

Iron status in relation to morbidity when considering iron supplementation among urban pregnant women in South Africa

C Goodchild



orcid.org 0000-0003-0309-5781

BSc Dietetics

Dissertation submitted in partial fulfillment of the requirements for the degree Magister Scientiae in Dietetics at the North-West University

Supervisor: Dr L Malan
Co-supervisor: Mrs EA Symington

Graduation: May 2018

Student number: 23490209

<http://dspace.nwu.ac.za/>

The financial assistance of the National Research Foundation (NRF) towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at, are those of the author and are not necessarily to be attributed to the NRF.

PREFACE

“Welcome to the grind, the battle royale between you and your mind, your body and the devil on your shoulder telling you this is just a waste of time.”

Acknowledgements

I am most grateful to the Lord for blessing me with two amazing years and smooth sailing throughout this study. "Let us run with endurance the race that is set before us, fixing our eyes on Jesus, the author and finisher of our faith." Hebrews 12:1-2

To my supervisor, Dr Linda Malan, who constantly invested time in helping me, and encouraging me during my MSc. Dr Malan, thank you that you were always available, always timeous and always friendly and calm. You helped me get through this dissertation with very little anxiety and with joy.

To Elize Symington, you took on such an immense task of co-ordinating this study. Thank you for how organised you were with each aspect of NuPED, and for always handling tasks with happiness. You can be incredibly proud of what you have achieved.

To the CEN staff, thank you for being approachable and so prestigious in your fields. It is truly admirable. Tannie Ronel, thank you that you know everything and that we could always count on you for an answer.

To the NuPED team, thank you for all the hours of hard work, from the planning to the execution. What a lovely team to be a part of.

To our statistician, Cristian Ricci, thank you for teaching us new skills and making us laugh.

To Katlego Kekana, you were an absolute star with all the data entering, without complaining. Thank you for all your hard work.

A big thank you to the women who took part in this study. Thank you for your vital role in allowing the field of health sciences to expand.

I would like to thank my family, Begley, Trudie, Roxanne and Shannon for always sticking together and being so incredibly strong, no matter what storm we face. The Lord has been good to us, through it all.

To those I grew with during my past six years in Potch: Bakang Olifant, Bibi Amina Joosub, Claudine Jordaan, EJ Crews, Joanna Skoczynski, Kolwin Louw, Kristen Lee, Madimabe Tebele, Nicole Jansen and Sunita Tobias. Also to the Jozi squad (Alex, Caitlin, James, Kate, Kevin, Tarryn, James and Virasha) who always ensure to keep in touch.

ABSTRACT

Background: Anaemia is a common and challenging nutritional problem worldwide and iron deficiency (ID) contributes to about half of all anaemia. In 2002, ID anaemia was considered to be one of the most important contributing factors to the global burden of disease. The prevalence of anaemia in South Africans older than 15 years of age is 17.5%. During pregnancy it is especially important to prevent anaemia and ID. In an attempt to do this, various countries provide routine iron supplementation to pregnant women. These supplementation regimes are not homogenous worldwide and may not be appropriate for iron replete women. In South Africa (SA), a standard quantity of ~60 mg of elemental iron is provided daily to all pregnant women, regardless of a large proportion of them being iron-sufficient. These women may experience a greater incidence of adverse effects due to excess iron intake or a high iron status. These adverse effects include hypertension, gestational diabetes mellitus (GDM), oxidative stress, decreased zinc absorption and increased infectious morbidity. Furthermore, these adverse effects may be modulated by n-3 polyunsaturated fatty acid (PUFA) status.

Aim: The overall aim of this dissertation was to assess whether iron status and anaemia at < 18 weeks of gestation is associated with morbidity during the course of pregnancy in urban pregnant women receiving routine iron supplementation in SA. More specifically, investigating associations with the incidence of morbidity symptoms, blood pressure and the incidence of hypertension, as well as blood glucose and the incidence of GDM during pregnancy. Furthermore, the association of morbidity symptoms and blood pressure with n-3 PUFA status, were also examined.

Design: In a prospective observational analysis, pregnant women (at < 18 weeks of gestation) attending antenatal care facilities in selected clinics within the City of Johannesburg in SA, were recruited. The first phase data collection took place at Rahima Moosa Mother and Child Hospital along with further follow-up visits at 22 and 36 weeks of pregnancy, and birth. Morbidity symptoms were recorded by the women for the duration of pregnancy. Additionally, blood pressure, blood glucose and n-3 PUFA status were measured and compared between women with and without ID and anaemia, which were based on ferritin and haemoglobin concentrations respectively.

Results: ID and anaemia prevalence among all women was 11.9% and 20.8%, respectively. Diastolic blood pressure was higher and mean arterial pressure tended to be higher in the iron-sufficient group at 36 weeks gestation, compared to the group with ID, when corrected for age, height, weight, ethnicity and n-3 PUFA status ($p = 0.045$ and $p = 0.075$,

respectively). Morbidity symptoms, namely fever, extreme tiredness, coughing and diarrhoea were higher in the iron-sufficient group than the ID group between 18 and 40 weeks gestation ($p < 0.001$, $p = 0.043$, $p < 0.001$ and $p = 0.014$, respectively). Between 18 and 40 weeks, extreme tiredness, runny nose and diarrhoea were higher in the anaemic group ($p = 0.031$, $p = 0.010$ and $p = 0.024$, respectively) than in the non-anaemic group. Also in this duration, women who were compliant to iron supplementation had a higher incidence of fever, extreme tiredness, runny nose and vomiting ($p < 0.001$, $p = 0.013$, $p = 0.025$, $p = 0.008$, respectively) compared to the iron non-compliant women. Throughout pregnancy, women with a higher n-3 PUFA status, experienced a lower incidence of fever, extreme tiredness, headache, runny nose, coughing, diarrhoea, vomiting, constipation and heartburn ($p < 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.001$, $p = 0.018$, $p = 0.008$, $p = 0.045$, $p < 0.001$ and $p = 0.037$, respectively).

Conclusion: This study showed that iron-sufficient pregnant women receiving daily routine iron supplementation of ~60 mg elemental iron experienced a higher incidence of morbidity symptoms, including infectious morbidity symptoms. These women also had a higher diastolic blood pressure at 36 weeks gestation, which may increase the risk of hypertension. Our study, however, showed greater associations with morbidity in iron-sufficient women, than in non-anaemic women. This indicated that iron sufficiency should be evaluated when providing iron supplementation to pregnant women. Furthermore, women with an n-3 PUFA status above the median experienced less fever, extreme tiredness, headache, runny nose, coughing, diarrhoea, vomiting, constipation and heartburn during pregnancy, compared to women with an n-3 PUFA status below the median. It may be important to re-evaluate the iron supplementation strategy during pregnancy in SA.

OPSOMMING

Agtergrond: Anemie is 'n algemene en uitdagende voedingsprobleem wêreldwyd en ystertekort dra by tot die helfte van alle anemieë. In 2002 is anemie as gevolg van ystertekort beskou as een van die belangrikste bydraende faktore tot die wêreldwye las van morbiditeit. Die voorkoms van anemie in Suid-Afrikaners ouer as 15 jaar is 17,5%. Gedurende swangerskap is dit veral belangrik om anemie en ystertekort te voorkom. In 'n poging om dit te doen, bied verskeie lande roetine-ysteraanvulling aan swanger vroue. Hierdie aanvulling strategieë is nie wêreldwyd homogeen nie en is moontlik nie geskik vir vroue met 'n voldoende ysterstatus nie. In Suid-Afrika (SA) word 'n standaard hoeveelheid van ~60 mg elementêre yster daaglik aan alle swanger vroue verskaf, ongeag van hulle ysterstatus. Hierdie vroue kan 'n groter voorkoms van nadelige effekte ervaar as gevolg van oormatige yster inname of 'n hoë yster status. Hierdie nadelige effekte sluit hoë bloeddruk, swangerskap diabetes mellitus (SDM), oksidatiewe stres, verminderde sink absorpsie en verhoogde aansteeklike siektes in. Verder kan hierdie nadelige effekte gemoduleer word deur n-3 poli-onversadigde vetsuur (POV) status.

Doel: Die oorhoofse doel van hierdie verhandeling was om te bepaal of ysterstatus en anemie teen < 18 weke van swangerskap assosieer met morbiditeit tydens swangerskap in stedelike swanger vroue wat roetine-ysteraanvulling in SA ontvang. Meer spesifiek, is die assosiasie met die voorkoms van morbiditeitsimptome, bloeddruk en die voorkoms van hipertensie, asook bloedglukose en die voorkoms van SDM tydens swangerskap ondersoek. Verder is die verband tussen morbiditeitsimptome en bloeddruk met n-3 POV status ook ondersoek.

Metodes: In 'n voornemende observasie-analise is swanger vroue (< 18 weke van swangerskap) wat voorgeboortelike sorgfasiliteite in geselekteerde klinieke in die Stad van Johannesburg in SA bygewoon het, geworf. Die eerste fase van data-insameling het by die Rahima Moosa Moeder- en Kinderhospitaal plaasgevind, asook verdere opvolgbesoeke op 22 en 36 weke van swangerskap en by geboorte. Morbiditeit simptome is self deur die vroue aangeteken vir die duur van swangerskap. Daarbenewens is bloeddruk, bloedglukose en n-3 POV status gemeet en vergelyk tussen vroue met en sonder ystertekort en anemie, wat onderskeidelik gebaseer was op ferritien- en hemoglobienkonsentrasies.

Resultate: Ystertekort en anemie voorkoms onder alle vroue was onderskeidelik 11,9% en 20,8%. Diastoliese bloeddruk was hoër en gemiddelde arteriële druk was geneig om hoër te wees in die yster-voldoende groep teen 36 weke van swangerskap, vergeleke met die groep met ystertekort, wanneer dit gekorrigeer is vir ouderdom, lengte, gewig, etnisiteit en n-3 POV

status ($p = 0,045$ en $p = 0,075$, onderskeidelik). Morbiditeitsimptome, naamlik koors, ekstreme moegheid, hoes en diarree was hoër in die ystervoldoende groep as die ystergebrekkige groep tussen 18 en 40 weke van gestasie ($p < 0.001$, $p = 0.043$, $p < 0.001$ en $p = 0.014$, onderskeidelik). Tussen 18 en 40 weke was ekstreme moegheid, loopneus en diarree hoër in die anemiese groep ($p = 0.031$, $p = 0.010$ en $p = 0.024$ onderskeidelik) as in die nie-anemiese groep. Ook tydens hierdie gestasie tyd het vroue wat meesal yster aanvullings geneem het, 'n hoër voorkoms van koors, uiterste moegheid, loopneus en braking ($p < 0.001$, $p = 0.013$, $p = 0.025$, $p = 0.008$, onderskeidelik) gehad in vergelyking met vroue wat meesal nie die ysteraanvullings geneem het nie. Gedurende swangerskap het vroue met 'n hoër n-3 POV status 'n laer voorkoms van koors, uiterste moegheid, hoofpyn, loopneus, hoes, diarree, braking, hardlywigheid en sooibrand ($p < 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.001$, $p = 0.018$, $p = 0.008$, $p = 0.045$, $p < 0.001$ and $p = 0.037$, onderskeidelik) gehad.

Samevatting: Hierdie studie het getoon dat ystervoldoende swanger vroue wat daaglikse roetine-ysteraanvulling van ~60 mg elementêre yster ontvang, 'n hoër voorkoms van morbiditeitsimptome ervaar, insluitende infeksie-morbiditeitsimptome. Hierdie vroue het ook 'n hoër diastoliese bloeddruk gehad teen 36 weke van swangerskap, wat die risiko van hipertensie verhoog. Ons studie het egter groter assosiasies met morbiditeit in ystervoldoende vroue as in nie-anemiese vroue getoon. Dit dui aan dat yster genoegsaamheid die oorheersende faktor moet wees wat in ag geneem moet word wanneer yster aanvullings aan swanger vroue verskaf word. Verder het vroue met 'n n-3 POV status 'n hoër mediaan minder koors, ekstreme moegheid, hoofpyn, loopneus, hoes, diarree, braking, hardlywigheid en sooibrand tydens swangerskap ondervind, in vergelyking met vroue met 'n n-3 PUFA status onder die mediaan. Dit kan dus belangrik wees om die yster aanvullingstrategie tydens swangerskap in SA te herevalueer.

KEY TERMS

AGP:	α 1-acid glycoprotein
ANC:	antenatal care
ANCOVA:	analysis of covariance
ARV:	antiretroviral
CEN:	Centre of Excellence for Nutrition
COJ:	City of Johannesburg
CRH:	corticotropin-releasing hormone
CRP:	C-reactive protein
DHA:	docosahexaenoic acid
DoH:	Department of Health
ELISA:	enzyme-linked immunosorbent assay
EPA:	eicosapentaenoic acid
FAME:	fatty acid methyl ester
GDM:	gestational diabetes mellitus
GI:	gastrointestinal
GOS:	galacto-oligosaccharides
Hb:	haemoglobin
HIV:	human immunodeficiency virus
HPCSA:	Health Professions Council of South Africa
HREC:	Health Research Ethics Committee
Ht:	haematocrit
ID:	iron deficiency
IDA:	iron deficiency anaemia
LBW:	low birth weight
LSM:	living standards measure
MAP:	mean arterial pressure
MCH:	mean corpuscular haemoglobin

MCV:	mean corpuscular volume
MNP:	micronutrient powders
MRM:	multiple reaction monitoring
MUAC:	mid-upper arm circumference
n-3 PUFA:	omega-3 polyunsaturated fatty acid
NE:	norepinephrine
NHANES:	National Health and Nutrition Examination Survey
NuPED:	Nutrition during Pregnancy and Early Development
NWU:	North-West University
OGTT:	oral glucose tolerance test
PUFA:	polyunsaturated fatty acid
RBC:	red blood cell
RMMCH:	Rahima Moosa Mother and Child Hospital
SA:	South Africa
SAARF:	South African Audience Research Foundation
SAS:	Statistical Analysis System
SANHANES:	South African National Health and Nutrition Examination Survey
SPSS:	Statistical Package for the Social Sciences
TB:	tuberculosis
USA:	United States of America
WHO:	World Health Organisation

TABLE OF CONTENTS

PREFACE	I
ABSTRACT	III
OPSOMMING	V
KEY TERMS	VII
 CHAPTER 1 INTRODUCTION	 1
1.1 Problem statement	1
1.2 Background and rationale	1
1.2.1 Iron deficiency anaemia in South Africa and southern Africa (e.g. Botswana, Zimbabwe)	1
1.2.2 Negative effects of iron supplementation.....	2
1.2.3 Omega-3 fatty acid status	3
1.2.4 Justification of the feasibility, novelty and relevance of the project	4
1.3 The study site	4
1.4 Aim	5
1.5 Objectives	5
1.6 Ethical approval	6
1.7 Research team	6
1.8 Structure of this mini-dissertation	7
1.9 References	8
 CHAPTER 2 LITERATURE REVIEW	 11
2.1 Iron deficiency and anaemia	11
2.2 Iron deficiency and anaemia in southern Africa	12
2.3 Clinical significance of iron deficiency and iron deficiency anaemia in pregnancy	13
2.4 Supplementation of iron during pregnancy	14
2.5 Adverse effects of high iron status and supplementation during pregnancy	15

2.5.1	High iron status, supplementation and gestational diabetes mellitus during pregnancy	17
2.5.2	High iron status, supplementation and hypertension during pregnancy	17
2.5.3	Iron supplementation and oxidative stress during pregnancy	18
2.5.4	Iron supplementation and decreased zinc absorption during pregnancy	19
2.5.5	Iron supplementation and morbidity symptoms	20
2.5.5.1	Side effects	20
2.5.5.2	Infectious morbidity symptoms	21
2.6	Omega-3 fatty acid status and iron supplementation	28
2.7	Strategies to improve the safety of iron supplementation in pregnancy	29
2.8	References	33
CHAPTER 3 METHODOLOGY		43
3.1	Research design and methods.....	43
3.1.1	Study population and sample	43
3.1.1.1	Sample size calculation	44
3.1.2	Recruitment and informed consent.....	44
3.1.3	Research procedures and data collection	44
3.1.3.1	Data collection at different phases during pregnancy.....	47
3.1.3.1.1	Medical history	47
3.1.3.1.2	Morbidity data.....	47
3.1.3.1.3	Anthropometrical data.....	47
3.1.3.1.4	Diet history.....	47
3.1.3.1.5	Brachial blood pressure.....	47
3.1.3.1.6	Oral Glucose Tolerance Test.....	48
3.1.3.2	Specimens and biochemical analysis.....	48
3.1.3.2.1	Biochemical analysis.....	49
3.1.3.2.2	Iron and inflammation markers.....	49

3.1.3.2.3	Fatty acids	49
3.1.4	Data capturing, quality assurance and statistical analysis	50
3.2	Ethical and legal considerations	51
3.2.1	Legal authorisation	51
3.2.2	Expertise, skills and legal competencies	53
3.2.3	Privacy and confidentiality	53
3.2.4	Benefits	54
3.2.4.1	Direct benefits	54
3.2.4.2	Indirect benefits	54
3.2.5	Risks/discomforts, precautions and safety plan	55
3.2.6	Incentive and reimbursement	56
3.2.7	Announcements of results	56
3.2.8	Storage and archiving of data	56
3.3	References	58
CHAPTER 4	MANUSCRIPT	60
4.1	Manuscript	60
CHAPTER 5	CONCLUSION AND RECOMMENDATIONS	99
5.1	Conclusion	99
5.2	Recommendations	100
5.3	References	102
ANNEXURES		104
Annexure A		
Annexure B		
Annexure C		
Annexure D		

LIST OF TABLES

Table 1:	Team members and their roles	6
Table 2:	Studies of iron supplementation and adverse effects in pregnant women	23
Table 3-1:	Summary of inclusion and exclusion criteria at sampling	43
Table 3-2:	Schedule of study activities during pregnancy	46
Table 4-1:	Characteristics of pregnant women participating in the study at entry (gestational age < 18 weeks)	67
Table 4-2:	Supplemental iron intake during pregnancy	69
Table 4-3:	Hypertension and gestational diabetes prevalence, as well as their markers by iron status and anaemia	71
Table 4-4:	Correlations between markers of iron status, anaemia, hypertension and gestational diabetes	72

LIST OF FIGURES

Figure 1:	Simple framework of the topic under investigation	4
Figure 2:	The seven regions of the City of Johannesburg	5
Figure 3:	Diagrammatic illustration of the recruitment and data collection process	52
Figure 4.1:	Bi-plot of correspondence analysis of the incidence of morbidity symptoms during pregnancy	74
Figure 4.2:	Symptoms of total population over time	75
Figure 4.3:	Morbidity symptoms over time in iron-deficient and iron-sufficient pregnant women	78
Figure 4.4:	Morbidity symptoms over time in anaemic and non-anaemic pregnant women	80
Figure 4.5:	Morbidity symptoms over time in iron non-compliant and iron compliant pregnant women	83
Figure 4.6:	Morbidity symptoms over time pregnant women with n-3 PUFA status below the median status and n-3 PUFA status above the median status	87

CHAPTER 1 INTRODUCTION

1.1 Problem statement

Due to the existing problem of iron deficiency (ID) and iron deficiency anaemia (IDA) in South Africa (SA), routine iron supplementation is supplied to all pregnant women. This supplementation may have adverse effects on their health if they are iron-sufficient but may be modulated by n-3 polyunsaturated fatty acid (PUFA) status. The study intends to investigate whether iron status associates with the incidence of infectious morbidity, hypertension and diabetes. This will give insight as to whether or not routine iron supplementation during pregnancy is still preferable in SA.

1.2 Background and rationale

1.2.1 Iron deficiency anaemia in South Africa and southern Africa (e.g. Botswana, Zimbabwe)

Anaemia is a very common and challenging nutritional problem worldwide (WHO, 2016). In 2002, IDA was considered to be one of the most important contributing factors to the global burden of disease (WHO, 2002). According to the South African National Health and Nutrition Examination Survey (SANHANES-1), the prevalence of anaemia in South Africans older than 15 years of age was found to be 17.5%, with females contributing 22% thereof. A major contributing factor of anaemia is ID (Shisana *et al.*, 2013). The World Health Organisation (WHO) defines IDA as haemoglobin (Hb) ≤ 12 g/dL and ferritin ≤ 15 μ g/L (WHO, 2011). In females of reproductive age, 9.7% were found to have IDA. This is a national public health concern which needs to be resolved with a multifactorial and multisectoral approach (Shisana *et al.*, 2013).

With regards to southern Africa, the proportion of Zimbabwean women of reproductive age with severe anaemia (Hb < 7 g/dL) did not exceed 2.5% per age group (WHO, 2008). In Botswana, the proportion of severe anaemia was 1.3% in a survey of non-pregnant women. In pregnant women, 21.3% of the population had anaemia (Hb < 11 g/dL). This indicates that anaemia in pregnant women is a moderate public health problem in southern Africa (WHO, 2008).

With pregnant women having increased iron needs, due to the growing foetus and due to their increased erythrocyte mass, ID may become a health problem during pregnancy, even with adequate status before pregnancy (Milman *et al.*, 2005). Iron status is an essential indicator of health during pregnancy, for both the mother and her infant. Studies have shown that maternal

IDA could result in the infant being born prematurely, resulting in a low birth weight (LBW). There is also an association between maternal iron status and the infant's iron status (Allen, 2000). A study by Idjradinata and Pollitt (1993) found that infants with IDA had a lower mental and motor development compared to infants without the disease. The WHO recommends a daily dose of iron supplementation during pregnancy of 30-60 mg of elemental iron to prevent IDA, where 30 mg of elemental iron is obtained from 150 mg of ferrous sulphate heptahydrate (WHO, 2012). It is recommended to begin this supplementation as early as possible, and take it throughout pregnancy (WHO, 2012).

The provision of quality health care for pregnant women is emphasized in various national policies. These policies include: Strategic Plan for Maternal, Newborn, Child and Women's Health and Nutrition in South Africa, as well as the Department of Health's Maternal, Child and Women's Health policy proposal, published in regulation 655 (2012). In SA, routine supplementation of iron (as well as calcium and folic acid) is provided to pregnant women with an Hb $\geq$ 10g/dL to prevent anaemia while attending primary health care. Their daily regime consists of 200 mg ferrous sulphate (equal to ~60 mg elemental iron), 5 mg folic acid and 1000 mg calcium carbonate (DoH, 2015). Women with mild anaemia (Hb of 8-9.9 g/dL) receive a more aggressive oral treatment of 200 mg ferrous sulphate three times daily and 5 mg folic acid daily. If they are < 36 weeks pregnant, their Hb is again assessed after four weeks. If they are $\geq$ 36 weeks pregnant or if they do not respond to the given oral treatment, they are referred to a district hospital. If ID is then confirmed, intravenous iron therapy is considered. Women with moderate to severe anaemia (Hb of $\leq$ 7.9 g/dL) also receive 200 mg ferrous sulphate three times daily and 5 mg folic acid daily. If the women have a Hb < 6 g/dL and display symptoms such as tachycardia, dizziness or shortness of breath, then a blood transfusion is administered (DoH, 2015).

1.2.2 Negative effects of iron supplementation

Although iron supplementation during pregnancy improves the health of women and their infants, routine iron supplementation to all women, whether they are iron-deficient or not, may be problematic. Various morbidity outcomes have been shown with iron supplementation in non-anaemic pregnant women (Lao *et al.*, 2001; Ziaei *et al.*, 2007), but has even been shown to increase infectious morbidity in anaemic children (Malan *et al.*, 2015; Sazawal *et al.*, 2006). The increase in infectious morbidity might be dependent on the pathogen load in the environment (Murray *et al.*, 1978; Sazawal *et al.*, 2006). It was found that women in the third trimester of pregnancy with increased ferritin levels have an increased risk of intrauterine infection (Scholl, 2005). Ziaei *et al.* (2007) found that the number of pregnant women with hypertensive disorder

was higher in the women supplemented with 150 mg ferrous sulphate, containing 50 mg of elemental iron, than women receiving the placebo. A study by Lao *et al.* (2001) found that excess iron can affect insulin synthesis and secretion, decrease glucose utilisation in the muscles and increase gluconeogenesis in the liver, which can result in liver mediated insulin resistance. In a review by Beard (2000), it was stated that the routine provision of therapeutic doses of iron had been queried due to excess iron possibly causing chronic disease. O'Brien *et al.* (1999) also found that in pregnant women a daily supplement of 60 mg of ferrous iron resulted in a significant decrease in zinc absorption. It was also found that iron supplementation results in an increased concentration of free radicals in the intestinal milieu. This can cause epithelial damage in the intestine (Lund *et al.*, 1999).

These findings prompt the need for further investigation whether routine prophylactic iron with a daily dose of 200 mg ferrous sulphate, as performed in SA to prevent anaemia, is still the best approach.

1.2.3 Omega-3 fatty acid status

Iron supplementation increases the state of oxidative stress and inflammation (Milman, 2006; Malan *et al.*, 2016). Therefore, an adequate n-3 PUFA status or even supplementation, is a suggested strategy to reduce detrimental effects of high iron supplementation doses when needed (Casanueva & Viteri, 2003; Malan *et al.*, 2016). Thus, along with iron intake, antioxidant and n-3 PUFA intake and status must also be considered. Many studies have demonstrated n-3 PUFA to act as antioxidants, which counteract iron-induced oxidative stress (Ali *et al.*, 2014; Barden *et al.*, 2004; Brand *et al.*, 2008; Kones, 2010; Pacheco *et al.*, 2014). Combining n-3 PUFA supplementation with iron supplementation in 6-11-year-old rural iron-deficient children, prevented the increase in respiratory morbidity caused by the iron supplementation (Malan *et al.*, 2015), while simultaneously improving the n-3 PUFA-derived anti-inflammatory lipid mediator profile of the children (Malan *et al.*, 2016).

This information indicates that iron status, especially amongst pregnant women, is an important indicator of health, but excessive and unnecessary supplementation might increase morbidity. This includes infectious morbidity, hypertension and insulin resistance. We, therefore, wish to examine whether there is an association between iron status and morbidity in pregnant women receiving routine iron supplementation and living in Johannesburg, SA. We furthermore want to investigate whether n-3 PUFA status plays a role in this relationship. A simple framework of the study can be seen in Figure 1.

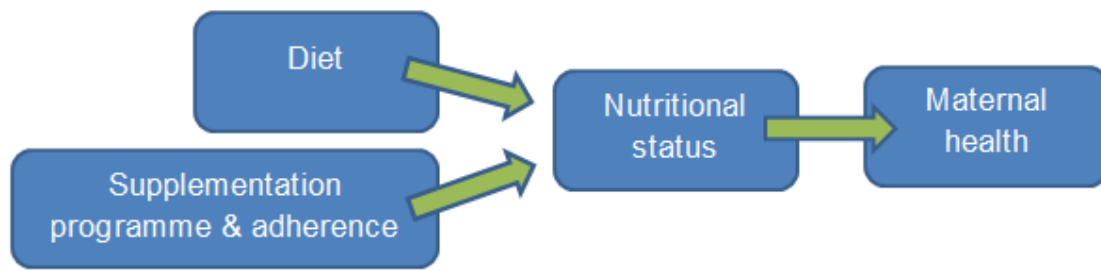


Figure 1: Simple framework of the topic under investigation

1.2.4 Justification of the feasibility, novelty and relevance of the project

Iron status, especially amongst pregnant women, is an important indicator of health, but excessive and unnecessary supplementation might increase morbidity. This includes infectious morbidity, hypertension and insulin resistance. Furthermore, it is unclear from existing data, whether iron status in early pregnancy associates with these morbidities, and whether n-3 PUFA status plays a role in this possible association. Therefore, it is relevant and novel to assess whether the current practice of routine iron supplementation during pregnancy, is associated with an increased risk of morbidity.

1.3 The study site

The setting of the research, and specifically recruitment, took place in Primary Health Care clinics within municipal Regions B and C of the COJ (City of Johannesburg, 2015a, 2015b) in the Gauteng Province. The COJ is divided into seven regions (See Figure 2) (City of Johannesburg, 2015c). RMMCH (formerly known as Coronation Hospital) is a provincial hospital focusing on maternal and paediatric healthcare and is situated in Coronationville, which falls in Region B. This is the only government hospital in SA focusing on maternal and paediatric care and therefore served as an ideal point of focus to address the research aim. Sampling subjects from this region, therefore, represents most urban areas in SA. After recruitment of pregnant women at the Primary Health Care clinics and from ANC at RMMCH, participants were followed-up at RMMCH.

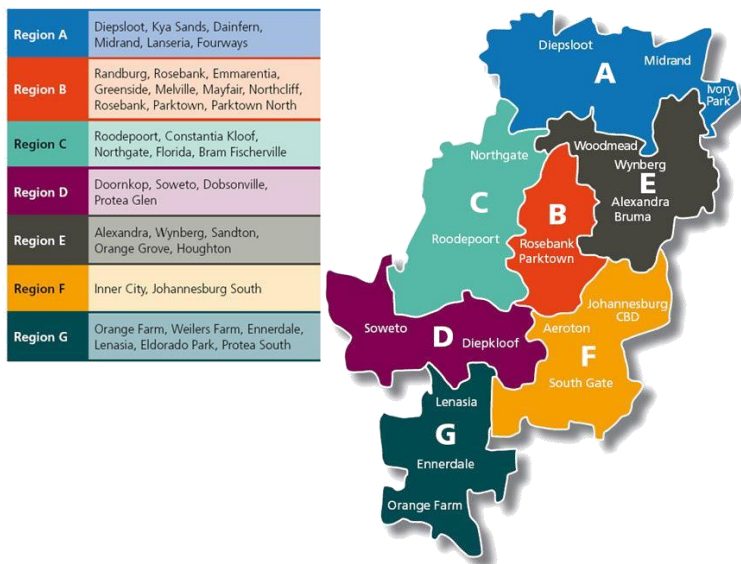


Figure 2: The seven regions of the City of Johannesburg (obtained from <http://www.htxt.co.za/wpcontent/uploads/2014/06/jra-map.jpg>)

1.4 Aim

The study aims to assess whether the iron status at < 18 weeks of gestation is associated with morbidity during the course of pregnancy in urban pregnant women receiving routine iron supplementation in SA.

1.5 Objectives

The objectives to reach this aim are to:

- Determine whether iron status and anaemia at < 18 weeks of gestation are associated with the incidence of morbidity symptoms during the course of pregnancy in urban pregnant women receiving routine iron supplementation in SA.
- Determine whether iron status and anaemia at < 18 weeks of gestation are associated with the incidence hypertension and gestational diabetes mellitus (GDM), and blood pressure and blood glucose, during the course of pregnancy in urban pregnant women receiving routine iron supplementation in SA.
- Determine whether n-3 PUFA status at < 18 weeks of gestation is associated with the incidence of morbidity symptoms and blood pressure during pregnancy in urban pregnant women receiving routine iron supplementation in SA.

1.6 Ethical approval

Ethical approval for this study has been obtained from the Health Research Ethics Committee (HREC) of the Faculty of Health Sciences of the North-West University (NWU), Potchefstroom (NWU-00186-15-A1-02) as well as from the University of Witwatersrand Human Research Ethics Committee (Medical), M150968. Approval was obtained from the RMMCH research review committee, the Gauteng Department of Health (which includes the Johannesburg Health District's District Research Committee), RMMCH ANC clinic, Zandspruit clinic, Sophia Town clinic, Florida clinic and Bosmont clinic.

1.7 Research team

The members of the research team and their roles are listed in Table 1. None of the team members has a conflict of interest.

Table 1: Team members and their roles

Partner name	Team member	Qualification	Professional registration	Role and responsibility
North-West University	Prof Marius Smuts	PhD Biochemistry	HPCSA: Nutritionist	Principal investigator
North-West University	Dr Linda Malan	MSc Biochemistry, PhD Nutrition	HPCSA: Medical Biological Scientist	Supervisor, immune function and laboratory expertise
North-West University (PhD student)	Elize Symington	BSc (Dietetics) M (Dietetics)	HPCSA: Dietitian	Co-Supervisor and dietary data expertise
North-West University	Dr Cristian Ricci	PhD Statistics	None	Biostatistician
North-West University	Caylin Goodchild	BSc (Dietetics)	HPCSA: Dietitian	Student: data collection, creating data input masks, capturing and cleaning of data, and performing statistics under supervision, writing of a dissertation.

HPCSA: Health Professions Council of South Africa

1.8 Structure of this mini-dissertation

Chapter one comprise of an introduction of the dissertation. The following literature review (Chapter two) provides an understanding of the prevalence of ID and anaemia, globally as well as in SA. The clinical significance of these prevalences during pregnancy will then be discussed, as well as the use of iron supplementation as a means of prevention thereof. The adverse effects, along with the interaction between n-3 PUFA status and iron supplementation will then be explored, using evidence from previous studies. Furthermore, possible effective ways of improving the safety of this supplementation will be provided. Chapter three describes the methodology used throughout the prospective longitudinal observational analysis. Chapter four is a manuscript with the title "Routine iron supplementation in iron-sufficient urban pregnant women in South Africa is associated with increased infectious morbidity and blood pressure". It was prepared for the Maternal and Child Health Journal and possible presentation of the results at national and international conferences. The dissertation ends with conclusions and recommendations in Chapter five.

1.9 References

- Allen, L.H. 2000. Anemia and iron deficiency: effects on pregnancy outcome. *The American Journal of Clinical Nutrition*, 71(5):1280s-1284s.
- Ali, H.A., Afifi, M., Abdelazim, A.M. & Mosleh, Y.Y. 2014. Quercetin and omega 3 ameliorate oxidative stress induced by aluminium chloride in the brain. *The Journal of Molecular Neuroscience*, 53(4):654-660.
- Barden, A.E., Mori, T.A., Dunstan, J.A., Taylor, A.L., Thornton, C.A., Croft, K.D., Beilin, L.J. & Prescott, S.L. 2004. Fish oil supplementation in pregnancy lowers F 2-isoprostanes in neonates at high risk of atopy. *Free Radical Research*, 38(3):233-239.
- Beard, J.L. 2000. Effectiveness and strategies of iron supplementation during pregnancy. *The American Journal of Clinical Nutrition*, 71(5):1288S-1294S.
- Brand, A., Schonfeld, E., Isharel, I. & Yavin, E. 2008. Docosahexaenoic acid-dependent iron accumulation in oligodendroglia cells protects from hydrogen peroxide-induced damage. *The Journal of Neurochemistry*, 105(4):1325-1335.
- Casanueva, E. & Viteri, F.E. 2003. Iron and oxidative stress in pregnancy. *The Journal of Nutrition*, 133(5):1700S-1708S.
- Department of Health. see South Africa. Department of Health
- Docosahexanoic acid antagonizes TNF-alpha-induced necroptosis by attenuating oxidative stress, ceramide production, lysosomal dysfunction, and autophagic features. *Inflammation Research*, 63(10):859-871.
- Idjradinata, P. & Pollitt, E. 1993. Reversal of developmental delays in iron-deficient anaemic infants treated with iron. *The Lancet*, 341(8836):1-4.
- Kones, R. 2010. Mitochondrial therapy for Parkinson's disease: neuroprotective pharmaconutrition may be disease-modifying. *Clinical Pharmacology*, 2:185-198.
- Lao, T.T., Chan, P.L. & Tam, K.F. 2001. Gestational diabetes mellitus in the last trimester—a feature of maternal iron excess? *Diabetic Medicine*, 18(3):218-223.

Lund, E.K., Wharf, S.G., Fairweather-Tait, S.J. & Johnson, I.T. 1999. Oral ferrous sulfate supplements increase the free radical-generating capacity of feces from healthy volunteers. *The American Journal of Clinical Nutrition*, 69(2):250-255.

Malan, L., Baumgartner, J., Calder, P.C., Zimmermann, M.B. & Smuts, C.M. 2015. n-3 Long-chain PUFAs reduce respiratory morbidity caused by iron supplementation in iron-deficient South African schoolchildren: a randomized, double-blind, placebo-controlled intervention. *The American Journal of Clinical Nutrition*, 101(3):668-79.

Malan, L., Baumgartner, J., Zandberg, L., Calder, P.C. & Smuts, C.M. 2016. Iron and a mixture of DHA and EPA supplementation, alone and in combination, affect bioactive lipid signalling and morbidity of iron deficient South African school children in a two-by-two randomised controlled trial. *Prostaglandins, Leukotrienes and Essential Fatty Acids (PLEFA)*, 105:15-25.

Milman, N. 2006. Iron prophylaxis in pregnancy—general or individual and in which dose? *Annals of Hematology*, 85(12):821-828.

Milman, N., Bergholt, T., Eriksen, L., Byg, K.E., Graudal, N., Pedersen, P. & Hertz, J. 2005. Iron prophylaxis during pregnancy—how much iron is needed? A randomized dose-response study of 20–80 mg ferrous iron daily in pregnant women. *Acta Obstetrica et Gynecologica Scandinavica*, 84(3):238-247.

Murray, M.J., Murray, A.B., Murray, M.B. & Murray, C.J. 1978. The adverse effect of iron repletion on the course of certain infections. *British Medical Journal*, 2(6145):1113-1115.

WHO. 2016. Micronutrient deficiencies: Iron deficiency anaemia. *Geneva, Switzerland: World Health Organization*.

O'Brien, K.O., Zavaleta, N., Caulfield, L.E., Yang, D.X. & Abrams, S.A. 1999. Influence of prenatal iron and zinc supplements on supplemental iron absorption, red blood cell iron incorporation, and iron status in pregnant Peruvian women. *The American Journal of Clinical Nutrition*, 69(3):509-515.

Pacheco, F.J., Almaguel, F.G., Evans, W., Rios-Colon, L., Filippov, V., Leoh, L.S., Rook-Arena, E., Mediavilla-Varela, M., De Leon, M. & Casiano, C.A. 2014. Docosahexanoic acid antagonizes TNF- α -induced necroptosis by attenuating oxidative stress, ceramide production, lysosomal dysfunction, and autophagic features. *Inflammation Research*, 63(10):859-871.

Sazawal, S., Black, R.E., Ramsan, M., Chwaya, H.M., Stoltzfus, R.J., Dutta, A., Dhingra, U., Kabole, I., Deb, S., Othman, M.K. & Kabole, F.M. 2006. Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community-based, randomised, placebo-controlled trial. *The Lancet*, 367(9505):133-143.

Scholl, T.O. 2005. Iron status during pregnancy: setting the stage for mother and infant. *The American Journal of Clinical Nutrition*, 81(5):1218S-1222S.

Shisana, O., Labadarios, D., Rehle, T., Simbayi, L., Zuma, K., Dhansay, A., Reddy, P., Parker, W., Hoosain, E., Naidoo, P., Hongoro, C., Mchiza, Z., Steyn, N.P., Dwane, N., Makoe, M., Maluleke, T., Ramlagan, S., Zungu, N., Evans, M.G., Jacobs, L., Faber, M. & SANHANES-1 Team. 2013. South African National Health and Nutrition Examination Survey (SANHANES-1). Cape Town: HSRC Press.

South Africa. Department of Health. 2015. Guidelines for maternity care in South Africa. A manual for clinics, community health centres and district hospitals. https://www.health-e.org.za/wp-content/uploads/2015/11/Maternal-Care-Guidelines-2015_FINAL-21.7.15.pdf Date of access: 2 Nov. 2016.

WHO (World Health Organization). 2002. The world health report 2002: reducing risks, promoting healthy life. http://www.who.int/entity/whr/2002/en/whr02_en.pdf?ua=1 Date of access: 3 Feb. 2017.

WHO (World Health Organization). 2008. Worldwide prevalence of anemia, WHO Vitamin and Mineral Nutrition Information System. www.who.int/nutrition/publications/micronutrients/PHNmay2008.pdf Date of access: 4 Feb. 2017.

WHO (World Health Organization). 2011. Serum ferritin concentrations for the assessment of iron status and iron deficiency in populations. www.who.int/vmnis/indicators/serum_ferritin.pdf Date of access: 17 Feb. 2017.

Ziaei, S., Norrozi, M., Faghihzadeh, S. & Jafarbegloo, E. 2007. A randomised placebo-controlled trial to determine the effect of iron supplementation on pregnancy outcome in pregnant women with haemoglobin ≥ 13.2 g/dl. *BJOG: An International Journal of Obstetrics and Gynaecology*, 114(6):684-688.

CHAPTER 2 LITERATURE REVIEW

2.1 Iron deficiency and anaemia

ID is defined as a decrease in total body iron to the extent that iron stores are depleted, and some degree of tissue ID is present (Cook, 2005; Skikne, *et al.*, 1990). ID can result due to many reasons, categorised into physiological and pathological causes. Physiological ID results due to an imbalance between the physiological requirements for iron and the maximal absorption of iron from the diet (Cook, 2005). Iron requirements are higher in premenopausal women due to loss of blood during menstruation. Iron requirements are also increased during pregnancy, where red blood cells (RBC) need additional iron to increase in mass, blood plasma needs to increase in volume, and developmental growth is necessary for the foetus and placenta (Yip, 2001). Blood loss may also be caused by the chronic use of non-steroidal anti-inflammatory drugs, which increase gastrointestinal (GI) blood loss. The risk of ID is also increased in premenopausal women who donate blood regularly (Cook, 2005). The pathological causes of ID involve excessive blood loss, which has been found to usually involve blood loss from the GI tract. This also includes the hindered ability of the GI tract to absorb iron. Another common cause is uterine blood loss, while more rare causes include pulmonary haemosiderosis and chronic haemoglobinuria (Cook, 2005).

Serum iron tests are performed to assess the quantity of iron carried in the blood, while a ferritin test can be used to evaluate current iron stores of the body (Brugnara *et al.*, 2013). Serum ferritin ≤ 15 $\mu\text{g/L}$, also in pregnancy, has been defined by the WHO as ID (WHO 2001). It is important that ferritin is corrected for inflammation, as inflammation causes an elevation in ferritin measurements. Thurnham *et al.* (2015) developed calculations using C-reactive protein (CRP) and $\alpha 1$ -acid glycoprotein (AGP) to adjust ferritin appropriately, according to the impact of inflammation.

ID may precede IDA, in which iron stores are absent, or ID can continue without progression (WHO, 2001). IDA is a more severe condition in which low levels of iron are associated with anaemia, along with the presence of microcytic hypochromic RBC (WHO, 2001). The WHO defines IDA as Hb ≤ 12 g/dL and ferritin ≤ 15 $\mu\text{g/L}$ (WHO, 2011). A major contributing factor of anaemia is ID (Shisana *et al.*, 2013). In a study of healthy South African adults, the prevalence of anaemia was 12.6%, and ID was found in 78% of anaemic subjects (Phatlhane *et al.*, 2016). Other causes of anaemia include micronutrient deficiencies (e.g. riboflavin, folate, vitamins A and B₁₂), infections and disorders regarding Hb synthesis, RBC production or RBC survival. The concentration of blood Hb is the most reliable screening indicator of anaemia in a public health setting (WHO, 2015). In clinical practice in SA, a low Hb at the initial screening then requires a

complete blood count (CBC) to determine haematocrit (Ht) and Hb (Gunther, 2008). It must be noted that these measures alone do not establish the cause of the anaemia (WHO, 2015). To adequately diagnose IDA, it is necessary to measure Hb or Ht response to iron administration, together with serum ferritin, erythrocyte protoporphyrin, transferrin saturation and transferrin receptor (WHO, 2001).

ID and IDA have many clinical manifestations which occur at various phases. These include the depletion of tissue iron, which results in decreased levels of serum ferritin, ferritin and hemosiderin in tissue macrophages and iron in bone marrow macrophages. There are also changes in serum iron, whereas it decreases and as unsaturated transferrin increases, total iron binding capacity increases. If iron stores continue towards depletion, anaemia develops. This results in impairment of Hb synthesis. The bone marrow, therefore, produces fewer RBC to maintain its iron stores. Furthermore, there is a reduced mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV). Lastly, tissue changes occur due to the decreasing levels of iron-dependent enzymes (Besa *et al.*, 1992).

2.2 Iron deficiency and anaemia in southern Africa

Anaemia is a pervasive and challenging nutritional problem worldwide (WHO, 2016). In 2002, IDA was considered to be one of the most important contributing factors to the global burden of disease (WHO, 2002). According to the SANHANES-1, the prevalence of anaemia in South Africans older than 15 years of age was 17.5%. Females had a prevalence of 22% and males 12.2%. In the SANHANES-1 report on the severity of anaemia among females aged 16 to 35 years (based on data from 2012), the prevalence of severe, moderate and mild anaemia was 1.2%, 10.1% and 11.8%, respectively. The mean Hb was 12.8 g/dL and prevalence of anaemia was 23.1%. It was also found that women living in KwaZulu-Natal had the highest prevalence of anaemia at 35.9% (Shisana *et al.*, 2013). It should be noted that these prevalences were based on a relatively small population size of 1359 females. SANHANES-1 also reported on the iron status of females of reproductive age, which found 5.9% to be iron depleted and 9.7% to have IDA. Urban informal areas had the highest IDA prevalence with 14.7%. In addition to these reports, a study done in the North Coast in KwaZulu-Natal found that 32% of girls aged 13 to 16 years had anaemia (Govender, 2016). Furthermore, similar to the study mentioned above, in healthy adults born in SA (n = 651), 39.8% had ID, with females and black Africans displaying the highest prevalence. In this retrospective study, the exact location within SA was not defined (Phatlhane *et al.*, 2016). This demonstrates that ID and anaemia are national public health concerns which need to be resolved with a multifactorial and multisectoral approach (Shisana *et al.*, 2013). Furthermore, ID and anaemia are variable throughout SA, making a uniform

treatment challenging and potentially dangerous (Mwangi *et al.*, 2017; Weinberg, 2009).

The WHO Global Prevalence of Anaemia in 2011 included data by country on prevalence of anaemia and mean Hb concentration. In these data, it is evident that the prevalence of anaemia extends to southern Africa as a whole. In Botswana, it was found that the mean Hb concentration for non-pregnant women aged 15 to 49 years was 12.7 g/dL, with 28% and 1.5% of these women with Hb concentrations less than 12 g/dL and 8 g/dL, respectively. In pregnant women from the same population, mean Hb concentration was 11.8 g/dL, with 32% and 0.5% of these women with Hb concentrations less than 11 g/dL and 7 g/dL, respectively. This was noted to be of moderate public health significance. Similar findings occurred in Zimbabwe, with a mean Hb concentration of 12.8 g/dL for non-pregnant women aged 15 to 49 years. 28% and 1.6% of these women had Hb concentrations less than 12 g/dL and 8 g/dL, respectively, whereas the respective percentages of Hb concentrations less than 12 g/dL and 8 g/dL in pregnant women were 34% and 0.5%. This too was noted as having moderate public health significance (WHO, 2015). In the Demographic and Health Survey of Zimbabwe (2005-2006), women aged 15 to 19 years, 20 to 29 years and 30 to 39 years had a prevalence of anaemia of 34.5%, 35.1% and 41.4% respectively. In pregnant women specifically, 47% were found to have anaemia, with 27.4% with mild anaemia, 18.1% with moderate anaemia and 1.5% with severe anaemia (CSO Zimbabwe, 2007). This demonstrates that although there has been an improvement regarding Zimbabwe's anaemia prevalence, there is still cause for concern. Other countries in southern Africa found an even greater anaemia prevalence, such as Mozambique, where 48% of pregnant women aged 15 to 49 years had Hb concentrations below 11 g/dL. This was labelled to be of severe importance for public health (WHO, 2015).

The prevalence of anaemia in SA and Botswana is similar, while in Zimbabwe and Mozambique it is much higher. The extent to which food fortification plays a role in this prevalence is still unknown. Food fortification programmes are mandatory for wheat flour and maize flour in SA, Zimbabwe and Mozambique, yet not in Botswana (Food Fortification Initiative, 2017). It may be of importance to investigate the effect of such programmes on ID and anaemia in southern Africa.

2.3 Clinical significance of iron deficiency and iron deficiency anaemia in pregnancy

With pregnant women having increased iron needs, due to the growing foetus and due to their increased erythrocyte mass, ID and IDA may become a health problem during pregnancy, even with adequate status before pregnancy (Milman *et al.*, 2005). It has been shown that maternal IDA can result in the infant being born prematurely, resulting in a LBW. Allen (2001) suggested

three possible ways that this may occur, namely infection, oxidative stress and hypoxia. Regarding infection, due to a decrease in immune functioning during pregnancy, there is a more extensive production of cytokines and prostaglandins, as well as an increased secretion of corticotropin-releasing hormone (CRH). The second possible mechanism, oxidative stress, may cause damage to the foetus, which may lead to an early delivery (Allen, 2001). ID may cause oxidative stress as it creates an increased susceptibility to oxidative damage in erythrocytes. This damage contributes to microcytic erythrocytes having a decreased survival period (Diez-Ewald & Layrisse, 1968; Vettore & Griffin, 1975). Additionally, excess globin chains autoxidise and release heme and produce superoxide at increased rates. The presence of IDA also results in lower concentrations of erythrocyte pyrimidine 5'-nucleotidase, the enzyme most sensitive to sulfhydryl group damage in vivo (Vives Corrons *et al.* 1995). Lastly, hypoxia may cause onset of a stress response, thereby releasing CRH. This happens because ID increases norepinephrine (NE) concentrations, which stimulates the secretion of CRH. Chronic hypoxia can result due to low Hb concentrations. This state can be worsened during pregnancy due to increased oxygen needs of the mother and foetus (Allen, 2001). Viteri (1994) stated that when the mother's Hb decreases, it is probable that oxygen transferred to the foetus is reduced.

There is also an association between maternal iron status and the infant's iron status (Allen, 2000). Furthermore, a study by Idjradinata and Pollitt (1993) found that infants with IDA had lower mental and motor development compared to infants without IDA. Along with this evidence, Allen (2001) concluded that maternal IDA increases the risk of preterm delivery and LBW. It is clear that maternal IDA has major adverse health impacts on the foetus. It is therefore vital to prevent this by improving maternal iron status, which is commonly achieved via the provision of iron supplements.

2.4 Supplementation of iron during pregnancy

Additional iron is only necessary when one's daily needs are not met via the combination of recycled iron from senescent RBC and the diet or if there is an increased need for iron. The absorption of iron depends on the availability and quality of iron in the diet and supplementation (Dainty *et al.*, 2014) and will, therefore, affect iron status. The initial standard supplemental dose of elemental iron for pregnant women was 60 mg, which was established in 1959 by the WHO. In 2012, the WHO published an article containing guidelines on the recommended daily iron and folic acid supplementation in pregnant women, suggesting 30 to 60 mg elemental iron daily. A dose of 150 mg of ferrous sulphate heptahydrate, 90 mg of ferrous fumarate or 250 mg of ferrous gluconate each delivers 30 mg of elemental iron. It was recommended to start these supplements early and to take them throughout pregnancy. The WHO also stated that the upper

range should be given in settings where anaemia during pregnancy is a severe public health problem (40% or higher). It was recommended that women diagnosed with anaemia in a clinical setting, should be treated with 120 mg elemental iron daily until their Hb levels are normal. They can then be given the standard dosage (WHO, 2012). However, according to The National Academies (2001), who composed the Dietary Reference Intakes, the tolerable upper-level intake for adults is 45 mg of iron per day. According to The National Academies, individuals treated with a higher dose of iron should be kept under strict medical supervision (The National Academies, 2001).

Currently, in SA, routine supplementation of iron (as well as calcium and folic acid) is provided to all pregnant women to prevent anaemia while attending primary health care. Women with an Hb ≥ 10 g/dL receive 200 mg ferrous sulphate daily, those with mild anaemia (Hb 8–9.9 g/dL) receive 200 mg ferrous sulphate three times daily, while moderate to severe anaemia cases (Hb ≤ 7.9 g/dL) are referred to a hospital or high-risk clinic for further investigation and treatment. This routine iron supplementation is provided without consideration of iron markers (DoH, 2015). The entire antenatal daily regime consists of 200 mg ferrous sulphate (equal to ~60 mg elemental iron), 5 mg folic acid and 1000 mg calcium carbonate (DoH, 2015).

2.5 Adverse effects of high iron status and supplementation during pregnancy

Although iron supplementation has been shown to improve the health of the mother during pregnancy as well as the health of the baby, adverse effects have also resulted. Large doses of iron via supplements when ID or anaemia is not present may be of concern. Not only do these adverse effects have an impact on the mother, but also the foetus and its development (Afkhami-Ardekani and Rashidi, 2007; Bloxam *et al.*, 1989; Bo *et al.*, 2009; Dawson *et al.*, 1989; Lachili *et al.*, 2001; Lao *et al.*, 2001; Murphy *et al.*, 1986; O'Brien *et al.*, 1999; Rehema *et al.*, 2004; Rush 2000; Sharifi *et al.*, 2010; Tripathi *et al.*, 2013; Viteri *et al.*, 2012; Zhou *et al.*, 2009; Ziaei *et al.*, 2007).

With regards to the foetus, the additional iron in the mother's stores may impair the utero-placental flow of blood due to the greater viscosity of the blood, or may result in other toxic responses (Rush, 2000). A retrospective cohort analysis by Scanlon *et al.* (2000) found that high Hb levels (higher than 14.4 g/dL) during the first and second trimester were associated with a greater risk (5–79%) of small gestational age (SGA), however, was not associated with a greater risk for premature delivery. Another study considered Ht as well as Hb, and found that a high Ht was negatively correlated with placental and birth weight (Hemminki & Rimpela, 1991). This study provided routine and selective iron supplements to non-anaemic women. The

particular group only received iron when Hb decreased below 100 g/L or Ht decreased below 30% after 33 weeks gestation, for two consecutive visits. Iron supplementation consisted of 100 mg elemental iron administered from at least 16 weeks gestation until delivery (Hemminki & Rimpela, 1991). A study by Goldenberg *et al.* (1996) measured the relationship between plasma ferritin, along with Ht, preterm delivery and birth weight. Plasma ferritin is widely used as a convenient measure to assess iron stores of the body, specifically iron overload and deficiency (Finch *et al.*, 1986). It was found that Ht had no significant correlation with preterm delivery or birth weight, but high plasma ferritin was strongly associated with these two outcomes. This association was found to be greatest at 26 weeks gestation. All women in this study were given a daily multivitamin-mineral supplement containing iron (Goldenberg *et al.*, 1996). Another study also measuring serum ferritin during pregnancy, found that high serum ferritin (≥ 41.5 $\mu\text{g/L}$) at week 28, which after that increased further (compared to those with declined ferritin), was associated with preterm and very preterm delivery, LBW, chorioamnionitis and markers of maternal infection (Scholl, 1998). This study did not mention if the women received iron supplementation. Both studies, Goldenberg *et al.* (1996) and Scholl (1998) did not state whether or not ferritin was corrected for inflammation, which may result in inaccurate markers of iron status.

A further concern includes women infected with HIV. A study in Nigeria, done in 80 HIV-positive patients, found that iron supplementation contributed to the derangement in iron metabolism. Additionally, serum iron was negatively correlated with CD4 cells. This study compared newly diagnosed HIV-positive patients, at different stages of the infection, with 50 sero-negative controls. Concentrations of serum total iron, transferrin, total iron binding capacity (TIBC), transferrin saturation and number of CD4 cells were estimated. It was concluded to discourage supplementation with iron in HIV infection (Banjoko *et al.*, 2012). These findings agreed with Traore and Meyer (2004), who found that iron overload associated with HIV infection is detrimental to the host, whilst benefiting the pathogen. An overview by Gordeuk *et al.* (2001) investigated clinical studies regarding iron status and HIV infection, and also concluded that high iron status may have an adverse influence on HIV-infected patients. When women take antiretroviral (ARV) treatment containing protease inhibitors, however, maternal tissue iron depletion often results (Widen *et al.*, 2015). Widen *et al.* (2015) concluded that further randomised controlled trials are necessary to investigate the effects of extended ARV treatment on iron status. This would provide insight regarding appropriate provision of ARV treatment, alongwith iron supplementation, for pregnant women (Widen *et al.*, 2015).

Numerous studies have investigated the effect of increased iron markers on maternal health. This literature review will focus on morbidity in pregnant women, specifically GDM, hypertension and oxidative stress, as well as decreased zinc absorption, which may contribute to increased

infectious morbidity. Studies which have looked at these three outcomes have been summarised in Table 2.

2.5.1 High iron status, supplementation and gestational diabetes mellitus during pregnancy

Limited studies have investigated iron supplementation in relation to GDM. Three case-control studies described adverse effects in groups taking iron supplementation (Lao *et al.*, 2001; Sharifi *et al.*, 2010; Bo *et al.*, 2009). These studies varied in daily elemental iron dosages: 29 mg, 50 mg and the other which is unknown (Table 2). Bo *et al.* (2009) did not report on iron status. They assumed the usual hospital practice of iron supplementation with 60 to 100 mg elemental iron to treat IDA and no supplementation provided to prevent IDA. Studies by Lao *et al.* (2001) and Sharifi *et al.* (2010) were done in pregnant women who were non-anaemic at the start of the study. These three studies had similar durations of iron supplement provision, starting from mid-pregnancy until birth. The findings showed that women who developed GDM during pregnancy had a greater s-ferritin or were taking iron supplementation. DeFronzo (1988) speculated that excess iron could affect insulin production and secretion, and increase lipid oxidation. This causes decreased glucose utilisation in the muscles and increased gluconeogenesis in the liver, which can result in liver mediated insulin resistance (DeFronzo, 1988).

A study agreeing with these results was done by Afkhami-Ardekani and Rashidi (2007), which compared the iron status of women with and without GDM, at 24 to 28 weeks gestation. This case-control study identified an association between GDM and increased iron status. The group with GDM had significantly higher concentrations of plasma ferritin, transferrin saturation and Hb, iron, MCH and MCV, with significantly lower TIBC. Familial history of diabetes and GDM had no significant association (Afkhami-Ardekani and Rashidi, 2007). These results may suggest that high iron status and stores may play a role in the development of GDM.

2.5.2 High iron status, supplementation and hypertension during pregnancy

Along with GDM, studies investigating the association between hypertension and iron intake during pregnancy have also been done (Ziaei *et al.*, 2007; Murphy *et al.*, 1986; Bo *et al.*, 2009). According to the statement from the International Society for the Study of Hypertension in Pregnancy (Brown *et al.*, 2001), four categories of hypertension exist: preeclampsia, chronic hypertension, gestational hypertension (also known as pregnancy-induced hypertension (PIH))

and preeclampsia superimposed on chronic hypertension. Preeclampsia and gestational hypertension start after 20 weeks gestation, while chronic hypertension is present before conception or during the first half of pregnancy. Preeclampsia superimposed on chronic hypertension is diagnosed when new symptoms of preeclampsia appear after 20 weeks gestation, in women with chronic hypertension (Brown *et al.*, 2001).

The observational study by Murphy *et al.* (1986) and the case-control study by Bo *et al.* (2009) did not specify the dosage of daily iron received. Bo *et al.* (2009) did not report anaemia or iron status before the intervention. However, the majority of subjects in the study by Murphy *et al.* (1986) had an initial Hb of between 10.4 and 13.2 g/dL. Ziaei *et al.* (2007) did a randomised controlled trial and reported pregnancy outcomes in women with Hb ≥ 13.2 g/dL prior to receiving iron supplementation of 50 mg elemental iron daily. The incidence of hypertensive disorder during pregnancy was greater in those receiving iron supplementation or those with an increased Hb, by 35% (Murphy *et al.*, 1986), 16.1% (Bo *et al.*, 2009) and 1.9% (Ziaei *et al.*, 2007). Murphy *et al.* (1986) suggested to avoid routine iron supplementation for mothers whose Hb does not decrease early in pregnancy.

2.5.3 Iron supplementation and oxidative stress during pregnancy

Another adverse consequence due to excess iron intake is increased oxidative stress. Pregnancy favours oxidative stress, due to the high concentration of mitochondria as well as iron in the placenta (Casanueva & Viteri, 2003). There is, therefore, an increase in the production of free radicals during pregnancy. Oxidative stress is greatest beyond the second trimester, with free radical damage having a greater impact on the development of gestational hypertension, insulin resistance and GDM in the third trimester (Casanueva & Viteri, 2003). Due to intestinal mucosal cells being exposed to unabsorbed excess non-heme iron, it is essential for the dose of supplemental iron to be carefully considered as to avoid free radical damage (Casanueva & Viteri, 2003).

A few studies found an increased measure of oxidative stress in pregnant women receiving iron supplementation compared to those not receiving supplements (Tripathi *et al.*, 2013; Lachili *et al.*, 2001; Rehema *et al.*, 2004; Viteri *et al.*, 2012). Tripathi *et al.* (2013) measured lipid peroxidation in pregnant women who were non-anaemic or mildly anaemic prior to receiving supplementation. Supplementation was then provided for 2 to 4 weeks, containing 100 mg elemental iron. Findings showed that lipid peroxidation was higher in the supplemented group (9.76 ± 0.05 nmol/g Hb) compared to the unsupplemented group (8.89 ± 0.27 nmol/g Hb). It was also found that antioxidant enzymes, namely superoxide dismutase (SOD) and catalase (CAT)

declined more in the supplemented group than in the unsupplemented group (Tripathi *et al.*, 2013). Lachili *et al.* (2001) also strongly suggested that supplemental non-heme iron is a pro-oxidant. This study gave pregnant women, with a normal Hb, 100 mg fumarate iron (combined with vitamin C) during the last trimester of pregnancy. A considerable increase in thiobarbituric acid reactive substances (TBARS) was found in the supplemented group ($3.62 \pm 0.56 \mu\text{mol/l}$) compared to the unsupplemented group ($3.01 \pm 0.37 \mu\text{mol/l}$). Viteri *et al.* (2012) also reported an increase in TBARS when supplementation was given as 60 mg iron daily or 120 mg iron weekly, starting from 20 weeks gestation. Women in this study were also non-anaemic prior to receiving supplementation. An increase in TBARS only resulted when supplementation was given compared to the unsupplemented control group (Viteri *et al.*, 2012). Lastly, a study by Rehema *et al.* (2004) showed that women receiving iron had elevated levels of oxidised glutathione. Glutathione disulphide (GSSG) measures changed from $81.5 \pm 28.4 \mu\text{g/ml}$ to $152.4 \pm 41.1 \mu\text{g/ml}$ ($p = 0.001$) after receiving 36 mg ferrous iron daily for four weeks (Rehema *et al.*, 2004).

2.5.4 Iron supplementation and decreased zinc absorption during pregnancy

Zinc plays an important role in the immune system, and people with a zinc deficiency have an increased susceptibility to various pathogens (WHO, 1996; Shankar & Prasad, 1998). Multiple randomised controlled trials have found that iron supplementation resulted in decreased zinc absorption in pregnant women (O'Brien, 1999; Dawson *et al.*, 1989; Bloxam *et al.*, 1989). In the study by O'Brien (1999), subjects were grouped to receive daily antenatal supplements, either 60 mg of iron as ferrous sulphate and 250 μg of folic acid, or the same quantities of iron and folic acid with an additional 15 mg of zinc as zinc sulphate, while others received no supplementation. The supplements were administered starting from 10 to 24 weeks gestation until delivery. Subjects receiving iron had a zinc absorption of 26.5% lower than those not receiving iron supplementation ($p < 0.05$). It is evident that even though zinc supplementation was received, zinc absorption remained more poorly absorbed. There was also a significant positive relationship between maternal plasma zinc concentrations and zinc concentrations in the cord blood (O'Brien, 1999).

Another study with congruent results involved the administration of multivitamins containing 18 mg iron daily to pregnant teenagers from 20 to 38 weeks of gestation, until 12 weeks postpartum (Dawson *et al.*, 1989). Findings showed that those receiving iron had a 35% decrease in plasma zinc concentrations from pre-study to the end of the third trimester ($p \leq 0.02$), whereas no statistically significant changes were found in those not receiving iron (Dawson *et al.*, 1989). Bloxam *et al.* (1989) did a similar study finding the zinc concentration in

the supplemented group to be $8.9 \pm 0.4 \mu\text{mol/l}$ while the control group was $10.1 \pm 0.4 \mu\text{mol/l}$ ($p = 0.006$) after 12 weeks. Daily supplementation was also in the form of a multivitamin, containing 94 mg of elemental iron as ferrous sulphate, while the control group received the same multivitamin without iron (Bloxam *et al.*, 1989).

Lower levels of plasma zinc may be associated with pre-eclampsia (Memon *et al.*, 2017; Zhu *et al.*, 2015). Pre-eclampsia is one of the most significant causes of increased maternal and perinatal morbidity as well as mortality (Memon *et al.*, 2017). A study by Memon *et al.* (2017) found that the serum zinc concentration among women with pre-eclampsia was lower than women with a normal blood pressure ($p < 0.001$). This study used 40 case and 40 control subjects to measure serum zinc concentrations of each group, with gestational age falling within the range of 31 to 40 weeks. Scheplyagina's study (2005) found a powerful negative correlation ($r = -0.93$) between serum zinc concentrations and the rate of disease exacerbation of the duodenum and stomach, the transformation of thyroid gland goitres and allergopathies of the respiratory system.

2.5.5 Iron supplementation and morbidity symptoms

2.5.5.1 Side effects

It is known that side effects such as heartburn, constipation, diarrhoea, tiredness, headaches, nausea and vomiting occur throughout pregnancy (Zhou *et al.*, 2009). However, similar side effects may be enhanced as a result of iron supplementation, although these studies were mainly performed in children (Paganini *et al.*, 2017; Malan *et al.*, 2015; Kumar *et al.*, 2009). Zhou *et al.* (2009) did a study where women in mid-pregnancy were randomly assigned to receive 20, 40 or 80 mg of daily elemental iron for eight weeks. The incidence of GI symptoms in the group receiving 80 mg iron was significantly higher than the group receiving 20 mg iron. When comparing the 20 mg group to the 80 mg group, the odds ratio for nausea, stomach pain and vomiting was 0.4 ($p = 0.014$), 0.3 ($p = 0.005$) and 0.4 ($p = 0.023$), respectively. Other symptoms such as heartburn, hard stools, less than three bowel movements per week and feeling unwell were experienced by a larger portion of women in the group receiving 80 mg iron compared to the group receiving 20 mg iron. Although the higher dose improved iron status more effectively than other groups, it did not statically differ in improving the incidence of anaemia (Zhou *et al.*, 2009).

2.5.5.2 Infectious morbidity symptoms

It is important to differentiate between pregnancy side effects and infectious morbidity, since infectious morbidity such as respiratory and GI infections are also potential effects of iron supplementation (Paganini *et al.*, 2017; Malan *et al.*, 2015; Kumar *et al.*, 2009). A review by Mwangi *et al.* (2017) reported that infections result from the movement of iron from circulation into intracellular storage. GI infections usually causes diarrhoea and may also cause symptoms such as nausea and vomiting (Malan *et al.* 2015, Kumar *et al.*, 2009). Respiratory infections may cause coughing and a running nose. Other morbidity symptoms include fever, which could be associated with extreme tiredness and headaches (Malan *et al.*, 2015).

Although few studies have investigated iron and morbidity symptoms during pregnancy, and no studies could be found that investigated infectious morbidity symptoms, several studies have been performed in children and infants (Filteau *et al.*, 2010; Malan *et al.*, 2015; Paganini *et al.*, 2017). The Chilenge Infant Growth, Nutrition and Infection Study (CIGNIS) gave 6-month old infants a porridge which was fortified with a micronutrient containing 12.5 mg iron. These infants had a greater incidence of lower respiratory tract infections ($p = 0.02$) (Filteau *et al.*, 2010). Another study gave infants micronutrient powders (MNP) containing 2.5 mg iron as sodium iron EDTA. These infants had a significantly greater number of days experiencing coughing and dyspnoea (Barth-Jaeggi *et al.*, 2015). In addition to these studies with infants, research was done on schoolchildren by de Gier *et al.* (2016). This study gave three types of micronutrient-fortified rice, containing approximately 8 mg iron in each portion, to schoolchildren. These children had a significantly increased risk of new hookworm infection (de Gier *et al.*, 2016). Prentice *et al.* (2016) assumed that these findings were as a result of iron which caused the pathology, rather than the other micronutrients, due to the known role of iron in microorganisms. Malan *et al.* (2015) found that iron supplementation in children increased the number of days of illness caused by respiratory symptoms. Agreeing with these results, Paganini *et al.* (2017) reported that infants receiving a high dose of daily iron supplementation may have increased respiratory tract infections, enteropathogens and diarrhoea.

Other possible contributors to the incidence of infectious morbidity may be oxidative stress and decreased zinc absorption. As previously discussed, oxidative stress and decreased zinc absorption have been investigated during pregnancy, even though infectious morbidity has not. An increase in oxidative stress results in an increase in inflammation. This can be followed by an immunosuppressed state, increasing one's susceptibility to secondary infection (Biswas, 2016; Calder, 2007). Zinc is an essential micronutrient required in adaptive and innate immune responses. The homeostasis of zinc is vital to ensure proper functioning of the immune system (Gammoh and Rink, 2017). With decreased zinc absorption during pregnancy resulting from

iron supplementation, pregnant women may be more susceptible to infectious morbidity (Gammoh and Rink, 2017).

Table 2: Studies of iron supplementation and adverse effects in pregnant women

Author, year	Adverse effect	Study design	Country	n	Gestational age	Iron status prior to receiving iron supplements	Dosage of iron supplemented	Duration of supplement provision	Findings & statistical significance
Tripathi <i>et al.</i> , 2013	Oxidative stress	Case-control	India	• 90 pregnant women (30 in each Trimester T1, T2, T3). T2 & T3 divided into supplemented & unsupplemented groups. • Control group = 50 non-pregnant women	Trimester 1, 2 or 3	Non-anaemic / mildly anaemic (Hb > 10g/dL)	• Supplemented group = 100 mg elemental (and 500 µg folic acid) daily during Trimester 2 and 3, not Trimester 1 • Unsupplemented group = nothing • Control group (non-pregnant) = nothing	2-4 weeks	• MDA (the measure of lipid peroxidation) increased more throughout pregnancy in the supplemented group (9.76 ± 0.05 nmol/g Hb) compared to the unsupplemented group (8.89 ± 0.27 nmol/g Hb), $p < 0.05$. • SOD and CAT (antioxidant enzymes) declined steadily throughout pregnancy more in the supplemented group (SOD = 811.62 ± 14.10 ig/g Hb; CAT = 7.77 ± 0.14 ig/g Hb) as compared to the unsupplemented group (SOD = 857.36 ± 13.35 ig/g Hb; CAT = 8.22 ± 0.13 ig/g Hb), both $p < 0.05$. • FRAP (a marker of overall antioxidant capacity) increased steadily throughout pregnancy in both the supplemented and unsupplemented groups, but was not significantly different between both groups.
Lachili <i>et al.</i> , 2001	Oxidative stress	Randomised controlled trial	Algeria	• Supplemented group = 27 pregnant women • Unsupplemented group = 27 pregnant women			• Supplemented group = 100 mg ferrous fumarate (and 500 mg of vitamin C as ascorbate) daily • Control group = nothing	Trimester 3 of pregnancy	• Increase in TBARS plasma levels in the supplemented group (3.62 ± 0.56 µmol/l) compared to the unsupplemented group (3.01 ± 0.37 µmol/l), $p < 0.005$. • Increase in TBARS/cholesterol in supplemented group (1.53 ± 0.43 µmol/g) compared to the unsupplemented group (1.23 ± 0.24 µmol/g), $p < 0.005$. This strongly suggests a pro-oxidant role for iron supplementation.

Author, year	Adverse effect	Study design	Country	n	Gestational age	Iron status prior to receiving iron supplements	Dosage of iron supplemented	Duration of supplement provision	Findings & statistical significance
Rehema <i>et al.</i> , 2004	Oxidative stress	Randomised controlled trial	Estonia	- Supplemented group = 13 pregnant women - Unsupplemented group = 6 pregnant women		Hb between 9.7 and 11.1 g/dL (borderline anaemia)	- Supplemented group = 36 mg ferrous iron daily - Unsupplemented group = nothing	4 weeks	The group receiving iron had significantly different values for GSSG after the intervention compared to the unsupplemented group (152.4 ± 41.1 µg/ml compared to 105.3 ± 24.8 µg/ml, $p = 0.02$). GSSG in the supplemented group before intervention was 81.5 ± 28.4 µg/ml, and changed to 152.4 ± 41.1 µg/ml after the intervention ($p = 0.001$). The glutathione system is important in cellular defence, therefore in oxidative stress this may be clinically significant.
Viteri <i>et al.</i> , 2012	Oxidative stress	- Randomised controlled trial - Cross-over	Mexico	- Daily-weekly group = 43 pregnant women - Weekly-daily group = 41 pregnant women	First hospital consultation at ≤ 20 weeks gestation	Non-anaemic, 30% iron-deficient at week 20	- Daily-weekly group = daily tablet containing 60 mg elemental iron, 0.2 mg folic acid, and 1 µg vitamin B12, then weekly supplementation with two tablets (providing 120 mg elemental iron, 0.4 mg folic acid, and 2 µg vitamin B12). - Weekly-daily group = the scheme was inverted, first weekly then daily.	Gestational week 20 to 28, then to week 36 for the inverted scheme	Oxidative stress (indicated by elevated plasma TBARS concentration) occurred only when supplements were taken daily, therefore weekly supplementation appeared to be safer. Serum iron and TBARS was directly and significantly correlated at 36 weeks gestation ($r = 0.59$; $p < 0.05$). Serum iron and TBARS were not statistically significant at other gestational ages.
Ziaei <i>et al.</i> , 2007	Hypertension	Randomised, double-blind, placebo-controlled trial	Iran	- Supplemented group = 370 pregnant women - Placebo group = 357 pregnant women	Early stage of Trimester 2	Non-anaemic (Hb ≥ 13.2 g/dl)	- Supplemented group = Ferrous sulphate tablet containing 50 mg elemental iron daily - Placebo group = placebo in similar wrapping, daily	Throughout pregnancy	Hypertensive disorder was greater in the supplemented group (2.7%) compared to the placebo group (0.8%), $p = 0.05$.

Author, year	Adverse effect	Study design	Country	n	Gestational age	Iron status prior to receiving iron supplements	Dosage of iron supplemented	Duration of supplement provision	Findings & statistical significance
Murphy <i>et al.</i> , 1986	Hypertension	Observational	Wales	54 382 pregnant women	≤24 weeks	Majority Hb between 10.4 and 13.2 g/dL	No supplementation		There was a strong relation between booking Hb and subsequent frequency of hypertension ($p < 0.001$). Frequency of subsequent hypertension ranged from 7% at Hb values < 10.5 g/dl to 42% at Hb concentrations > 14.5 g/dl, in primiparas.
Lao <i>et al.</i> , 2001	GDM	• Prospective • Case control	Hong Kong	• With GDM = 97 pregnant women • Non-diabetic controls = 194 pregnant women	Women with an antenatal booking before 20 weeks gestation	Non-anaemic	Multivitamin preparation containing 29 mg of elemental iron offered to all patients daily (compliance not monitored)	From 20 weeks of gestation until delivery	Women with GDM had a greater s-ferritin (47.4 vs 22.5 pmol/l, $p = 0.0000$), s-iron (14.9 vs 12.6 umol/l, $p = 0.0073$), and transferrin saturation (22.0 vs 17.2% , $p = 0.0004$) than controls without GDM. The OGTT 2-h (8.9 ± 0.9 vs 6.4 ± 0.9 mmol/l) and fasting (4.5 ± 0.5 vs 4.3 ± 0.4 mmol/l, $p = 0.0000$) values were higher in the GDM group.
Sharifi <i>et al.</i> , 2010	GDM	• Prospective • Case control	Iran	• With GDM = 64 pregnant women • Non-diabetic controls = 64 pregnant women	24-28 weeks gestation	No IDA (Baseline Hb GDM 12.8 ± 0.8 g/dL, Control 12.5 ± 0.58 g/dL)	50 mg elemental iron daily (continued by all, based on national policy)	After 4th month of pregnancy until delivery	Women with GDM had a ferritin concentration of 112 ± 28.4 pmol/L, whilst women without GDM had a ferritin concentration of 65 ± 16.9 pmol/L, $p < 0.001$. There was a positive correlation between ferritin concentration and fasting glucose during mid-pregnancy. Women in the highest ferritin quartile had a more than two-fold increased GDM risk. No significant correlation existed between ferritin concentrations and early postpartum OGTT measures.

Author, year	Adverse effect	Study design	Country	n	Gestational age	Iron status prior to receiving iron supplements	Dosage of iron supplemented	Duration of supplement provision	Findings & statistical significance
Bo <i>et al.</i> , 2009	GDM and hypertension	Case-control	Italy	• With GDM = 500 pregnant women • Non-diabetic controls = 500 pregnant women	Mid-pregnancy (Screened at 24-28 weeks gestation)	Iron status and Hb levels not available.	The most common dosage was 525 mg ferrous sulphate daily (105 mg of elemental iron).	Iron supplementation duration was retrieved from the obstetric clinical records, which ranged between 2-10 weeks.	Iron supplementation (taken by 212/1000 women) was significantly associated with increased insulin resistance, hyperglycaemia and metabolic syndrome. GDM in iron supplemented women was 70.8%, while in non-supplemented it was 44.4% ($p < 0.001$). Hypertension in iron supplemented women was 25.9%, whilst in non-supplemented it was 9.8% ($p < 0.001$).
Afkhami-Ardekani and Rashidi, 2007	GDM	Case-control	Iran	• With GDM = 34 pregnant women • Non-diabetic controls = 34 pregnant women	24-28 weeks				The group with GDM had significantly higher concentrations of s-ferritin (73.34 ± 31.73 vs. 41.55 ± 28.31 ng/ml, $p < 0.001$), transferrin saturation ($26.49 \pm 5.94\%$ vs $12.77 \pm 5.67\%$, $p < 0.001$) and Hb (13.39 ± 1.10 vs. 11.75 ± 1.43 g/dl, $p < 0.001$), s-iron (100.44 ± 22.09 vs 56.85 ± 23.03 µg/dl, $p < 0.001$), MCH (46.94 vs. 22.06 pg) and MCV (85.34 ± 10.80 vs. 77.69 ± 6.46 fl, $p = 0.001$). TIBC was significantly lower in the GDM group (383.09 ± 30.55 vs. 457.79 ± 58.20 µg/dl in the control group, $p < 0.001$).
O'Brien <i>et al.</i> , 1999	Decreased zinc absorption	Randomised controlled trial	Peru	• Iron supplemented = 16 pregnant women • Iron & zinc supplemented = 14 pregnant women • No supplement = 17 pregnant women	During Trimester 3 (33 ± 1 weeks gestation)	• Hb of iron supplemented group = 11.18 ± 1.34 g/dL • Hb of iron & zinc supplemented group = 11.46 ± 1.08 g/dL	• Iron supplemented = 60 mg iron as ferrous sulphate and 250 µg of folate daily • Iron & zinc supplemented = Iron and folic acid as above, with additional 15 mg Zn sulphate daily • No supplementation	From 10–24 weeks of pregnancy until delivery	Fasting women taking iron supplements had a decreased zinc absorption compared to those who did not receive iron supplements (20.5 ± 6.4 vs 20.2 ± 4.6 vs. $47.0 \pm 12.6\%$ absorption for iron, iron & zinc and control groups respectively, $p < 0.0001$). Plasma zinc concentrations were lower in the iron supplemented group than the control group (8.2 ± 2.2 vs. 9.2 ± 2.2 vs. 10.9 ± 1.8 µmol/L for iron, iron & zinc and control groups respectively, $p = 0.002$). It was also found that cord zinc concentrations were significantly related to levels of maternal plasma zinc ($r = 0.486$, $p = 0.002$).

Author, year	Adverse effect	Study design	Country	n	Gestational age	Iron status prior to receiving iron supplements	Dosage of iron supplemented	Duration of supplement provision	Findings & statistical significance
Dawson <i>et al.</i> , 1989	Decreased zinc absorption	Randomised controlled trial	Texas	• Iron supplemented = 20 pregnant teenagers • Non-iron supplemented = 21 pregnant teenagers	8-17 weeks (Average: 13 weeks)	Hb > 11 g/dL or Ht > 0.33. • s-Iron of iron supplemented group = 13 ± 2 $\mu\text{mol/L}$ (Mean $\pm$ SEM) • s-Iron of non-iron supplemented group = 16 ± 2 $\mu\text{mol/L}$ (Mean $\pm$ SEM)	• Iron supplemented = multivitamin with 18 mg iron daily • Non-iron supplemented = multivitamin without iron, daily	From 20 to 38 weeks	In trimester 3, subjects receiving iron supplementation showed a 35% decrease in zinc concentration from pre-study ($p \leq 0.02$). Adjusted serum zinc in the group receiving iron decreased from pre-study until 32, 38, 40 weeks gestation, by 35% ($p < 0.02$), 30% ($p < 0.05$) and 34% ($p < 0.02$) respectively. No significant changes in serum zinc occurred in the group not receiving iron.
Bloxam <i>et al.</i> , 1989	Decreased zinc absorption	Randomised controlled trial	London	• Iron supplemented = 14 pregnant women • Control group = 8 pregnant women	16 weeks	Hb > 10.4 g/dl	• Iron supplemented = multivitamins with 94 mg iron as ferrous sulphate daily • Control group = multivitamins without iron, daily	During trimester 2 and 3	Oral iron supplementation during pregnancy decreased maternal plasma zinc concentration. Zn concentrations of the control group after 12 weeks was 10.1 ± 0.4 $\mu\text{mol/l}$, whereas the supplemented group was 8.9 ± 0.4 $\mu\text{mol/l}$, $p = 0.006$.

Abbreviations: CAT, catalase, FRAP, ferric reducing antioxidant power, GDM, gestational diabetes mellitus, GSSG, glutathione disulphide, Hb, haemoglobin, MCH, mean corpuscular haemoglobin, MDA, malondialdehyde, OGTT, oral glucose tolerance test, SEM, standard error of the mean, SOD, superoxide dismutase, T, Trimester, TBARS, thiobarbituric acid reactive substances, TIBC, total iron-binding capacity.

2.6 Omega-3 fatty acid status and iron supplementation

Along with the adverse effects resulting from an excess iron intake, the interaction between n-3 PUFA and iron could also be of importance. Dietary sources of n-3 PUFA include marine sources, fish oil supplements, and certain vegetable oils, such as flaxseed, canola and soybean (CanolaInfo, 2015). n-3 PUFA such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) play a vital role in the central nervous system, the structure of human cells, as well as the immune response (Greenberg *et al.*, 2008; Malan *et al.*, 2015). Within immune cells, fatty acids have various functions. These include the provision of fuel for energy, modification of protein structure, regulation of gene expression, forming part of structural components of cell membranes and acting as precursors to synthesise bioactive lipid mediators (Calder, 2008). The role of n-3 PUFA transcends to the growth and development of the foetus. Adequate fatty acid intake during pregnancy as well as lactation is therefore critical (Mennitti *et al.*, 2015).

Studies investigating interactions between n-3 PUFA and iron are limited, especially human studies. A study by Smuts (1995) found that iron status improved with n-3 PUFA supplementation in South African children aged 6 to 10 years in KwaZulu-Natal. This supplementation consisted of 11 ml of oil, which contained evening primrose, sunflower and high DHA fish oil and vitamin E, given for six months. There was a decline in the prevalence of children with Hb < 11 g/dl and with ferritin < 20 ng/ml in the group receiving the oil mixture, compared to the control group which received sunflower oil. This could indicate an improved utilisation of iron in children receiving the DHA oil mixture rather than sunflower oil alone (Smuts, 2012). Similar findings were reported by Jamieson *et al.* (2013), where a higher n-3 PUFA intake was associated with a lower risk of iron depletion. Jamieson *et al.* (2013) found this association in food insecure women in Arctic Canada in a cross-sectional study. It was concluded that the anti-inflammatory properties of long-chain polyunsaturated fatty acids (LCPUFA) might be an important factor for the iron status in this population (Jamieson *et al.*, 2013).

Furthermore, Zaman *et al.* (2013) did a study investigating the fatty acid desaturase activity in young women receiving iron supplementation. It is known that iron is involved in the desaturation process of fatty acids (Berg *et al.*, 2002). In Zaman *et al.*'s study, the supplemented group received 37,4 mg elemental iron daily, for 12 weeks, while the control group received no iron supplements. It was found that fatty acid and desaturase activities were not significantly affected by the iron supplementation. However, a non-statistically significant positive correlation was found between changes in serum ferritin and changes in fatty acid $\Delta 6$ -desaturation activity in this group. These fatty acid desaturation activities

ultimately lead to an increase in longer chain n-3 PUFA (Zaman *et al.*, 2013). In agreement with this association mentioned above are the findings of Smuts *et al.* (1995). Smuts *et al.* (1995) demonstrated increased concentrations of n-3 PUFA in RBC membranes, after consuming soup fortified with iron. It should be noted that this study was done in malnourished children who showed symptoms of ID, therefore a normal iron status might not yield similar results. In addition to this, a cross-sectional study of the general population of Slovakia found that subjects with low iron levels had significantly reduced $\Delta 6$ - and $\Delta 5$ -desaturase activity (Krajčovičová-Kudláčková *et al.*, 2004).

A study regarding iron and n-3 PUFA supplementation was performed by Malan *et al.* (2015), specifically investigating morbidity in iron-deficient schoolchildren in SA. It was found that morbidity, mainly respiratory morbidity, increased in the group receiving iron supplements, but was prevented when iron was given along with 420 mg DHA and 80 mg EPA for 8.5 months, four times a week (Malan *et al.*, 2015). Malan *et al.* (2016) also concluded that supplementation with DHA/EPA changed the immune modulator profile to being anti-inflammatory. However, with supplementation of iron, a pro-inflammatory profile resulted. The combination of DHA/EPA and iron was found to maintain the anti-inflammatory effect (Malan *et al.*, 2016). This demonstrates that n-3 PUFA supplementation may be beneficial and reduce respiratory morbidity through its anti-inflammatory properties.

2.7 Strategies to improve the safety of iron supplementation in pregnancy

It is evident that various aspects need to be considered when giving iron supplementation to pregnant women. These include the type of iron, the frequency, duration and dosage of supplementation, as well as iron and possibly n-3 PUFA status. Viteri *et al.* (2012) did a study to compare oxidative stress resulting from daily versus weekly iron supplementation in non-anaemic women in Mexico. The study involved 100 pregnant women at 20 weeks gestation. Iron supplements were given daily for eight weeks, then weekly for the same duration, or in the reversed order. The tablet received daily contained 60 mg of elemental iron, while the weekly supplementation regime consisted of two of these tablets. Findings showed that TBARS, an indicator of oxidative stress, increased significantly with the daily supplementation, along with increased s-ferritin, iron and Hb near term. Weekly supplementation appeared to be safer, as there was no excessive increase of iron markers, yet still anaemia was prevented, and TBARS were decreased (Viteri *et al.*, 2012). The British Committee for Standards in Haematology reported that researchers have examined taking supplementation on alternate days or weekly. This was done to prevent the constant exposure to excess unabsorbed iron and oxidation experienced by the mucosal cells of the

intestine. It appeared that this intermittent regime was as effective as the daily regime (Pavord *et al.*, 2011) and may also be a strategy to reduce side effects.

Along with considering the frequency of taking iron supplements, the dosage of iron is also important. Makrides *et al.* (2013) assessed the effects on IDA and ID of pregnant women receiving a daily placebo or 20 mg of iron as ferrous sulphate (4 mg elemental iron), as opposed to the current WHO-recommended dose of 30 to 60 mg of elemental iron per day (WHO, 2006). The effect of the 20 mg ferrous sulphate on iron status was determined at time of delivery and six months postpartum. Side effects were also considered and included nausea, vomiting, heartburn, stomach pain, rashes, bowel movements and zinc concentrations. It was found that these side effects were not significantly different between the iron supplemented group and the placebo group. Makrides *et al.* (2013) concluded that a daily supplement with 20 mg ferrous sulphate, initiated from 20 weeks gestation to delivery, is effective for prevention of ID and IDA without additional side effects.

Furthermore, it may be beneficial to provide iron supplementation along with other compounds, apart from n-3 PUFA as discussed in a previous section. Baqui *et al.* (2003) investigated this phenomenon in infants in Bangladesh, providing iron and zinc supplementation weekly. They evaluated the incidence of acute lower respiratory infection and diarrhoea. Various experimental groups received different supplement combinations, with the intermittent simultaneous supplementation of iron and zinc giving the most promising results. This consisted of 20 mg iron and 20 mg zinc provided weekly for six months. This group was associated with 19% lower risk of severe diarrhoea overall and 30% lower risk in infants with a lower nutritional status (weight-for-age Z-score less than -1). Furthermore, infants of a lower nutritional status had 40% less risk of severe acute lower respiratory infection (Baqui *et al.*, 2003). As previously discussed, the provision of iron supplementation decreases the absorption of zinc (O'Brien, 1999; Dawson *et al.*, 1989; Bloxam *et al.*, 1989). It may therefore be necessary to counteract this by increasing zinc intake.

The addition of prebiotic galacto-oligosaccharides (GOS) to iron-containing MNP was investigated by Paganini *et al.* (2017). This was a randomised controlled trial in 155 Kenyan infants done for four months. Subjects were grouped and received daily supplementation of either a MNP containing 5 mg iron (consisting of 2.5 mg as sodium iron ethylenediaminetetraacetate and 2.5 mg as ferrous fumarate), or the same MNP containing iron plus 7.5g GOS, or MNP with no iron. It was found that anaemia was reduced in infants taking the MNP containing iron. It was also found that the provision of GOS decreased the adverse effects, commonly caused by iron, on the gut microbiome as well as morbidity

(Paganini *et al.*, 2017).

Also, the method of iron administration may affect the safety of supplemented iron. Bhavi and Jaju (2017) found that administration of daily intravenous iron sucrose had a greater effect in increasing maternal iron stores, compared to daily 200 mg ferrous fumarate received orally. The group receiving intravenous iron showed an increase in Hb by 22 ± 11.5 g/L, compared to 12 ± 9.1 g/L in the group receiving oral iron, both after four weeks of treatment (Bhavi & Jaju, 2017). Like Bhavi & Jaju (2017), Al *et al.* (2005) also found that treating IDA of pregnancy with intravenous iron resulted in faster and more efficient restoration of iron stores, compared to oral iron. Intravenous iron treatment was also said to cause "no serious adverse reactions" (Al *et al.*, 2005). Bayoumeu *et al.* (2002) did a similar study but did not find significant results regarding Hb between the two groups. It was, however, concluded that iron sucrose appears not to have serious side effects when administered to treat anaemia during pregnancy or to treat depletion of iron stores (Bayoumeu *et al.*, 2002).

In the United Kingdom, routine iron supplements are not recommended. Pregnant women are screened for IDA, to possibly predict adverse birth outcomes. This is ideally done by testing serum ferritin levels during the first trimester. If this is found to be below the cut-off range, iron supplementation may be recommended, along with advice on how to increase iron and its absorption via the diet (Alwan, 2015). Agreeing with this approach, Murphy *et al.* (1986) stated that it "would seem reasonable to avoid routine prescription of iron to mothers who do not show the physiological fall in Hb early in pregnancy".

The fall in Hb early in pregnancy may influence the effect of administered iron supplementation. This provides reason to investigate the initial Hb status of pregnant women. Sloan *et al.* (2002) did a meta-analysis to review the efficacy of iron supplementation on Hb concentrations in pregnant women in developing countries. It was found that the effect on these women was greater (by 1.13 ± 0.120 g/dL) if their initial Hb was < 11 g/dL, compared to those not receiving iron supplements ($p < 0.001$). Initial Hb between 10 and 11 g/dL had an effect of 1.10 ± 0.045 g/dL ($p < 0.001$), while initial Hb between 11 and 12 g/dL had an effect of 0.85 ± 0.018 g/dL ($p < 0.01$), when compared to women receiving no iron supplementation. Data were not available for initial Hb ≥ 12 g/dL. In developed countries, initial Hb between 11 and 12, and ≥ 12 was 1.17 ± 0.022 g/dL ($p < 0.01$) and 1.16 ± 0.045 ($p < 0.001$) g/dL greater than measures in the supplemented women, respectively (Sloan *et al.*, 2002). Alizadeh and Salehi (2016) specifically investigated iron supplementation in pregnant women with Hb > 13.2 g/dL at 16 to 20 weeks gestation. The experimental group received a daily supplement containing 50 mg of elemental iron, while

the control received a placebo, until delivery. The Hb levels in the two groups were significantly different ($p = 0.03$), yet there was no difference in incidence of anaemia between the two groups. These data support the idea that iron status should be considered prior to the provision of maternal iron supplementation (Alizadeh & Salehi, 2016).

Therefore, a range of aspects exist which influence the effect of iron supplementation on the body during pregnancy. Provision of iron supplementation becomes challenging, as there is a need to prevent ID and IDA, yet safety of these supplements also need to be examined. Further research is necessary to determine the safest strategy to improve iron status, and this approach will probably also have to be suited to each specific setting. This study aims to find the relation between iron status at < 18 weeks gestation and morbidity when considering iron supplementation among urban pregnant women in SA. Currently, in SA, 60 mg of elemental iron is routinely provided to all pregnant women from their first antenatal visit (usually before 18 weeks gestation) until delivery. This study will investigate the association of iron status and anaemia in early pregnancy with morbidity, including blood pressure/hypertension, fasting glucose/GDM, side effects and infectious morbidity symptoms. Compliance of the women to take the supplements will be taken into consideration. Results from this study should give a better indication of the possible adverse effects of iron supplementation when taken by women with ID or anaemia, compared to those with an optimal iron status in urban SA.

2.8 References

- Afkhami-Ardekani, M. & Rashidi, M. 2009. Iron status in women with and without gestational diabetes mellitus. *Journal of Diabetes and its Complications*, 23(3):194-198.
- Al, R.A., Unlubilgin, E., Kandemir, O., Yalvac, S., Cakir, L. & Haberal, A. 2005. Intravenous versus oral iron for treatment of anemia in pregnancy: a randomized trial. *Obstetrics and Gynecology*, 106(6):1335-1340.
- Alizadeh, L. & Salehi, L. 2016. Is routine iron supplementation necessary in pregnant women with high hemoglobin? *Iranian Red Crescent Medical Journal*, 18(1).
- Allen, L.H. 2001. Biological mechanisms that might underlie iron's effects on fetal growth and preterm birth. *The Journal of Nutrition*, 131(2):581S-589S.
- Alwan, N.A., Cade, J.E., McArdle, H.J., Greenwood, D.C., Hayes, H.E. & Simpson, N.A. 2015. Maternal iron status in early pregnancy and birth outcomes: insights from the Baby's Vascular health and Iron in Pregnancy study. *British Journal of Nutrition*, 113(12):1985-1992.
- Anon. 2017. Food Fortification Initiative, Africa.
http://www.ffinetwork.org/regional_activity/africa.php Date of access: 17 Oct. 2017.
- Banjoko, S.O., Oseni, F.A., Togun, R.A., Onayemi, O., Emma-Okon, B.O. & Fakunle, J.B. 2012. Iron status in HIV-1 infection: implications in disease pathology. *BioMed Central Clinical Pathology*, 12(1):26.
- Barth-Jaeggi T., Moretti D. & Kvalsvig J. 2015. In-home fortification with 2.5 mg iron as NaFeEDTA does not reduce anaemia but increases weight gain: a randomised controlled trial in Kenyan infants. *Maternal and Child Nutrition*, 11:151–162.
- Baqui, A.H., Zaman, K., Persson, L.A., El Arifeen, S., Yunus, M., Begum, N. & Black, R.E. 2003. Simultaneous weekly supplementation of iron and zinc is associated with lower morbidity due to diarrhea and acute lower respiratory infection in Bangladeshi infants. *The Journal of Nutrition*, 133(12):4150-4157.

Bayoumeu, F., Subiran-Buisset, C., Baka, N.E., Legagneur, H., Monnier-Barbarino, P. & Laxenaire, M.C. 2002. Iron therapy in iron deficiency anemia in pregnancy: intravenous route versus oral route. *American Journal of Obstetrics and Gynecology*, 186(3):518-522.

Berg J.M., Tymoczko J.L., Stryer L. 2002. Elongation and unsaturation of fatty acids are accomplished by accessory enzyme systems. New York, NY: W.H. Freeman.
<https://www.ncbi.nlm.nih.gov/books/NBK22401/> Date of access: 31 July 2017.

Besa, E.C., Catalano, P.M., Kant, J.A. & Jefferies, L.C. 1992. Hematology. National medical series for independent study.

Bhavi, S.B. & Jaju, P.B. 2017. Intravenous iron sucrose v/s oral ferrous fumarate for treatment of anemia in pregnancy: a randomized controlled trial. *BMC Pregnancy and Childbirth*, 17(1):137.

Biswas, S.K. 2016. Does the interdependence between oxidative stress and inflammation explain the antioxidant paradox? *Oxidative Medicine and Cellular Longevity*, 2016(2016):9.

Bloxam, D.L., Williams, N.R., Waskett, R.J., Pattinson-Green, P.M., Morarji, Y. & Stewart, S.G. 1989. Maternal zinc during oral iron supplementation in pregnancy: a preliminary study. *Clinical Science*, 76(1):59-65.

Bo, S., Menato, G., Villois, P., Gambino, R., Cassader, M., Cotrino, I. & Cavallo-Perin, P. 2009. Iron supplementation and gestational diabetes in midpregnancy. *American Journal of Obstetrics and Gynecology*, 201(2):158-e1.

Brown, M.A., Lindheimer, M.D., de Swiet, M., Assche, A.V. & Moutquin, J.M. 2001. The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy.
<https://doi.org/10.3109/10641950109152635> Date of access: 13 June 2017.

Brugnara, C., Adamson, J., Auerbach, M., Kane, R., Macdougall, I. & Mast, A. 2013. Iron deficiency: what are the future trends in diagnostics and therapeutics? *Clinical Chemistry*, 59(5):740-745.

CanolaInfo. 2015. Canola oil information.
<http://www.canolainfo.org/health/index.php?page=12> Date of access: 10 Aug. 2017.

Calder, P.C. 2007. Immunonutrition in surgical and critically ill patients. *British Journal of Nutrition*, 98(S1):S133-S139.

Calder, P.C. 2008. The relationship between the fatty acid composition of immune cells and their function. *Prostaglandins, Leukotrienes and Essential Fatty Acids (PLEFA)*, 79(3):101-108.

Casanueva, E. & Viteri, F.E. 2003. Iron and oxidative stress in pregnancy. *The Journal of Nutrition*, 133(5):1700S-1708S.

Central Statistical Office (CSO) Zimbabwe. 2007. Macro International Inc. Zimbabwe Demographic and Health Survey 2005-06. Calverton, MD.

Cook, J.D. 2005. Diagnosis and management of iron-deficiency anaemia. *Best Practice & Research Clinical Haematology*, 18(2):319-332.

Dainty, J.R., Berry, R., Lynch, S.R., Harvey, L.J. & Fairweather-Tait, S.J. 2014. Estimation of dietary iron bioavailability from food iron intake and iron status. *PLoS One*, 9(10):e111824.

Dawson, E.B., Albers, J. & McGanity, W.J. 1989. Serum zinc changes due to iron supplementation in teen-age pregnancy. *The American Journal of Clinical Nutrition*, 50(4):848-852.

de Gier B., Ponce M.C. & Perignon M. 2016. Micronutrient-fortified rice can increase hookworm infection risk: a cluster randomized trial. *PLoS One*. 11:e0145351.

DeFronzo, R.A. 1988. The triumvirate: β -cell, muscle, liver. A collusion responsible for NIDDM. *Diabetes*, 37(6):667-687.

Diez-Ewald, M. & Layrisse, M. 1968. Mechanisms of hemolysis in iron deficiency anemia. *Further Studies. Blood*, 32:884-894.

Filteau, S., Kasonka, L., Gibson, R., Gompels, U.A., Jaffar, S., Kafwembe, E., Monze, M., Sinkala, M., Tomkins, A., Zulu, R. & Chisenga, M. 2010. Micronutrient fortification to

improve growth and health of maternally HIV-unexposed and exposed Zambian infants: a randomised controlled trial. *PloS One*, 5(6):e111165.

Finch, C.A., Stray, S., Huebers, H.A., Bellotti, V., Lipschitz, D.A., Cook, J.D. & Pippard, M.J. 1986. Plasma ferritin determination as a diagnostic tool. *Western Journal of Medicine*, 145(5):657.

Gammoh, N.Z. & Rink, L. 2017. Zinc in Infection and Inflammation. *Nutrients*, 9(6):624.

Goldenberg, R.L., Tamura, T., DuBard, M., Johnston, K.E., Copper, R.L. & Neggers, Y. 1996. Plasma ferritin and pregnancy outcome. *American Journal of Obstetrics and Gynecology*, 175(5):1356-1359.

Gordeuk, V.R., Delanghe, J.R., Langlois, M.R. & Boelaert, J.R. 2001. Iron status and the outcome of HIV infection: an overview. *Journal of Clinical Virology*, 20(3):111-115.

Govender, T. 2016. The impact of access to antenatal care on maternal health outcomes among young adolescents on the North coast of KwaZulu-Natal, South Africa. KwaZulu-Natal: DUT. (Dissertation - Masters).

Greenberg, J.A., Bell, S.J. & Van Ausdal, W. 2008. Omega-3 fatty acid supplementation during pregnancy. *Reviews in Obstetrics and Gynecology*, 1(4):162.

Gunther, L. 2008. Diagnosing and managing iron deficiency anaemia in adults: A practical, clinical approach to iron deficiency anaemia. *Continuing Medical Education*, 26(5):232-234.

Hemminki E., & Rimpela U. 1991. Iron supplementation, maternal packed cell volume and fetal growth. *Arch Dis Child*, 66:422–425.

Idjradinata, P. & Pollitt, E. 1993. Reversal of developmental delays in iron-deficient anaemic infants treated with iron. *The Lancet*, 341(8836):1-4.

Jamieson, J.A., Kuhnlein, H.V., Weiler, H.A. & Egeland, G.M. 2013. Higher n3-fatty acid status is associated with lower risk of iron depletion among food insecure Canadian Inuit women. *BMC Public Health*, 13(1):289.

Krajčovičová-Kudláčková, M., Klvanova, J. & Dušinská, M. 2004. Polyunsaturated fatty acid plasma content in groups of general population with low vitamin B6 or low iron serum levels. *Annals of Nutrition and Metabolism*, 48(2):118-121.

Kumar, N., Chandhiok, N., Dhillon, B.S. & Kumar, P. 2009. Role of oxidative stress while controlling iron deficiency anemia during pregnancy - Indian scenario. *Indian Journal of Clinical Biochemistry*, 24:5–14.

Lachili, B., Hininger, I., Faure, H., Arnaud, J., Richard, M.J., Favier, A. & Roussel, A.M. 2001. Increased lipid peroxidation in pregnant women after iron and vitamin C supplementation. *Biological Trace Element Research*, 83(2):103-110.

Lao, T.T., Chan, P.L. & Tam, K.F. 2001. Gestational diabetes mellitus in the last trimester— a feature of maternal iron excess? *Diabetic Medicine*, 18(3):218-223.

Makrides, M., Crowther, C.A., Gibson, R.A., Gibson, R.S. & Skeaff, C.M. 2003. Efficacy and tolerability of low-dose iron supplements during pregnancy: a randomized controlled trial. *The American Journal of Clinical Nutrition*, 78(1):145-153.

Malan, L., Baumgartner, J., Calder, P.C., Zimmermann, M.B. and Smuts, C.M. 2015. n–3 Long-chain PUFAs reduce respiratory morbidity caused by iron supplementation in iron-deficient South African schoolchildren: a randomized, double-blind, placebo-controlled intervention. *The American Journal of Clinical Nutrition*, 081208:1-12.

Malan, L., Baumgartner, J., Zandberg, L., Calder, P.C. & Smuts, C.M. 2016. Iron and a mixture of DHA and EPA supplementation, alone and in combination, affect bioactive lipid signalling and morbidity of iron deficient South African school children in a two-by-two randomised controlled trial. *Prostaglandins, Leukotrienes and Essential Fatty Acids (PLEFA)*, 105:15-25.

Memon, A.R., Memon, F.W., Akram, M., Memon, P.J. & Rahman, I. 2017. Association of serum zinc level with pre eclampsia. *Journal of the Liaquat University of Medical and Health Sciences*, 16(1):58-61.

Mennitti, L.V., Oliveira, J.L., Morais, C.A., Estadella, D., Oyama, L.M., do Nascimento, C.M.O. & Pisani, L.P. 2015. Type of fatty acids in maternal diets during pregnancy and/or

lactation and metabolic consequences of the offspring. *The Journal of Nutritional Biochemistry*, 26(2):99-111.

Milman, N., Bergholt, T., Eriksen, L., Byg, K.E., Graudal, N., Pedersen, P. & Hertz, J. 2005. Iron prophylaxis during pregnancy—how much iron is needed? A randomized dose–response study of 20–80 mg ferrous iron daily in pregnant women. *Acta Obstetrica et Gynecologica Scandinavica*, 84(3):238-247.

Murphy, J.F., Newcombe, R.G., O'riordan, J., Coles, E.C. & Pearson, J.F. 1986. Relation of haemoglobin levels in first and second trimesters to outcome of pregnancy. *The Lancet*, 327(8488):992-995.

Mwangi, M.N., Prentice, A.M. & Verhoef, H. 2017. Safety and benefits of antenatal oral iron supplementation in low-income countries: a review. *British Journal of Haematology*, 177:884-895.

O'Brien, K.O., Zavaleta, N., Caulfield, L.E., Wen, J. & Abrams, S.A. 2000. Prenatal iron supplements impair zinc absorption in pregnant Peruvian women. *The Journal of Nutrition*, 130(9):2251-2255.

Paganini, D., Uyoga, M.A., Kortman, G.A., Cercamondi, C.I., Moretti, D., Barth-Jaeggi, T., Schwab, C., Boekhorst, J., Timmerman, H.M., Lacroix, C. & Karanja, S. 2017. Prebiotic galacto-oligosaccharides mitigate the adverse effects of iron fortification on the gut microbiome: a randomised controlled study in Kenyan infants. *Gut*, 66:1956–67.

Pavord, S., Myers, B., Robinson, S., Allard, S., Strong, J. & Oppenheimer, C. 2012. UK guidelines on the management of iron deficiency in pregnancy. *British Journal of Haematology*, 156(5):588-600.

Phatlhane, D.V., Zemlin, A.E., Matsha, T.E., Hoffmann, M., Naidoo, N., Ichihara, K., Smit, F. & Erasmus, R.T. 2016. The iron status of a healthy South African adult population. *Clinica Chimica Acta*, 460:240-245.

Prentice, A.M., Mendoza, Y.A., Pereira, D., Cerami, C., Wegmuller, R., Constable, A. & Spieldenner, J. 2016. Dietary strategies for improving iron status: balancing safety and efficacy. *Nutrition reviews*, 75(1):49-60.

Rehema, A., Zilmer, K., Klaar, U., Karro, H., Kullisaar, T. & Zilmer, M. 2004. Ferrous iron administration during pregnancy and adaptational oxidative stress (Pilot study). *Medicina (Kaunas)*, 40(6):547-552.

Rush, D. 2000. Nutrition and maternal mortality in the developing world. *The American Journal of Clinical Nutrition*, 72(1):212s-240s.

Scanlon K.S., Yip R., Schieve L.A., & Cogswell M.E. 2000. High and low haemoglobin levels during pregnancy: differential risks for preterm birth and small for gestational age. *Obstetrics and Gynecology*, 96:741–748.

Scholl TO. 1998. High third-trimester ferritin concentration: associations with very preterm delivery, infection, and maternal nutritional status. *Obstetrics and Gynecology*, 92:161–165.

Shankar, A.H. & Prasad, A.S. 1998. Zinc and immune function: the biological basis of altered resistance to infection. *The American Journal of Clinical Nutrition*, 68(2):447S-463S.

Sharifi, F., Ziaee, A., Feizi, A., Mousavinasab, N., Anjomshoaa, A. & Mokhtari, P. 2010. Serum ferritin concentration in gestational diabetes mellitus and risk of subsequent development of early postpartum diabetes mellitus. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, 3:413.

Schepliyagina, L.A. 2005. Impact of the mother's zinc deficiency on the woman's and newborn's health status. *Journal of Trace Elements in Medicine and Biology*, 19(1):29-35.

Shisana, O., Labadarios, D., Rehle, T., Simbayi, L., Zuma, K., Dhansay, A., Reddy, P., Parker, W., Hoosain, E., Naidoo, P., Hongoro, C., Mchiza, Z., Steyn, N.P., Dwane, N., Makoae, M., Maluleke, T., Ramlagan, S., Zungu, N., Evans, M.G., Jacobs, L., Faber, M. & SANHANES-1 Team. 2013. South African National Health and Nutrition Examination Survey (SANHANES-1). [www.hsrb.ac.za/uploads/pageNews/72/SANHANES-launch%20edition%20\(online%20version\).pdf](http://www.hsrb.ac.za/uploads/pageNews/72/SANHANES-launch%20edition%20(online%20version).pdf) Date of access: 7 July 2016.

Skikne, B.S., Flowers, C.H. & Cook, J.D. 1990. Serum transferrin receptor: a quantitative measure of tissue iron deficiency. *Blood*, 75(9):870-1876.

Sloan, N.L., Jordan, E. & Winikoff, B. 2002. Effects of iron supplementation on maternal hematologic status in pregnancy. *American Journal of Public Health*, 92(2):288-293.

Smuts, C.M. 2012. Morbidity data from 1999 fish oil intervention in rural primary school children in KwaZulu-Natal (personal communication).

Smuts, C.M., Tichelaar, H.Y., Van Jaarsveld, P.J., Badenhorst, C.J., Kruger, M., Laubscher, R., Mansvelt, E.P.G. & Benade, A.J.S. 1995. The effect of iron fortification on the fatty acid composition of plasma and erythrocyte membranes in primary school children with and without iron deficiency. *Prostaglandins, Leukotrienes and Essential Fatty Acids (PLEFA)*, 52(1):59-67.

South Africa. Department of Health. 2015. Guidelines for maternity care in South Africa. A manual for clinics, community health centres and district hospitals. https://www.health-e.org.za/wp-content/uploads/2015/11/Maternal-Care-Guidelines-2015_FINAL-21.7.15.pdf Date of access: 2 Nov. 2016.

The National Academies. 2001. Dietary Reference Intakes. National Academies Press. <http://www.nap.edu/catalog/10026.html> Date of access: 19 June 2017.

Thurnham, D.I., Northrop-Clewes, C.A. & Knowles, J. 2015. The use of adjustment factors to address the impact of inflammation on vitamin A and iron status in humans. *The Journal of Nutrition*, 145(5):1137S-1143S.

Traore H.N. & Meyer D. 2004. The effect of iron overload on in vitro HIV-1infection. *Journal of Clinical Virology*, 31(1):S92–S98.

Tripathi, R., Gupta, S., Rai, S. & Mittal, P.C. 2013. Short-term, high-dose iron supplementation to healthy pregnant women increases oxidative stress markers: Implications for use of phytonutrients. *Annals of Phytomedicine*, 2(2):71-78.

Vettore, L. & Griffin, T.C. 1975. Membrane lipid peroxidation in hypochromic red blood cells. *Haematologica*, 60(2):250-250.

Viteri, F.E. 1994. The consequences of iron deficiency and anemia in pregnancy. (*In* Allen L., King J., Lönnerdal., ed. Nutrient regulation during pregnancy, lactation, and infant growth. Boston: Springer. p. 127-139).

Viteri, F.E., Casanueva, E., Tolentino, M.C., Díaz-Francés, J. & Erazo, A.B. 2012. Antenatal iron supplements consumed daily produce oxidative stress in contrast to weekly supplementation in Mexican non-anemic women. *Reproductive Toxicology*, 34(1):125-132.

Vives Corrons, J. L., Miguel-García, A., Pujades, M. A., Miguel-Sosa, A., Cambiazzo, S., Linares, M., Dibarrart, M. T. & Calvo, M. A. 1995. Increased susceptibility of microcytic red blood cells to in vitro oxidative stress. *European Journal of Haematology*, 55:327-331.

Weinberg, E.D. 2009. Are iron supplements appropriate for iron replete pregnant women? *Medical Hypotheses*, 73(5):714-715.

WHO (World Health Organization). 2001. Iron deficiency anaemia: assessment, prevention and control: a guide for programme managers.

http://www.who.int/iris/bitstream/10665/66914/1/WHO_NHD_01.3.pdf?ua=1 Date of access: 29 Jan. 2017

WHO (World Health Organization). 2006. Iron and folate supplementation. Standards for maternal and neonatal care. *Geneva, Switzerland: World Health Organization*

WHO (World Health Organization). 2011. Serum ferritin concentrations for the assessment of iron status and iron deficiency in populations.

www.who.int/vmnis/indicators/serum_ferritin.pdf Date of access: 17 Feb. 2017.

WHO (World Health Organization). 2012. *Guideline: daily iron and folic acid supplementation in pregnant women. Geneva, Switzerland: World Health Organization.*

WHO (World Health Organization). 2015. The global prevalence of anaemia in 2011. *Geneva, Switzerland: World Health Organization.*

WHO (World Health Organization). 2016. Micronutrient deficiencies: Iron deficiency anaemia. *Geneva, Switzerland: World Health Organization.*

Widen, E.M., Bentley, M.E., Chasela, C.S., Kayira, D., Flax, V.L., Kourtis, A.P., Ellington, S.R., Kacheche, Z., Tegha, G., Jamieson, D.J. & Van Der Horst, C.M. 2015. Antiretroviral treatment is associated with iron deficiency in HIV-infected Malawian women that is mitigated with supplementation, but is not associated with infant iron deficiency during 24

weeks of exclusive breastfeeding. *Journal of Acquired Immune Deficiency Syndromes*, 69(3):319.

Yip R. 2001. Iron. (In Bowman, B., Russell, R.M., ed. Present knowledge in nutrition. 8th ed. Washington: ILSI Press. p 311–318).

Zaman, K., McArthur, J.O., Abboud, M.N., Ahmad, Z.I., Garg, M.L., Petocz, P. & Samman, S. 2013. Iron supplementation decreases plasma zinc but has no effect on plasma fatty acids in non-anemic women. *Nutrition Research*, 33(4):272-278.

Zhou S.J., Gibson R.A., Crowther C.A., & Makrides M. 2009. Should we lower the dose of iron when treating anaemia in pregnancy? A randomized dose-response trial. *European Journal of Clinical Nutrition*, 63(2):183-191.

Zhu, Q., Zhang, L., Chen, X., Zhou, J., Liu, J. & Chen, J. 2016. Association between zinc level and the risk of preeclampsia: a meta-analysis. *Archives of Gynecology and Obstetrics*, 293(2):377-382.

Ziaei, S., Norrozi, M., Faghihzadeh, S. & Jafarbegloo, E. 2007. A randomised placebo-controlled trial to determine the effect of iron supplementation on pregnancy outcome in pregnant women with haemoglobin $\geq$ 13.2 g/dl. *BJOG: An International Journal of Obstetrics and Gynaecology*, 114(6):684-688.

CHAPTER 3 METHODOLOGY

3.1 Research design and methods

This study is nested within the Nutrition during Pregnancy and Early Development (NuPED) study. The nested study is a prospective observational analysis. The study started with the recruitment of pregnant women (at <18 weeks of gestation) attending antenatal care (ANC) facilities in selected clinics within the COJ, Gauteng. The women were then referred for ANC at Rahima Moosa Mother and Child Hospital (RMMCH) for first phase data collection and further follow-up visits at 22 and 36 weeks of pregnancy, and birth.

3.1.1 Study population and sample

The study population was urban pregnant women attending ANC facilities in selected clinics within the COJ, which complied with the inclusion and exclusion criteria listed in Table 3-1. Human immunodeficiency virus (HIV) positive participants were also included in the study. The inclusion and exclusion criteria were dependent on the NuPED study. For this study, it was particularly important that existing hypertensive women or women with diabetes were excluded, regardless of the disease being controlled or not. Consecutive sampling was applied, thus, all available subjects at the recruitment site formed part of the sample.

Table 3-1: Summary of inclusion and exclusion criteria at sampling

Inclusion criteria	Exclusion criteria
Confirmed pregnancy and planning to deliver baby at RMMCH < 18 weeks gestational age	> 18 weeks gestational age
Born in SA, Lesotho, Swaziland, Zimbabwe, Botswana or Namibia	< 18 years and > 39 years
Able to communicate effectively in one of the following languages: English; Afrikaans; Sotho; Zulu or Xhosa	Multiple pregnancy
HIV-positive or HIV-negative	Women using illicit drugs (self-confessed)
	Smoking (current and/or in past year)
	Known non-communicable diseases such as diabetes, renal disease, history of high blood cholesterol, and hypertension
	Known infectious diseases such as tuberculosis (TB) and hepatitis
	Known serious illness such as cancer, lupus or psychosis.

3.1.1.1 Sample size calculation

For this nested study, the first 100 participants of the NuPED study were included, excluding women with prevalent hypertension or diabetes at enrolment. The number of subjects to sample from the clinics was calculated for the purpose of the NuPED study considering birth outcomes. This was done by means of a priori sample size calculation in the G*Power 3.1.9.2 statistical programme (Faul *et al.*, 2007). The statistical calculation involved was the multiple linear regression: fixed model, single regression coefficient. The calculation was based on a small effect size F^2 of 0.05; a probability of error (alpha) of 5%; a power of 80% and ten predictors on the birth outcome “LBW”. The result was that 160 subjects were required, which was adjusted to 250 to allow for a 30% rate of participant dropout and to improve power.

3.1.2 Recruitment and informed consent

The nursing operational managers of the identified clinics acted as mediators during the recruitment period with the approval of all necessary authorities. The first step in the recruitment was performed by the nursing staff (acting as mediators) informing the group of patients in the waiting area of the clinic about the project (after appropriate training). A detailed information brochure about the project, written in simple language (English, Afrikaans, Sotho, Xhosa and Zulu), was then handed out. Those eligible and interested were able to go to the allocated private room where the details of the study were explained by the fieldworker in a one-on-one setup. Inclusion and exclusion criteria were then checked (see Table 3-1). Any subjects who wanted to participate in the study but did not qualify in terms of the inclusion and exclusion criteria, received a pamphlet and a short description of the importance of healthy habits during pregnancy. Those eligible and interested then received a further detailed explanation with the informed consent form. They were given two options if they were interested to voluntarily participate: 1) they were given time alone or with any person of their choice to consider signing the written informed consent form; 2) they could return home with the informed consent form to discuss with those they wish to and return it on the next appointment. Nursing staff, the fieldworker(s) and a research team member were available for any questions. A few short questions were asked before signing the informed consent form to ensure the respondent understood the information supplied.

3.1.3 Research procedures and data collection

The study sample was assessed four times throughout pregnancy (see Table 3-2 for

details). Participants were recruited from the clinics and were referred for ANC at RMMCH for the duration of the study. Participants were reimbursed for the additional travelling at a standard amount. In the first phase subjects were assessed in early pregnancy (< 18 weeks) at RMMCH. The second phase of data collection was done at 22 weeks gestation, the third phase at 36 weeks gestation and the fourth phase at birth. The recruitment and data collection process is displayed in Figure 3.

Table 3-2: Schedule of study activities during pregnancy

Phase	1 ^a	1	2	3	4
Approximate gestational age	< 18 weeks	< 18 weeks	22 weeks	36 weeks	Birth
Window			± 1 week	± 1 week	
Informed consent	X				
Medical history		X	X ^b	X ^b	X
Demographics		X			
Inclusion/Exclusion		X			
Anthropometrical measurements^c		X ^d	X	X	
24-hour recall, including supplementation information		X	X	X	
Past week morbidity & supply sheets		X	X	X	X
Blood sample		X	X		
Blood pressure		X	X	X	X
OGTT			X ^e		
Reimburse for travelling		X	X	X	
Summarise pregnancy complications/adverse effects					X
Token of appreciation				X	

a: Recruitment was done at four different primary health care clinics: Florida clinic; Zandspruit clinic; Bosmont clinic; Sophiatown clinic as well as at the antenatal care clinic of RMMCH.

b: A medical history from Maternity Case Record was used to determine adverse outcomes or change in health status

c: Anthropometrical measurements included: height; weight; MUAC

d: Height was measured only with visit 1.

e: Subjects were in a fasting state and were scheduled between 24 and 28 weeks gestation

OGTT: Oral Glucose Tolerance Test

MUAC: Mid-Upper Arm Circumference

3.1.3.1 Data collection at different phases during pregnancy

All fieldworkers were trained females with additional training for data collection purposes. The study co-ordinators were responsible for overseeing the safe storage and transport of samples.

3.1.3.1.1 Medical history

Medical history was recorded in phases 1, 2, 3 and 4. It included the development of hypertension and diabetes, TB, HIV infection, bacterial vaginosis and smoking during pregnancy, as well as number of births. It was done as part of a routine medical assessment by the physicians at the RMMCH. The NuPED study had permission from the participants and ethics committees to transfer the stated hospital's medical data from the patients' medical files.

3.1.3.1.2 Morbidity data

Morbidity data were collected using a symptoms diary, as seen in Annexure A, and for this study was collected from enrolment until birth.

3.1.3.1.3 Anthropometrical data

Anthropometrical data, including weight, height and mid-upper arm circumference (MUAC) were collected at phase 1, 2 and 3. The procedures were performed according to the National Health and Nutrition Examination Survey (NHANES) Anthropometry Procedures Manual 2007.

3.1.3.1.4 Diet history

Diet history included a 24-hour recall, including supplement use. The 24-hour recall questionnaire was adapted with an additional section which collected data on the compliance of supplementation during pregnancy. The accuracy of this compliance was cross-checked with a section in the symptoms diary, also determining iron supplementation compliance. All 102 women, whether iron compliant or not, were included in the data analysis. Trained fieldworkers administered diet history questionnaires. Training of fieldworkers was done by an expert in diet history data collection.

3.1.3.1.5 Brachial blood pressure

Brachial blood pressure was also measured and recorded with Dinamap® ProCare, by trained fieldworkers, at all three phases. It was correctly calibrated if Pressure Transducer Verification was out of tolerance. The mean arterial pressure (MAP, calculated as double the

diastolic blood pressure and add the sum to the systolic blood pressure, then divide by 3) of > 90 mmHg was used to indicate an increased risk for preeclampsia (Cnossen *et al.*, 2008). Systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg, and systolic blood pressure between 120 and 139 mmHg or diastolic blood pressure between 80 and 89 mmHg, was used to define hypertension and prehypertension, respectively (Weber *et al.*, 2014).

3.1.3.1.6 Oral Glucose Tolerance Test

A fasting Oral Glucose Tolerance Test (OGTT) to diagnose GDM was done by a qualified professional at RMMCH laboratory at phase 2. The OGTT is the gold standard test for the diagnosis of GDM between 24 and 28 weeks gestation (WHO, 2013). All participants were requested to do the OGTT unless previously diagnosed with diabetes (random blood glucose of > 11 mmol/L). The patients were requested to fast from 10 pm the night before the appointment (resulting in approximately 10 hours of fasting). Water, as required, was permitted. The laboratory staff of RMMCH checked if the patients had fasted and that they understood to remain in the waiting area for 2 hours without eating, drinking or smoking. A fasting blood sample was taken peripherally (finger prick) as well as by venepuncture (± 3 ml) on arrival. Patients with high blood glucose values (> 7 mmol/L) were admitted to the hospital. If the blood glucose values were normal (< 5.3 mmol/L), the patients were requested to drink a cooled glucose solution (75 g) over a maximum of 15 minutes (ideally within 5 minutes). The patients then remained seated within the waiting area for 2 hours, without eating, drinking or smoking. The test was invalid if the participants did not comply with these requirements. They were, however, allowed to walk to the bathroom. Blood samples (± 3 ml each time), using a venepuncture, were drawn and again at 1 and 2 hours after glucose consumption. The laboratory staff recorded all the results. Standard medical care was provided depending on each patient's results. A fasting plasma glucose ≥ 7.0 mmol/l or two-hour plasma glucose ≥ 11.1 mmol/l following a 75 g OGTT was a positive indication of diabetes. Also, a diagnosis of diabetes by a medical doctor in the medical file of a participant at phase 3 or 4 was incorporated in the data as positive for diabetes.

3.1.3.2 Specimens and biochemical analysis

A qualified professional collected maternal venous bloods at the laboratory of the hospital. Venous blood samples (42 ml) were drawn from the women into EDTA-coated and trace element free evacuated tubes (Becton Dickinson) via antecubital venepuncture. Blood

samples were processed within 1 hour after the blood was drawn by a trained research team member at an allocated space in the medical facility for blood withdrawal. Plasma/serum was separated, and RBC washed with normal saline. Aliquots were stored at -20°C on site for one week at most and transported to the Unisa laboratories of the Sciences campus in Florida where they were stored in locked facilities at -80°C. There was restricted access to all on-site freezer facilities. After that, samples were transported on dry ice (or portable freezer) to NWU laboratories for storage at -80°C for up to 7 years. Storage temperatures were monitored and logged for the entire duration of the study. The Centre of Excellence of Nutrition (CEN) laboratory personnel were responsible for the storage and management of the samples at the NWU. There was restricted access to the CEN fridge-freezer facilities.

3.1.3.2.1 Biochemical analysis

Biomarkers of iron status (ferritin and Hb) and fatty acid status were assessed at phase 1 for this study.

3.1.3.2.2 Iron and inflammation markers

Ferritin, CRP and α 1-acid glycoprotein (AGP) were analysed with a sandwich enzyme-linked immunosorbent assay (ELISA) with the Quansys Q-View Imager Pro (Logan, Utah, USA) at the CEN laboratory. Haemoglobin was analysed with HemoCue® Hb 201⁺ System (Angelholm, Sweden), performed by the NuPED laboratory technician (Mathee *et al.*, 2014).

3.1.3.2.3 Fatty acids

Fatty acids were analysed at the NWU by trained laboratory technician/students. Phospholipids were extracted from plasma with chloroform: methanol (2:1 vol: vol; containing 0.01% butylated hydroxytoluene) by using a modification of the method of Folch *et al* (1957). Lipid extracts were concentrated, and the neutral lipids separated from the phospholipids by using thin-layer chromatography (silica gel 60 plates, 10x 20 cm; Merck) and eluted with diethyl ether: petroleum, ether: acetic acid (30:90:1 vol: vol: vol). The lipid band that contains phospholipids was removed from the thin-layer chromatography plate and trans- methylated with methanol: sulphuric acid (95:5 vol: vol) at 70 °C for 2 hours to yield fatty acid methyl esters (FAMES). The resulting FAMES were extracted with water and hexane. The organic layer was evaporated, re-dissolved in hexane, and analysed by using quadrupole gas chromatography–electron impact–mass spectrometry on an Agilent Technologies 7890A Gas Chromatograph system equipped with an Agilent Technologies 7000GC/MS triple quad mass selective detector (Agilent Technologies). The gas chromatography separation of FAMES was carried out on an HP88 capillary column (100 m

x 0.25 mm x 0.20 µm; Agilent) by using helium as the carrier gas at a flow rate of 2.2 mL/min. The gas chromatography injector was held at a temperature of 270°C, and the mass spectrometry source at 250°C. The injection volume of the sample solution was 1 µL by using a split ratio of 1:20. The oven temperature was programmed from 50° C to 170°C at 30°C/min, then from 170°C to 215°C at 2°C/min, and at 4°C/min to 230°C. After that, the temperature was held isothermally at 230°C for 7 min. The total analysis time was 38.25 min. Mass spectrometry with 70 eV electron ionisation was carried out in multiple reaction monitoring mode, with at least two transitions per compound. Quantification of FAME was done with Masshunter (B.06.00). FAME peaks were identified and calibrated against a standard reference mixture of 33 FAMES (Nu-Check-Prep) and two single FAME standards (Larodan Fine Chemicals AB). Relative percentages of fatty acids were calculated by taking the concentration of a given fatty acid derivative as a percentage of the total concentration of all fatty acids identified in the sample.

3.1.4 Data capturing, quality assurance and statistical analysis

Statistical analysis was performed by the student, who was trained and supervised by her supervisors and the NuPED study biostatistician. Data were captured in Access Software. Overall, data processing and statistical analysis of data were done using Statistical Package for the Social Sciences (SPSS) version 23 software (SPSS Inc, Chicago, IL, USA), Statistical Analysis System (SAS) and Excel Windows XP (Microsoft, Seattle, WA, USA). Data regarding iron supplements were recorded at < 18 weeks and 22 weeks. Morbidity data underwent a 10% check to ensure the data were clean. Common mistakes were identified and corrected in the entire data set.

Data were tested for outliers and normality using Q-Q plots, histograms and Shapiro-Wilk test. Subjects characteristics were described using the median and interquartile ranges, as well as the mean and standard deviation for continuous variables. Frequency and percentages were used for categorical variables. Differences between the ID and iron-sufficient, and anaemic and non-anaemic groups at study entry were examined by using independent t-tests for continuous variables and the chi-square test for categorical variables. Differences between hypertension and diabetes and their markers of the ID and iron-sufficient, and anaemic and non-anaemic groups were examined by using analysis of covariance (ANCOVA) for continuous variables, correcting for confounders including n-3 PUFA status, and the chi-square test for categorical variables. Non-normal data were log-transformed to perform statistical analysis. Correlations between markers of iron status, anaemia, hypertension and GDM were analysed with Spearman's correlations.

The frequencies of symptoms during pregnancy were analysed using a multiple correspondence analysis, to find an underlying structure of association between the symptoms during pregnancy (Greenacre, 2017; Greenacre & Blasius, 2006). Two main dimensions were retained and used to double scale variables by using a biplot. Explained variance was reported as dimension relative inertia. Variable points were aggregated to cluster and interpreted by biplot visual inspection.

The median number of symptoms during pregnancy was performed by week and analysed using an ordinary linear regression, with time as a covariate. The trend over time by symptoms was evaluated considering the slope of the relation of symptom frequencies over time. The frequency of symptoms over time was represented using a dispersion plot of symptom frequencies by week. The mobile average was also reported on the plots. The change of trends over time was evaluated using a joint-point analysis, further checked by visual inspection of the dispersion plot (Kim *et al.*, 2000). Participants with ID, anaemia, n-3 PUFA status below the median, and those who were not compliant to iron supplementation, were compared to their counterparts. This was done using the regression analysis reported above where group and gestational time per group interaction were also considered. The frequency of symptoms was transformed with an arcsine function to improve the distribution of the regression residuals. Model fitting was evaluated using the R-squared statistic. The level of significance was set at $p < 0.05$.

3.2 Ethical and legal considerations

3.2.1 Legal authorisation

Ethical approval for this study was obtained from the HREC of the Faculty of Health Sciences of the NWU, Potchefstroom (NWU-00186-15-A1-02). Ethical approval was obtained for the NuPED study: by the HREC of NWU, as well as from the University of Witwatersrand (Annexure B, C).

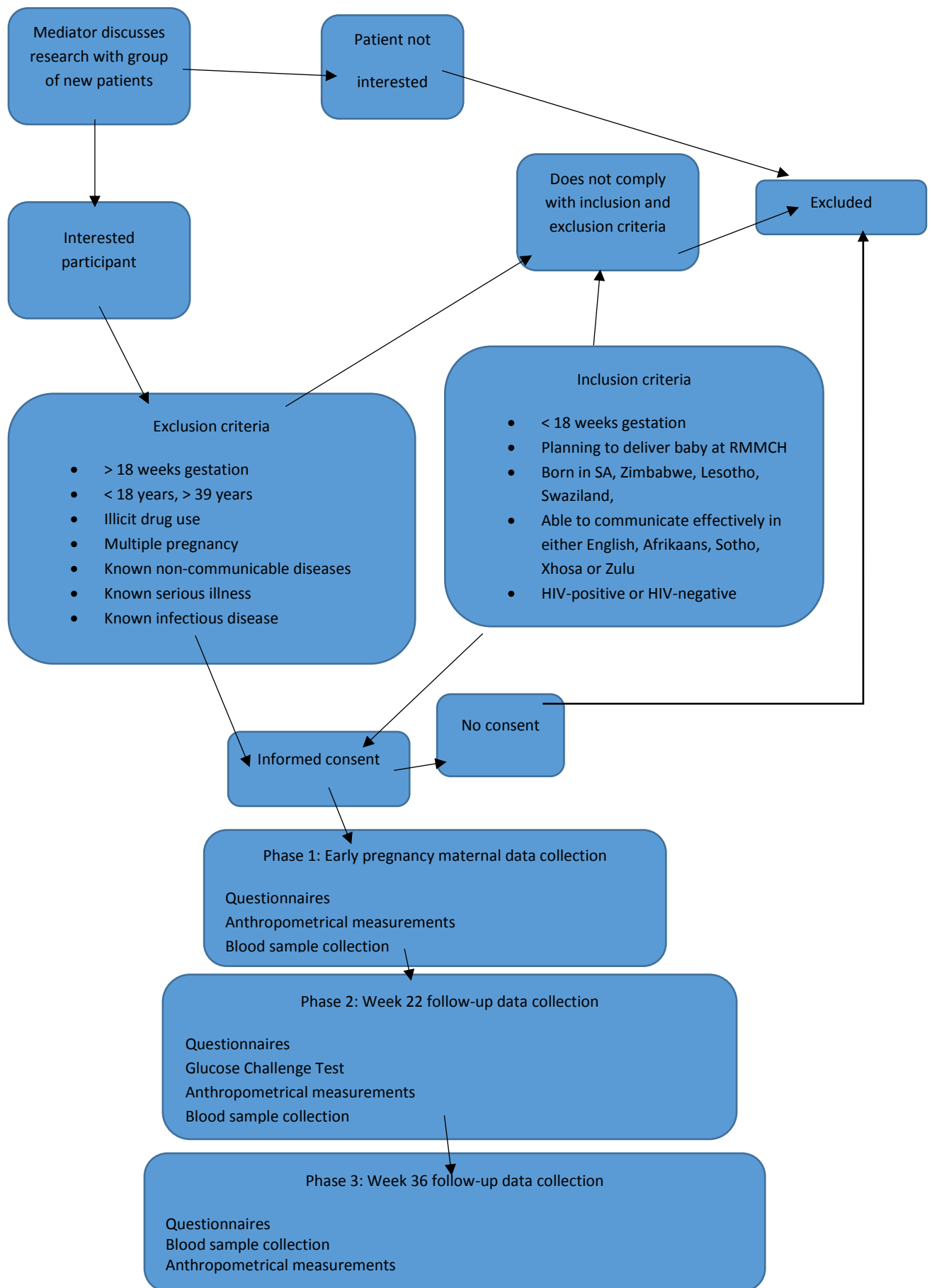


Figure 3: Diagrammatic illustration of the recruitment and data collection process

3.2.2 Expertise, skills and legal competencies

All the researchers and assistants (nurses, anthropometrists and students) part of this study are experienced in their fields. Dr L Malan, was responsible for infectious morbidity questionnaire training and monitoring of data collection. Also, all laboratory analyses performed at the NWU, were supervised by Dr L Malan. Mrs Elize Symington (study coordinator of the NuPED pregnancy study) acted as study coordinator. Dr C Ricci is an experienced biostatistician.

In the NuPED study, Dr Mieke Faber, an expert in diet history data collection, supervised dietary intake methodology and data. Dr Sola Sotunde (ISAK accredited anthropometrist) supervised anthropometry training and monitoring of data collection. Dr Amy Wise (RMMCH gynaecologist and Wits employee) supervised all medical aspects, such as data collected from medical history and notes and follow-ups of participants with abnormal results. Fieldworkers with at least one year of nutrition or research training or experience were recruited. All involved with data collection received training to ensure standardized data collection and completion of questionnaires. Physical and anthropometric measures were gathered by fieldworkers using standardised protocols. These fieldworkers were trained and tested on “mock” subjects to ensure that measured values between fieldworker and study coordinator were within an acceptable range. If, between fieldworker variation compared to the coordinator was unacceptable, the field worker was retrained.

Two days were spent on the background of the project, ethical matters and questionnaire completion only. Five days were spent on dietary intake data collection training specifically. Blood samples were collected and medical procedures performed by medical professionals, minimising risk and/or harm to the participants and themselves. Specific hands-on instruction was given to the laboratory technician preparing the biological samples for storage. For the first three months of data collection training and updates were done monthly. Thereafter, update training was done bi-monthly.

3.2.3 Privacy and confidentiality

Privacy of the participants was ensured by using either private rooms or dedicated areas that were closed off from the general research area. Privacy was further guaranteed by ensuring that the conversation between the participant and interviewee was not overheard by any other person or passer-by. To protect both the research team members and the participant, two members of the research team were present in the room or dedicated area when the

measurements required the participant to undress partially.

Each participant received a unique participant number during the initial data collection. This unique number was used in all stages of data gathering. Upon arrival on the day of the study, the participant had to produce her identification documentation or maternal case record, to verify the ID number against her unique participant number. It was done only by the project coordinators. No other researcher and/or assistant had access to this information or was involved in the process. Furthermore, participants were not required to provide their personal information to any other researcher or assistant, except in some cases where the date of birth was required. All the data captured was done by using the unique participant number.

Data was kept safe and secure by locking hard copies in locked cabinets at the clinic/hospital, until the baby was born. Thereafter, these documents are kept secure in locked cabinets in the researcher's office for seven years and electronic data are kept password protected. Reporting of findings will remain anonymous. Data shared on DropBox is only accessible to the study team members and students.

3.2.4 Benefits

3.2.4.1 Direct benefits

The participants benefited from the study as follow-up visits were done at the RMMCH clinic where a gynaecologist conducted assessments, and they received specialist care during their pregnancy. Secondly, they received additional medical evaluations which would not have been done otherwise, such as the ultrasound screens and OGTT.

Participants taking part in the study directly benefitted from the day's measurements as feedback was given to them regarding results that were immediately available (which was also part of the routine medical care), i.e. blood pressure. All results were recorded on the subject's clinic card, and therefore, the nurse exercised routine medical care in the case of any abnormalities or referred the patient if it was necessary.

3.2.4.2 Indirect benefits

Being involved at the local clinics may have sensitised nursing staff to refer high-risk patients sooner for ANC at RMMCH for additional screening and assessment. Therefore, more

patients were treated earlier when risks were detected. These results may also serve as a basis from which to plan intervention studies. Furthermore, depending on the results, the study may benefit the South African population since it may shed light on the current management of micronutrient and fatty acid status in pregnant women, which could guide health care policy.

3.2.5 Risks/discomforts, precautions and safety plan

Human research always involves some risk. The procedures in this study were mostly non-invasive. However, some may have caused some discomfort during measurement. A Safety Monitoring Board monitored the study. The study was also performed under the supervision of the clinical study member, a gynaecologist (Dr Amy Wise).

Blood sampling (which is an invasive procedure) from pregnant women on their first ANC was part of routine medical care, and therefore, for this study, merely a larger volume of blood in additional vacutainers was collected. Thus, there was no further puncturing required. Venous blood collection could have led to some bruising at the point of puncture. However, participants were recommended not to bend their arms after blood collection to lessen the risk. When blood pressure was taken, the subject might have felt some discomfort when the cuff was pumped tightly around the arm. During anthropometrical measurements, subjects were requested to wear minimal clothing to ensure accurate measurements. To minimise discomfort, all measurements were done in an allocated private space to ensure privacy.

During the OGTT women might have occasionally experienced nausea, vomiting or diarrhoea as the glucose drink is hyperosmolar. The laboratory staff supplied diluted lemon juice to combat the extreme sweetness of the solution. Patients were monitored for side effects by the laboratory phlebotomist. Blood drawn using venepuncture to assess the blood glucose values might have led to some bruising at the point of puncture. Participants were recommended not to bend their arms after blood collection.

Data collection at each phase of the project took more than 2 hours per day per subject and might have exhausted the participants. They were therefore provided with refreshments to eat and drink during the day.

Allocated stations were used for data collection at each data collection site to ensure privacy.

Biological sample preparation for transport and/or storage was done at dedicated points at each data collection site. Waste produced on the site was disposed of according to the biological waste protocol of the healthcare facility, and the researchers supplied biological waste bins and bags for these purposes.

Field workers and investigators obtained Hepatitis A and B and tetanus vaccinations before the commencement of data collection. Appropriate personal protective equipment, including gloves and a lab coat, was worn at all times, to ensure safe handling of samples. If a glove incurred a tear, it was removed and replaced immediately. Since the nursing staff of the healthcare facility were drawing the blood samples, they followed government policy if needle stick injuries occurred. The research team did not perform HIV testing. It was done during routine medical care (with the participant's consent). The participant was requested permission for the results to be used in the study and it was done confidentially.

3.2.6 Incentive and reimbursement

All study subjects received a token of appreciation (gift hamper to the value of R150) at the phase 3 visit ($\pm$ 36 weeks gestation). For adverse cases, such as miscarriages, still births etc., supermarket gift vouchers to the same value were given. At each visit, the women received a R10 cell phone voucher to enable them to make a call to the researchers or fieldworkers if needed.

All study subjects who attended RMMCH ANC for this study were reimbursed for travelling.

At each phase (phases 1 to 3), all study subjects received a food parcel from the fieldworker during/after data collection in the private room or every 2 hours of assessment.

3.2.7 Announcements of results

We aim to publish an article in the Maternal and Child Health Journal.

3.2.8 Storage and archiving of data

Data was kept safe and secure by locking hard copies in locked cabinets at the clinic/hospital until the baby was born. Thereafter, these documents are kept secure in locked cabinets in the researcher's office for seven years and electronic data are kept

password protected. Reporting of findings will remain anonymous. Data shared on a DropBox is only accessible to the study team members and students.

3.3 References

- CDC (Centers for Disease Control and Prevention). 2007. National Health and Nutrition Examination Survey (NHANES) anthropometry procedures manual. *USA: Centers for Disease Control and Prevention.*
- Cnossen, J.S., Vollebregt, K.C., de Vrieze, N., ter Riet, G., Mol, B.W., Franx, A., Khan, K.S. & van der Post, J.A. 2008. Accuracy of mean arterial pressure and blood pressure measurements in predicting pre-eclampsia: systematic review and meta-analysis. *British Medical Journal*, 336(7653):1117-1120.
- Faul, F., Erdfelder, E., Lang, A.G. & Buchner, A. 2007. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behaviour Research Methods*, 39(2):175-191.
- Folch, J., Lees, M. & Sloane-Stanley, G.H. 1957. Extraction of fatty acid. *Journal of Biological Chemistry*, 226:497-509.
- Greenacre, M. 2017. Correspondence analysis in practice. CRC press.
- Greenacre, M. & Blasius, J., eds. 2006. *Multiple correspondence analysis and related methods*. CRC press.
- Kim, H.J., Fay, M.P., Feuer, E.J. & Midthune D.N. 2000. Statistics in medicine.
- Mathee, A., Naicker, N., Kootbodien, T., Mahuma, T., Nkomo & P., Naik, I. 2014. A cross-sectional analytical study of geophagia practices and blood metal concentrations in pregnant women in Johannesburg, South Africa. *South African Medical Journal*, 104(8):568-573.
- Weber, M.A., Schiffrin, E.L., White, W.B., Mann, S., Lindholm, L.H., Kenerson, J.G., Flack, J.M., Carter, B.L., Materson, B.J., Ram, C.V.S. & Cohen, D.L. 2014. Clinical practice guidelines for the management of hypertension in the community. *The Journal of Clinical Hypertension*, 16(1):14-26.
- WHO (World Health Organization). 2013. Diagnostic criteria and classification of hyperglycaemia first detected in pregnancy.

apps.who.int/iris/bitstream/10665/85975/1/WHO_NMH_MND_13.2_eng.pdf Date of access:
13 Mar. 2017.

CHAPTER 4 MANUSCRIPT

4.1 Manuscript

Routine iron supplementation in iron-sufficient urban pregnant women in South Africa is associated with increased morbidity and blood pressure.

Caylin Goodchild, Elize Symington, Cornelius M Smuts, Jeannine Baumgartner, Cristian Ricci, Lizelle Zandberg, Linda Malan.

Centre of Excellence for Nutrition, Faculty of Health Sciences, North-West University, Potchefstroom Campus, Private Bag X6001, Potchefstroom, 2520, South Africa

Corresponding author: Linda Malan, Centre of Excellence for Nutrition, North-West University (Potchefstroom Campus), Private Bag X6001, Potchefstroom, 2520, South Africa.
E-mail: linda.malan@nwu.ac.za.

Source of funding: Funded by the South-African Medical Research Council, Tygerberg, South Africa (492421). The financial assistance of the National Research Foundation (UID: 107465) towards this research is hereby also acknowledged. Opinions expressed and conclusions arrived at, are those of the author and are not necessarily to be attributed to the NRF.

Short running head: Iron is associated with increased morbidity in pregnancy

Abbreviations used:

AGP, α 1-acid glycoprotein; ANC, antenatal care; COJ, City of Johannesburg; CRP, C-reactive protein; DoH, Department of Health; ELISA, enzyme-linked immunosorbent assay; FAME, fatty acid methyl ester; GDM, gestational diabetes mellitus; GI, gastrointestinal; Hb, haemoglobin; HIV, human immunodeficiency virus; ID, iron deficiency; IDA, iron deficiency anaemia; MAP, mean arterial pressure; MRM, multiple reaction monitoring; MUAC, mid-upper arm circumference; n-3 PUFA, omega-3 polyunsaturated fatty acid; NuPED, Nutrition during Pregnancy and Early Development; NWU, North-West University; PUFA, polyunsaturated fatty acid; RBC, red blood cell; SA, South Africa; SANHANES, South African National Health and Nutrition Examination Survey; WHO, World Health Organisation

Abstract

Anaemia and iron deficiency (ID) contribute to the global burden of disease and should be prevented during pregnancy. In South Africa (SA), ~60 mg of elemental iron is provided daily to all pregnant women. In this prospective observational analysis, we recruited 102 pregnant women (at < 18 weeks of gestation) from urban regions within Johannesburg. We found that iron sufficiency was associated with higher diastolic blood pressure at 36 weeks gestation ($p = 0.045$). Iron-sufficient women also experienced a higher incidence of fever, extreme tiredness, coughing and diarrhoea, between 18 and 40 weeks gestation ($p < 0.001$, $p = 0.043$, $p < 0.001$ and $p = 0.014$, respectively). Between 18 and 40 weeks, extreme tiredness, runny nose and diarrhoea were higher in the anaemic group ($p = 0.031$, $p = 0.010$ and $p = 0.024$, respectively) and women who were compliant to iron supplementation had a higher incidence of fever, extreme tiredness, runny nose and vomiting ($p < 0.001$, $p = 0.013$, $p = 0.025$, $p = 0.008$, respectively). Throughout pregnancy, women with a higher n-3 polyunsaturated fatty acid status, experienced a lower incidence of fever, extreme tiredness, headache, runny nose, coughing, diarrhoea, vomiting, constipation and heartburn ($p < 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.001$, $p = 0.018$, $p = 0.008$, $p = 0.045$, $p < 0.001$ and $p = 0.037$, respectively). Iron sufficiency is associated with increased morbidity during pregnancy and it may be necessary to re-evaluate the iron supplementation strategy during pregnancy in SA.

KEY WORDS: iron supplementation, pregnancy, infectious morbidity, hypertension

Introduction

The supplementation of iron during pregnancy is a controversial concept (Beard, 2000; Mwangi *et al.*, 2017; Ribot *et al.*, 2013; Weinberg, 2009). Various countries follow their own regimes, of various quantities and over various durations (Beard, 2000; Makrides 2003; Milman *et al.*, 2005). The World Health Organisation (WHO) recommends 60 mg of elemental iron, for a duration of 3 months, in countries where iron deficiency anaemia (IDA) is above 40% and therefore considered to be a severe problem (WHO, 2001). In South Africa (SA), routine supplementation of iron (along with calcium and folate) is administered to all pregnant women while attending primary health care (DoH, 2015), even though the prevalence of IDA and iron depletion in females of reproductive age is estimated to be only 9.7% and 5.9%, respectively (Shisana *et al.*, 2013). There are, however, certain regions in SA where the prevalence of anaemia is greater. Shisana *et al.* (2013) reported 35.9% anaemia in women in Kwa-Zulu Natal, and Govender *et al.* (2016) reported 32% anaemia in girls ages 13 to 16 years. This highlights the possible disadvantage of providing standard routine supplementation to all populations. The current supplementation regime which SA women receive includes 200 mg ferrous sulphate (equal to ~60 mg elemental iron) (DoH, 2015). This minimum regime is provided irrespective of the women's current iron status, and in the case of mild or severe anaemia the iron dosage is increased (DoH, 2015).

Although iron is important in preventing iron deficiency (ID) and anaemia, provision of a high daily oral dose of iron to pregnant women whose iron stores are sufficient has been shown to cause serious side effects. These include gestational diabetes mellitus (GDM), hypertension, oxidative stress and decreased zinc absorption. Not only do these have a negative impact on the mother, but the foetus too (Bo *et al.*, 2009; O'Brien *et al.*, 1999; Sharifi *et al.*, 2010; Tripathi *et al.*, 2013; Zhou *et al.*, 2009;).

A few studies have been done to determine the adverse effects involved in increased iron intake via oral supplementation (Mwangi *et al.*, 2017; Prentice *et al.*, 2016). There is still, however, little known about the relation between iron status and symptoms of infectious morbidity during pregnancy.

In this study, we investigated the relationship of iron status and anaemia in early pregnancy with infectious morbidity symptoms, potential adverse effects, as well as hypertension and GDM and their markers in 102 pregnant women in an urban region of SA.

Methods

Study design, population and setting

The study, nested within the Nutrition during Pregnancy and Early Development (NuPED) study, is an observational prospective analysis. The study recruited pregnant women (at < 18 weeks of gestation) attending antenatal care (ANC) facilities in selected clinics within the City of Johannesburg (COJ) in Gauteng, SA. The women were then referred for ANC at Rahima Moosa Mother and Child Hospital (RMMCH) for first phase data collection and follow-up visits at 22 and 36 weeks of pregnancy, and birth. 102 women were enrolled into the study, according to the following inclusion criteria: 1) confirmed pregnancy at < 18 weeks gestational age and planning to deliver baby at RMMCH; 2) born in SA, Lesotho, Swaziland, Zimbabwe, Botswana or Namibia; 3) able to communicate effectively in English, Afrikaans, Sotho, Zulu or Xhosa; 4) between 18 and 39 years of age; 5) with a single pregnancy; 6) not using recreational drugs or alcohol; 7) not smoking currently and in the past year; and 8) without a known non-communicable diseases or infectious disease or serious illness, except human immunodeficiency virus (HIV). Women eligible and interested signed a written informed consent after ensuring they understood the information supplied. The study was approved by the North-West University (NWU) Health Research Ethics Committee (NWU-00186-15-A1-02) and the University of the Witwatersrand Human Research Ethics Committee (Medical), M150968.

Living standards measure

The living standards measure (LSM) was developed by the South African Audience Research Foundation (SAARF). LSM scores were calculated for each participant using degrees of urbanisation and ownership of cars and major appliances in households, as seen in question 11 of the sociodemographic questionnaire (Annexure D). This was used to divide the population into 10 groups, with 10 being the highest LSM score, and 1 being the lowest (SAARF, 2017).

Morbidity

Morbidity data were collected using a symptoms diary given to each woman and explained by a fieldworker at each of the three study visits before birth. This included a checklist of morbidity symptoms experienced daily, namely diarrhoea, runny nose, coughing, fever, nausea, vomiting, extreme tiredness, headache, constipation and heartburn. This data were collected from enrolment until birth.

Iron supplementation

The 24-hour recall questionnaire was adapted with an additional section which collected data on the compliance of supplementation during pregnancy. The accuracy of this compliance was cross-checked with a section in the symptoms diary, also determining iron supplementation compliance. All 102 women, whether iron compliant or not, were included in the data analysis.

Laboratory analysis

Venous blood was collected from the women. Ferritin, C-reactive protein (CRP) and α 1-acid glycoprotein (AGP) were analysed with a multiplex enzyme-linked immunosorbent assay (ELISA) on the Quansys Q-View Imager Pro (Logan, Utah, USA). Hb was analysed with HemoCue® Hb 201 + System (Angelholm, Sweden). Ferritin concentrations were adjusted by using correction factors specific to each subject's inflammatory status (Thurnham *et al.*, 2015). The adjustment factors for ferritin were 0.77, 0.53, and 0.75 for the incubation (CRP > 5 mg/L, AGP $\leq$ 1 g/L), early convalescent (CRP > 5 mg/L, AGP > 1 g/L), and late convalescent (CRP $\leq$ 5 mg/L, AGP > 1 g/L) groups, respectively. Anaemia was defined as Hb $\leq$ 11 g/dL, whilst ID was defined as corrected ferritin $\leq$ 15 μ g/L. Hypertension and prehypertension was defined as systolic blood pressure $\geq$ 140 mmHg or diastolic blood pressure $\geq$ 90 mmHg, and systolic blood pressure between 120 and 139 mmHg or diastolic blood pressure between 80 and 89 mmHg, respectively. Women with a mean arterial pressure (MAP) > 90 mmHg during the second trimester of pregnancy were considered to have an increased risk for preeclampsia (Cnossen *et al.*, 2008). MAP is used to estimate overall blood flow, and is calculated as double the diastolic blood pressure added to systolic blood pressure, divided by 3. As defined by the International Society for the Study of Hypertension in Pregnancy, preeclampsia is the new onset of hypertension after gestational week 20, along with proteinuria $\geq$ 300 mg/day or a spot urine protein/creatinine ratio $\geq$ 30 mg/mmol (Brown *et al.*, 2001).

Red blood cell (RBC) total phospholipid fatty acids were extracted as previously described (Baumgartner *et al.*, 2012). Shortly, phospholipids were extracted from RBC with chloroform: methanol (2:1 vol: vol; containing 0.01% butylated hydroxytoluene) by using a modification of the method of Folch (Folch *et al.*, 1956). Lipid extracts were concentrated, and the neutral lipids were separated from the phospholipids by using thin-layer chromatography (silica gel 60 plates, 10x 20 cm; Merck) and eluted with diethyl ether: petroleum, ether: acetic acid (30:90:1, vol: vol: vol). The lipid band that contains phospholipids were removed from the thin-layer chromatography plate and trans- methylated with methanol: sulphuric acid (95:5 vol: vol) at 70°C for 2h to yield fatty acid methyl esters (FAMES). The resulting FAMES were

extracted with water and hexane. The organic layer was evaporated and re-dissolved in hexane. FAMES were analyzed with a gas chromatograph triple quadrupole mass spectrometer with electron impact ionization on an Agilent Technologies 7890A Gas Chromatograph system equipped with an Agilent Technologies 7000GC/MS triple quad mass selective detector (Agilent Technologies). The gas chromatography separation of FAMES was carried out on a HP88 capillary column (100 m x 0.25 mm x 0.20 μ m; Agilent) by using helium as the carrier gas at a flow rate of 2.2 mL/min. The gas chromatography injector was held at a temperature of 270°C, and the mass spectrometry source at 250°C. The injection volume of the sample solution was 1 μ L by using a split ratio of 1:80. The oven temperature was programmed to rise from 50° C to 170 °C at 30 °C/min, then from 170 °C to 215 °C at 2 °C/min, and lastly at 4 °C/min to 230 °C. After that the temperature was held isothermally at 230 °C for 7 min. The total analysis time was 38.25 min. Mass spectrometry was carried out in multiple reaction monitoring (MRM) mode, with at least two transitions per compound. Quantification of FAMES was performed with Masshunter (B.06.00). FAME peaks were identified and calibrated against a standard reference mixture of 33 FAMES (Nu-Check-Prep) and two single FAME standards (Larodan Fine Chemicals AB). Relative percentages of fatty acids were calculated by taking the concentration of a given fatty acid derivative as a percentage of the total concentration of all fatty acids identified in the sample.

Statistical analysis

Subjects characteristics were described using mean and standard deviation (parametric data) or median and interquartile ranges (non-parametric data) for continuous variables while frequency and percentages were used for categorical variables. Differences between the ID and iron-sufficient, and anaemic and non-anaemic groups at study entry were examined by using independent t-tests for continuous variables and the chi-square test for categorical variables. Differences between hypertension and diabetes and their markers of the ID and iron-sufficient, and anaemic and non-anaemic groups were examined by using analysis of covariance (ANCOVA) for continuous variables, correcting for confounders including n-3 PUFA status, and the chi-square test for categorical variables. Non-normal data were log-transformed to perform statistical analysis. Correlations between markers of iron status, anaemia, hypertension and GDM were analysed with Spearman's correlations.

Frequencies of symptoms during pregnancy were analysed using multiple correspondence analysis to find an underlying structure of association between symptoms during pregnancy (Greenacre, 2017; Greenacre & Jorg, 2006). Two main dimensions were retained and used to double scale variable using a biplot. Explained variance was reported as dimension

relative inertia. Variable points were aggregated to cluster and interpreted by biplot visual inspection.

Median number of symptoms during pregnancy were performed by week and analysed using an ordinary linear regression having time as covariates. The trend over time by symptoms was evaluated considering the slope of the relation of symptom frequencies over time. Frequency of symptoms over time was represented using dispersion plot of symptom frequencies by week. Mobile average was also reported on the plots. Change of trends over time was evaluated using a joint-point analysis further checked by visual inspection of the dispersion plot (Kim *et al.*, 2000). Joint-points were identified for fever, vomiting and heartburn, at 32, 24 and 29 weeks gestation, respectively. Participants with ID, anemia, compliant iron intake and n-3 PUFA status lower than median were compared to their counterparts over the total duration of gestation depending on availability of data, using the regression analysis where group and gestational time per group interaction were also considered. Differences between the groups were also estimated for gestation between 18 and 40 weeks to exclude effects of extremities between 8 and 18 weeks gestation. Frequency of symptoms were transformed with arcsine function to improve the distribution of the regression residuals. Model fitting was evaluated using the R-squared statistic.

Results

At entry (Table 4-1) ID and anaemia prevalence among all women was 11.9% and 20.8%, respectively. Eighty-eight percent of the population were Black and 25.5% were HIV-positive. More HIV-positive women than HIV-negative women were anaemic ($p = 0.014$). The iron-sufficient group had higher ferritin and Hb concentrations compared to the ID group ($p < 0.001$ and $p = 0.044$, respectively) and the non-anaemic group had higher ferritin and Hb concentrations compared to the anaemic group ($p = 0.041$ and $p < 0.001$, respectively). The percentage of monounsaturated fatty acids was higher in the anaemic group than the non-anaemic group ($p = 0.025$). There were more primigravida, but less multigravida women in the iron-deficient group ($p = 0.017$).

As seen in Table 4-2, 67.3% of women reported to take 60 mg or more supplemental iron daily. The remaining women took less than 60 mg daily, with 15.8% of women taking no iron supplements throughout pregnancy. The average total amount of iron supplementation taken during pregnancy among the women was 9 375 mg (data not shown), being 11 160 mg in the participants taking 60 mg or more per day and 1 414 in participants taking less than 60 mg per day. The median duration of taking iron supplementation was 174 days (~6 months).

Table 4-1: Characteristics of pregnant women participating in the study at entry (gestational age < 18 weeks)¹

	Total (n = 96-102)	Iron-deficient (n = 11-12)	Iron-sufficient (n = 87-89)	p-value	Anaemic (n = 19-20)	Non-anaemic (n = 76)	p-value
Age (y)	28.5 (24.0, 32.0) ²	28.5 (21.3, 31.8)	28.0 (24.5, 32.0)	0.295	30.5 (24.3, 32.8)	28.0 (24.0, 31.8)	0.307
<i>Anthropometry</i>							
Weight (kg)	70.4 ± 15.6 ³	66.9 ± 11.9	70.9 ± 16.1	0.416	74.8 ± 18.7	69.9 ± 14.0	0.197
N Height (cm)	158.8 ± 6.7	161.8 ± 7.4	158.4 ± 6.5	0.094	158.6 ± 6.5	158.6 ± 6.8	0.982
MUAC (cm)	30.3 ± 4.6	28.4 ± 3.7	30.6 ± 4.7	0.127	31.5 ± 5.3	30.1 ± 4.1	0.204
<i>Blood pressure</i>							
Systolic blood pressure (mmHg)	109.5 (103.0, 117.0)	104.5 (101.3, 122.3)	109.5 (103.5, 117.0)	0.913	106.0 (102.3, 118.9)	110.3 (103.1, 117.0)	0.593
Diastolic blood pressure (mmHg)	69.5 (63.5, 75.5)	66.0 (63.1, 75.3)	70.0 (64.5, 76.0)	0.891	68.3 (64.1, 76.0)	70.3 (63.1, 75.5)	0.410
Mean arterial pressure (mmHg)	82.6 (77.0, 89.0)	79.3 (76.1, 91.9)	83.0 (77.0, 89.0)	0.886	80.0 (77.9, 88.2)	83.2 (76.6, 89.8)	0.611
<i>Iron, anaemia and inflammatory status</i>							
Ferritin (µg/L) ⁴	53.8 (25.0, 117.7)	10.2 (7.5, 11.8)	61.0 (30.8, 136.0)	< 0.001	25.0 (10.8, 53.3)	59.8 (28.8, 122.5)	0.041
Haemoglobin (g/dL)	12.2 ± 1.7	11.4 ± 1.8	12.4 ± 1.6	0.044	10.0 ± 1.0	12.0 (11.4, 12.9)	<0.001
ID prevalence (Ferritin < 15 µg/L)	12 (11.9)						
Anaemia prevalence (Hb < 11 g/dL)	20 (20.8) ⁵						
C-reactive protein (mg/L)	25.0 (14.1, 51.7)	16.4 (7.3, 41.7)	26.1 (14.9, 52.8)	0.179	25.0 (11.3, 62.5)	25.3 (14.7, 50.6)	0.443
Alpha-1-acid glycoprotein (g/L)	0.80 (0.66, 0.99)	0.64 (0.56, 1.02)	0.81 (0.69, 0.99)	0.149	0.81 (0.65, 1.00)	0.80 (0.65, 0.98)	0.911
Acute inflammation prevalence (CRP > 5 mg/L)	92 (91.1)	10 (83.3)	82 (92.1)	0.315	16 (84.2)	72 (94.7)	0.116
Chronic inflammation prevalence (AGP > 1 g/L)	16 (15.8)	3 (25.0)	13 (14.6)	0.355	4 (21.1)	12 (15.8)	0.583

	Total (n = 96-102)	Iron-deficient (n = 11-12)	Iron-sufficient (n = 87-89)	p-value	Anaemic (n = 19-20)	Non-anaemic (n = 76)	p-value
<i>Ethnicity prevalence and socio-demography</i>							
Black	89 (88.1)	12 (100)	76 (86.4)	0.602	18 (90.0)	65 (86.7)	0.843
Coloured	9 (8.9)	0 (0.0)	9 (10.2)		2 (10.0)	7 (9.3)	
White	2 (2.0)	0 (0.0)	2 (2.3)		0 (0.0)	2 (2.7)	
Other	1 (1.0)	0 (0.0)	1 (1.1)		0 (0.0)	1 (1.3)	
Living standards measure	7.0 (6.0, 8.0)	7.0 (6.0, 8.0)	7.0 (6.0, 8.0)	0.896	7.5 (6.0, 9.0)	7.0 (6.0, 8.0)	0.983
HIV-positive status	24 (25.5)	4 (36.4)	19 (23.2)	0.341	9 (50.0)	15 (21.1)	0.014
Primigravida	23 (22.5)	6 (50.0)	17 (19.1)	0.017	6 (30.0)	16 (21.1)	0.397
Multigravida	79 (77.5)	6 (50.0)	72 (80.9)		14 (70.0)	60 (78.9)	
<i>Red blood cell total phospholipid fatty acid composition (% of total fatty acid composition)</i>							
Saturated	45.8 (44.2, 48.2)	46.7 (44.5, 49.4)	45.7 (44.2, 48.2)	0.755	45.7 (43.6, 46.9)	46.0 (44.4, 49.0)	0.135
MUFA	14.6 ± 1.4	14.3 ± 1.4	14.6 ± 1.4	0.399	15.2 ± 1.4	14.5 ± 1.4	0.025
PUFA	39.8 (36.7, 41.3)	39.9 (36.8, 40.7)	39.9 (36.5, 41.3)	0.959	39.4 (37.8, 40.8)	39.9 (35.9, 41.4)	0.596
n-6 PUFA	32.4 (29.8, 33.8)	32.8 (29.4, 34.6)	32.3 (29.8, 33.8)	0.480	32.1 (29.9, 34.3)	32.4 (29.5, 33.7)	0.328
n-3 PUFA	7.2 ± 1.4	6.6 ± 1.4	7.3 ± 1.4	0.175	7.0 ± 1.7	7.3 ± 1.3	0.482
n-6 Long-chain PUFA	21.1 (19.2, 22.4)	21.3 (19.1, 23.3)	21.1 (19.3, 22.4)	0.663	21.6 (18.2, 23.2)	20.9 (19.3, 22.0)	0.710
n-3 Long-chain PUFA	7.1 ± 1.4	6.5 ± 1.3	7.2 ± 1.4	0.176	6.9 ± 1.7	7.2 ± 1.3	0.476
n-6:n-3 PUFA ratio	4.5 (3.9, 5.0)	4.7 (4.3, 5.9)	4.5 (3.9, 4.9)	0.088	4.8 (3.7, 5.8)	4.4 (3.9, 4.8)	0.203
n-6:n-3 Long-chain PUFA ratio	2.9 (2.6, 3.3)	3.2 (2.8, 3.7)	2.9 (2.6, 3.3)	0.100	3.3 (2.3, 3.9)	2.9 (2.6, 3.2)	0.160

¹Differences between the iron-deficient and iron-sufficient, and anaemic and non-anaemic groups were examined by using independent t tests for continuous variables and the chi-square test for categorical variables. Ferritin was lower in the iron-deficient and anaemic groups (p < 0.001 and 0.041, respectively), haemoglobin was lower in the anaemic and ID groups (p < 0.001 and 0.044, respectively) and mono-unsaturated fatty acids were higher in the anaemic group (p = 0.025). More HIV positive women were anaemic (p = 0.014) and more primigravida, but less multigravida women were iron-deficient (p = 0.017). HIV, human immunodeficiency virus; MUAC, mid upper arm circumference; MUFA, mono-unsaturated fatty acid; PUFA, polyunsaturated fatty acid.

²Median, 25th and 75th percentile in parentheses (all such values).

³Mean ± SD (all such values)

⁴Ferritin concentrations were adjusted by using correction factors specific to each subject's inflammatory status (Thurnham *et al.*, 2015).

⁵n (%)

Table 4-2: Supplemental iron intake during pregnancy

Daily supplemental iron (mg)	n (%)	Duration of taking iron supplements (days)	Total supplemental iron taken during pregnancy (mg)
≥ 60 mg (1)	68 (67.3%)	174 (158, 197)	11 160 (9 660, 12 900)
< 60 mg (2)	33 (32.7%)	173 (157, 182)	1414 (0, 5 606)

Diastolic blood pressure tended to be higher in the iron-sufficient group at 36 weeks gestation when correcting for age, height, weight and ethnicity ($p = 0.079$) and was significantly higher when correcting additionally for n-3 PUFA status ($p = 0.045$), as seen in Table 4-3. MAP tended to be higher in the iron-sufficient group at 36 weeks gestation ($p = 0.075$), when corrected for age, height, weight, ethnicity and n-3 PUFA status.

Table 4-4 shows that ferritin correlated positively with Hb ($r = 0.228$, $p = 0.026$) and tended to correlate with AGP ($r = 0.188$, $p = 0.060$). A small positive correlation also existed between Hb and total iron supplementation intake during pregnancy ($r = 0.165$, $p = 0.109$), but did not reach significance. Diastolic blood pressure at 22 weeks gestation tended to correlate negatively with total iron supplementation intake during pregnancy ($r = -0.188$, $p = 0.065$).

Table 4-3: Hypertension and gestational diabetes prevalence, as well as their markers by iron status and anaemia¹

	Iron-deficient (n = 8-12)	Iron-sufficient (n = 62-89)	B- value	p-value	Anaemic (n = 16-20)	Non-anaemic (n = 53-76)	B-value	p-value
Hypertension								
Hypertension incidence [n (%)]	3 (27.3)	6 (6.8)	-	0.026	2 (10.5)	8 (10.5)	-	1.000
Pre-hypertension & hypertension incidence [n (%)]	3 (27.3)	28 (31.8)	-	0.759	6 (31.6)	25 (32.9)	-	0.913
<i>Risk for pre-eclampsia (MAP > 90 in second trimester)</i>								
< 18 weeks	3 (27.3)	28 (31.8)	-	0.948	4 (21.1)	19 (25.0)	-	0.641
22 weeks	2 (18.2)	13 (14.8)	-	0.876	4 (21.1)	10 (13.2)	-	0.455
36 weeks	0 (0.0)	14 (15.9)	-	0.114	4 (21.1)	11 (14.5)	-	0.550
<i>Systolic blood pressure</i>								
22 weeks	110.0 (100.5, 122.5) ²	109.0 (100.0, 116.1)	1.37 (-5.94, 8.68)	0.710	110.3 (101.3, 116.0)	109.0 (101.0, 16.5)	-0.67 (-6.46, 5.11)	0.818
36 weeks	102.0 (101.0, 108.5)	108.5 (102.0, 114.1)	-3.15 (-8.87, 2.58)	0.362	111.0 (101.3, 115.3)	107.5 (101.6, 113.8)	3.10 (-2.83, 9.04)	0.347
36 weeks*			-3.10 (-9.12, 2.91)	0.274			3.32 (-2.64, 9.28)	0.342
<i>Diastolic blood pressure</i>								
22 weeks	65.0 (58.0, 71.0)	67.8 (63.0, 72.1)	-0.81 (-6.32, 4.69)	0.769	65.5 (58.1, 75.4)	68.5 (64.0, 72.3)	-2.51 (-6.89, 1.87)	0.258
36 weeks	62.5 (58.5, 70.0)	68.3 (63.9, 73.5)	-4.06 (-8.35, 0.22)	0.079	65.5 (62.5, 75.3)	68.3 (63.6, 72.9)	1.18 (-3.24, 5.60)	0.596
36 weeks*			-4.52 (-8.97, -0.66)	0.045			1.43 (-3.00, 5.87)	0.691
<i>Mean arterial pressure (mmHg)</i>								
22 weeks	76.8 (74.5, 88.2)	81.6 (76.3, 86.0)	-0.09 (-5.77, 5.59)	0.976	80.9 (75.3, 88.3)	81.5 (76.4, 86.1)	-1.87 (-6.36, 2.62)	0.341
36 weeks	75.5 (74.0, 83.5)	81.8 (76.6, 86.8)	-3.76 (-8.15, 1.08)	0.113	80.7 (75.3, 89.6)	81.8 (75.5, 86.8)	1.82 (-2.87, 6.52)	0.521
36 weeks*			-4.05 (-8.61, 0.32)	0.075			2.06 (-2.64, 6.77)	0.514
Gestational diabetes								
Gestational diabetes incidence [n (%)]	0 (0.0)	3 (3.4)	-	0.534	0 (0.0)	3 (4.0)	-	0.379
Fasting blood glucose (mmol/L)	4.20 (3.93, 4.45)	4.10 (3.90, 4.40)	0.04 (-0.38, 0.45)	0.861	4.10 (3.93, 4.45)	4.10 (3.90, 4.40)	-0.21 (-0.52, 0.11)	0.193
Blood glucose at 120 min [#] (mmol/L)	4.90 (4.50, 5.50)	5.00 (4.30, 6.20)	-0.26 (-1.15, 0.612)	0.555	5.20 (4.30, 5.70)	5.00 (4.20, 6.28)	-0.34 (-1.03, 0.36)	0.333

¹ Differences between the iron-deficient and iron-sufficient, and anaemic and non-anaemic groups were examined by using ANCOVA for continuous variables and the chi-square test for categorical variables. Hypertension, prehypertension and mean arterial pressure were corrected for maternal age, height, weight and ethnicity, except time points marked with*, which were corrected additionally for n-3 PUFA. Gestational diabetes, fasting blood glucose and blood glucose at 120 min were corrected for maternal age, weight and ethnicity. Non-normal data were log-transformed to perform statistical analysis.

² Median, 25th to 75th percentile in parentheses (all such values).

[#] Blood glucose at 120 min after consumption of 20 ml glucose in a fasting state
MAP, mean arterial pressure.

Table 4-4: Correlations between markers of iron status, anaemia, hypertension and gestational diabetes¹

	Ferritin (µg/L)		Haemoglobin (g/dL)		Total iron supplementary intake during pregnancy (mg)	
	r-value	p-value	r-value	p-value	r-value	p-value
Ferritin (µg/L)			0.228	0.026	0.117	0.250
Haemoglobin(g/dL)	0.228	0.026			0.165	0.109
C-reactive protein (mg/L)	0.129	0.199	-0.16	0.878	-0.83	0.878
Alpha-1-acid glycoprotein (g/L)	0.188	0.060	-0.66	0.526	-0.111	0.276
Mean arterial pressure (mmHg)						
< 18 weeks	-0.109	0.281	0.131	0.204	-0.021	0.839
22 weeks	-0.041	0.689	0.086	0.413	-0.169	0.098
36 weeks	-0.097	0.377	0.115	0.306	0.009	0.934
Systolic blood pressure (mmHg)						
< 18 weeks	-0.110	0.280	0.112	0.275	-0.017	0.868
22 weeks	-0.139	0.174	0.029	0.782	-0.132	0.198
36 weeks	-0.087	0.429	0.076	0.500	0.029	0.792
Diastolic blood pressure						
< 18 weeks	-0.116	0.252	0.133	0.197	-0.025	0.808
22 weeks	0.025	0.807	0.110	0.292	-0.188	0.065
36 weeks	-0.076	0.488	0.135	0.229	-0.027	0.803
Fasting blood glucose (mmol/L)	0.009	0.942	0.150	0.218	0.014	0.906
Blood glucose at 120 min (mmol/L) ²	0.025	0.838	0.179	0.154	-0.112	0.367

¹ Correlations were determined with Spearman correlation analysis

² Blood glucose at 120 min after consumption of 20 ml glucose in a fasting state

A visual representation of the relationship between the incidence of the separate morbidity symptoms with each other during pregnancy is presented in Figure 4.1. The bi-plot explains 40% of the total variance in the data. Five distinct clusters of symptoms were identified: Cluster 1: headache and extreme tiredness; Cluster 2: runny nose, coughing and diarrhoea; Cluster 3: heartburn; Cluster 4: nausea and vomiting; and Cluster 5: fever and constipation. Cluster 1: Nearest to the axis intersect is the cluster of symptoms which had the most frequent incidence, namely headache and extreme tiredness. Cluster 2: On the positive side of dimension 1, we have the incidence of diarrhoea, coughing and runny nose. Cluster 3: In opposition, on the negative side of dimension 1, we find Cluster 3, consisting of a singular symptom namely heartburn. Cluster 4: Contributing to dimension 2 we find vomiting and nausea in opposition to cluster 1 and 5. Cluster 5: On the negative side of dimension two is Cluster 5, consisting of fever and constipation. These symptoms are in close proximity to Cluster 1, and therefore are related closely to Cluster 1, even though they are opposite sides of the y-axis. Cluster 1, 2 and 5 can be attributed to represent mostly infectious morbidity, and are separated from the other clusters, which can be attributed to represent mostly pregnancy side effects.

A visual representation of the relationship between the separate morbidity symptoms during pregnancy, for frequencies greater or equal to the third quartile is given in Figure 1b. The bi-plot explains 36% of the total variance in the data. Five distinct clusters of symptoms were identified: Cluster 1: headache, extreme tiredness, fever and constipation; Cluster 2: vomiting and nausea; Cluster 3: runny nose and coughing; Cluster 4: heartburn; and Cluster 5: diarrhoea. Cluster 1: Nearest to the axis intersect is the cluster of symptoms which had the most frequent incidence, namely headache, extreme tiredness, fever and constipation. Cluster 2: On the positive side of dimension 2, we have the incidence of vomiting and nausea. Cluster 3: Also on the positive side of dimension 2, we find Cluster 3, consisting of runny nose and coughing. Cluster 4: Opposite Cluster 2 and 3 we find heartburn. Cluster 5: On the negative side of dimension two, and opposite Clusters 2 and 3 is Cluster 5, consisting of diarrhoea. Cluster 1, 3 and 5 can be attributed to represent mostly infectious morbidity, and are separated from the other clusters, which can be attributed to represent mostly pregnancy side effects. Cluster 3 specifically represents respiratory infectious morbidity, whilst Cluster 5 represents gastrointestinal (GI) infectious morbidity.

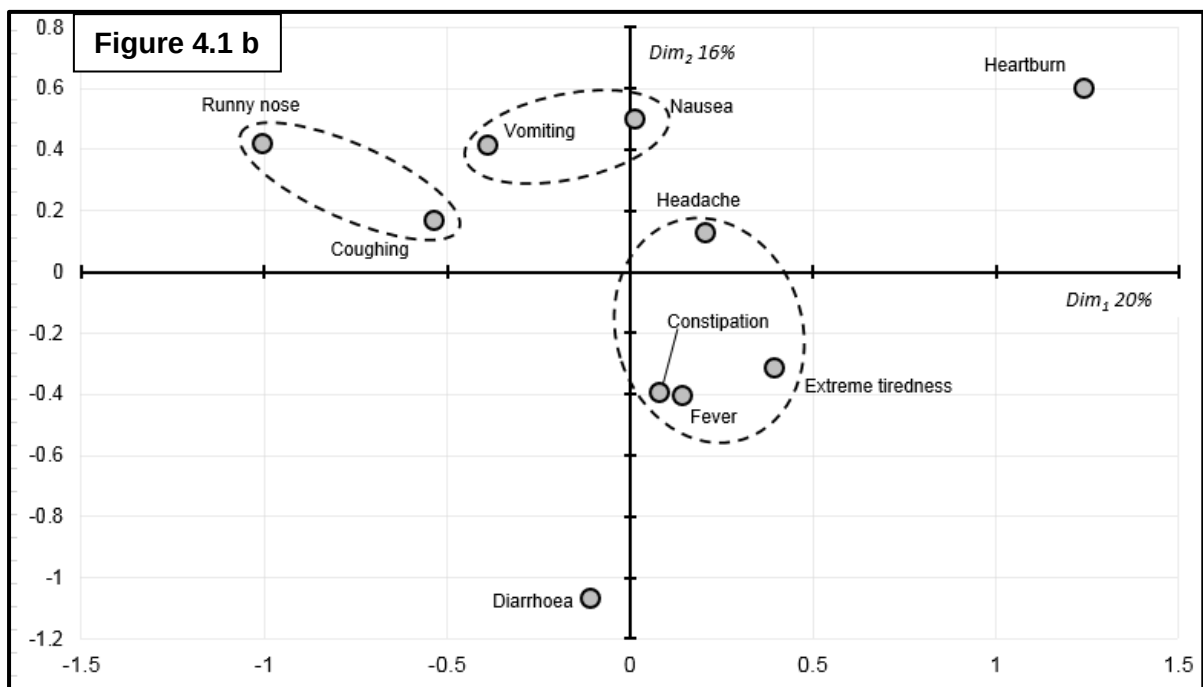
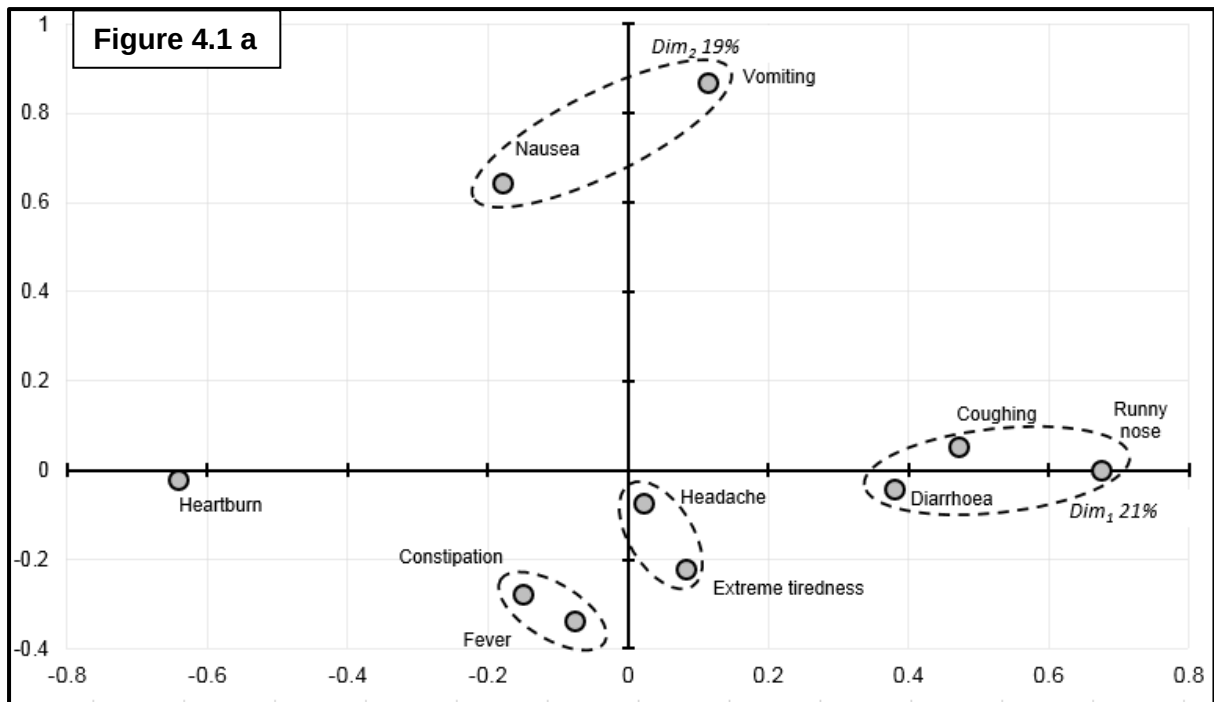
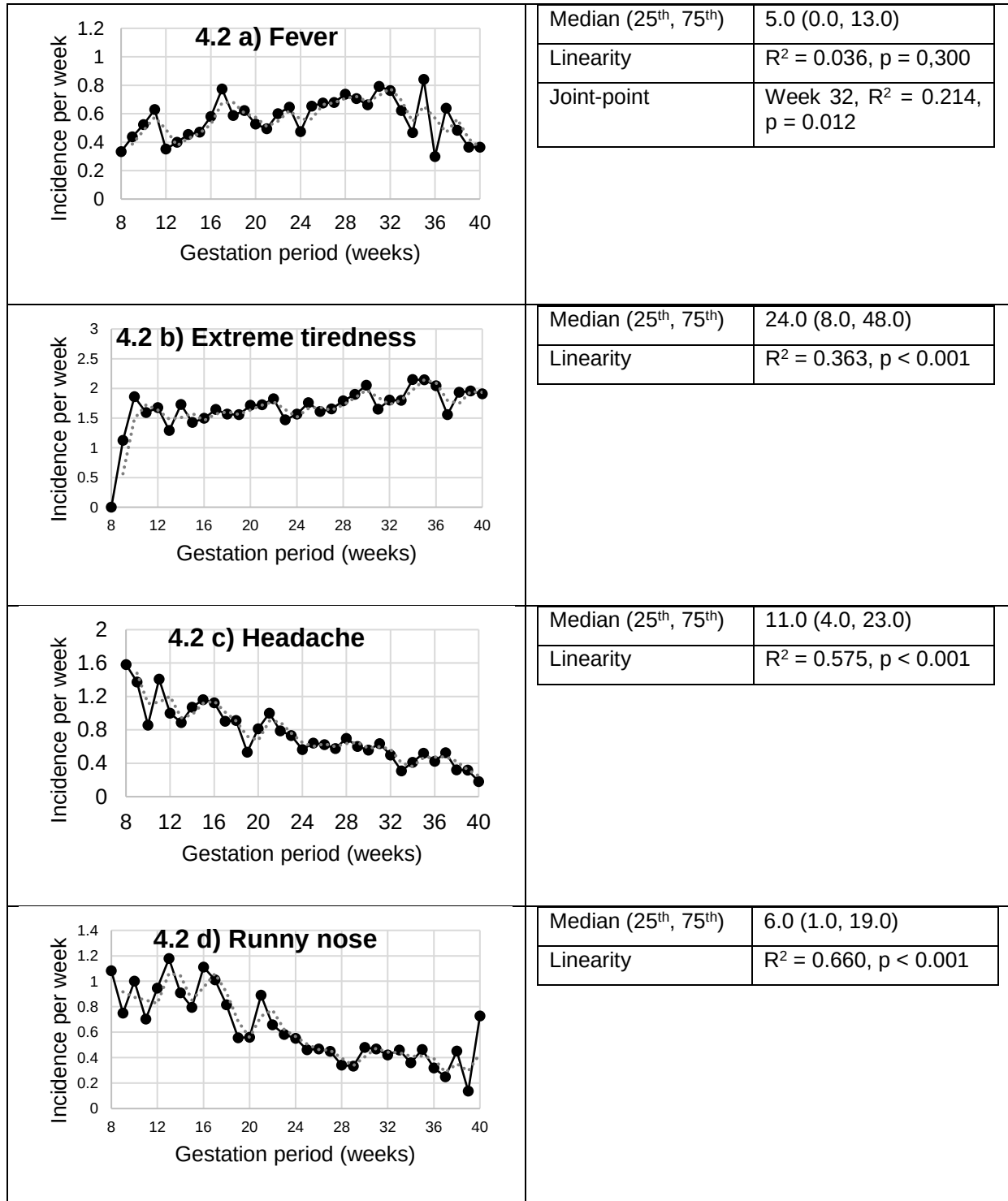
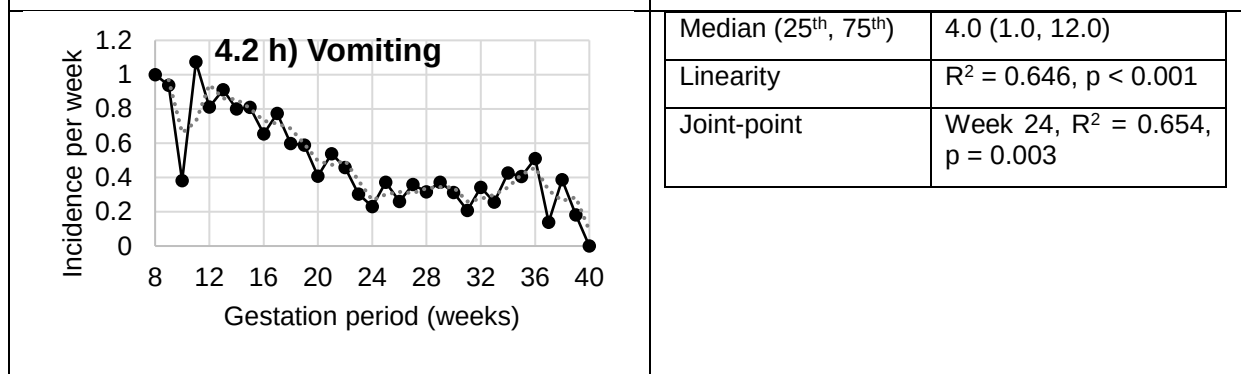
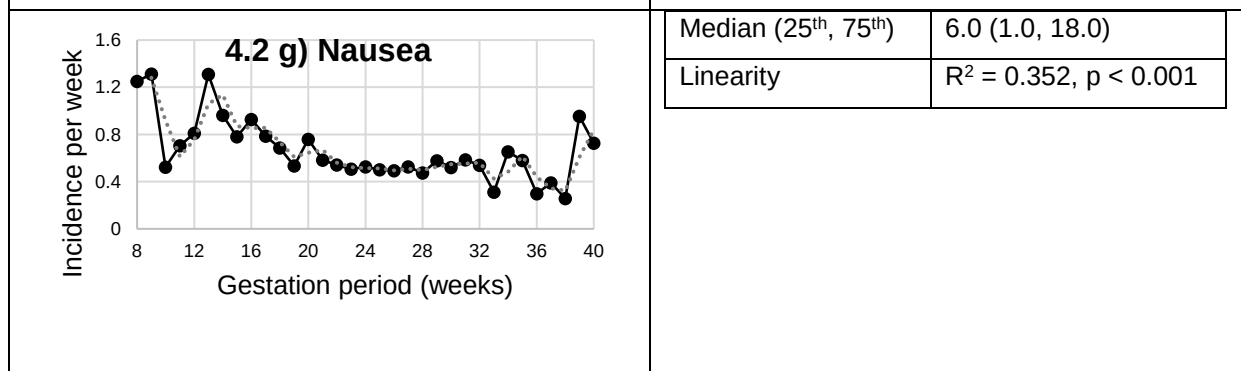
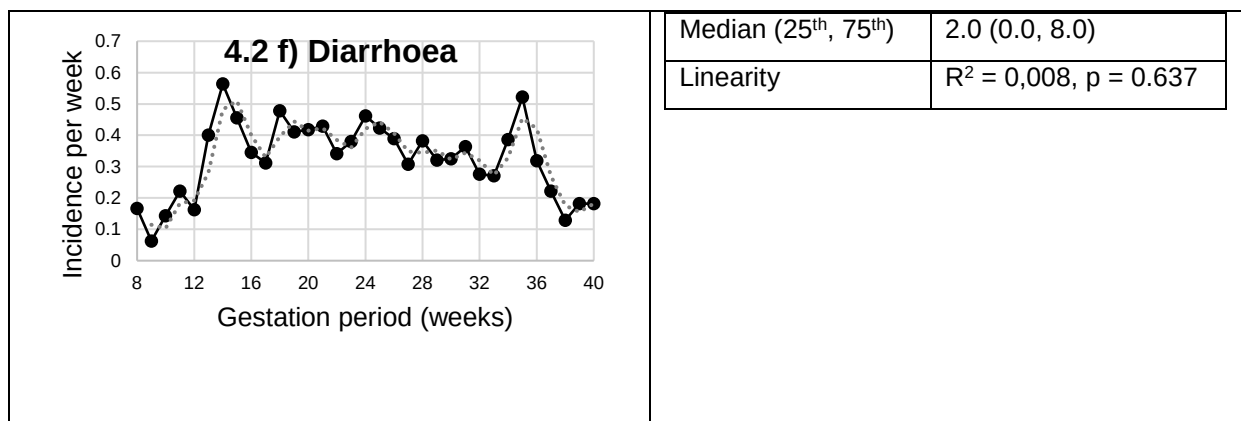
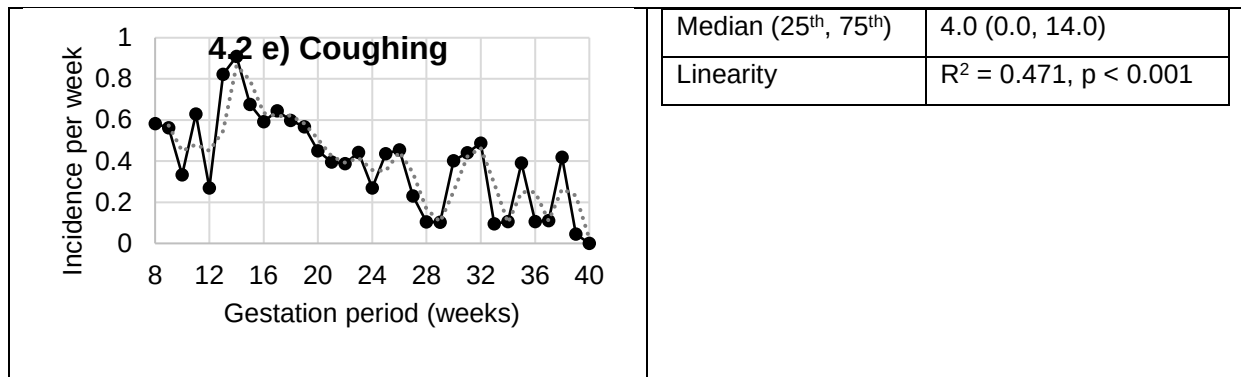


Figure 4.1: Bi-plot of correspondence analysis of the incidence of morbidity symptoms during pregnancy. a) all symptoms, b) symptoms with frequency distribution equal or greater than the third quartile.

The morbidity symptoms experienced by all the women (Figure 4.2) followed a linear trend over the duration of pregnancy for headaches, runny nose, coughing, nausea, and vomiting which all decreased (all $p < 0.001$), while extreme tiredness increased ($p < 0.001$). The

percentage of women who experienced at least one incidence of diarrhoea, runny nose, coughing, fever, nausea, vomiting, extreme tiredness, headache, constipation and heartburn during pregnancy was 73%, 83%, 77%, 77%, 81%, 81%, 97%, 93%, 84% and 86%, respectively (data not shown).





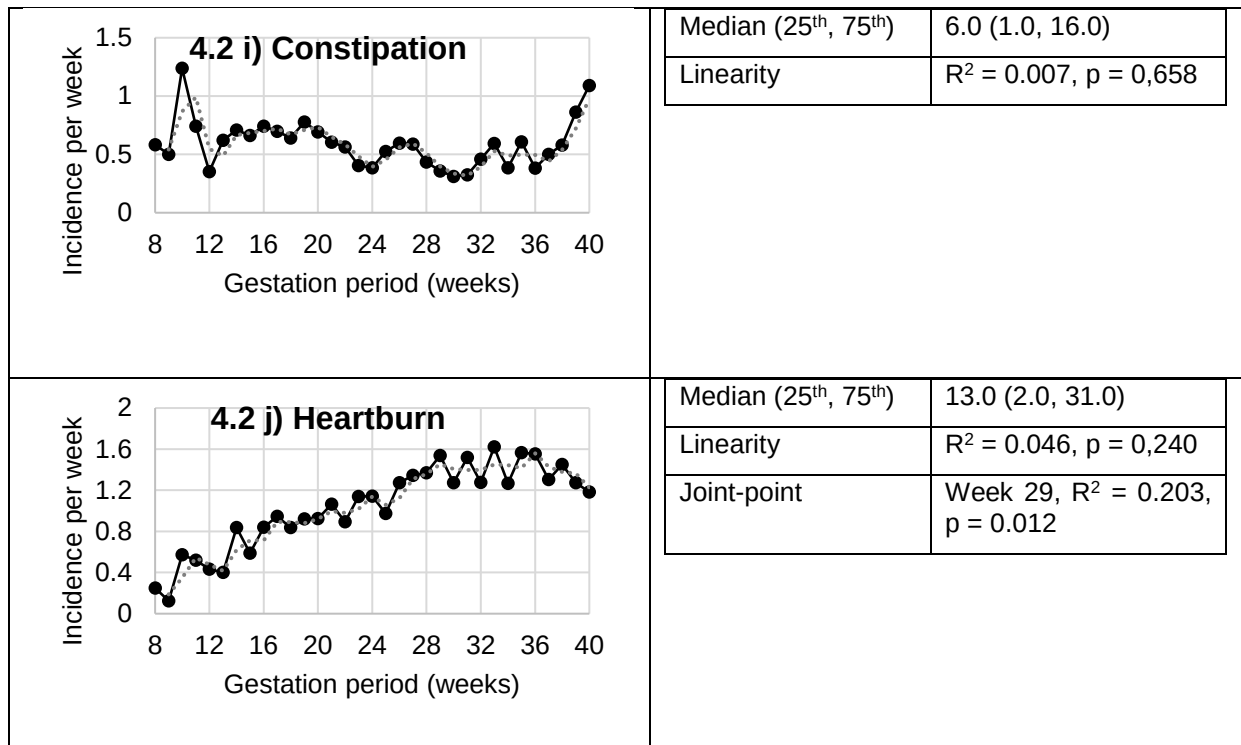
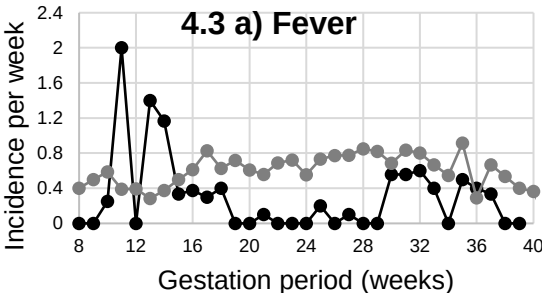
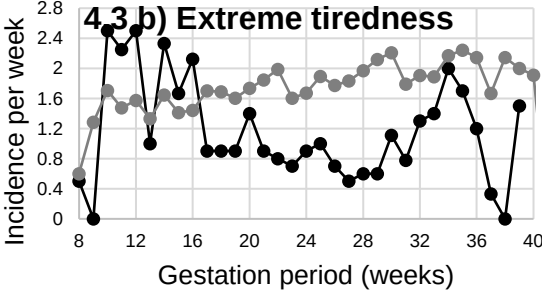
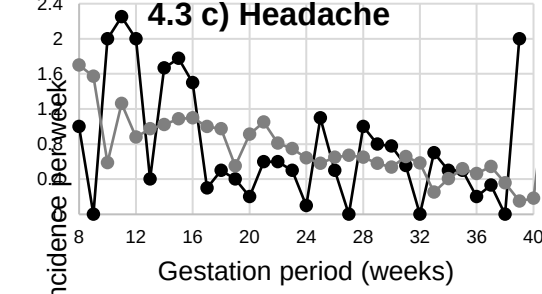
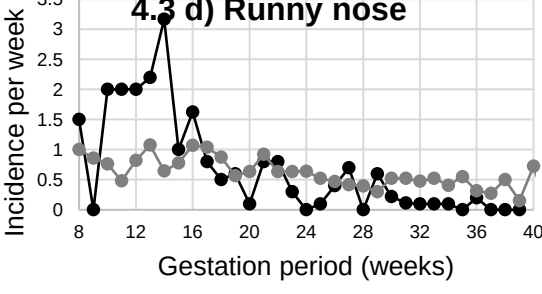
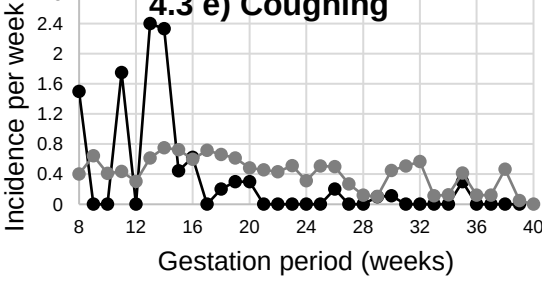
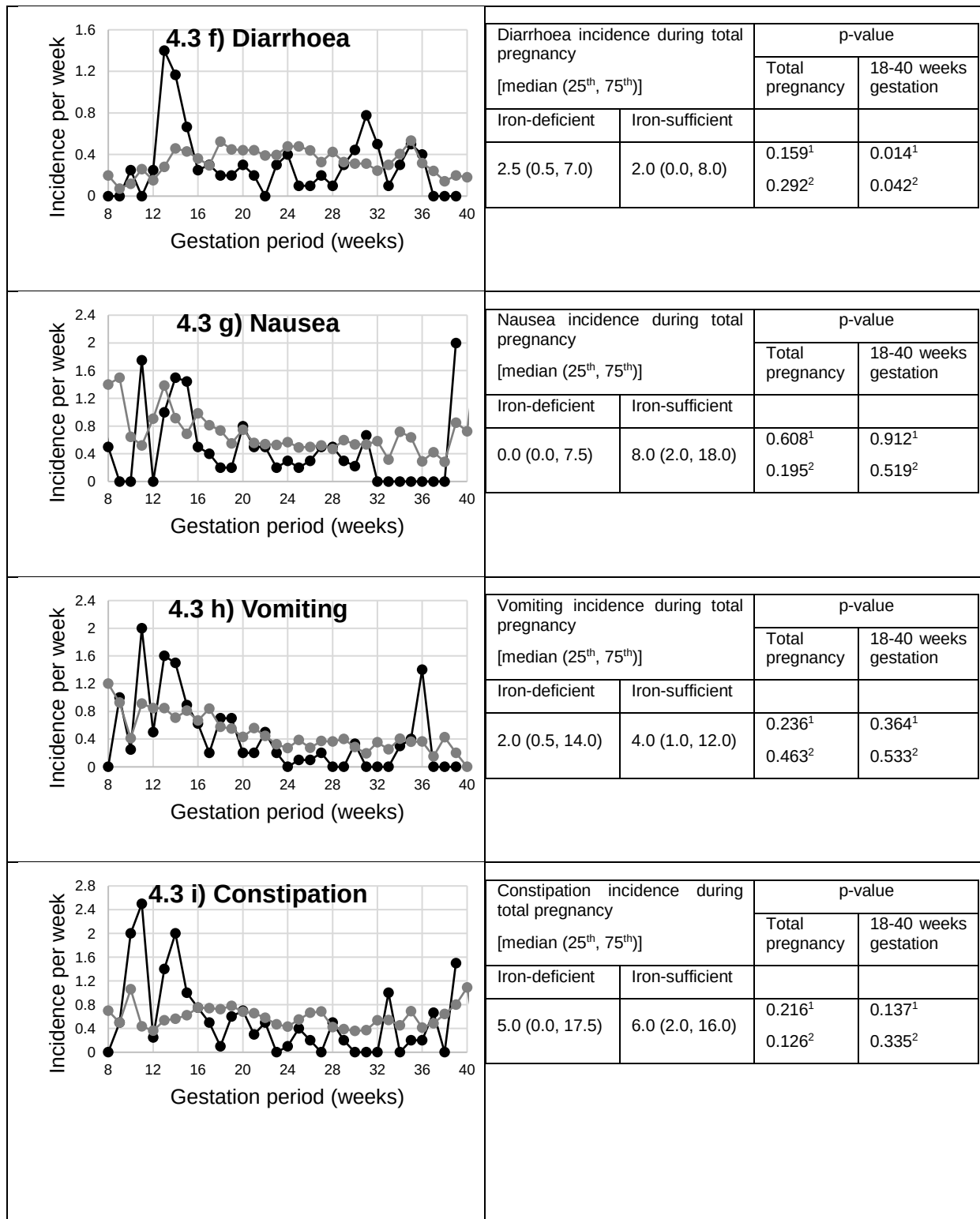


Figure 4.2: Symptoms of total population over time.
(Black line = median, dotted line = mobile average).

When grouping the women that were ID and iron-sufficient (Figure 4.3), the incidence per week of runny nose and coughing over the total gestation period were higher in the iron-sufficient group ($p < 0.001$ and $p = 0.014$, respectively). The incidence over time of runny nose and coughing decreased in the ID group, whereas it stayed constant in the iron-sufficient group, causing a difference in group by gestation time interaction ($p < 0.001$ and $p = 0.006$, respectively). The ID group seemed visually to have higher morbidity (runny nose, coughing, fever, headache, extreme tiredness, nausea, vomiting, constipation and heartburn) from the start of data collection (~8 weeks) until between 16-18 weeks. Variance was generally high in the ID group, especially up to 18 weeks gestation. Visually, the incidence of fever was double and of extreme tiredness was more than double in the iron-sufficient group than in the ID group from ~18 to 28 weeks of gestation.

Between 18 and 40 weeks, the iron-sufficient group experienced more fever, extreme tiredness, coughing and diarrhoea compared to the ID group ($p < 0.001$, $p = 0.043$, $p < 0.001$ and $p = 0.014$, respectively). The overall median of diarrhoea was, however, higher in the ID group, but this was likely due to the extreme values noted between 8 to 18 weeks. For the duration of gestation between 18 and 40 weeks, significant differences between group by gestation time occurred in fever, coughing and diarrhoea ($p = 0.013$, $p = 0.022$ and $p = 0.042$).

4.3 a) Fever  Incidence per week Gestation period (weeks)	<table><tr><th colspan="2" rowspan="2">Fever incidence during total pregnancy [median (25th, 75th)]</th><th colspan="2">p-value</th></tr><tr><th>Total pregnancy</th><th>18-40 weeks gestation</th></tr><tr><td>Iron-deficient</td><td>Iron-sufficient</td><td></td><td></td></tr><tr><td>0.5 (0.0, 6.0)</td><td>6.0 (1.0, 14.0)</td><td>0.210¹ 0.846²</td><td>< 0.001¹ 0.013²</td></tr></table>	Fever incidence during total pregnancy [median (25 th , 75 th)]		p-value		Total pregnancy	18-40 weeks gestation	Iron-deficient	Iron-sufficient			0.5 (0.0, 6.0)	6.0 (1.0, 14.0)	0.210 ¹ 0.846 ²	< 0.001 ¹ 0.013 ²
Fever incidence during total pregnancy [median (25 th , 75 th)]				p-value											
		Total pregnancy	18-40 weeks gestation												
Iron-deficient	Iron-sufficient														
0.5 (0.0, 6.0)	6.0 (1.0, 14.0)	0.210 ¹ 0.846 ²	< 0.001 ¹ 0.013 ²												
4.3 b) Extreme tiredness  Incidence per week Gestation period (weeks)	<table><tr><th colspan="2" rowspan="2">Extreme tiredness incidence during total pregnancy [median (25th, 75th)]</th><th colspan="2">p-value</th></tr><tr><th>Total pregnancy</th><th>18-40 weeks gestation</th></tr><tr><td>Iron-deficient</td><td>Iron-sufficient</td><td></td><td></td></tr><tr><td>10.5 (8.0, 1.5)</td><td>26.0 (8.0, 8.0)</td><td>0.355¹ 0.582²</td><td>0.043¹ 0.496²</td></tr></table>	Extreme tiredness incidence during total pregnancy [median (25 th , 75 th)]		p-value		Total pregnancy	18-40 weeks gestation	Iron-deficient	Iron-sufficient			10.5 (8.0, 1.5)	26.0 (8.0, 8.0)	0.355 ¹ 0.582 ²	0.043 ¹ 0.496 ²
Extreme tiredness incidence during total pregnancy [median (25 th , 75 th)]				p-value											
		Total pregnancy	18-40 weeks gestation												
Iron-deficient	Iron-sufficient														
10.5 (8.0, 1.5)	26.0 (8.0, 8.0)	0.355 ¹ 0.582 ²	0.043 ¹ 0.496 ²												
4.3 c) Headache  Incidence per week Gestation period (weeks)	<table><tr><th colspan="2" rowspan="2">Headache incidence during total pregnancy [median (25th, 75th)]</th><th colspan="2">p-value</th></tr><tr><th>Total pregnancy</th><th>18-40 weeks gestation</th></tr><tr><td>Iron-deficient</td><td>Iron-sufficient</td><td></td><td></td></tr><tr><td>12.0 (5.0, 1.0)</td><td>11.0 (4.0, 3.0)</td><td>0.103¹ 0.187²</td><td>0.091¹ 0.119²</td></tr></table>	Headache incidence during total pregnancy [median (25 th , 75 th)]		p-value		Total pregnancy	18-40 weeks gestation	Iron-deficient	Iron-sufficient			12.0 (5.0, 1.0)	11.0 (4.0, 3.0)	0.103 ¹ 0.187 ²	0.091 ¹ 0.119 ²
Headache incidence during total pregnancy [median (25 th , 75 th)]				p-value											
		Total pregnancy	18-40 weeks gestation												
Iron-deficient	Iron-sufficient														
12.0 (5.0, 1.0)	11.0 (4.0, 3.0)	0.103 ¹ 0.187 ²	0.091 ¹ 0.119 ²												
4.3 d) Runny nose  Incidence per week Gestation period (weeks)	<table><tr><th colspan="2" rowspan="2">Runny nose incidence during total pregnancy [median (25th, 75th)]</th><th colspan="2">p-value</th></tr><tr><th>Total pregnancy</th><th>18-40 weeks gestation</th></tr><tr><td>Iron-deficient</td><td>Iron-sufficient</td><td></td><td></td></tr><tr><td>5.5 (2.5, 20.5)</td><td>7.0 (2.0, 18.0)</td><td>< 0.001¹ < 0.001²</td><td>0.863¹ 0.400²</td></tr></table>	Runny nose incidence during total pregnancy [median (25 th , 75 th)]		p-value		Total pregnancy	18-40 weeks gestation	Iron-deficient	Iron-sufficient			5.5 (2.5, 20.5)	7.0 (2.0, 18.0)	< 0.001 ¹ < 0.001 ²	0.863 ¹ 0.400 ²
Runny nose incidence during total pregnancy [median (25 th , 75 th)]				p-value											
		Total pregnancy	18-40 weeks gestation												
Iron-deficient	Iron-sufficient														
5.5 (2.5, 20.5)	7.0 (2.0, 18.0)	< 0.001 ¹ < 0.001 ²	0.863 ¹ 0.400 ²												
4.3 e) Coughing  Incidence per week Gestation period (weeks)	<table><tr><th colspan="2" rowspan="2">Coughing incidence during total pregnancy [median (25th, 75th)]</th><th colspan="2">p-value</th></tr><tr><th>Total pregnancy</th><th>18-40 weeks gestation</th></tr><tr><td>Iron-deficient</td><td>Iron-sufficient</td><td></td><td></td></tr><tr><td>2.0 (0.5, 6.5)</td><td>4.0 (0.0, 14.0)</td><td>0.014¹ < 0.001²</td><td>< 0.001¹ 0.022²</td></tr></table>	Coughing incidence during total pregnancy [median (25 th , 75 th)]		p-value		Total pregnancy	18-40 weeks gestation	Iron-deficient	Iron-sufficient			2.0 (0.5, 6.5)	4.0 (0.0, 14.0)	0.014 ¹ < 0.001 ²	< 0.001 ¹ 0.022 ²
Coughing incidence during total pregnancy [median (25 th , 75 th)]				p-value											
		Total pregnancy	18-40 weeks gestation												
Iron-deficient	Iron-sufficient														
2.0 (0.5, 6.5)	4.0 (0.0, 14.0)	0.014 ¹ < 0.001 ²	< 0.001 ¹ 0.022 ²												



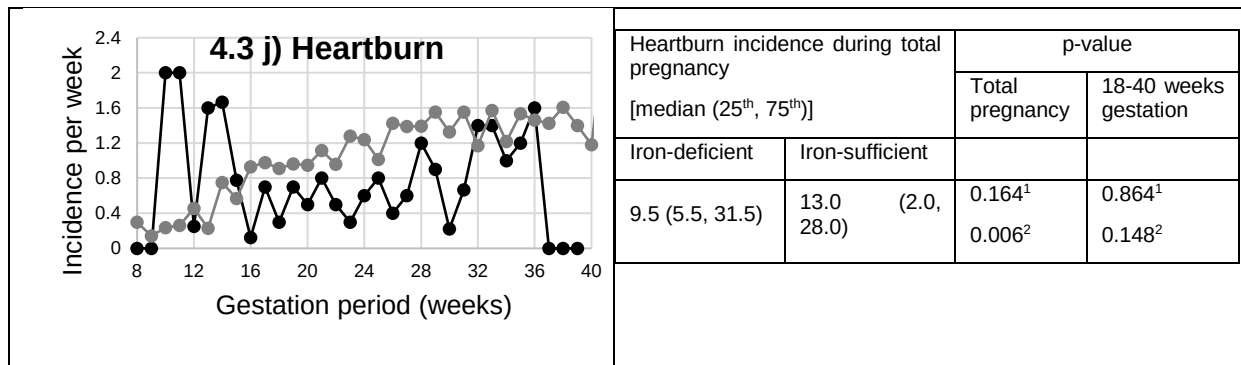
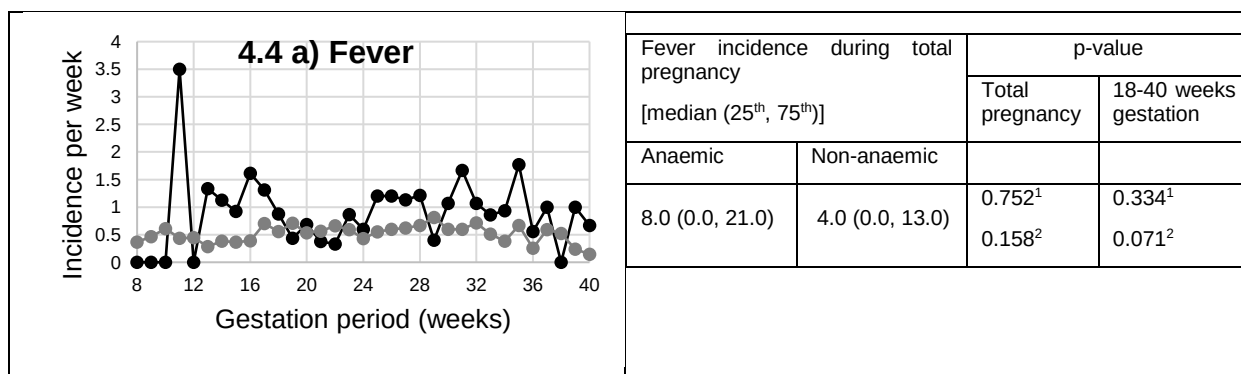
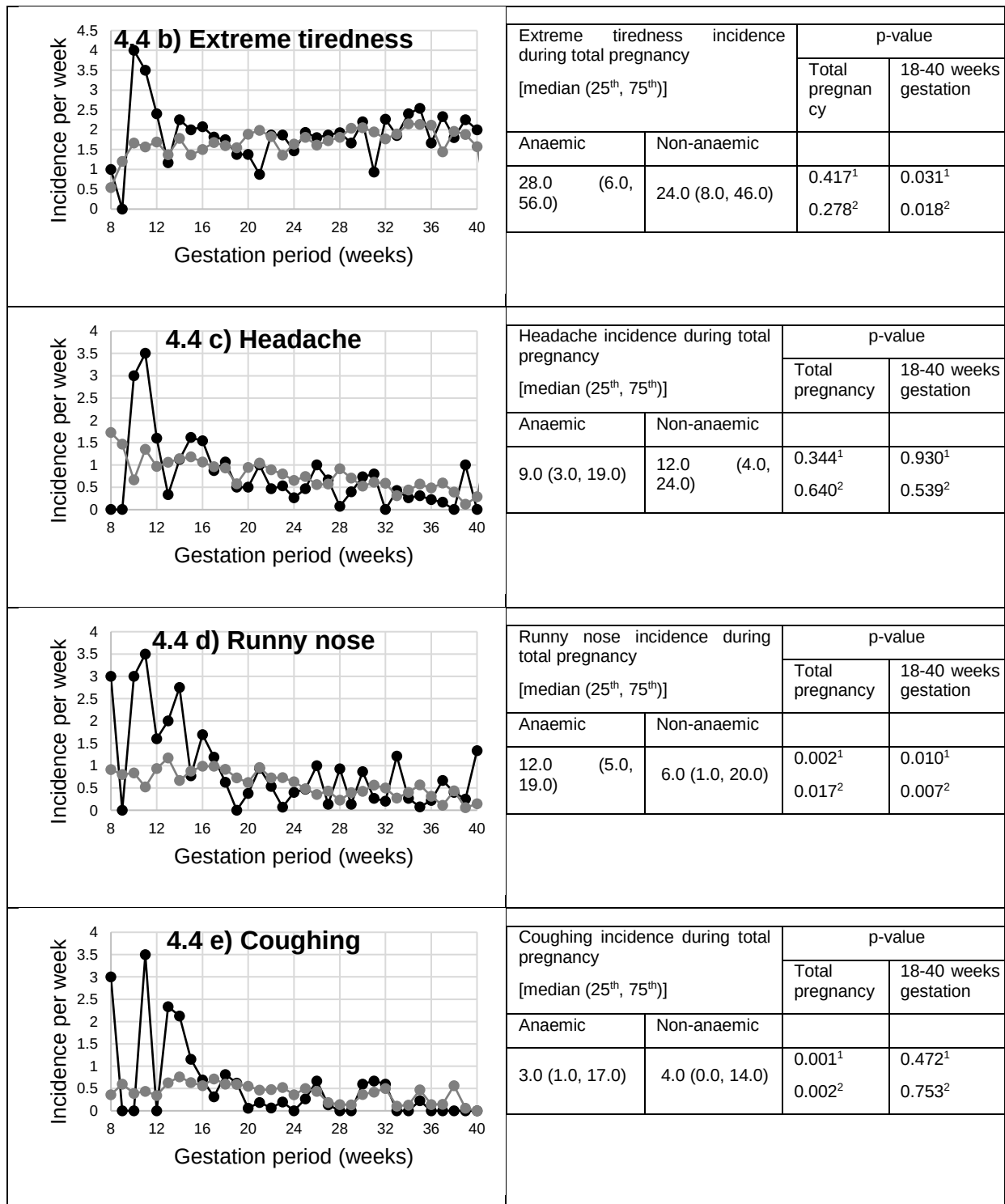


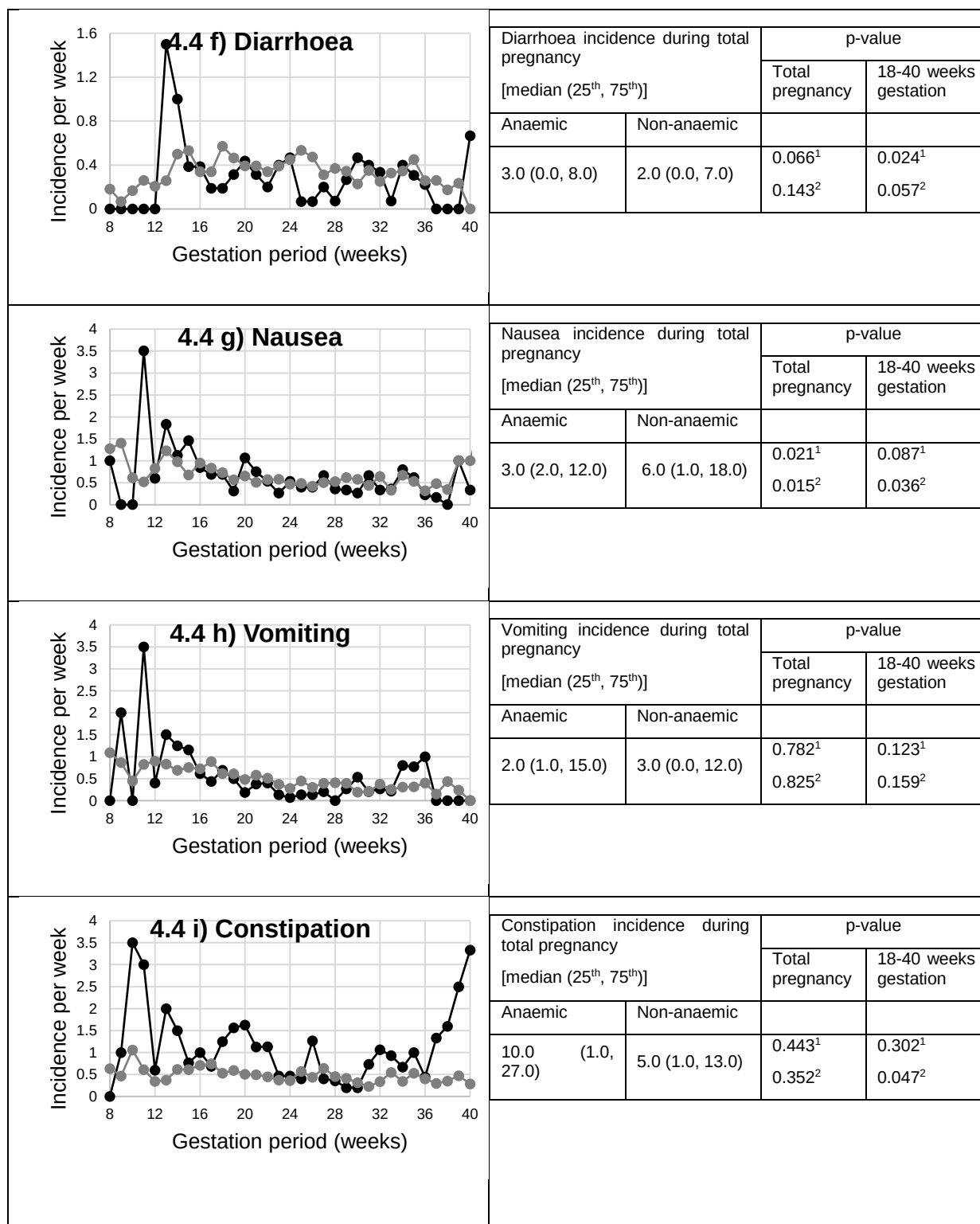
Figure 4.3: Morbidity symptoms over time in iron-deficient and iron-sufficient pregnant women. (Black line = iron-deficient, grey line = iron-sufficient; ¹difference between groups. ²difference between group by gestation time interaction).

When observing the anaemic and non-anaemic groups, the anaemic group visually had higher morbidity (fever, extreme tiredness, headache, runny nose, coughing, diarrhoea, nausea, vomiting, constipation and heartburn) from the start of data collection at ~8 weeks until between 12 to 16 weeks gestation (Figure 4.4). Variance was generally higher in the anaemic group than the non-anaemic group, especially up to ~16 weeks gestation for all symptoms. The incidence per week of coughing and nausea was higher in the non-anaemic group ($p = 0.001$ and $p = 0.021$, respectively) when compared to the anaemic group. However, runny nose was higher ($p = 0.002$) and diarrhoea incidence showed a trend to be higher in the anaemic group ($p = 0.066$). The incidence over time of runny nose, coughing and nausea decreased in the anaemic group, whereas it stayed constant in the non-anaemic group, causing differences between the group by gestation time interactions ($p = 0.017$, $p = 0.002$ and $p = 0.015$, respectively).

Between 18 and 40 weeks, extreme tiredness, runny nose and diarrhoea were higher in the anaemic group ($p = 0.031$, $p = 0.010$ and $p = 0.024$, respectively) than in the non-anaemic group. The group by gestation time interaction differed significantly between groups for extreme tiredness ($p = 0.018$), runny nose ($p = 0.007$), nausea ($p = 0.036$) and constipation ($p = 0.047$).







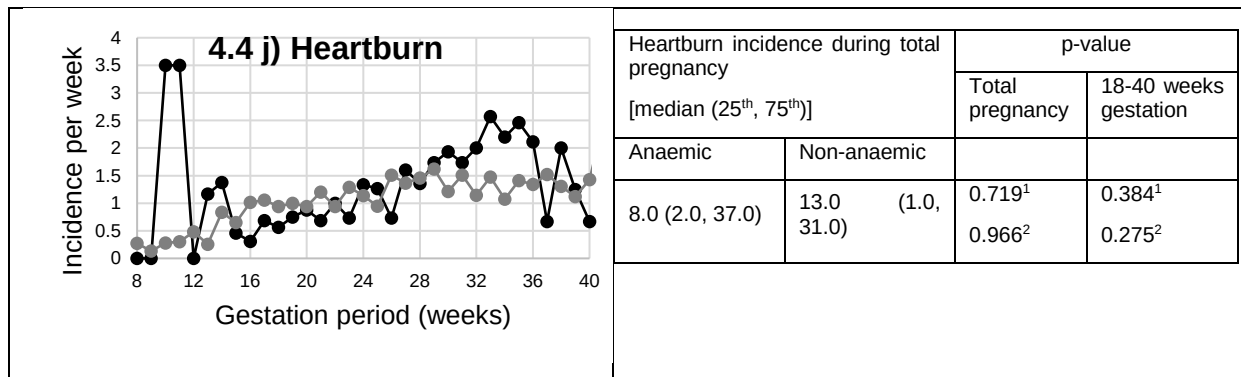
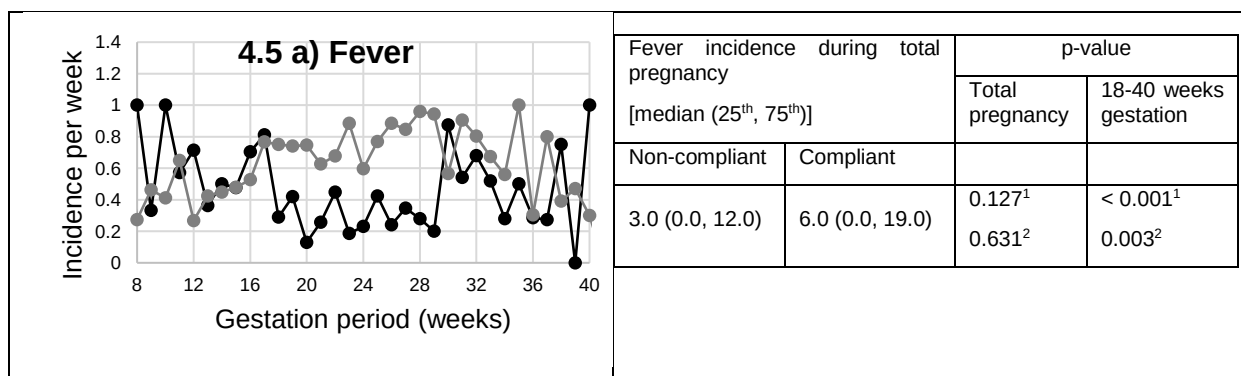
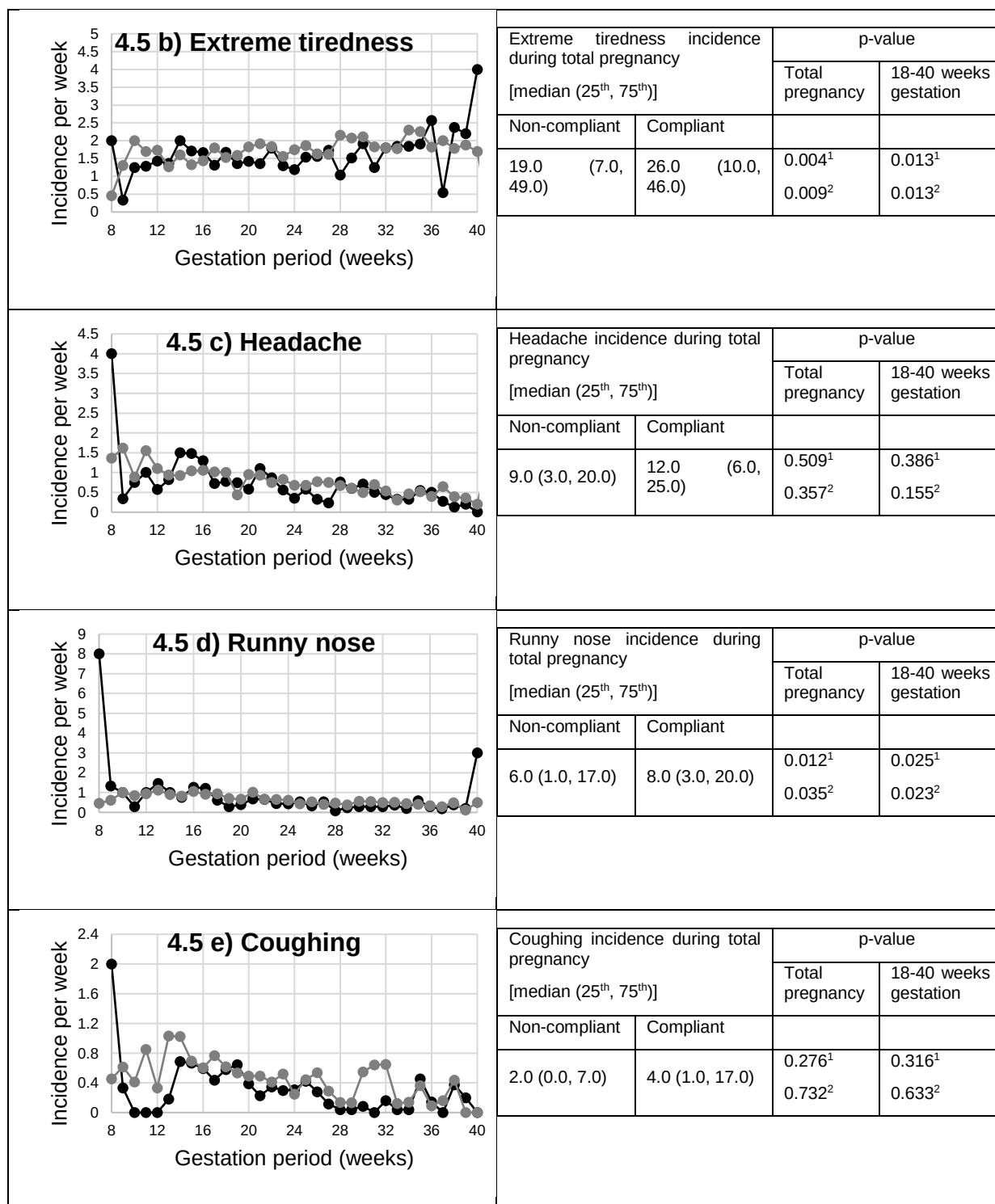


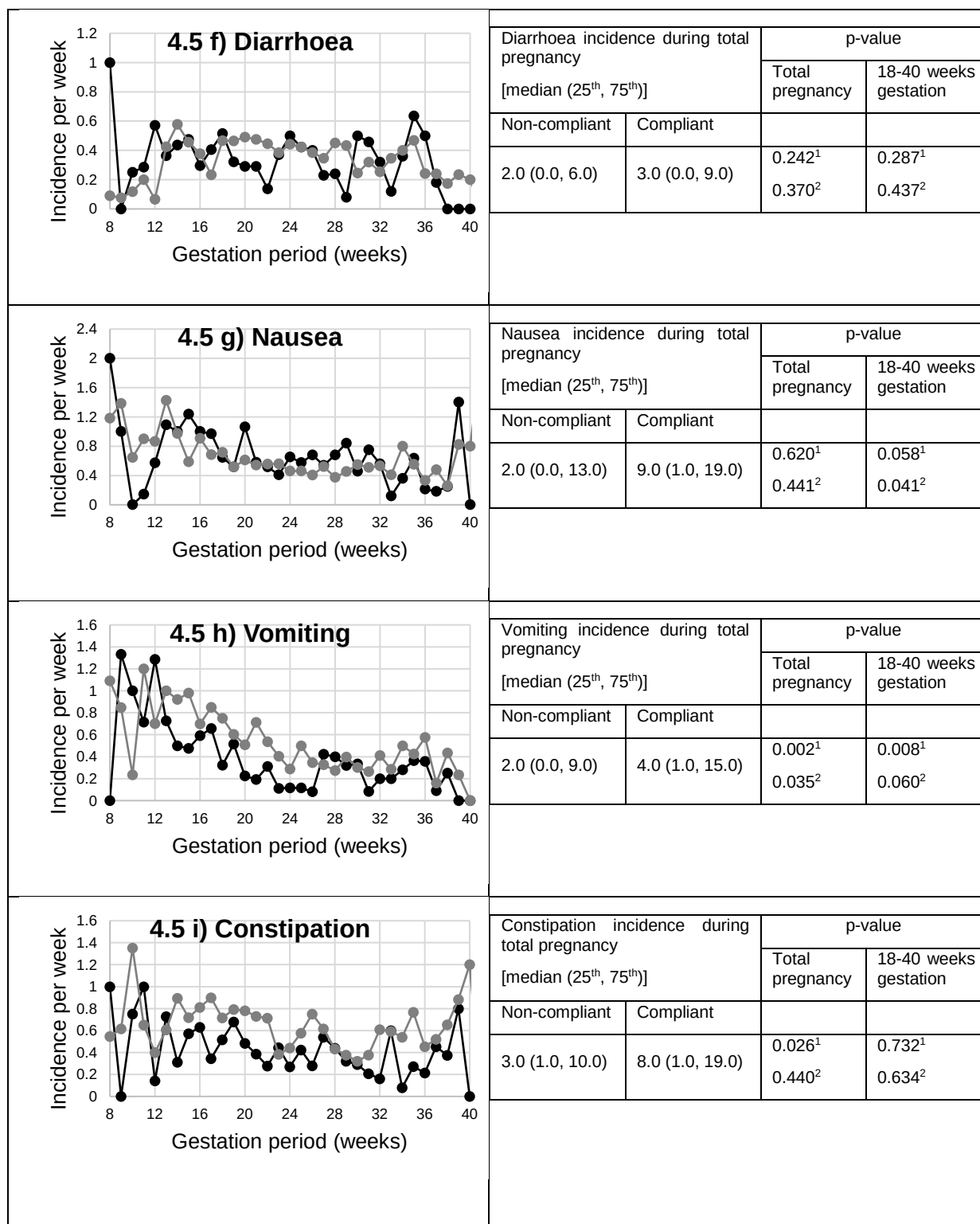
Figure 4.4: Morbidity symptoms over time in anaemic and non-anaemic pregnant women. (Black line = anaemic, grey line = non-anaemic; ¹difference between groups. ²difference between group by time interaction).

Figure 4.5 shows that the incidence per week of extreme tiredness, runny nose, vomiting, constipation and heartburn were higher in the compliant group throughout gestation ($p = 0.004$, $p = 0.012$, $p = 0.002$, $p = 0.026$ and $p = 0.037$, respectively). The group by gestation time interaction differed significantly for extreme tiredness, runny nose and vomiting ($p = 0.009$, $p = 0.035$ and $p = 0.035$, respectively). The incidence of fever between the two groups showed the greatest difference, with higher fever in the compliant group, between week 18 and 30, but this did not cause the overall model to reach significance ($p = 0.127$).

When specifically observing gestation week 18 to 40, the iron compliant group had a higher incidence of fever, extreme tiredness, runny nose and vomiting ($p < 0.001$, $p = 0.013$, $p = 0.025$ and $p = 0.008$, respectively) compared to the iron non-compliant group. The group by gestation time interaction of fever ($p = 0.003$), extreme tiredness ($p = 0.013$), runny nose ($p = 0.023$) and nausea ($p = 0.041$) also differed between groups.







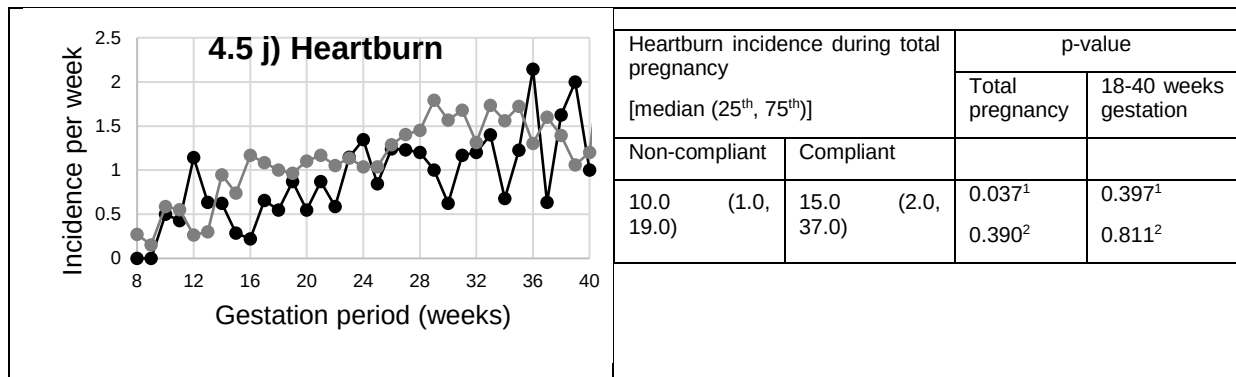
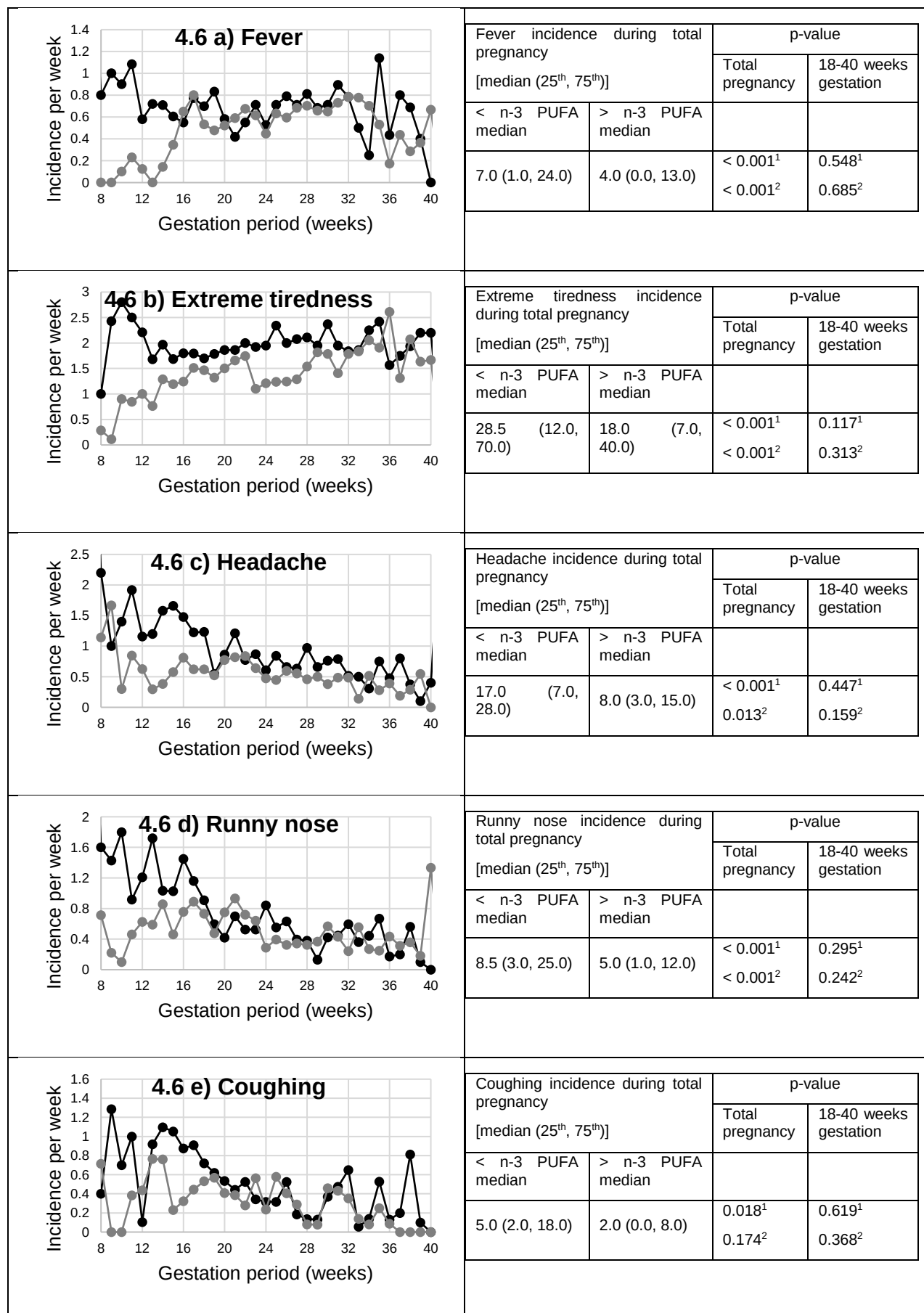
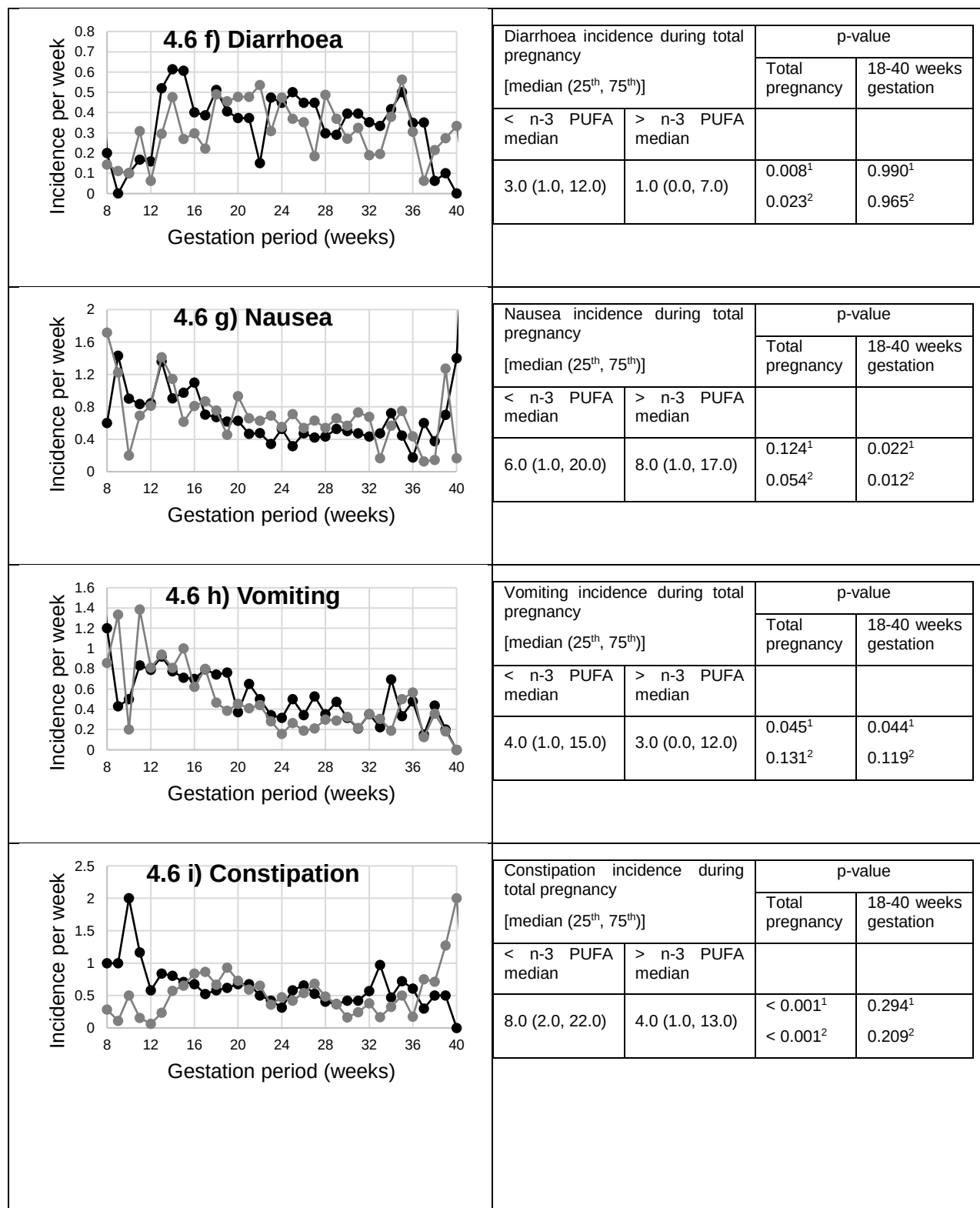


Figure 4.5: Morbidity symptoms over time in iron non-compliant and iron compliant pregnant women. (Black line = non-compliant, grey line = compliant; ¹difference between groups. ²difference between group by time interaction).

The final grouping of women was based on their n-3 PUFA status, above or below the median status of all the women (Figure 4.6). The incidence of fever, extreme tiredness, headache, runny nose, coughing, diarrhoea, vomiting, constipation and heartburn ($p < 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.001$, $p = 0.018$, $p = 0.008$, $p = 0.045$, $p < 0.001$ and $p = 0.037$, respectively) was lower in the higher n-3 PUFA status group compared to the lower n-3 PUFA status group. Incidence over time of headache and runny nose decreased in the lower n-3 PUFA status group whereas it stayed the same in the higher n-3 PUFA group. This caused the group by gestation time interaction to differ between groups ($p = 0.013$ and $p < 0.001$, respectively). The incidence over time of extreme tiredness increased more in the higher n-3 PUFA group than the lower n-3 PUFA group, resulting in a difference in group by gestation time interaction ($p < 0.001$). The incidence of fever and constipation decreased in the lower n-3 PUFA group and increased in the higher n-3 PUFA group during week 8 to ~16, causing an overall group by gestation time interaction (both $p < 0.001$).

Between 18 and 40 weeks gestation, nausea was higher in the higher n-3 PUFA group, but vomiting was lower ($p = 0.022$ and $p = 0.044$, respectively), compared to women with a poorer n-3 PUFA status. The group by gestation time interaction of nausea also differed between groups ($p = 0.012$).





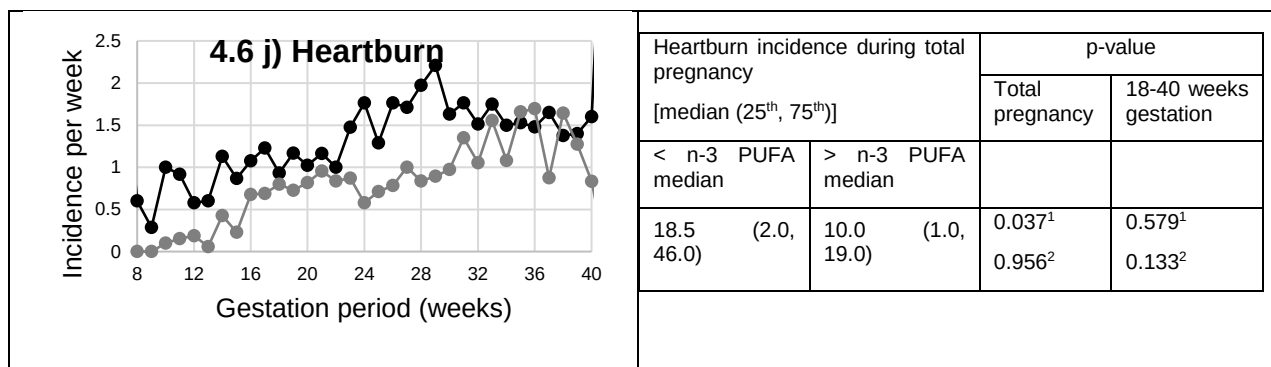


Figure 4.6: Morbidity symptoms over time pregnant women with n-3 PUFA status below the median status and n-3 PUFA status above the median status. (Black = n-3 PUFA status below the median (< n-3 PUFA median), grey = n-3 PUFA status above the median status (> n-3 PUFA median); ¹difference between groups. ²difference between group by time interaction).

Discussion

In urban SA pregnant women, 67% of the study population took on average 60 mg of daily iron for 5.6 months. In this population, iron sufficiency at early pregnancy was associated with higher blood pressure by 36 weeks gestation in women with an n-3 PUFA status below the median. This is also the first study to our knowledge to show that a higher incidence of self-reported fever, respiratory symptoms and diarrhoea were associated with iron sufficiency in pregnant women, during routine iron supplementation. Iron sufficiency was further associated with a higher incidence of extreme tiredness. Furthermore, women with an n-3 PUFA status above the median experienced less fever, extreme tiredness, headache, runny nose, coughing, diarrhoea, vomiting, constipation and heartburn during pregnancy, compared to women with an n-3 PUFA status below the median.

Iron deficiency and anaemia prevalence among the women was 11.9% and 20.8%, respectively. An acute inflammation prevalence of 91.1% and chronic inflammation prevalence of 15.8% were observed in the population, probably causing highly elevated ferritin concentrations. Although ferritin was adjusted for inflammation, it is possible we could still have underestimated the prevalence of ID. All women, even those without ID and anaemia, received ~60 mg of iron daily from the clinic. Along with this, some women bought additional iron supplementation. Women that took 60 mg or more daily, had a median total iron intake of 11 160 mg during pregnancy. The anaemia prevalence found in our study was similar to that of the South African National Health and Nutrition Examination Survey (SANHANES-1), which reported a 22% anaemia prevalence in females. The highest prevalence of females with anaemia was in KwaZulu-Natal, at 35.9% (Shisana *et al.*, 2013). It may be argued that the population size used in this survey was too small to give a clear indication of overall anaemia prevalence in SA (n = 1 359 females). Furthermore, the WHO does not define this as a severe

public health problem, as anaemia prevalence is less than 40% (WHO, 2012). It can therefore be questioned whether or not SA should abide by the supplementation guidelines of the WHO, of 30-60 mg elemental iron throughout pregnancy, especially considering that maize flour is mandatorily fortified with iron. This demonstrates the complexity of the problem, making it challenging to determine whether or not a standard iron supplementation dosage should be provided to all pregnant women in SA.

Additionally, similar to our findings, others have found increased hypertension prevalence in iron-sufficient and also in non-anaemic pregnant women who were supplemented with iron (Bo *et al.*, 2009, Ziaei *et al.*, 2007). Ziaei *et al.* (2007) found adverse effects in non-anaemic pregnant women in Iran (Hb ≥ 13.2 g/dl) after being supplemented with 50 mg of elemental iron daily. Hypertensive disorder was greater in the supplemented group (2.7%) compared to the placebo group (0.8%). Bo *et al.* (2009) found that hypertensive disorder in iron supplemented pregnant women was 25.9%, while in non-supplemented women it was 9.8%. This study, done in Italy, did not provide any data on iron markers. In our study, both systolic and diastolic blood pressure tended to be higher at 36 weeks gestation in the iron-sufficient group. Our study, however, showed less associations with morbidity in non-anaemic women (only with coughing and nausea), than in iron-sufficient women, indicating that iron sufficiency is the aspect which should acquire more regard when routinely supplementing pregnant women with iron. Additionally, it should be noted that of women with anaemia, approximately only 1% was contributed by IDA, indicating that other types of anaemias existed.

According to the findings of others, it was probable that women that are iron-sufficient or non-anaemic would have an increased incidence of GDM after iron supplementation. In our study, we found no association between iron sufficiency or non-anaemia and GDM. A possible reason for this is that our sample population was too small ($n = 102$). Previous prospective case-control studies in Iran ($n = 128$) and Hong Kong ($n = 291$) have found that non-anaemic women with GDM had a higher serum ferritin concentration than those without GDM (Sharifi *et al.*, 2010; Lao *et al.*, 2001). A retrospective case-control study also found that prevalence of GDM is lessened in IDA (Lao and Ho., 2004). This study also took place in Hong Kong, comparing 242 women with IDA, with 484 non-anaemic women. Conflicting results from a randomised controlled trial, again in Hong Kong, showed that no significant difference existed between GDM incidence in a group of pregnant women ($n = 1\ 164$) receiving 60 mg of iron supplementation daily compared to the placebo group. These women had an Hb between 8 and 14 g/dL (Chan *et al.*, 2009).

A correspondence analysis made it possible to distinguish pregnancy side effects and infectious morbidity symptoms from one another (Figure 1a). Clusters one and five associated closely, and could be interpreted to be due mostly to infectious morbidity. Cluster five, however,

contained both a pregnancy side effect (constipation) and an infectious morbidity symptom (fever), making this cluster an exception. When looking at symptoms experienced at a higher incidence (Figure 1b), specific types of infectious morbidity symptoms, namely respiratory and GI, could be differentiated from one another. Along with this, nausea and vomiting, which both decreased throughout pregnancy (Figure 2), were in the same cluster, which was plotted at a distance from diarrhoea. This also makes it evident that nausea and vomiting can be classified as pregnancy side effects, whereas diarrhoea is classified as a GI morbidity symptom.

The symptoms experienced by the overall population (Figure 3) indicate that while vomiting and nausea decreased through the gestation period, heartburn increased. The patterns of these side effects are expected throughout pregnancy (National Collaborating Centre for Women's and Children's Health, 2008). Extreme tiredness (which could either be a pregnancy side effect, infectious morbidity symptom or both) also increased throughout pregnancy. In our study, it was found that only 5.9% of women were diagnosed with asthma and 13.9% with rhinitis. This also strengthens our assumption that women with runny nose and coughing had infectious morbidity, rather than allergies (unpublished results). Other symptoms that decreased over the duration of pregnancy included coughing, headaches and a runny nose. Fever and constipation stayed reasonably constant for most of the gestation period, while diarrhoea peaked at the beginning and end of gestation.

When specifically observing infectious morbidity symptoms, both respiratory and GI infectious morbidity symptoms were higher in the iron-sufficient group compared to the ID group, from gestation week 18 to 40. These included fever, coughing and diarrhoea. These results are in congruence with iron supplementation studies performed in children, which showed to increased gastric and respiratory infectious morbidity symptoms (Paganini *et al.*, 2017, Malan *et al.*, 2016). This is the first study that we know of that compared infectious morbidity symptoms, based on iron status and markers, in pregnant women. Studies have, however, been conducted to investigate the effect of different iron dosages on GI symptoms. Zhou *et al.*, 2009 found that women receiving 20 mg of elemental iron daily had a significantly lower incidence of GI symptoms, compared to women receiving 80 mg elemental iron daily. These GI symptoms were specifically stomach pain, vomiting and nausea. Makrides *et al.* (2003) also found that a low dosage of 20 mg of iron daily was effective in preventing ID and IDA in Australian women, without aggravating GI symptoms. Since SA women receive ~60 mg of iron daily possibly for longer than three months, it was evident that this higher dosage, taken by iron replete women, enhanced the symptoms of infectious and other morbidities.

Another factor which influenced both hypertension and morbidity symptoms was n-3 PUFA status. Women with an n-3 PUFA status below the median had a higher diastolic blood pressure

than those above the median. It was also generally found that an n-3 PUFA status above the median status of the women, was associated with a lower incidence of diarrhoea, runny nose, coughing, fever, vomiting, extreme tiredness, headache, constipation and heartburn throughout gestation. These results agree with previous findings from our group and are likely due to the known anti-inflammatory effect of n-3 PUFA (Malan *et al.*, 2015; Malan *et al.*, 2016). Interestingly, only nausea was higher in women with a higher n-3 PUFA status.

Women who were compliant to iron supplementation experienced a higher incidence of morbidity symptoms. These included runny nose, fever, vomiting, extreme tiredness, constipation and heartburn. It is evident that not only pregnancy side effects, of which some are also known side effects of iron supplementation, but also morbidity symptoms were worsened due to iron supplementation (Hyder *et al.*, 2002). Women with hereditary haemochromatosis should also be considered. These women have elevated levels of serum ferritin and transferrin saturation, making iron supplementation a contributing factor of iron overload (Allen *et al.*, 2008). This gives rise to the concern of potential harmful effects caused by iron supplementation.

It may therefore be of value for SA to re-consider the current iron supplementation regime during pregnancy. This could be done by assessing the iron status of all women, prior to the provision of iron supplementation, ensuring that only women requiring a high dosage of iron receive it. A disadvantage of this option is that it is timeous and expensive. Another approach could entail the provision of a lower daily dosage of iron to all women. Studies have shown that a lower dosage of iron effectively treats or prevents ID and IDA during pregnancy, without enhancing the side effects (Zhou *et al.*, 2009, Makrides *et al.*, 2013). Alternatively, iron supplementation could be administered weekly, instead of daily, as this too was deemed safer (Pavord *et al.*, 2011; Viteri *et al.*, 2012). These regimes may be more cost effective and practically acceptable, but certainty of preventing ID and IDA is not guaranteed. Lastly, pregnant women should be encouraged to improve their n-3 PUFA status, as this has shown to reduce morbidity symptoms (Malan *et al.*, 2016). This can be done by means of dietary counselling, to inform women which food sources are high in n-3 PUFA. The practicality and financial implications of these alternatives will have to be evaluated to decide which will be most effective in ensuring the health of all pregnant women in SA.

A merit of our study is that the patterns of the side effects in the total population are patterns which are expected throughout pregnancy, which confirms the integrity of the data. Furthermore, the specific clustering of infectious morbidity symptoms made it possible to distinguish between morbidity symptoms and pregnancy side effects.

A limitation of our study was that the population was relatively small for the purpose of grouping the women into iron status and anaemia prevalence groups. Another limiting factor was that ID was defined only on the basis of ferritin. This is an acute phase protein which is affected by inflammation, and was therefore adjusted using Thurnham *et al.*'s calculation (2015). Although this adjustment was made, an underestimation of the true ID prevalence among this population may still have occurred. However for this reason, ID was strictly defined, which may have contributed to a more distinct difference between ID and iron-sufficient groups, which actually may have resulted in clearer differences between these groups. Transferrin receptor, which is another important marker in the assessment of iron status and not really affected by inflammation, was not measured (Désidéri-Vaillant *et al.*, 2011). Additionally, aspirin was prescribed by doctors if the women had a MAP > 90 mmHg in the second trimester. This was done as a precautionary measure to prevent the development of preeclampsia. This would have, therefore, decreased the number of preeclamptic women thus confounding our findings with regards to hypertension. Furthermore, morbidity symptoms were self-reported by each woman, and were not clinically diagnosed.

Conclusion

In conclusion, in this study, we showed that iron-sufficient women had a higher incidence of morbidity symptoms during pregnancy. Iron-sufficient women also tended to have an increased diastolic blood pressure which may increase the risk of developing hypertension. This association was even stronger in women with a lower n-3 PUFA status. Additionally, women with an n-3 PUFA status above the median had a lower incidence of morbidity symptoms, except for nausea. It may be prudent to reassess the current iron supplementation regime during pregnancy in SA.

Acknowledgments

We thank all the participants in the study; the entire research team for executing the study; and the staff of the antenatal clinic of the Rahima Moosa Mother and Child Hospital.

The authors' responsibilities were as follows: CMS, LM and JB were the principal investigators of the study, developed the study design, were overall responsible for data collection and data analysis. LM had the primary responsibility for final content of the manuscript. ES contributed to study protocols contributed to the study design and edited the draft manuscript. LM and CG wrote the first draft of the manuscript. CR was the study statistician, and analysed data and interpreted statistical analysis. ES was the study co-ordinator, helped to execute the study and edit the draft manuscript. LZ helped to execute the study.

References

- Allen, K.J., Gurrin, L.C., Constantine, C.C., Osborne, N.J., Delatycki, M.B., Nicoll, A.J., McLaren, C.E., Bahlo, M., Nisselle, A.E., Vulpe, C.D. & Anderson, G.J. 2008. Iron-overload-related disease in HFE hereditary hemochromatosis. *New England Journal of Medicine*, 358(3):221-230.
- Baumgartner, J., Smuts, C.M., Malan, L., Kvalsvig, J., van Stuijvenberg, M.E., Hurrell, R.F. & Zimmermann M.B. 2012. Effects of iron and n-3 fatty acid supplementation, alone and in combination, on cognition in school children: a randomized, double-blind, placebo-controlled intervention in South Africa. *The American Journal of Clinical Nutrition*, 96(6):1327-1338.
- Beard, J.L. 2000. Effectiveness and strategies of iron supplementation during pregnancy. *The American Journal of Clinical Nutrition*, 71(5):1288S-1294S.
- Chan, K.K.L., Chan, B.C.P., Lam, K.F., Tam, S. & Lao, T.T. 2009. Iron supplement in pregnancy and development of gestational diabetes - a randomised placebo-controlled trial. *BJOG: An International Journal of Obstetrics and Gynaecology*, 116(6):789-798.
- Cnossen, J.S., Vollebregt, K.C., de Vrieze, N., ter Riet, G., Mol, B.W., Franx, A., Khan, K.S. & van der Post, J.A. 2008. Accuracy of mean arterial pressure and blood pressure measurements in predicting pre-eclampsia: systematic review and meta-analysis. *British Medical Journal*, 336(7653):1117-1120.
- Department of Health. see South Africa. Department of Health
- Désidéri-Vaillant, C., Galinat, H., Sapin-Lory, J., Valero, E., Perennec, V. & Lefevre, F. 2011. Serum transferrin receptor in the assessment of iron status. *Transfusion Clinique et Biologique: journal de la Societe francaise de transfusion sanguine*, 18(1):36-39.
- Folch, J., Lees, M. & Sloane-Stanley, G.H. 1957. Extraction of fatty acid. *Journal of Biological Chemistry*, 226:497-509.
- Greenacre, M. 2017. Correspondence analysis in practice. CRC press.
- Greenacre, M. & Blasius, J. eds. 2006. *Multiple correspondence analysis and related methods*. CRC press.

Govender, T. 2016. The impact of access to antenatal care on maternal health outcomes among young adolescents on the North coast of KwaZulu-Natal, South Africa. KwaZulu-Natal: DUT. (Dissertation - Masters).

Hyder, S.Z., Persson, L.Å., Chowdhury, A.M.R. & Ekström, E.C. 2002. Do side-effects reduce compliance to iron supplementation? A study of daily-and weekly-dose regimens in pregnancy. *Journal of Health, Population and Nutrition*, 20(20):175-179.

Kim, H.J., Fay, M.P., Feuer, E.J. & Midthune D.N. 2000. Statistics in medicine.

Lao, T.T., Chan, P.L. & Tam, K.F. 2001. Gestational diabetes mellitus in the last trimester—a feature of maternal iron excess? *Diabetic Medicine*, 18(3):218-223.

Lao, T.T. & Ho, L.F. 2004. Impact of iron deficiency anemia on prevalence of gestational diabetes mellitus. *Diabetes Care*, 27(3):650-656.

Makrides, M., Crowther, C.A., Gibson, R.A., Gibson, R.S. & Skeaff, C.M. 2003. Efficacy and tolerability of low-dose iron supplements during pregnancy: a randomized controlled trial. *The American Journal of Clinical Nutrition*, 78(1):145-153.

Malan, L., Baumgartner, J., Calder, P.C., Zimmermann, M.B. and Smuts, C.M. 2015. n–3 Long-chain PUFAs reduce respiratory morbidity caused by iron supplementation in iron-deficient South African schoolchildren: a randomized, double-blind, placebo-controlled intervention. *The American Journal of Clinical Nutrition*, 081208:1-12.

Malan, L., Baumgartner, J., Zandberg, L., Calder, P.C. & Smuts, C.M. 2016. Iron and a mixture of DHA and EPA supplementation, alone and in combination, affect bioactive lipid signalling and morbidity of iron deficient South African school children in a two-by-two randomised controlled trial. *Prostaglandins, Leukotrienes and Essential Fatty Acids (PLEFA)*, 105:15-25.

Milman, N., Bergholt, T., Eriksen, L., Byg, K.E., Graudal, N., Pedersen, P. & Hertz, J. 2005. Iron prophylaxis during pregnancy—how much iron is needed? A randomized dose–response study of 20–80 mg ferrous iron daily in pregnant women. *Acta Obstetricia et Gynecologica Scandinavica*, 84(3):238-247.

Mwangi, M.N., Prentice, A.M. & Verhoef, H. 2017. Safety and benefits of antenatal oral iron supplementation in low-income countries: a review. *British Journal of Haematology*, 177:884-895.

National Collaborating Centre for Women's and Children's Health UK. 2008. Antenatal care.

O'Brien, K.O., Zavaleta, N., Caulfield, L.E., Wen, J. & Abrams, S.A. 2000. Prenatal iron supplements impair zinc absorption in pregnant Peruvian women. *The Journal of Nutrition*, 130(9):2251-2255.

Paganini, D., Uyoga, M.A., Kortman, G.A., Cercamondi, C.I., Moretti, D., Barth-Jaeggi, T., Schwab, C., Boekhorst, J., Timmerman, H.M., Lacroix, C. & Karanja, S. 2017. Prebiotic galacto-oligosaccharides mitigate the adverse effects of iron fortification on the gut microbiome: a randomised controlled study in Kenyan infants. *Gut*, 66:1956–67.

Pavord, S., Myers, B., Robinson, S., Allard, S., Strong, J. & Oppenheimer, C. 2012. UK guidelines on the management of iron deficiency in pregnancy. *British Journal of Haematology*, 156(5):588-600.

Prentice, A.M., Mendoza, Y.A., Pereira, D., Cerami, C., Wegmuller, R., Constable, A. & Spieldenner, J. 2016. Dietary strategies for improving iron status: balancing safety and efficacy. *Nutrition Reviews*, 75(1):49-60.

Ribot, B., Aranda, N., Giralt, M., Romeu, M., Balaguer, A. & Arijia, V. 2013. Effect of different doses of iron supplementation during pregnancy on maternal and infant health. *Annals of Hematology*, 92(2):221-229.

Sharifi, F., Ziaee, A., Feizi, A., Mousavinasab, N., Anjomshoa, A. & Mokhtari, P. 2010. Serum ferritin concentration in gestational diabetes mellitus and risk of subsequent development of early postpartum diabetes mellitus. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, 3:413.

Shisana, O., Labadarios, D., Rehle, T., Simbayi, L., Zuma, K., Dhansay, A., Reddy, P., Parker, W., Hoosain, E., Naidoo, P., Hongoro, C., Mchiza, Z., Steyn, N.P., Dwane, N., Makoe, M., Maluleke, T., Ramlagan, S., Zungu, N., Evans, M.G., Jacobs, L., Faber, M. & SANHANES-1

Team. 2013. South African National Health and Nutrition Examination Survey (SANHANES-1). [www.hsrc.ac.za/uploads/pageNews/72/SANHANES-launch%20edition%20\(online%20version\).pdf](http://www.hsrc.ac.za/uploads/pageNews/72/SANHANES-launch%20edition%20(online%20version).pdf) Date of access: 7 July 2016.

South Africa. Department of Health. 2015. Guidelines for maternity care in South Africa. A manual for clinics, community health centres and district hospitals. https://www.health-e.org.za/wp-content/uploads/2015/11/Maternal-Care-Guidelines-2015_FINAL-21.7.15.pdf Date of access: 9 Nov. 2016.

South African Audience Research Foundation. 2017. Living Standards Measure. <http://www.saarf.co.za/lsm/lsm.asp> Date of access: 11 Sep. 2017.

Thurnham, D.I., Northrop-Clewes, C.A. & Knowles, J. 2015. The use of adjustment factors to address the impact of inflammation on vitamin A and iron status in humans. *The Journal of Nutrition*, 145(5):1137S-1143S.

Tripathi, R., Gupta, S., Rai, S. & Mittal, P.C. 2013. Short-term, high-dose iron supplementation to healthy pregnant women increases oxidative stress markers: Implications for use of phytonutrients. *Annals of Phytomedicine*, 2(2):71-78

Viteri, F.E., Casanueva, E., Tolentino, M.C., Díaz-Francés, J. & Erazo, A.B. 2012. Antenatal iron supplements consumed daily produce oxidative stress in contrast to weekly supplementation in Mexican non-anemic women. *Reproductive Toxicology*, 34(1):125-132.

Weinberg, E.D. 2009. Are iron supplements appropriate for iron replete pregnant women? *Medical Hypotheses*, 73(5):714-715.

WHO (World Health Organization). 2001. Iron deficiency anaemia: assessment, prevention and control: a guide for programme managers. http://www.who.int/iris/bitstream/10665/66914/1/WHO_NHD_01.3.pdf?ua=1 Date of access: 29 Jan. 2017

Zhou S.J., Gibson R.A., Crowther C.A., & Makrides M. 2009. Should we lower the dose of iron when treating anaemia in pregnancy? A randomized dose-response trial. *European Journal of Clinical Nutrition*, 63(2):183-191.

Ziaei, S., Norrozi, M., Faghihzadeh, S. & Jafarbegloo, E. 2007. A randomised placebo-controlled trial to determine the effect of iron supplementation on pregnancy outcome in pregnant women with haemoglobin $\geq$ 13.2 g/dl. *BJOG: An International Journal of Obstetrics and Gynaecology*, 114(6):684-688.

CHAPTER 5 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The aim of this mini-dissertation was to assess whether the iron status at < 18 weeks of gestation is associated with morbidity during the course of pregnancy in urban pregnant women receiving routine iron supplementation in SA. Our objectives were to:

- Determine whether iron status and anaemia at < 18 weeks of gestation are associated with the incidence of morbidity symptoms during the course of pregnancy in urban pregnant women receiving routine iron supplementation in SA.
- Determine whether iron status and anaemia at < 18 weeks of gestation are associated with the incidence of hypertension and gestational diabetes mellitus (GDM) and their respective biological markers during the course of pregnancy in urban pregnant women receiving routine iron supplementation in SA.
- Determine whether n-3 PUFA status at < 18 weeks of gestation is associated with the incidence of morbidity symptoms and blood pressure during pregnancy in urban pregnant women receiving routine iron supplementation in SA.

This study was one of the first to evaluate infectious morbidity symptoms during pregnancy, based on iron status and anaemia. We found that iron-sufficient women experienced a higher incidence of morbidity symptoms. Morbidity symptoms with a higher incidence in the iron-sufficient group included fever, extreme tiredness, coughing and diarrhoea. Iron-sufficient women also tended to have a higher blood pressure at 36 weeks gestation, and affected women with a lower n-3 PUFA more. We found a greater association of morbidity in iron-sufficient women, than in non-anaemic women receiving routine iron supplements during pregnancy. This indicated that iron sufficiency should be evaluated when providing iron supplementation to pregnant women. We also found that women with an n-3 PUFA status above the median experienced less fever, extreme tiredness, headache, runny nose, coughing, diarrhoea, vomiting, constipation and heartburn during pregnancy, compared to women with an n-3 PUFA status below the median.

Our study agreed with the findings of Bo *et al.* (2009) and Ziaei *et al.* (2007), who also found increased blood pressure, more specifically hypertension, in iron-sufficient and non-anaemic pregnant women. Our study, however, showed less associations with morbidity in non-anaemic women, than in iron-sufficient women, indicating that iron sufficiency is the aspect which should be dominantly considered when routinely supplementing pregnant women with iron. Along with this, women who were compliant to iron supplementation experienced a higher incidence of

morbidity symptoms. Studies in children have also found increased infectious morbidity symptoms due to iron supplementation (Paganini *et al.*, 2017; Malan *et al.*, 2016). Our study found that morbidity symptoms, as well as common pregnancy side effects, were associated with iron supplementation. An unexpected outcome of our study was that iron sufficiency and non-anaemia were not associated with GDM, fasting blood glucose and blood glucose 2 hours after taking a dose of 20 ml glucose in a fasted state at ~22 weeks gestation. This may be due to the small sample population of our study (n = 102), having only three women with GDM. Most studies have found that an association between increased iron status and GDM (Sharifi *et al.*, 2010; Lao *et al.*, 2001; Lao & Ho., 2004).

Due to the impact of zinc on infectious morbidity, it may have been beneficial to have measured plasma zinc levels, as well as oxidative stress. These may have provided further insight regarding the effect of iron on these two measures and their link to infectious morbidity and pregnancy side effects. Furthermore, various factors that are able to affect the oxidative status of the blood should be investigated, as well as investigating the intake of vitamin C, fruit and leafy vegetables by the pregnant women. The effect of HIV on these findings should also be explored.

5.2 Recommendations

It would be advantageous to increase the sample size. This would decrease the variance in morbidity incidence, particularly at the start and end of pregnancy where the number of participants were fewer. The accuracy of the study could also be improved by measuring transferrin receptor as an additional iron status marker, as it is an important marker in the assessment of iron status which are less affected by inflammation than ferritin (Désidéri-Vaillant *et al.*, 2011).

A further recommendation from this study to the Public Health Association of South Africa (PHASA) would be to consider reassessing the current supplementation programme. Due to the harmful effects caused by the current iron dosage administered to all women, it may be beneficial to adapt this supplementation regime. An ideal solution would be assessing the iron status of all women, prior to providing iron supplementation. In this manner, only women who require this high dosage of iron would receive it. This, however, is expensive and timeous, making this option less viable. Another approach could entail the provision of a lower daily dosage of iron to all women. Studies have shown that a lower dosage of iron effectively treats or prevents ID and IDA during pregnancy, without enhancing the side effects (Zhou *et al.*, 2009, Makrides *et al.*, 2013). Alternatively, iron supplementation could be administered weekly, instead of daily, as this too was deemed safer (Pavord *et al.*, 2011; Viteri *et al.*, 2012). These

regimes may be more cost effective and practically acceptable, but certainty of preventing ID and IDA is not guaranteed. It may still be necessary to provide anaemic women with a higher or more frequent iron supplementation regime. Lastly, pregnant women should be encouraged to improve their n-3 PUFA status, as this has shown to reduce morbidity symptoms (Malan *et al.*, 2016). This can be done by means of dietary counselling, informing the women which food sources are high in n-3 PUFA. The practicality and financial implications of these alternatives will have to be evaluated to decide which will be most effective in ensuring the health of all pregnant women in SA.

This study demonstrated the need for further research to be done on this topic. Randomised placebo-controlled trials, specifically, would increase the reliability and validity of these findings. These trials could be done by grouping iron-sufficient women, providing each group with either a high iron dosage, low iron dosage or placebo. Alternatively, the women could be grouped to receive either daily or weekly iron supplementation. Another possible future study, would be to provide a group of women receiving iron supplementation, with an additional dietary source which is high in n-3 PUFA, whilst the other group receives iron supplementation and a placebo. The incidence of morbidity symptoms, blood pressure and the incidence of hypertension, as well as blood glucose and the incidence of GDM during pregnancy should then be investigated between the various groups. Continued research regarding iron supplementation could attain an alteration in the current iron supplementation regime, in order to improve the health of pregnant women.

5.3 References

- Bo, S., Menato, G., Villois, P., Gambino, R., Cassader, M., Cotrino, I. & Cavallo-Perin, P. 2009. Iron supplementation and gestational diabetes in midpregnancy. *American Journal of Obstetrics and Gynecology*, 201(2):158.e1-6.
- Désidéri-Vaillant, C., Galinat, H., Sapin-Lory, J., Valero, E., Perennec, V. & Lefevre, F. 2011. Serum transferrin receptor in the assessment of iron status. *Transfusion Clinique et Biologique: journal de la Societe francaise de transfusion sanguine*, 18(1):36-39.
- Lao, T.T., Chan, P.L. & Tam, K.F. 2001. Gestational diabetes mellitus in the last trimester—a feature of maternal iron excess? *Diabetic Medicine*, 18(3):218-223.
- Lao, T.T. & Ho, L.F. 2004. Impact of iron deficiency anemia on prevalence of gestational diabetes mellitus. *Diabetes Care*, 27(3):650-656.
- Makrides, M., Crowther, C.A., Gibson, R.A., Gibson, R.S. & Skeaff, C.M. 2003. Efficacy and tolerability of low-dose iron supplements during pregnancy: a randomized controlled trial. *The American Journal of Clinical Nutrition*, 78(1):145-153.
- Malan, L., Baumgartner, J., Zandberg, L., Calder, P.C. & Smuts, C.M. 2016. Iron and a mixture of DHA and EPA supplementation, alone and in combination, affect bioactive lipid signalling and morbidity of iron deficient South African school children in a two-by-two randomised controlled trial. *Prostaglandins, Leukotrienes and Essential Fatty Acids (PLEFA)*, 105:15-25.
- Paganini, D., Uyoga, M.A., Kortman, G.A., Cercamondi, C.I., Moretti, D., Barth-Jaeggi, T., Schwab, C., Boekhorst, J., Timmerman, H.M., Lacroix, C. & Karanja, S. 2017. Prebiotic galacto-oligosaccharides mitigate the adverse effects of iron fortification on the gut microbiome: a randomised controlled study in Kenyan infants. *Gut*, 66:1956–67.
- Pavord, S., Myers, B., Robinson, S., Allard, S., Strong, J. & Oppenheimer, C. 2012. UK guidelines on the management of iron deficiency in pregnancy. *British Journal of Haematology*, 156(5):588-600.
- Sharifi, F., Ziaee, A., Feizi, A., Mousavinasab, N., Anjomshoaa, A. & Mokhtari, P. 2010. Serum ferritin concentration in gestational diabetes mellitus and risk of subsequent development of early postpartum diabetes mellitus. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, 3:413.

Viteri, F.E., Casanueva, E., Tolentino, M.C., Díaz-Francés, J. & Erazo, A.B. 2012. Antenatal iron supplements consumed daily produce oxidative stress in contrast to weekly supplementation in Mexican non-anemic women. *Reproductive Toxicology*, 34(1):125-132.

Zhou S.J., Gibson R.A., Crowther C.A., & Makrides M. 2009. Should we lower the dose of iron when treating anaemia in pregnancy? A randomized dose-response trial. *European Journal of Clinical Nutrition*, 63(2):183-191.

Ziaei, S., Norrozi, M., Faghihzadeh, S. & Jafarbegloo, E. 2007. A randomised placebo-controlled trial to determine the effect of iron supplementation on pregnancy outcome in pregnant women with haemoglobin ≥ 13.2 g/dl. *BJOG: An International Journal of Obstetrics and Gynaecology*, 114(6):684-688.

ANNEXURES

Annexure A



NuPED

Nutrition during Pregnancy and Early Development

WEEKLY SYMPTOMS for the PREGNANT MOTHER

Participant number:

Gestational week number:

Fieldworker's name: _____

Indicate what you experienced by ticking ✓ the symptoms for the day and write down cravings, aversions and medicine. Please remember to fill in the date

Day	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
Date (dd/mm/yyyy):							
Healthy							
Gastro							
Runny nose							
Coughing							
Hot Body							
Nauseous							
Vomiting							
Strange cravings? Please write them down							
Very tired							
Headache							
Constipation							
Heartburn							
Any aversions? Please write them down							
Did you take your antenatal supplementation?							
Did you use any medicine or traditional medicine? Please write them down							

Annexure B



NORTH-WEST UNIVERSITY
YUNIBESITHI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT

Private Bag X6001, Potchefstroom,
South Africa, 2520

Tel: (018) 299-4900

Faks: (018) 299-4910

Web: <http://www.nwu.ac.za>

Institutional Research Ethics Regulatory Committee

Tel: +27 18 299 4849

Email: Ethics@nwu.ac.za

ETHICS APPROVAL CERTIFICATE OF STUDY

Based on approval by Health Research Ethics Committee (HREC) on 01/09/2017 after being reviewed at the meeting held on 19/07/2017, the North-West University Institutional Research Ethics Regulatory Committee (NWU-IRERC) hereby approves your study with conditions as indicated below. This implies that the NWU-IRERC grants its permission that provided the special conditions specified below are met and pending any other authorisation that may be necessary, the study may be initiated, using the ethics number below.

Study title: Iron status in relation to morbidity when considering iron supplementation among urban pregnant women in South Africa.

Study Leader/Supervisor: Dr L. Malan

Student: C Goodchild-23490209

Ethics number:

N	W	U	-	0	0	1	8	6	-	1	5	-	A	1	-	0	2
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

Institution Study Number Year Status Sub-study

Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

Application Type: Sub-study

Commencement date: 01-09-2017

Risk: Medium

Continuation of the study is dependent on receipt of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation.

Special conditions of the approval (if applicable):

- The researchers have indicated that they will be able to analyse the plasma samples within the laboratory at the Centre of Excellence for Nutrition, however, it is indicated that if for any reason the plasma samples need to be sent to Dr Erhardt's laboratory in Germany for analysis, the researchers will have to apply for an amendment request to obtain permission to send the samples overseas, as well as providing the HREC with a copy of an updated export permit from the Department of Health, when it is obtained.

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, please note the following:

- The study leader (principle investigator) must report in the prescribed format to the NWU-IRERC via HREC:
 - annually (or as otherwise requested) on the monitoring of the study, and upon completion of the study
 - without any delay in case of any adverse event or incident (or any matter that interrupts sound ethical principles) during the course of the study.
- Annually a number of studies may be randomly selected for an external audit.
- The approval applies strictly to the proposal as stipulated in the application form. Would any changes to the proposal be deemed necessary during the course of the study, the study leader must apply for approval of these amendments at the HREC, prior to implementation. Would there be deviation from the study proposal without the necessary approval of such amendments, the ethics approval is immediately and automatically forfeited.
- The date of approval indicates the first date that the study may be started.
- In the interest of ethical responsibility the NWU-IRERC and HREC retains the right to:
 - request access to any information or data at any time during the course or after completion of the study;
 - to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process.
 - withdraw or postpone approval if:
 - any unethical principles or practices of the study are revealed or suspected,
 - it becomes apparent that any relevant information was withheld from the HREC or that information has been false or misrepresented,
 - the required amendments, annual (or otherwise stipulated) report and reporting of adverse events or incidents was not done in a timely manner and accurately,
 - new institutional rules, national legislation or international conventions deem it necessary.
- HREC can be contacted for further information or any report templates via Ethics-HRECAppl@nwu.ac.za or 018 299 1206.

The IRERC would like to remain at your service as scientist and researcher, and wishes you well with your study. Please do not hesitate to contact the IRERC or HREC for any further enquiries or requests for assistance.

Yours sincerely

Prof LA
Du Plessis

Digitally signed by
Prof LA Du Plessis
Date: 2017.09.15
08:15:44 +02'00'

Prof Linda du Plessis

Chair NWU Institutional Research Ethics Regulatory Committee (IRERC)

Annexure C



R14/49 Prof Marius Smuts et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M150968

NAME: Prof Marius Smuts et al
(Principal Investigator)
DEPARTMENT: Centre of Excellence for Nutrition
University of the Witwatersrand and North West University
Region B and C, City of Johannesburg
Florida Clinic, Bosmont Clinic, Sophiatown
and Rahima Moosa Mother and Child Hospital

PROJECT TITLE: Nutrition during Pregnancy and Early Development:
The NuPED Study

DATE CONSIDERED: 02/10/2015

DECISION: Approved unconditionally

CONDITIONS:

SUPERVISOR:

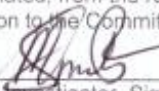
APPROVED BY: 
Professor P Cleaton-Jones, Chairperson, HREC (Medical)

DATE OF APPROVAL: 08/02/2016

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 10004, 10th floor, Senate House/2nd Floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. I agree to submit a yearly progress report


Principal Investigator Signature

Date 18/02/2016

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Annexure D

Socio-demographic questionnaire

Participant nr:

Date: 2 0 Y Y M M D D

Fieldworker:

1. Date of birth:

----------------------	----------------------	----------------------	----------------------	----------------------	----------------------	----------------------	----------------------

2. How would you describe yourself in terms of population group?

1	2	3	4	5
Black	Coloured	Indian	White	Other. Specify:
1	2	3	4	5

3. What is your home language?

English	Xhosa	Zulu	Sotho	Other. Specify:
---------	-------	------	-------	--------------------

4. In which country were you born?

1	2	3	4	5	6	7
South Africa	Zimbabwe	Lesotho	Swaziland	Botswana	Namibia	Other. Specify:

5. How long have you been staying in South Africa?

1	2	3	4
Less than 1 year	1 - 2 years	2 - 5 years	More than 5 years

6. What is your highest formal educational level?

1	2	3	4	5
None	Primary	Std 6-8/Gr 6-8	Std 9-10/Gr 11&12	Tertiary Education

7. What is your marital status?

1	2	3	4	5	6	7	8
Unmarried	Married	Divorced	Separated	Widowed	Living Together	Traditional Marriage	Other. Specify:

8. What is your employment status?

1	2	3	4	5	6
Unemployed	Homemaker by choice	Self-Employed	Wage-Employed	Self-employed Professional	Other. Specify:

9. How many people live in your household most days of the week (including children and elderly)?

10. Do any members of the household receive any grants?

1	2	3	4	5	6
None	Child support	Social relief	Disability	Old age pension	Other. Specify

11. To determine your living standards measure, please indicate which of the following you currently have in your household:

X = Yes ; - = No

X	Metropolitan dweller (250 000+)		DVD Player / Blu Ray Player
	~~Living in a non-urban area~~		Refrigerator or combined fridge/freezer
	House / Cluster House / Town House		Electric Stove
	Tap water in house / on plot		Microwave oven
	Flush Toilet inside house		Deep Freezer - Free Standing
	Hot running water		Washing machine
	Built in Kitchen Sink		Tumble dryer
	No Domestic Workers or Gardeners		Dishwashing Machine
	Home security service		PayTV (M-net / DSTV / TopTV) Subscription
	2 Cell phones in Household		Home Theatre System
	3 or more Cell phones in Household		Vacuum Cleaner
	Zero or One Radio set in Household		Motor Vehicle
	Air conditioner (excl. fans)		Computer - Desktop / Laptop
	Have TV set(s)		Land line (excl. Cellphone)
	Swimming Pool		