best wishes for a successful project.

Associate Professor Alison Ferguson

Chair, Human Research Ethics Committee

For communications and enquiries:

Human Research Ethics Administration

Research Services Research Office The University of Newcastle Callaghan NSW 2308

T +61 2 492 18999  F +61 2 492 17164  Human-Ethics@newcastle.edu.au

Linked University of Newcastle administered funding:

<table>
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<tr>
<th>Funding body</th>
<th>Funding project title</th>
<th>First investigator</th>
<th>Grant Ref</th>
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<tr>
<td>Human Nutrition Research Program</td>
<td>The effects of non-anaemic iron deficiency on cognition, fatigue and mental health: a blinded randomised controlled trial of iron supplementation in women of childbearing age</td>
<td>Patterson Amanda, Jane</td>
<td>G1000419</td>
</tr>
</tbody>
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Appendix 2: Study flyer
Find out your knowledge of dietary iron, and if iron deficiency is affecting your concentration or memory

Are you interested in participating in a research study which involves questionnaires, and having your iron status and cognition tested?

Details
We are conducting a research study to investigate:
- The effects of iron deficiency and change in iron status on measures of cognitive functioning, including memory and concentration
- Nutrition knowledge surrounding dietary iron and dietary iron intake (questionnaire phase)

Who can volunteer?
For the cognition testing phase we need females aged 18 to 35 years who are:
- A healthy body weight
- Not currently taking iron supplements
- Able to provide at least one blood sample
- Willing to complete a computerised test of cognitive functioning, that takes about 60 minutes
- Available to be involved in the study over a four month period

For the questionnaire phase of the study, all females aged 18-35 are eligible

How do I find out more information?
If you are interested in participating in the study, please contact Ms Alecia Greig on 49215690 or iron.cognition@newcastle.edu.au for further information

This project has been approved by The University of Newcastle Human Research Ethics Committee, Approval Number H-2010-1079. The research team: Dr Kerry Chalmers, Prof. Clare Collins, Dr Amanda Patterson, Ms Alecia Greig, Hannah Lucas.
Appendix 3: Screening form
Iron and cognition project
Eligibility Screen
Please complete for all potential participants to assess eligibility for entry into the study.

Name (surname) _______________ (given) __________________
Address ________________________________________________
Suburb _______________ Postcode _______________________
Phone Contact Day __________________ Evening _____________
Mobile __________________ Preferred contact number ________ Day / Evening / Mobile
E-mail _____________________________
If the subject asks why this is important, their staff/student status provides demographic data on the study sample.

1. Age _____ Is their age within the required range (18-35 years)?
   Yes ☐ No ☐
   * If they are under 18 or over 35, advise they are ineligible to participate in the study.

2. Anthropometrics
   Height (cm) ☐☐☐☐
   Weight (kg) ☐☐☐☐
   Body Mass Index (kg/m²) ☐☐☐☐
   Is their BMI within range (18-30 kg/m²)?
   Yes ☐ No ☐
   * If they have a BMI outside of the required range, advise that they are ineligible to participate in the study.

If the participant is unsure of their anthropometric measures, provide the option to organise a brief assessment time to enable accurate calculation of BMI.

3. Have you been diagnosed with iron deficiency within the last 12 months?
   Yes ☐ No ☐
   *If yes, excluded from study

4a. Are you currently on any form of iron supplementation (or within the last 3 months)?
   Yes ☐ No ☐
   * If they answer yes to this question, advise that they are ineligible to participate in the study as this will interfere with baseline iron studies.

4b. Are you taking a multivitamin (MV)?
   Brand of MV __________________________
   Yes ☐ No ☐
   *Those on standard MV’s are eligible to participate.

4c. Are you willing to take iron supplements for a 4 month intervention period if you are found to have iron deficiency without having anaemia?
   Yes ☐ No ☐
   Reason, if no ________________________________
5. Do you have a chronic medical condition or allergies?  

Yes ☐  No ☐  

*Refer to list of conditions excluded from study

6. Are you taking any regular medication?  

Yes ☐  No ☐  

*Refer to list of medications excluded from study

7. Do you have amenorrhea for any reason other than pharmaceutical contraception?  

Yes ☐  No ☐  

* If yes, ineligible to participate in this study

8. Are you able to provide blood samples for the iron and inflammation studies?  

Yes ☐  No ☐  

Reason, if no__________________________________________________________________________  

*If they answer no, ineligible to participate in the study

9. Are you pregnant, or planning to become pregnant within the following 4 months?  

Yes ☐  No ☐  

* If yes, ineligible to participate in this study

10. Have you been pregnant within the last 12 months and/or are you breastfeeding?  

Yes ☐  No ☐  

* If yes, ineligible to participate in this study

11. Have you made a recent blood donation (within the last 3 months?)  

Yes ☐  No ☐  

* If yes, ineligible to participate in this study

12. Will you be available to participate in this study for the following 4 months?  

Yes ☐  No ☐  

* If no, ineligible to participate in this study
If the participant is eligible for inclusion, say that we will be providing them an information pack about the study which includes an information sheet and consent form. Advise them of the times available to have blood testing at HAPS and the dates and times available to complete the IntegNeuro tests.

If they would like to be involved in the study after reading the information they will need to fill out and sign the consent form, and then return the form to Alecia Greig in HE25 Hunter Building, University of Newcastle

Or send it via internal mail or via the post in the reply paid envelope provided to:

Ms Alecia Greig
c/o School of Health Sciences
HE25 Hunter Building, University of Newcastle
University Drive
Callaghan NSW 2308

Refer participant to the study’s chief investigator
Dr Kerry Chalmers (49215757) if any further information is required.

Chronic medical conditions excluded from study: Inflammatory conditions, Arteriosclerosis, Psoriasis, Inflammatory bowel disease, Rheumatoid arthritis, Chronic Obstructive Pulmonary Disease, Osteoarthritis, Uncontrolled asthma, uncontrolled diabetes (hyper ad hypo glycaemia can affect cognition).

Medications excluded from the study: Anti-inflammatory medications, antacids, histamine receptor antagonists (e.g., cimetidine, ranitidine), proton pump inhibitor (e.g., omeprazole, lansoprazole),

Take 2 hours apart from iron supps: levodopa, levothyroxine, methyl dopa, penicillamine, quinolones, tetracyclines, and bisphosphonates. Cholestyramine resin, used to lower blood cholesterol levels, Gout medication (Allopurinol)

Medications that decrease stomach acidity, such as antacids, histamine (H2) receptor antagonists (e.g., cimetidine, ranitidine), and proton pump inhibitors (e.g., omeprazole, lansoprazole), may impair iron absorption.

Taking iron supplements at the same time as the following medications may result in decreased absorption and efficacy of the medication: levodopa, levothyroxine, methyl dopa, penicillamine, quinolones, tetracyclines, and bisphosphonates. Therefore, it is best to take these medications two hours apart from iron supplements. Cholestyramine resin, used to lower blood cholesterol levels, should also be taken two hours apart from iron supplements because it interferes with iron absorption. Allopurinol, a medication used to treat gout, may increase iron storage in the liver and should not be used in combination with iron supplements.

Ref: Micronutrient Research Centre
http://lpi.oregonstate.edu/infocenter/minerals/iron/
Appendix 4: Information statement
Information Statement for the Research Project:

The effects of non-anemic iron deficiency on cognitive functioning: a blinded randomised controlled trial of iron supplementation in women of childbearing age

Version 6; dated 30/9/2011

You are invited to participate in the research project identified above which is being conducted by University of Newcastle researchers: Dr Kerry Chalmers, Dr Amanda Paterson, Prof Clare Collins, Ms Alecia Greig, and Mrs Hannah Lucas. This project forms part of the PhD research for Alecia Greig and is funded by a research grant from Meat and Livestock Australia.

Why is the research being done?

Iron deficiency is the most prevalent nutritional deficiency worldwide. Women of childbearing age are at particular risk due to increased requirements caused by menstruation and pregnancy. Iron deficiency refers to a total decrease in the level of stored iron and is normally not treated. Iron deficiency anaemia occurs when iron stores have been depleted and the individual is not able to build enough healthy red blood cells to have a normal haemoglobin level. This condition is treated with iron supplementation. Research on the effects of iron deficiency on the general health and well-being and day to day functioning of women is limited. Currently, women who have iron deficiency (without having anaemia) are often not fully assessed or treated.

There are two main aims of this research project: firstly, to provide a better understanding of the effects of iron deficiency on cognitive functioning (including memory, concentration, and mental processing speed) and to assess the effects of change in iron status on measures of cognitive functioning. Secondly, to determine the level of knowledge in young women regarding dietary iron.

The results of this research study will also inform the design of a future study which will examine the effects of iron deficiency on cognition, mental health and fatigue. The current study will assist with confirmation of the methodology and processes required for the future study.

What is involved in the research?

This project will be conducted in two parts:
- Phase one involves the completion of questionnaires
- Phase two involves a randomised controlled trial to measure iron status and cognitive function

Who can participate in the research?

You can participate in phase one (questionnaire) of this project if you are:
- Female
- Aged 18-35 years

You can participate in the phase two (trial) of this project if you are:
- Female
- Aged 18-35 years
- Are of a healthy body weight
- Are able to provide at least one blood sample to assess your iron status
- Able to take iron supplementation during the intervention stage of the study if found to be iron deficient (without anaemia)
You will not be eligible to participate if you:

- Have been diagnosed with iron deficiency within the last 12 months
- Are currently taking iron supplements or have been within the last 3 months
- Are taking any regular prescription medications that may be interfered with by taking iron supplementation or that may interfere with the effect of iron supplementation (e.g. antibiotics, Penicillamine (Cuprimine, Depen), Levothyroxine).
- Are pregnant, or planning to become pregnant within the next 4 months
- Have been pregnant within the last 12 months and/or are breastfeeding
- Have a chronic medical condition
- Have amenorrhea for any reason other than pharmaceutical contraception
- Have made a recent blood donation (within last 3 months)

**What choice do you have?**

Participation in this research is entirely your choice. You will be only included in the project if you have given your informed written consent. Whether or not you decide to participate, your decision will not disadvantage you. If you do decide to participate, you may withdraw from the project at any time without giving a reason and have the option of withdrawing any data that identifies you.

**What would you be asked to do?**

Phase one (questionnaires) involves completing two individual questionnaires, a food frequency questionnaire (FFQ) and a questionnaire designed to assess knowledge and practice regarding dietary iron (NKQ).

- FFQ: this questionnaire aims to assess dietary intake, data will be used to identify amount of dietary iron in your diet
- NKQ: this questionnaire aims to determine what young women know about iron and what their practices are regarding iron intake (including dietary and supplementation). The nutrition knowledge questionnaire can be completed in paper format and takes 5-10 minutes to complete.

There are three stages in phase two (trial) of the study.

i) In the initial stage of the study you will be asked to complete some brief questions related to demographical information, medical history and psychological measures. Cognition testing will be conducted at the University of Newcastle Callaghan campus. The cognitive tests are designed to assess memory, concentration and mental processing speed. These tasks are computer based, with responses recorded via a touch screen computer monitor. The computer tasks take approximately 50-60 minutes to complete. You will also be required to attend the Hunter Area Pathology Service (HAPS) located on the University of Newcastle Callaghan campus to provide an approximate 20ml blood sample. This sample will be sent for analysis of iron and inflammation status and results will be provided directly to the research team. Markers of iron status can be altered during illness when there is inflammation present; therefore it is important that markers of inflammation are measured.

On completion of the cognitive testing and the iron and inflammation studies, participants who have been identified as having iron deficiency anaemia will be notified. If you are found to have iron deficiency anaemia you will be provided with your blood test results and advised to discuss your results with your general practitioner and you will no longer be eligible to participate in the study.

If you are not anaemic you will be invited to participate in the intervention phase of the study. Your iron status will be revealed at the end of the study. For the intervention phase of the study, we require approximately 32 participants, the majority of those included in this phase will be iron deficient, but you may be iron sufficient and still be asked to take placebo treatment. **If you are iron sufficient you will not be given iron supplements.**

If you do not wish to participate in the intervention phase or you are not among the first 32 participants to volunteer your involvement in this phase, you will be provided with your blood test results and will no longer be involved in the study.

ii) The intervention stage involves iron deficient participants being randomised to one of three groups (one of two doses of iron supplementation or placebo tablets) and iron sufficient participants being placed in the placebo group (you will not know which treatment you are taking). All participants are required to take the
appropriate treatment for that group over a 16 week period. You will have the option of receiving an SMS reminder service (i.e., an SMS can be sent to your mobile phone three times per week) during the 16 week intervention. You will be able to email Alecia Greig to cease or commence this service as you please. To ensure accuracy of the results it is essential that no iron supplementation is taken in addition to treatment distributed in the study. You will be surveyed by phone every four weeks about compliance with the treatment regimen and any side effects you may have experienced. At the conclusion of the treatment period, you will be asked to guess which treatment you believe you have been taking.

iii) The follow-up stage is the final phase and commences after completion of the 16 week intervention phase. During the follow-up phase, iron and inflammation studies will again be conducted and you will again be requested to complete the cognitive function tests.

How much time will it take?

Phase one (questionnaires):

- It is estimated that the questionnaires will each take approximately 10-15 minutes to complete (total estimated time 30 minutes)

Phase two (trial):

- The initial stage
  - Screening phone call- 2-5 minutes
  - Blood testing- 10-20 minutes
  - Cognitive functioning test approximately 50 minutes
- Intervention stage
  - Treatment- 16 weeks
  - Survey by phone every 4 weeks (approximately 5-10 minutes)
- Follow-up stage
  - Blood testing- 10-20 minutes
  - Cognitive functioning test approximately 50 minutes

What are the benefits and risks of participating in the questionnaire phase?

Benefits
- You will be contributing to research
- You will have the results of the study available upon its conclusion

Risks
- You may find the completion of questionnaires a burden

What are the benefits and risks of participating in the randomised controlled trial?

Benefits
- You will find out your current iron status
- You will be contributing to research
- If iron deficiency anaemia is detected, you will be referred to a general practitioner
- You will have the results of the study available upon its conclusion
- You will receive course credit towards either Psychology classes PSYC1010 or PSYC1020; OR Nursing classes NURS1101 or NURS1201.

Risks
- As a result of blood collection some individuals may feel light headed
- You may experience some bruising around the site of the blood collection
- High dose iron supplements can cause side effects including constipation, nausea and darkening of stools in some people

How will your privacy be protected?

Data will be de-identified by replacing names with numerical codes. Once the information is entered on the data file, all raw data will be shredded and no person will be identifiable in the data files or published report. De-identified data and consent forms will be retained for at least 5 years at the University of Newcastle by
University staff. No individual will be identifiable in any reports arising from the study; only summarised data will be presented. De-identified data may be used for further analysis in a future project.

**How will the information collected be used?**

The results of the research will be reported and disseminated via national and international conferences, peer reviewed publications, and will contribute towards Ms Alecia Greig’s PhD thesis. You will not be identified in any reports arising from the study. At the conclusion of the study, you will receive an email from the project officer summarising the results of the study.

**What do you need to do to participate?**

* Please read this Information Statement carefully and be sure you understand its contents before you consent to participate.
* If you are willing to participate in this study, **please complete the accompanying consent form and return it to Ms Alecia Greig in Room HA06 Hunter building via internal mail or send it via the post in the reply paid envelope provided.** If there is anything you do not understand, or you have questions, please contact Ms Alecia Greig (details below).
* If you consent to participate in the questionnaire phase of the research Ms Alecia Greig will then contact you to obtain a postal address or to arrange a convenient time to hand over the questionnaires. If you agree to participate in the randomised controlled trial, Ms Alecia Greig will contact you to arrange a suitable time for testing.

**Further information**

If you would like further information please contact Ms Alecia Greig on 49215690
[lon.cognition@newcastle.edu.au](mailto:lon.cognition@newcastle.edu.au)

Thank you for considering this invitation.

Dr Kerry Chalmers PhD

**The Research Team**

Dr Kerry Chalmers¹ Prof. Clare Collins² Dr Amanda Patterson² Ms Alecia Greig² Mrs Hannah Lucas²

¹ School of Psychology
² School of Health Sciences

**Complaints about this research**

This project has been approved by the University’s Human Research Ethics Committee, Approval No.H-2010-1079. Should you have concerns about your rights as a participant in this research, or you have a complaint about the manner in which the research is conducted, it may be given to the researcher, or if an independent person is preferred, to the Human Research Ethics Officer, Research Office, The Chancellery, The University of Newcastle. University Drive, Callaghan NSW 2308, Australia, telephone (02) 49216333. Email [Human-Ethics@newcastle.edu.au](mailto:Human-Ethics@newcastle.edu.au)
Appendix 5: Consent form
Consent Form for the Research Project:

The effects of non-anaemic iron deficiency on cognitive functioning: a blinded randomised controlled trial of iron supplementation in women of childbearing age

Dr Kerry Chalmers, Prof. Clare Collins, Dr Amanda Patterson, Ms Alecia Greig, Mrs Hannah Lucas.

Version 3; dated 27/09/2011

I agree to participate in the above research project and give my consent freely. I understand that the project will be conducted as described in the Information Statement, a copy of which I have retained. I understand I can withdraw from the project at any time and do not have to provide any reason for withdrawing. I consent to (please tick as many as applicable):

☐ Providing blood samples to enable the assessment of iron status
☐ Completing the approximately 50 minute computerised touch screen cognitive functioning test
☐ Complying with the iron supplementation requirements during the intervention phase of the project
☐ Not taking additional iron supplementation to that supplied during the study
☐ Providing responses to the nutrition knowledge questionnaire
☐ Providing responses to the food frequency questionnaire

I understand that my personal information will remain confidential to the researchers and that data collected from my participation will be used in journal publications and conference presentations, will contribute towards Ms Alecia Greig’s PhD thesis and may be used in future research projects. My refusal to participate or withdraw from the study will not affect my relationship with the University of Newcastle. I have had the opportunity to have questions answered to my satisfaction. By signing below I am indicating my consent to participate in the research project conducted by Dr Kerry Chalmers, Prof. Clare Collins, Dr Amanda Patterson, Ms Alecia Greig, and Mrs Hannah Lucas as it has been described to me in the Information Statement, a copy of which I have retained.

I am aware that I will receive my blood test results when my participation in the study has concluded.

If found eligible, I do / do not wish to participate in the intervention and follow-up phases of the study (please circle your preference).

Print Name: ____________________________

Contact Details: e-mail ____________________________

Phone ____________________________

Signature: ____________________________ Date: ____________________________

Please return the completed consent form with the completed questionnaire to Ms Alecia Greig at the University of Newcastle, in person or in the return envelope provided. Your cooperation is greatly appreciated.
Appendix 6: Food frequency questionnaire

This document was removed for copyright reasons.
Please contact the Cancer Council of Victoria for more information
Appendix 7: Nutrition knowledge of iron questionnaire
Iron, women and diet: what do we know, and does it matter?

Please answer every question by ticking the most applicable option or writing an answer in the space provided

Section A-Demographic

1. What is your country of origin?
   □ Australia
   Other (please specify)____________________

2. What is your date of birth? (Day/month/year)
   ___ ___ / ___ ___ / ___ ___ ___

3. What is your current occupation? (Please tick one option only)
   □ Manager or administrator
     (e.g. magistrate, farm manager, general manager, director of nursing, school principal)
   □ Professional
     (e.g. scientist, doctor, registered nurse, allied health professional, teacher, artist)
   □ Associate professional
     (e.g. technician, manager, youth worker, police officer)
   □ Tradesperson or related worker
     (e.g. hairdresser, gardener, florist)
   □ Advanced clerical or service worker
     (e.g. secretary, personal assistant, flight attendant, law clerk)
   □ Intermediate clerical, sales or service worker
     (e.g. typist, word processing/data entry operator, receptionist, child care worker, nursing assistant, hospitality worker)
   □ Elementary clerical, sales or service worker
     (e.g. filing/mail clerk, parking inspector, sales assistant, telemarketer, housekeeper)
   □ Labourer or related worker
     (e.g. cleaner, factory worker, general farm hand, kitchen hand)
   □ No paid job
   □ Don’t know or not applicable

PLEASE NOTE: QUESTIONS ARE ON BOTH SIDES OF EACH PAGE
4. What is the highest level of education you have completed? *(Please tick one option only)*

- No formal qualifications
- Year 10 or equivalent (e.g., school certificate)
- Year 12 or equivalent (e.g., higher school certificate)
- Trade/apprenticeship (e.g., hairdresser, chef)
- Certificate/diploma (e.g., child care, technician)
- University degree
- Higher university degree (e.g., Grad Dip, Masters, PhD)

*For post year 12 study please list your program of study:* ____________________
Section B-Nutrition knowledge of dietary iron

5. Do you think your knowledge of dietary iron is? *(Please tick one option only)*
- Low
- Moderate
- High

6. What is the main function of iron in the body? *(Please tick one option only)*
- Forms part of haemoglobin
- Muscle stimulant
- Production of energy
- Don’t know
- As an antioxidant
- Don’t know
- Supports the immune system

7. Which of these foods are good sources of iron? *(Please tick as many options as applicable)*
- Chickpeas
- Green beans
- Apples
- Chicken
- Lamb
- Don’t know
- Milk

8. Which of the following are features of haem iron (as opposed to non-haem iron)? *(Please tick as many options as applicable)*
- Iron is attached to a protein
- More easily absorbed by humans
- Found in plant foods
- Less easily absorbed by humans
- Found in animal foods
- Don’t know

9. Which of these vegetarian foods are good sources of iron? *(Please tick as many options as applicable)*
- Onions
- Spinach
- Baked beans
- Eggs
- Lentils
- Cheese
- Vegetable stock
- Don’t know
10a. For adults aged 19-30 years, is the recommended daily intake of iron higher for men or women? (Please tick one option only)

☐ Men
☐ Women
☐ Don’t know

10b. Why do you believe this is the case? (Please tick as many options as applicable)

☐ Increased muscle mass
☐ Menstrual blood loss
☐ Production of testosterone
☐ Losses during childbirth
☐ To build iron stores during pregnancy
☐ Production of oestrogen
☐ Don’t know

11. Which of the following statements is true/false with regard to iron in the diet? (Please circle one number on each line)

<table>
<thead>
<tr>
<th></th>
<th>True</th>
<th>False</th>
<th>Unsure</th>
</tr>
</thead>
<tbody>
<tr>
<td>a)</td>
<td>Red meat has more than double the amount of iron of chicken or fish (gram for gram)</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>b)</td>
<td>The absorption of iron from meat foods is increased by Vitamin C consumed at the same meal</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>c)</td>
<td>Tea and coffee inhibit iron absorption</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>d)</td>
<td>Calcium enhances iron absorption</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>
Section C- Iron status and practices surrounding iron

12. In your household, how much cooking do you do? *(Please tick one option only)*

- None
- Less than 2 times per week
- Between 2 and 5 times per week
- More than 5 times per week

13. Are you vegetarian? *(Please tick one option only)*

- Yes
- Partially vegetarian (occasionally eat non-vegetarian options)
- No

14. Do you have any dietary restrictions? *(e.g. allergy/ intolerance/ religion)* *(Please tick one option only)*

- Yes
- No

If yes, what is the restriction? ______________________________________________________

15. Have you ever been told by a doctor that you have low iron levels (iron deficiency or anaemia)? *(Please tick one option only)*

- Yes Go to question 16
- No Go to section D

16. When was the last time you received a diagnosis of low iron?

- Less than 3 months ago
- Between 3 and 12 months ago
- More than 12 months ago

17. What symptoms were you experiencing? *(Please tick as many options as applicable)*

- Tiredness
- Impaired memory
- Neck pain
- None
- Inability to concentrate
- Other
- Nausea
- *(please specify)____________________
18. Has your doctor ever recommended that you alter your dietary intake or take an iron supplement in response to a diagnosis of iron deficiency? (Please tick one option only)

☐ Increase dietary iron Go to question 20
☐ Take iron supplementation Go to question 19
☐ Both Answer relevant questions from 19-23
☐ Neither Go to section D

19. What duration of time were you directed to take the supplements for? (Please tick one option only)

☐ Not specified by doctor ☐ 1-3 months
☐ Less than 1 month ☐ More than 3 months

20. Did you take the iron supplements for the prescribed length of time? (Please tick one option only)

☐ Yes Go to question 23
☐ No Go to question 21

21. What was the reason for this? (Please tick as many options as applicable)

☐ Could not remember to take the supplement
☐ Did not feel an improvement while taking the supplement
☐ Gastrointestinal disturbance (e.g. nausea/diarrhea/constipation/vomiting)
☐ Did not like taking a regular supplement
☐ Other ____________________________

If you had no dietary advice provided by your doctor, go to question 23

22. Did you think the dietary advice provided by your doctor was helpful?

☐ Yes
☐ No

23. Did your doctor refer you for a follow-up blood test to re-test your iron levels?

☐ Yes
☐ No
Section D - Health and wellbeing

24. In the last 12 months have you had any of the following? (Please tick as many options as applicable)

☐ Heavy periods
☐ Severe tiredness
☐ Episodes of intense anxiety
☐ Feelings of depression
☐ Regular blood donation
☐ I have had none of these problems

25. Have you received a diagnosis of depression from your doctor?

☐ Less than 3 months ago
☐ Between 3 and 12 months ago
☐ More than 12 months ago
☐ Never

26. In the past 3 months how many times have you had a menstrual period? (Please tick one option only)

☐ None
☐ One
☐ Two
☐ Three
☐ Four
☐ Five or more

27. Do you use any form of hormonal contraceptive?

☐ Yes Go to question 28
☐ No Go to question 29

28. What type hormonal contraceptive?

☐ Oral contraceptive pill (OCP)
☐ Implanon
☐ Intrauterine Device (IUD)
☐ Intramuscular injection (Depo Provera)

29. Do you have any medical condition affecting you blood? (e.g. hemophilia/leukemia/haemochromatosis)

☐ Yes What is the condition?
☐ No

Thank you for completing the survey

Please use the enclosed self addressed, reply paid envelope to return the completed questionnaire to the research team
Appendix 8: Tips and reminders for taking capsules
Iron and Cognition Study

Tips and reminders for taking capsules

- Take one capsule per day
- Leave your container of capsules next to your tooth brush
- Keep one or two capsules in the small container provided and leave this in your handbag in case you forget to take it in the morning and then remember part way through the day
- Use the calendar to cross off each day once you have taken your capsule. This help you to keep a track of how regularly you are remembering to take them
- Take the capsule two hours apart from any other regular medication (except the oral contraceptive pill which can be taken at the same time)
- Do not take two capsules on the same day to compensate for missing your capsule the previous day - Take only one capsule per day
- Return any left over capsules in your container when you return for your follow up testing
- Please take note of your compliance with the treatment regimen and any side effects you may have experienced and report this to the research team when you are contacted by phone every four weeks.

If you have any concerns during the intervention period please do not hesitate to get in contact with the research team.

Ms Alecia Greig: Ph. 49215690 or 0419544742
Iron.cognition@newcastle.edu.au
Appendix 9: Tips to assist with nausea and constipation
Iron and Cognition Study

Tips to assist with nausea and constipation

**Nausea**
To assist with nausea, sip dry ginger ale or iced water slowly and frequently. This may help settle your stomach.

As nausea becomes more controlled, try sips of more nourishing drinks: chilled diluted fruit juices or nectars, dilute vegetable juices. Soup may also be an option. Try snacking on dry biscuits or dried fruit. Eat small amounts frequently – aim to not get too full or too empty.

**Constipation**
To assist with constipation, select a larger amount of high fibre foods each day (see suggestions below). This is aimed to stimulate movement of the bowel. **Drinking several extra glasses of water each day is also needed.**

**High Fibre Foods**

**Bread**
Wholemeal, multigrain, high fibre white bread and fruit breads.

**Cereals**
Choose unprocessed cereals e.g. All Bran, Bran Flakes, Weetbix, Muesli, rolled oats, Health wise, and Sustain, instead of refined cereals e.g. Coco Pops, Rice Bubbles, and Cornflakes. Adding wheat bran and psyllium husks to cereals is another way of increasing soluble fibre.

**Rice and Pasta**
Choose wholemeal spaghetti, noodles and pasta, and brown rice.

**Biscuits**
Choose wholegrain biscuits e.g. Shredded Wheatmeal, Ryvita, Vita-Weat, Oatmeal Biscuits.

**Fruit**
Eat at least 3 serves of fruit each day; fresh, tinned or dried fruit.
Fruit juice contains NO fibre.

**Vegetables**
Increase servings of all salad and cooked vegetables and include the skins where possible

**Legumes**
Increase servings of baked beans, soya beans, red kidney beans, lima beans and lentils.

If symptoms persist or if you have any other concerns during the intervention period please do not hesitate to get in contact with the research team.

Ms Alecia Greig; Ph. 49216420
Iron.cognition@newcastle.edu.au
Appendix 10: Symptom pro forma
### Intervention Pro Forma

**Participant name**____________________  **Subject number**_____

**Phone number**_____________________

**Intervention week:** 4  8  12  16

<table>
<thead>
<tr>
<th>Symptom</th>
<th>No</th>
<th>Yes</th>
<th>Frequency</th>
<th>Severity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nausea</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vomiting</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dark stools</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constipation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhoea</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Comments/issues:**

<table>
<thead>
<tr>
<th>Reported compliance</th>
<th>Days missed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comments/issues:</td>
<td></td>
</tr>
</tbody>
</table>

Do you wish to change preference on SMS reminder service? **Receive / Not receive**
Appendix 11: End of intervention letter
Thank you for participating in the Iron and Cognition Study

Our research team hopes that you have enjoyed being part of a research study at the University of Newcastle. Your participation during the study has been greatly appreciated.

Please see enclosed your pathology results.

If you have any concerns with your results please make an appointment to discuss this with your general practitioner. If you intend to do so, it is suggested that you take your initial and follow-up pathology results. Also please take note of the group you were randomised to during the intervention phase (circled at bottom of page) as this may be useful information for your doctor.

You will receive an email at the end of the project to give you an overview of the project findings.

You were in group:  Control (Placebo)  Placebo
                             60mg Ferritin (Iron)  80mg Ferritin (Iron)

Your status at the point of initial testing was iron sufficient, you were placed in the control group and were taking placebo capsules during the intervention.

Sincerely,
The Iron and Cognition project team,

Dr Kerry Chalmers, Dr Amanda Patterson, Ms Alecia Greig, Prof. Clare Collins.

If you require any further information please do not hesitate to get in contact with the research team.

Ms Alecia Greig; Ph. 49215690
Iron.cognition@newcastle.edu.au
Appendix 12: IntegNeuro test description
1. Test procedures, functions measured, associated brain regions and what each test means (its potential practical significance)

1.1 Cognition

1.1.1 Memory Recall and Recognition

*Test procedure:* The client is asked to recall a set of words after various time intervals and later recognize the words from a list of repeated and new words.
*Putative brain regions involved:* Involvement of a fronto-parietal networks, including premotor, left prefrontal, left precuneus and left parietal regions.
*Selected references:* 15, 16
*Practical significance:* Ability to learn and remember new tasks based on verbal information. Critical, central everyday skill.

1.1.2 Digit Span

*Test procedure:* The client is presented with a sequence of digits and then has to repeat them in either forward or backward order.
*Functions measured:* Short term verbal memory (Score Forwards), working memory operations (Score Backwards).
*Putative brain regions involved:* Prefrontal, temporal and inferior parietal cortex.
*Selected references:* 10, 11
*Practical significance:* Ability to hold, retain and operate on new verbal information. Skills crucial to most everyday, verbal tasks requiring memory. Everyday examples include remembering telephone numbers and shopping lists.

1.1.3 Span of Visual Memory

*Test procedure:* The client is required to press a series of squares on the screen in the order in which they previously lit up.
*Functions measured:* Short term visuospatial memory and attention.
*Putative brain regions involved:* Parietal, motor and prefrontal cortex.
*Selected references:* 8, 9
*Practical significance:* Ability to hold and retain new spatial information. Skills crucial to most everyday, non-verbal tasks requiring memory. Examples include navigation, operating industrial machines.
1.1.4 Sustained Attention (CPT)

Test procedure: The client is presented with letters one by one, pressing a button if the same letter appears twice in a row.
Functions measured: Sustained attention, target detection.
Putative brain regions involved: Dorsolateral prefrontal and medial frontal cortex, thalamus, basal ganglia, posterior parietal and superior temporal lobe.
Selected references: 19, 20
Practical significance: Ability to detect and respond to significant change under conditions requiring vigilance. Fundamental everyday skills e.g. train, plane, automobile, computer and equivalent machine operations.

1.1.5 Switching of Attention

Test procedure: The test has two parts. Part 1: Numbers are connected up sequentially in chronological order. Part 2: Numbers and letters are connected up sequentially in chronological order.
Functions measured: Parts 1 and 2: Visuomotor tracking, simple attention. Part 2 only: Ability to shift the course of ongoing mental activity.
Putative brain regions involved: Dorsolateral frontal cortex (Part 2 only).
Selected references: 13, 14 (Part 2 only)
Practical significance: Part 1: Simple ability to attend. Part 2: Ability to sustain and control the direction of attention. Critical activity for everyday multitasking skills e.g. management, driving.

1.1.6 Motor Tapping

Test procedure: The client is required to tap a circle with the index finger of each hand in turn, as fast as possible.
Functions measured: Hand eye coordination and fine movement speed (manual dexterity).
Putative brain regions involved: Motor cortex, basal ganglia and cerebellum.
Selected references: 1, 2
Practical significance: Everyday motor skills such as typing and machine operation.
1.1.7 Choice Reaction Time

Test procedure: One of four circles lights up and the client is required to press the lit circle as quickly as possible.

Functions measured: Visuomotor coordination, speed and accuracy of selecting an appropriate response.

Putative brain regions involved: Occipital, parietal, frontal and motor cortices, diencephalon.

Selected reference: 3

Practical significance: Visual discriminative judgment and response. Examples: visual monitoring tasks requiring choice and reaction such as air traffic control, driving judgment.

1.1.8 Time Estimation

Test procedure: A circle appears on the screen for 1 to 12 seconds and the client is required to indicate the correct duration.

Functions measured: Ability to accurately estimate time duration.

Putative brain regions involved: Hippocampus and cerebellum.

Selected references: 4, 5

Practical significance: Time organization.

1.1.9 Verbal Interference

Test procedure: The test has two parts. The first part requires the client to read color words which are printed in colored ink. The second part requires the client to name the ink color in which the same color words are written.

Functions measured: The first part measures reading speed/accuracy for individual words. The second part measures the ability to inhibit inappropriate well-learned impulsive automatic responses.


Selected reference: 12 (Part 2 only)

1.1.10  Spot the Real Word

Test procedure: A real word is presented simultaneously with a nonsense word. The client is required to select the real word.

Functions measured: English language recognition.

Putative brain regions involved: Broad cortical involvement but particularly left perisylvian regions (e.g. Wernicke area).

Selected references: 6, 7

Practical significance: Language skill; correlates with premorbid intelligence.

1.1.11  Word Generation

Test procedure: The client is required to say as many words as possible (in 1 minute) which start with given letters and then state as many animals as possible.

Functions measured: Verbal fluency and thinking ability.

Putative brain regions involved: Include left inferior frontal cortex, left dorsolateral prefrontal cortex, supplementary motor cortex, the anterior cingulate cortex and the cerebellum.

Selected references: 17, 18

Practical significance: Ability to generate and articulate thoughts and ideas in a systematic manner.

1.1.12  Maze

Test procedure: The client is required to discover (by trial and error) a hidden path through a maze and remember it.

Functions measured: Planning, abstraction, foresight, error correction, the ability to choose, try, reject and adapt alternative courses of thought and action; visuospatial learning and memory.

Putative brain regions involved: Widespread brain networks.

Selected reference: 21

Practical significance: Ability to plan, strategize and implement complex tasks involving visuospatial information.
Appendix 13: IntegNeuro scoring system
2. How the scores are derived

2.1 Cognition

2.1.1 Memory Recall

Immediate recall trial n: The number of words correctly recalled within 30 seconds in trial n. Repeated words are counted only once.
Total immediate recall trials 1-4: The sum of the scores in trials 1, 2, 3 and 4.
Learning rate trials 1-4: The slope of the linear regression of the scores in trials 1-4.
Total intrusions errors trials 1-4: The number of times a word not in the list was recalled in trials 1-4.
Total perseveration errors trials 1-4: The number of times a word was repeated in trials 1-4.
Distractor recall trial 5: The score for the words recalled from the new list used in the fifth trial.
Short delay recall trial 6: The number of words recalled from the first list (after the recall of the distractor list).
Long delay recall trial 7: The number of words recalled approximately 40 minutes after trials 1-6.
Intrusion errors trial n: The number of times a word not in the list was recalled in trial n.
Interference errors trial n: The number of times a word was recalled from the other list in trial n (first list for trial 5, second list for trial 6 and 7).

2.1.2 Memory Recognition

Recognition Accuracy: The number of words from the memory recall list that were correctly recognized.
Rejection Accuracy: The number of words that were correctly rejected as not being in the memory recall list.

2.1.3 Digit Span

Recall span: Length of the longest sequence correctly recalled in forward or reverse order.
Total score: Total number of correct trials attempted in forward or reverse order.

2.1.4 Span of Visual Memory

Recall span: Length of the longest sequence correctly identified twice.
Total score: Total number of correct trials attempted.

2.1.5 Sustained Attention (CPT)

Reaction time: The average reaction time to identify the repeated letters.
False alarms: The number of incorrect responses.
False misses: The number of targets that the client did not respond to.
2.1.6 Switching of Attention

*Completion time:* The total time to connect the sequence of digits or digits and letters.

*Avg. connection time:* The average time needed to connect two neighboring fields when no error was made.

*Errors:* The number of errors that the client made.

2.1.7 Motor Tapping

*Number of taps:* The number of times the client tapped the touch screen within 30 seconds with their right or left hand.

*Variability of reaction time:* The standard deviation between taps.

2.1.8 Choice Reaction Time

*Reaction time:* The average time that the client took to tap a lit circle.

2.1.9 Time Estimation

*Accuracy:* The value of the average difference between the actual length of the stimulus (in) and the clients estimate (li) weighted by the length of the stimulus: \[ \frac{\sum_{i=1}^{n} |l_i - i|}{\sum_{i=1}^{n} i} \].

2.1.10 Verbal Interference

*Accuracy:* The number of correct responses in recognizing the color or the text of the displayed word.

*Errors:* The number of incorrect responses.

*Reaction time:* The average time to identify a stimulus when the response was correct.

2.1.11 Spot the Real Word

*Accuracy:* Number of words correctly recognized.

2.1.12 Word Generation

*Number of words generated (FAS):* The average number of words generated in one minute that began with a specific letter.

*Number of animal names generated:* The number of animal words generated in one minute.

2.1.13 Executive Function (maze)

*Trials completed:* The number of trials that the client completed before the task ended or a timeout occurred.

*Completion time:* The time the client took to complete the task twice without error (or until a timeout occurred after 8 minutes).

*Path learning time:* The time the client took to discover the hidden path. If no timeout occurred, this is the total time excluding the time needed for the last two trials (otherwise it is equal to the total time).
Total errors: The total number of errors that the client made including overruns.
Number of overruns: The total number of overrun errors that the client made. An overrun error occurs if the client goes in the same direction on a subsequent move but should have changed direction.

2.1.14 Oddball (Selective Attention)

Reaction time: The average time that the client took to respond to the targets.
False alarms: The number of incorrect responses.
False misses: The number of targets that the client did not respond to.

2.1.15 Go-NoGo (Inhibition)

Reaction time (Go): The average time that the client took to respond to the targets (word PRESS in green).
False alarms (NoGo): The number of incorrect responses.
False misses (Go): The number of targets that the client did not respond to.
Appendix 14: Statement of contribution and collaboration for Chapter Three
I attest that Research Higher Degree candidate Alecia J Leonard (nee Greig) contributed to the following paper:


Alecia J Leonard contributed to the study design, data analysis and manuscript preparation. Dr Amanda Patterson, Dr Kerry Chalmers and Professor Clare Collins contributed to the study design and manuscript preparation within their capacity as PhD supervisors.

Mrs Alecia Leonard 01/07/2014

Dr Amanda Patterson 05/06/2014

Professor Clare Collins 17/06/2014

Dr Kerry Chalmers 26/06/2014

Professor Robert Callister 01/07/2014

(Assistant Dean Research Training)
Appendix 15: Statement of contribution and collaboration for Chapter Four
I attest that Research Higher Degree candidate Alecia J Leonard contributed to the following paper:


Alecia J Leonard contributed to the study design, data analysis and manuscript preparation. Dr Amanda Patterson, Dr Kerry Chalmers and Professor Clare Collins contributed to the study design and manuscript preparation within their capacity as PhD supervisors.

Mrs Alecia Leonard 01/07/2014

Dr Kerry Chalmers 26/06/2014

Professor Clare Collins 17/06/2014

Dr Amanda Patterson 05/06/2014

Professor Robert Callister 01/07/2014

(Assistant Dean Research Training)
Appendix 16: Statement of contribution and collaboration for Chapter Six
I attest that Research Higher Degree candidate Alecia J Leonard contributed to the following paper:


Alecia J Leonard contributed to the study design, data analysis and manuscript preparation. Dr Amanda Patterson, Dr Kerry Chalmers and Professor Clare Collins contributed to the study design and manuscript preparation within their capacity as PhD supervisors.

Mrs Alecia Leonard  01/07/2014

Dr Kerry Chalmers  26/06/2014

Professor Clare Collins  17/06/2014

Dr Amanda Patterson  05/06/2014

Professor Robert Callister  01/07/2014

(Assistant Dean Research Training)
Appendix 17: Statement of contribution and collaboration for Chapter Seven
I attest that Research Higher Degree candidate Alecia J Leonard contributed to the following paper:


Alecia J Leonard contributed to the study design, data analysis and manuscript preparation. Dr Amanda Patterson, Dr Kerry Chalmers and Professor Clare Collins contributed to the study design and manuscript preparation within their capacity as PhD supervisors.

Mrs Alecia Leonard 01/07/2014

Dr Kerry Chalmers 26/06/2014

Professor Clare Collins 17/06/2014

Dr Amanda Patterson 05/06/2014

Professor Robert Callister 01/07/2014

(Assistant Dean Research Training)
Appendix 18: Statement of contribution and collaboration for Chapter Eight
I attest that Research Higher Degree candidate Alecia J Leonard contributed to the following paper:


Alecia J Leonard contributed to the study design, data analysis and manuscript preparation. Dr Amanda Patterson, Dr Kerry Chalmers and Professor Clare Collins contributed to the study design and manuscript preparation within their capacity as PhD supervisors.

Mrs Alecia Leonard 01/07/2014

Dr Amanda Patterson 05/06/2014

Professor Clare Collins 17/06/2014

Dr Kerry Chalmers 26/06/2014

Professor Robert Callister 01/07/2014

(Assistant Dean Research Training)
Appendix 19: Statement of contribution and collaboration for Chapter Nine
I attest that Research Higher Degree candidate Alecia J Leonard contributed to the following paper:


Alecia J Leonard contributed to the study design, data analysis and manuscript preparation. Dr Melinda Hutchesson contributed data from her research to this manuscript and contributed to the study design and manuscript preparation. Dr Amanda Patterson, Dr Kerry Chalmers and Professor Clare Collins contributed to the study design and manuscript preparation within their capacity as PhD supervisors.

Mrs Alecia Leonard  01/07/2014

Dr Melinda Hutchesson  04/07/2014

Dr Amanda Patterson  05/06/2014

Dr Kerry Chalmers  26/06/2014

Professor Clare Collins  17/06/2014
Professor Robert Callister  01/07/2014

(Assistant Dean Research Training)