AN INVESTIGATION INTO THE IMPACT OF VITAMIN SUPPLEMENTATION, MATERNAL CHARACTERISTICS AND LIFESTYLE CHOICES ON THE DEVELOPMENT OF PRE-ECLAMPSIA

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Student Declaration

I declare that the study presented in this paper

has been conducted by myself independent of any other person or group.

I also declare that the work presented here have been composed by

myself with the exception of feedback from my supervisors.

Turnitin score = 17%

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In memory of my beloved

mom Hazel Edwards - 1940 -2019

Contents

List of Tables

List of Figures

List of Abbreviations

BAME - Black, Asian and Minority Ethnic groups

BMI – Body mass index

CASP- Critical appraisal skills programme

CI – Confidence interval

CMACE- Centre for Maternal and Child Enquiries

CEMACH – The confidential enquiry into maternal and child health

DNA – Deoxyribonucleic acid

DSE – Display screen equipment

GCP – Good clinical practice

GP- General Practitioner

HRA – Health research Authority

ID – Identification number

MBRRACE – Mothers and Babies: Reducing risk through audits and Confidential Enquiries.

NHS – National Health Service

NICE- National Institute for Health and Care Excellence

NIGB – National information Governance board

NMC – Nursing and Midwifery Council

NPEU – National Perinatal Epidemiology Unit

NREC- National Resource Evaluation Centre

NRES- National Research Ethics Service

OR – Odds ratio

PE – Pre-eclampsia

PET – Pre-eclampsia toxaemia

PGCert – Post graduate certificate

PHE – Public Health England

RDA – Recommended daily allowance

PICO – Population, Intervention, Comparison, Outcome

RCOG – Royal College of Obstetricians and Gynaecologists

RCT – Randomised control trial

RR- Risk ratio/ relative risk

SPSS – Statistical Package for social sciences.

UK – United Kingdom

UV – Ultra violet light

UVB – Ultra violet B rays

WHO – World Health Organisation

Glossary

1,25 dihydroxyvitamin - The form of metabolised active hormone of vitamin D found in the blood. Metabolised from the 25-hydroxyvitamin D absorbed through ultra violet ray exposure. The level of 1,25-hydroxyvitamin in the blood is used to determine sufficiency, insufficiency or deficiency of vitamin D.

Binary Regression - A logistic statistical model for testing used to estimate parameters or correlations between predictor variables and a binary dependant variable.

Chorionic Villi - Finger like projections from the trophoblast, which embed into the decidua to anchor the placenta and enable nutrient exchange to the fetus.

Cholecalciferol - Otherwise known as D3 and derived within animals and from sun exposure.

Cytotrophoblastic layer - Lies adjacent to the syncitiotrophoblast layer of the villi and prevents mixing of the maternal and fetal blood circulation but permits nutrient exchange

Decidua - The lining of the uterus during pregnancy

Eclampsia/Eclamptic - A potentially fatal condition during pregnancy resulting from pre-eclampsia taking the form of seizures.

Ergocalciferol - The form of vitamin known as D2, said to be less efficient at raising serum vitamin D levels. Derived from plants and absorbed from the diet rather than sun exposure.

Fetus - A baby in utero prior to birth.

Folic acid – May be referred to as folate or vitamin B9. Part of the vitamin B group but in manufactured form. It is found naturally in plants and vegetables and is used in pregnancy to prevent neural tube defects in the fetus.

Gravida - The number of pregnancies experienced

Heterogeneity – being diverse. Can arise with different populations or within research due to differences in methodology.

Liquid Chromatography – A laboratory technique for separating and testing mixtures such as blood.

Multipara - A woman experiencing her second or subsequent pregnancy

Nullipara - A woman experiencing her first pregnancy

Parity - The number of live births over 24 weeks gestation expressed in numbers. Pregnancy losses are added with a plus e.g. P2 +1

Pre-eclampsia - A multi systemic condition presenting only during pregnancy. Symptoms can vary but characterised by raised blood pressure, proteinuria and oedema (swelling of the body and face).

Primigravida - A woman experiencing her first birth/newborn

Proteinuria - Protein found in the urine on testing. Can be due to infection or presence of blood. In pre-eclampsia, protein excretion is due to damage to the kidneys.

Syncitiotrophoblast - A membrane layer, which secretes enzymes to break down maternal blood vessels at the site of the placenta enabling transfer of nutrients. Lies adjacent to the cytotrophoblastic layer of the chorionic villi.

Trophoblast - Forms from specialist cells arising from the blastocyst following cell division after fertilization.

Vascular Endothelial Dysfunction – When damage occurs to the thin cell lining (endothelium) of blood vessels. Often a precursor to cardiovascular disease.

Vitamin D – A fat soluble vitamin which promotes bone growth and calcium absorption. Also helps immune function. Main source is from sunlight but also found in eggs, fish oils and liver**.**

Abstract

Background: Pre-eclampsia remains in the top five leading causes of maternal mortality and morbidity in the United Kingdom (Knight 2018). For decades, research has striven to establish the aetiology, improved ways of managing the condition and ways to prevent its occurrence. Recent studies have turned their attention to the physiological processes in vitamin metabolism, in particular vitamin D and folic acid, and associated links with a reduction in pre-eclampsia. With a Public Health England (PHE) initiative encouraging women to take the multivitamin supplement Healthy Start, providing a combination of these two vitamins as well as vitamin C, there was opportunity to explore the impact of combined supplementation and the outcome of pre-eclampsia. In addition, some have identified maternal characteristics and socio-economic factors such as age and parity as possible precursors to pre-eclampsia, though not always conclusively.

Methods: A retrospective cross-sectional cohort study was conducted on 952 women (including *n* = 599 (63%) non-pre-eclampsia women and *n* = 353 (37%) of women diagnosed with a range of severity of pre-eclampsia. All gave birth at a tertiary maternity unit in the West Midlands. Data collected from medical records regarded uptake of vitamins, along with lifestyle choices and maternal characteristics, such as body mass index (BMI), ethnicity and age. Non-parametric testing and binary regression was conducted using the Statistical Package for Social Sciences (SPSS). Ethical approval was ensured.

Findings: Whilst a combination of vitamins given later in pregnancy demonstrated no correlation between them and pre-eclampsia, folic acid supplementation in particular demonstrated a strong statistical correlation with the incidence of pre-eclampsia $p>0.001$ when taken pre-conception and during the first trimester. Women experiencing their first pregnancy, with raised BMI giving birth during winter or spring seasons in particular, showed a greater risk of developing pre-eclampsia.

Conclusion: Though no correlation was found between multi vitamins taken later in pregnancy there is evidence from this study to suggest that dual vitamin supplementation could have the potential to decrease the incidence of pre-eclampsia but more likely when administered pre-conception and during the first trimester. It is therefore proposed, that further research into the supplementation of multivitamins pre conception and early pregnancy, could be beneficial and this warrants further investigation.

Introduction and Literature Review

1 Background and literature review

1.1 Introduction

Pre-eclampsia is a condition affecting between 2 and 7 % of pregnant women (Webster, Dodd and Waugh 2013 p34) and continues to remain a leading cause of maternal morbidity and mortality both globally and within the United Kingdom; despite continued efforts by health professionals to diagnose and manage the condition (Knight et al 2016). Alongside three primary symptoms of raised blood pressure, protein in the urine and oedema, plus the potential for other symptoms including altered vision, headaches and epigastric pain, pre-eclampsia has the potential to affect all pregnant women: some significantly, sometimes fatally. Though most commonly presenting towards the later weeks of pregnancy, predominantly in the third trimester, there are significant risks of premature birth and the possibility of the mother developing eclamptic fits, which in turn can prove fatal. A key challenge facing clinicians is that symptoms may not present until the condition has fully established, if at all, and more puzzling still is the issue that women may experience very different degrees of severity. Subsequently there is a continued desire to investigate and explore this condition and minimise these potentials.

Previous high profile triennial reports into maternal deaths from the Centre for Maternal and Child Enquiries (CEMACH) (Lewis 2007 and 2011) and annual reports by MBRRACE (Knight et al 2016 and 2017) have indicated that the number of deaths from pre-eclampsia has fallen from second to fourth in the leading four direct causes over the decades. Even with the potential of the next report from MBRRACE, due for publication after the time of writing in November 2019, demonstrating a fluctuation in occurrence, there has been a notable decrease over the years. These encouraging results continue to spur on the determination to win the battle over what remains a notable threat to pregnant women and their babies. Whilst there may be a number of reasons why there has been such a drop in ranking, including the increase in other causes such as thromboembolism and sepsis, arguably the reduction reflects the importance of the impact decades of research has had. Continued research into pre-eclampsia strives to not only confirm the aetiology of the condition, but to find ways of predicting and preventing it, or at the very least limiting its impact. Despite

these ongoing investigations however, there remain unanswered questions (Dekker and Sibai 2001).

Indeed, the findings of studies thus far, including work by for example Purswani et al (2017) have successfully provided a much clearer picture of the aetiology of pre-eclampsia; linked to the ineffective implantation and development of the placenta. Other leading researchers are devoting time to exploring the development of tools for assessing and identifying women at risk. The remaining broad spectrum of studies have their focus primarily on a possible cure or prevention, though with varied degrees of success.

Pre-eclampsia has been labelled as a 'diseases of theories' (Redman and Walker 1992) and to date many studies have explored the association between traits such as age, ethnicity, height or factors such as climate and season, with women developing this condition. Many of these studies provide tenuous associations between characteristics such as drinking excessive amounts of tea or low fibre diets. However, one characteristic, which is gaining significant momentum, is the lack of certain vitamins in a mother's diet during and before pregnancy.

The association with vitamin D deficiency and the development of pre-eclampsia is proving to be key with prominent researchers such as Hyppönen (2005) and Hyppönen et al (2014) and Bodnar et al (2007a and 2014) leading substantial studies in this field. Alongside this, others including Makedos et al (2007) and Wu Wen et al (2008) have been considering the link between folic acid and pre-eclampsia. To date however, the advice given to pregnant women in the majority of cases is to take folic acid only or take vitamin D as well if seen as important, but generally at very separate times during their pregnancy. Routinely only women with a raised body mass index (BMI) are recommended vitamin D supplementation (National Institute for Health Care Excellence (NICE) 2014). Despite acknowledgement that a significant proportion of the British population are deficient or insufficient in vitamin D, testing for this is not routine, nor is the prescribing of vitamin D. Indeed, Palacios and Gonzales (2014) provides clear evidence that vitamin D insufficiency and deficiency is a global issue, which adds further support to the need to consider this for the diverse ethnic

groups found in the catchment area covered by this doctoral study. Promoted in the vitamin D deficiency guidance (National Institute for Health and Care Excellence NICE 2014) a public health programme will be focussing their attention on reversing this. Traditionally folic acid is taken prior to conception and for the first few weeks of pregnancy and unless women opt to take other supplements then vitamin C is not considered.

One such strategy to correct deficiency in at risk groups is to prescribe free Healthy Start vitamins containing vitamin D, folic acid and vitamin C to women during their pregnancy within the local region captured by the centre used for this research.

The main driver underpinning this initiative is to prevent and or reduce the incidence of rickets in the case of vitamin D and as clinically proven by (Lassi et al 2013) continue to reduce the incidence of neural tube defects with the use of folic acid. However, when considering the supposed association between pre-eclampsia and these vitamins, there is a potential that supplementing them simultaneously could have a positive impact on the development of, or at least the severity of pre-eclampsia.

Subsequently with these concepts in mind, this study intended to examine if such an association between supplementation and women who develop pre-eclampsia exists. Taking a positivist stance and using a quantitative methodology, this study had the intention of ascertaining whether the introduction of supplementation of a multivitamin such as 'Healthy Start vitamins' (containing both vitamin D, folic acid along with vitamin C) during pregnancy, could limit the onset or severity of the condition.

Following an introduction to the principal components of the study, an exploration of the methods used and presentation of results, an in depth analysis will aim to establish a clearer answer to the question of interest

1.2 Background and Literature

Pre- eclampsia remains a leading cause of maternal mortality worldwide (Duley 2009). Once ranked as the second highest cause of death during pregnancy in the United Kingdom (UK), with 22 deaths in the triennial report "Saving mother's lives: Reviewing maternal deaths to make motherhood safer (Centre for Maternal and Child Enquiries CEMACH (Lewis 2011). More recent findings from the Mothers and Babies: Reducing risk through audits and Confidential Enquiries into maternal deaths (MBRRACE), indicates a significant reduction in deaths from pre-eclampsia, but highlight continued efforts are needed to minimize the impact of this condition on the pregnant population (Knight et al 2016 and 2018). Subsequently ongoing research strives to continue this downward trend. Available literature has explored a number of factors, and some have shown interest in the effect of vitamin supplementation and the potential impact they may have, be that from increasing risk of disease development or from the reduction of such risks.

The intention of this review using a systematic approach of the available literature was therefore to explore vitamin supplementation alongside several variables, in pregnancy and the incidence of pre-eclampsia. Its purpose was to establish an appropriate basis to support this doctoral study and in addition identify some of the risk factors associated with women and pre-eclampsia, which could benefit from the introduction of supplementation should a positive correlation be observed. Amongst these risk factors for consideration are BMI status and lifestyle choices including smoking, age and ethnicity.

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A primary research question aided the focus of the literature review and also identified where a clear gap in current knowledge was present.

"Does vitamin supplementation in pregnant women within a regional maternity unit, reduce the risk of developing pre-eclampsia?"

Or the null hypothesis of - Vitamin supplementation has no impact on the reduction of risk of pre-eclampsia.

A key component of Healthy Start vitamins, vitamin D, is unique in that it is not only absorbed from dietary intake but also through synthesis within the body after sun exposure. Vitamin D from either source is essential for effective cell function, control of calcium and parathyroid

hormone levels (Mulligan et al 2010). Research has identified it as a potential major factor in effective placental function and fetal development (Evans et al 2014). Extensive studies into fluctuations in levels of serum vitamin D in pregnant populations have been conducted and in several cases in relation to pre-eclampsia. Despite food fortification and use of supplementation, vitamin D deficiency or insufficiency remains common in the female population of many countries worldwide, including the UK (Prentice 2008).

Other researchers have considered the potential for folic acid and vitamin C to impact on pre-eclampsia symptoms specifically, though with mixed results, and never in combination as Healthy Start are. Currently all women are advised to take folic acid in particular, taken ideally pre-conception and in the first trimester, independently from any other supplementation. The exception being for women with a raised BMI who are encouraged to take a larger dose of folic acid alongside vitamin D. In the preceding 4 years however, there has been a shift towards supplementing some women with a multi vitamin containing Vitamin D and C as well as folic acid in the form of Healthy Start vitamins. With suggested benefits arising from administering these vitamins separately, and with some literature showing signs of a positive impact on pre-eclampsia, this raises a question as to whether a combination of these vitamins in the form of the multivitamin, could have a greater effect on the outcomes for women at risk of developing pre-eclampsia

Alongside this, the incidence of obesity in populations worldwide is reaching epidemic proportions, predominantly in developed countries (Catov, Ness and Olsen 2007) leading to a higher prevalence of morbidity and mortality. This risk is further exacerbated by pregnancy, as demonstrated with the fact that 50% of the overall number of women who died in the aforementioned inquiry were obese and 11 of the overall totals, developed and died from pre-eclampsia (Lewis 2011). In addition, obesity affects 1 in 4 members of the overall population (Baker 2017).

Ongoing guidance from the National Institute for Health and Care Excellence (NICE 2008 and 2011), plus the Royal College of Obstetricians and Gynaecology (RCOG) (2014) has recommended supplementation for high-risk women, principally women with a high BMI. This initial decision however, originated from a limited number of studies, several of which are dated. The rationale for selecting this group of women, was based primarily on their proposed high risk of vitamin D deficiency and the prevention of rickets. NICE (2014) guidance, however, does encourage a more widespread application of the recommendation with any individual

who is at risk of deficiency having access to free supplementation. Should evidence suggest a correlation between a reduction in pre-eclampsia with supplementation then this guidance could perhaps be applied to women at risk of developing pre-eclampsia. For this practice to be adopted there needs to be supporting evidence, but as yet despite several papers, including work by Hyppönen (2005) and Hyppönen et al (2014) linking deficiency with pre-eclampsia, there has been limited literature demonstrating any associations between the correction of any deficiency and the reduction of risk in these specific groups of women.

1.3 Search strategy

A complete understanding of the available literature was required prior to compiling the research proposal. Reviewing this evidence helps to establish a full picture of the current thinking in order to identify where evidence is lacking, or where new avenues of enquiry are required. Prior to commencing the study a systematic search of the literature was undertaken alongside further annual literature searches into vitamin supplementation and pre-eclampsia between the years of 2012 and 2018. Systematic reviews and searches of the literature are seen as a robust way of developing insight into the chosen research question (Bell and Waters 2018) as well as making judgements regarding the evidence through critical appraisal (Cochrane database accessed November 2019). Bowling (2014) adds that a literature review should include all pertinent and valid available literature, which indicates the need for a systematic approach.

As Raich and Skelly (2013) write, it is important to specify a question on which to base a search and study. Therefore, the initial primary question reflecting the topic of interest here became **"Does vitamin supplementation in pregnant women reduce the risk of developing pre-eclampsia?"**

A PICO tool (Population/ Intervention/ comparison/ Outcome) was used to commence a systematic approach to searching, which aimed to encapsulate the concept of interest and aid development of a more structured and focused question, which is clinically relevant (Harvey and Land 2017 p26).

The resulting PICO became: -

Population/problem	Intervention	Counter intervention	Outcome
Childbearing women Primigravid and Multigravid Pregnant women	Healthy start vitamins	No vitamins	Pre-eclampsia and eclampsia
	Vitamin supplementation	Folic acid only	Mild, moderate and severe pre-eclampsia
	Vitamin D and C Folic acid		No pre-eclampsia

Though this study aimed to consider low risk women, it was acknowledged that women present with many different characteristics and traits, not just as a pregnancy. Some of these for example, their BMI, may raise their risk of developing conditions such as pre-eclampsia. The principle focus of the study was primarily regarding uptake of vitamin supplementation, however ignoring these additional characteristics could reduce the validity and generalisability of a study. It was pertinent therefore, to extend the literature search for evidence relating to other individual characteristics and to consider them as potential confounding variables. The characteristics selected were readily available within the medical records and were highlighted from the available literature as potential confounding variables. Conducting such investigation also aided deeper understanding of how these variables may interact with the development of pre-eclampsia and impact on the research findings as well as a more holistic view of the topic under investigation. Subsequently the search terms used were extended to include: -

- Age,
- season,
- body mass index (BMI)
- ethnicity,
- blood group,
- parity

- lifestyle factors, such as smoking, alcohol intake and use of recreational drugs, and their impact on pre-eclampsia.
- Alongside this, as there are two parents creating the genetic make-up of the fetus, paternal ethnicity was included as a potential confounding variable.

In turn, this generated the need for more supplementary questions: -

1. What effect does maternal and paternal ethnicity have on development of pre-eclampsia
2. Do correlations exist between maternal characteristics and lifestyle choices with the development of pre-eclampsia?
3. Does supplementation with vitamins alter the effect of these possible confounding variables on the development of pre-eclampsia and its severity?

Inclusion and exclusion criteria for the literature search.

The search intended to seek primary research relating to the aforementioned variables. To aid structure to this search a set of criteria were imposed.

Inclusion – Primary research of both quantitative and qualitative methods conducted in the UK between 2012 and 2018, guidelines, systematic reviews and meta-analysis, seminal work and grey literature. As the research progressed additional papers were added.

Exclusion – Papers older than 10 years, expert opinions, non- UK papers and journal articles were not included within the literature review, but it was acknowledged that any found may be useful for background information.

As the search progressed, these criteria were reviewed, and adjustments made as below.

The databases accessed included CINAHL, MEDLINE, PUBMED Mother and lnfant Care, Ethos, ASSIA and Science Direct. Other guidelines and policies were sourced from professional websites including the National Institute for Health Care Excellence (NICE) and the Royal College of Obstetricians and Gynaecologists (RCOG). An example of a search can be seen in appendix 4.

The use of the Boolean facility proved invaluable to focus search findings; as there were copious papers listed, though not all specifically aligned to the topic in question (Hart 2001).

Initially the use of filters and the inclusion criteria aimed to keep the literature to United Kingdom (UK) based studies only, as care in the United Kingdom can vary considerably from other countries; and studies within the last 10 years. When searches repeatedly generated the same literature and no new findings became evident, the perception was that saturation point had been reached.

However, after an initial review of the abstracts found thus far, this decision changed to not limiting the inclusion criteria to only UK studies. Despite sourcing large volumes of relevant papers, they failed to encapsulate a complete picture of vitamin deficiency amongst different ethnic groups. To consider representation of a wider populous, it was necessary therefore to consider the diversity within the United Kingdom, in addition to the local population attending the trust used within this study, and to source more globally produced literature. Furthermore, several leading studies conducted outside of the UK required inclusion and consideration to glean a more complete picture.

In addition, after studying a number of key pieces, it became evident that there was regular reference to several large, though dated, studies within them. Preliminary searches had purposefully confined to 2012 to 2018 but expanding the search to include work from the 1980's to present day subsequently encapsulated current and seminal literature.

Finally, to avoid the omission of any research of any value, hand searches took place, through reference lists contained within the literature, which resulted in the discovery of a few more relevant pieces of background information, as well as confirming saturation point.

Finally, once this search was completed, in order to further advance the researcher's understanding of the variables for consideration a review of the grey literature, journal articles, opinion statements, seminal midwifery texts and Google Scholar was undertaken. Several items accessed from this final search were used as background information only and were not included in the final literature review as they did not address the specific topic in question.

1.3.1 Management of literature

Initial findings prior to commencement of the study provided over 140 pieces of literature of varying quality and significance. Of these, seven items were not relevant as they did not focus on the topic of interest and 26 were marked for use as background information, because they did not relate to the question under exploration. Despite extensive searching and liaison with local and national libraries, some full text articles could not be retrieved, meaning abstracts

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containing limited information had to also be excluded, but were acknowledged as potential background information. The subsequent annual searches provided mixed amounts of new publications with on average one or two new pieces per year. Where necessary assistance from library services aided retrieval of papers not available through other routes. For some more recent publications, accessing ResearchGate and directly contacting the authors also proved valuable.

Due to the retrieval of a substantial amount of literature across all the variables, it proved necessary to establish some form of structure (O'Leary 2017). Therefore, to enable effective management of the literature and ensure the most relevant and best quality evidence were adopted, each find was scanned by title, then abstracts and categorized for relevance under key headings such as, vitamin D and pre-eclampsia. This resulted in the assignment of some further pieces into background reading and some not proving applicable to this study. Subdivision of the remaining literature using their main concept, into themes such as vitamin D deficiency, supplementation, pre-eclampsia and obesity plus vitamin D deficiency, folic acid and vitamin C completed the process.

Papers identified into the background or non-relevant categories, were generally studies relating to supplementation and their relationship with prevention of disorders in the wider population rather than pregnant women or proved to be not primary pieces of research.

Once this process was completed, a systematic review of each piece enabled analysis of the remaining evidence using the appropriate Critical Appraisal Skills Programme (CASP) tool (CASP a,b,c and d, 2013 available at <http// CASP-uk.net> accessed 2013) and using the tools developed by Rees, Beecroft and Booth (2015), of which the latter proved most effective. Transferring the findings from the critique into a summary table, provided a structured method for outlining each item, with consideration of the main findings of the study and key aspects of quality including assessment for bias, sample size, validity and reliability.

Qualitative pieces specifically relating to the proposed question of this study were very sparse, and those sourced, were predominantly included as supplementary information as they added little specifically to support this study.

The main purpose of conducting such a literature review is primarily to establish a gap in knowledge, establish expertise and inform (O'Leary 2017). After having completed the initial review of the literature, it became evident that there had been no consideration of how the three elements of Healthy Start vitamins given in combination, may impact on the development of pre-eclampsia, which this study now seeks to establish. Furthermore, the literature aided decisions regarding strategies for undertaking this study not least the choice of a quantitative methodology.

In addition, as research into this area is continuing to intrigue scientists, further annual reviews of the literature were required to establish any new findings regarding the association between pre-eclampsia and vitamin supplementation. Reassuringly there were no similar studies to this one, but those studies identified up until the present day, have added further supporting evidence towards a possible link between individual vitamin supplementation and pre-eclampsia. Further large studies and a Cochrane review regarding vitamin D and folic acid, have considered a possible link with vitamin D but relating to calcium supplementation (De-Regil et al 2016).

Subsequently, a critique of the available evidence was drafted into the following literature review, commencing with an introduction to the main elements of the study, pre-eclampsia and Healthy Start vitamins. These are followed by discussions around the individual components of Healthy Start and then identified potential risk factors.

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1.4 Pre-eclampsia

Formally known as pre-eclampsia toxaemia (PET), so called due to a perception that women suffered a toxic reaction to the fetus, has been a condition affecting women for centuries with mention in ancient Greek texts to fatal convulsions (Redman and Walker (1992). Fortunately, for the 2-7% of women who develop this potentially fatal condition, science has now progressed to greater understanding. Now more commonly entitled pre-eclampsia, research has proven that women with pre-eclampsia are of a significant risk of developing multi organ damage and systemic inflammation. In addition, that its aetiology relates more to altered placental implantation (Webster, Dodd and Waugh 2013) followed by inflammation at a cellular level.

Though the risks remain for a period of time after the birth, during the postnatal period, with the highest risk being in the first 72 hours post birth, the most effective method of halting the disease is to deliver the fetus, hence the aforementioned risk of premature birth. This fact does perhaps tie in with the current understanding of the aetiology of the condition regarding placental implantation whereby removing the placenta the maternal circulation can return to a more normal state.

Though some women may not develop any symptoms and can quickly deteriorate into eclamptic fits, for the majority of women who develop pre-eclampsia they demonstrate some degree of symptoms, and variant degrees of severity of the condition. Overall, only a few women who develop the condition, around 1:2000 go on to have full eclamptic fits and many of these survive (Robson and Waugh 2013).

Being multi-systemic by nature, these symptoms relate predominantly to the major organs of the body. Initially primary symptoms of raised blood pressure, proteinuria (protein in the urine) and oedema develop due to an affected circulatory function.

Following conception and cell division, a blastocyst develops from which finger like projections form, known as chorionic villi. These villi secrete enzymes that erode maternal blood vessels in the lining of the uterus (decidua). This results in reduced resistance between fetal and maternal circulations and enables exchange of gases and nutrients. In a normal healthy pregnancy, this process in unhindered and maternal blood pressure is unaffected. However, current thinking is that where implantation is affected maternal blood vessels remain constricted arising in raised blood pressure. In turn, the degree of affected implantation relates to the severity of the pre-eclampsia (Roberts and Gammill 2005). Damage to other organs arising intrinsically from the high circulating blood pressure and the resulting cellular damage leads to the additional symptoms of proteinuria, abnormal levels of oedema, visual impairment, epigastric pain (pain over the liver in the upper abdomen) and also hyperreflexia (extremely brisk reflexes). Furthermore, the severity of these symptoms relates to the severity of the pressure of circulating blood and build-up of fluid.

Taking this a step further, interest in the effect of the active form of vitamin D (1,25-dihydroxyvitamin D3) and placental development has been generated, whereby locally metabolised vitamin D3 affects the fetal-placental development and function (Evans et al

2004; Shin et al 2010). Furthermore, from personal communication, the researcher was informed of early findings from a study commenced 2017 at the University of Birmingham that is aiming to add support to this theory, associating a link between vitamin D and the immune response in the placenta. Alongside the evidence considered in Ganguly et al's (2018) literature review, there is clear support for further exploration of vitamin supplementation.

If conclusive evidence regarding correction of any vitamin insufficiency impacts on the key and most critical feature of pre-eclampsia [blood pressure], both at implantation and during pregnancy, then there could be a potential for impacting on the development of pre-eclampsia.

1.5 Healthy Start vitamins

Healthy Start vitamins are a relatively inexpensive supplement containing 10 micrograms of vitamin D, 400 micrograms of folic acid and 70 milligrams of vitamin C. Women are instructed to take one tablet orally per day.

Uptake of vitamin supplementation in England, however, can vary according to Jessiman et al (2013), for a number of reasons. Through a qualitative study of 13 primary care trusts, they identified that poor access, reduced promotion; lack of motivation and lack of awareness had an impact on uptake. Previously estimated figures provided by Public Health England (PHE), though from a local unpublished audit only, suggested an initial figure of around 23% uptake, the percentage used for the initial calculation of sample size for this study. However, a later published study by McGee and Shaw (2013), suggested a much lower rate, possibly as low as 10%. It is perhaps challenging however, to establish any accuracy specifically regarding pregnant women here, as the 2013 study (McGee and Shaw 2013) considers the wider population with only 7 of the sample being pregnant women but it is acknowledged by the researcher as the more accurate figure to use for later analysis. It is also acknowledged, that the work by McGee encapsulated only a small proportion of the wider populous, which is unlikely to be representative and there is potential for a much greater percentage of women who do take the vitamins, something this study may help to clarify.

No other evidence of studies, considering multiple supplementation uptake in pregnancy, were available, leaving little accurate data to work with. Usefully, however, a locally conducted evaluation by Filby, Lewis and Taylor (2014) concluded that there might well be a cost benefit in providing free vitamins, particularly vitamin D to pregnant women. This provides further enhancement of the rationale for the use of multivitamins in pregnancy, should a link between their use and a reduction in pre-eclampsia.

Costing as little as 75 pence per bottle of tablets, PHE introduced Healthy Start Vitamins to provide supplementation for people who were socio economically vulnerable and whose diet lacked many of the required nutrients (Dean 2012). At the time of development of this research, provision of these vitamins was free of charge and in many cases still are. Most likely through budget cuts, some areas are now charging women for them and in some cases as Lockyer, Porcellato and Gee (2011) discovered, health professionals are not encouraging uptake. This may account for a potential decline in women taking these vitamins suggested by the figures from PHE but should a possible correlation between Healthy Start vitamins and the reduction of pre-eclampsia exist, there could potentially be a positive shift in the percentage of uptake.

Despite extensive searching across numerous databases, no literature was found considering multivitamin supplements and pre-eclampsia nor hypertensive disorders, leaving a gap in knowledge and subsequent generation of this study.

Whilst the design of this study was principally to consider the possible impact of vitamin supplementation on the development of pre-eclampsia in pregnant women in general, it was important to consider any women with higher risk factors as women are complex in nature and these factors may affect the findings. It was therefore necessary to source evidence of any existing associations published in the literature, between pre-eclampsia, vitamin supplementation and variables including for example, obesity, which could have affected data collection, analysis and findings. The literature found also highlighted a number of other potential variables, which could confound any findings and analysis. This subsequently led to a decision to search for and critique available literature pertaining to principally ethnicity and season, BMI, blood group, gravida, parity, lifestyle choices and folic acid independently. In addition, this supported the rationale for including these variables in

the data collection in order to consider any correlations between them and pre-eclampsia and how introducing supplementation may alter this.

Initially, it was important to review and collate current evidence as to any association between the individual contents of Healthy Start vitamins (vitamin D, Folic acid and vitamin C) with their role from a physiological perspective and development of the condition. The literature review therefore will commence with vitamin D, followed by folic acid and vitamin C. Beyond this a review of literature pertaining to the other potential confounding variables will be undertaken.

1.5.1 Vitamin D - Background and physiology

Vitamin D is metabolised in the liver and kidneys from Vitamin D3 (cholecalciferol) obtained through UVB light into 25-hydroxyvitamin D (25(OH)D) and through the placenta during pregnancy (Karlsson 2013; Holick 2007). Its principal function is to modulate gene expression and principally calcium absorption in the intestine (Diaz et al 2002). It is measured by the blood serum level circulating form 25-hydroxyvitamin D (25(OH) D) and has a half-life of only 2 weeks (Robinson et al 2011). A measurement of 50nmol/L is considered adequate, 30 to 50nmol/L is insufficient and below that deficient (Purswani et al 2017). Though some vitamin D is metabolised through dietary means from such foods as eggs, especially through fortification or supplementation, the most effective way of maintaining levels is through sun exposure (O'Connor and Benelam 2011). Researchers including Hypönnen (2005); Hypönnen and Boucher 2010); Purswani et al 2017) highlight the prevalence of vitamin deficiency and insufficiency in many countries globally, including the United Kingdom (UK). This is affected especially when sunlight is reduced (for example in winter), when sun exposure is inhibited due to clothing; or with dark skinned individuals as the skin pigment minimises absorption. Indeed, Mostello et al (2008) identified a higher percentage of dark- skinned women developing pre-eclampsia in Nordic counties where sunlight is minimal for several months of the year. This corresponded with higher percentages of hypovitaminosis D. A small, but well conducted case control study, in Iran by Sadin, Pourghassem Gargari and Pourteymour Fard Tabrizi (2015), supports the findings regarding the impact of dark skin and ethnicity on serum levels of serum 25(OH) D. Using serum levels in weight matched cases and controls the higher incidence of deficiency found in the pre-eclampsia women, when compared to the controls, suggests an association.

Applying these findings to the different climate and population found in Britain, however, requires a degree of caution, not least due to confounding variables such as different diets and levels of sun exposure. Meanwhile a systematic review by Barrett and McElduff (2010), claims that blood serum levels of vitamin D will naturally drop and increase as pregnancy progresses and this is unrelated to external factors such as obesity. From the paper however, it is not clear how the authors have selected material for consideration, leaving questions of bias and unreliability. In addition, other authors, including Vandevijvere et al (2012) and McAree et al (2014), having used liquid chromatography studies of blood samples from 346 pregnant women representing a range of ethnicities and demographics within the UK; provided significant evidence of low levels of vitamin D in the majority percentage of the cohort, not just specific groups. Although there was higher prevalence in more obese women and those with darker skin. Arguably, these studies provide greater accuracy of interpretation, due to the use of laboratory studies and demonstrates greater reliability and validity.

From the evidence identified ethnic minority groups are at particular risk of deficiency, and efforts are already in place through the Department of Health projects to correct deficiencies in this population, including the prescription of Healthy Start vitamins (Moy et al 2012; McGee and Shaw 2013). However, in a proposal supported by the RCOG Scientific paper no 43 (2014) it is suggested that a universal approach to the wider populous rather than a targeted approach may have a greater benefit overall (Moy et al 2012). In addition, more recently a well conducted prospective cohort study by Kiely et al (2016) using blood serum level testing throughout pregnancies, ascertained similar findings to Mostello et al (2002) regarding ethnicity. In turn, Kiely et al (2014) identified the importance of the vitamin D metabolism on the fetus and placenta and highlights the protective effect of normal vitamin D levels on preventing pre-eclampsia. Further details regarding season and ethnicity are discussed later.

If the findings of the research reviewed so far were taken at face value, there would appear to be a case for preventing vitamin D deficiency and subsequently reducing the chance of women developing pre-eclampsia. Before coming to this conclusion however, consideration of all the available literature is required.

Studies pertaining to vitamin D deficiency and pre-eclampsia proved conflicting, not least due to differences in methodology and lack of clarity of the mechanisms involved (Fernández- Alonso et al 2011), but there appears to be more evidence to support an association than not. Two case control studies, one by the aforementioned Robinson et al (2011) and additionally Ringrose et al (2011), both demonstrated a higher incidence of pre-eclampsia in winter and in women with resulting deficiency. However, subject numbers were small at 56 and 197 respectively and it is not stated whether a power calculation was used, leading to possible issues of validity and reliability of the results. In addition, potentially such small samples are unlikely to be representative making these results difficult to generalise to the wider population. However, a systematic review by TePoel, Saftlas, Wallis (2011) endorses the same conclusion, as does an extensive robustly constructed meta-analysis by Aghajafari et al in 2013 and Wei et al 2013). Despite some heterogeneity within the included studies (though all are observational in nature), all of the studies demonstrated a link between low vitamin D levels (particularly during winter) and an increased risk of pre-eclampsia with pooled odds ratios of 1.79 (CI 95% 1.25 to 2.58) and 2.09 (CI 95% 1.50 to 2.90) for the respective studies.

With only 31 of the 3357 papers obtained through the search strategy implemented were used by Aghajafari et al (2013) and only 24 used by Wei et al 2013 for the final meta-analysis, there may be at risk of selection bias (Stewart 2016). However, consideration of the criteria used for selection and inclusion, outlined by the authors does appear reliable and valid with two independent researchers using agreed criteria. Furthermore, the findings are complimented by those identified by Khaing et al (2017) in another meta-analysis whereby similar inclusion criteria were used to assess the quality of the literature and those deemed to be of the better quality were analysed. In contrast a New Zealand study conducted by Boyle et al in 2016, using a relatively large sample of women, questions the conclusions of many aforementioned authors, as their findings provided no evidence of a connection between pre-eclampsia and low vitamin D levels.

Of the literature found, the study by Boyle et al (2016) was the only one that completely refuted the hypothesis connecting vitamin D and pre-eclampsia. However, the variables considered in the study by Boyle et al (2016) are similar variables to this study including seasonal, BMI status and lifestyle choices. As with McAree (2014) access to serum testing

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was utilised, which should raise the validity of the findings. However, there appears to be a degree of inconsistency in the screening process and the selection of the women. In addition, a number of women were taking alternative multivitamins, but it is not clear how this was accounted for as a possible confounding variable. What the results do suggest however, are associations between BMI, ethnicity and some lifestyle choices with lower vitamin D levels. The correlation with development of pre-eclampsia was less evident leaving room for more exploration within this study.

In contrast, a much quoted though now dated randomised control trial (RCT) by Marya et al (1987), and smaller studies published by Singla et al (2012 and 2015), suggest that correcting a deficiency does reduce the incidence of hypertension (raised blood pressure), but no link was found with reducing the incidence of specifically pre-eclampsia. In Singla et al's case (2015) this may be due to the limited sample size used, which is unlikely to be representative. However, the use of a well-established form of serum level testing and the age matched cases and controls brings greater validity to the study. It subsequently could be argued therefore that the effect on the blood pressure may impact on the severity of the condition as raised blood pressure is the primary symptom. However, these studies do associate pre-eclampsia with deficiency of vitamin D in some way.

Support for the potential of lowering blood pressure through supplementation was demonstrated in the findings of Shand et al (2010), Oken et al (2007) and Powe et al (2010). Significantly, however, these studies considered deficiency within mid trimester only, unlike some aforementioned studies, which involved supplementation and monitoring of women throughout pregnancy, generating quite different results. A recent systematic review by (Purswani et al (2017) drawing on several key papers, continues to highlight issues surrounding timing of supplementation and strongly recommends further research is needed in this area. Furthermore, as with other studies not included in their review, the conclusions drawn by Purswani et al (2017), again identify inconsistences in findings relating to the anti-inflammatory impact of vitamin D in pre-eclampsia, but intimate that there are some possible links. The narrow search using only one database within the reviews, however, perhaps limits the breadth of included evidence, but it does also provide the useful summary collated from the studies included presenting the suggested physiology of vitamin D's impact on pre-eclampsia as detailed in table 1.1

Stage of PE	Characteristic of PE	Effect of increased vitamin D level
Stage 1	Inflammation –linked abnormal placental implantation	↓predisposition to pro-inflammatory response ↑ regulation of genes associated with placental invasion and normal implantation
Stage 2	Vascular endothelial dysfunction Proteinuria mediated by renal vascular endothelial growth factor (VEGF)	↑ vascular structure, elasticity and thickness ↓ blood pressure (regulation of renin-angiotensin system ↓ oxidative stress ↑ Vascular smooth muscle cell proliferation by increasing VEGF gene transcription.

Table 1. 1 Summary of the effects of increased vitamin D on the pathogenesis of pre-eclampsia (extracted from Purswani et al 2017 p2).

Pre-eclampsia is a hot topic for research and a potential cure is ever being sought. Likewise, the use of vitamin D supplementation and its effect on pregnancy outcomes such as hypertension is an ongoing area of investigation. After undertaking annual searches for literature in preparation for this study and thesis, this became ever more apparent. Researchers continue to accept or reject hypotheses surrounding this topic. Bodnar et al (2007 and 2014) in particular, continue to strive to establish conclusive evidence that there is a direct association between vitamin D levels and pre-eclampsia. Having published two large sampled studies in 2007 and 2014, Bodnar and fellow researchers went some way to corroborate or contest the hypothesis that vitamin D helps reduce pre-eclampsia, with their findings. For both studies Bodnar et al selected to use a case control method, which may be

considered of lesser quality on the hierarchy of evidence (Harvey and Land 2017) but was appropriate for the investigation undertaken. The serum results were already available and potential for selection bias was reduced through the women volunteering for inclusion, as opposed to the researchers choosing them. Specified inclusion criteria were used in addition to appropriate non-parametric analysis. Like Bodnar et al (2014), Arisoy et al (2015) continue to add to the pool of knowledge in this field, though with a small cohort study with only 77 participants in the sample. Despite the small sample however, as with Bodnar et al's work, the study showed statistically significant results with *p* values of <0.0001.

Having considered the evidence it would seem logical to surmise that there is a potential of a physiological association between pre-eclampsia and vitamin D deficiency. Though some of the evidence could contest it, there are more studies of greater validity, reliability and generalisability demonstrating a link. Whether there is a benefit in using supplements throughout pregnancy or at conception is worth further investigation. It is possible that correcting levels of serum 25-hydroxyvitamin D [25(OH) D] for the prevention of rickets as the original intention, may also be beneficial in reducing the incidence or severity of a condition, which in turn could lead to further reductions in morbidity and mortality. Subsequently this leads in part to the aim of this study looking into multi-vitamin supplementation and pre-eclampsia.

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1.5.2 Timing of vitamin D to correct deficiency and placental implantation

Evidence demonstrates there is an extensive range of developing research aiming to prove the association of vitamin D and pre-eclampsia and overall, there does appear to be a compelling case. When the optimal time for supplementation would be however, is not clear, nor is the physiology underpinning this. To date researchers such as Bodnar et al (2014) have adopted different methods and strategies for supporting their hypotheses, often using laboratory testing to accurately assess levels of deficiency or insufficiency, but authors cannot seem to agree on the optimal time for correction of deficiency or the effect on the relevance of the coinciding gestation. Indeed, several papers including those by Dawodu and Akinbi (2013) and Fanos, Vierucci and Sagesse (2013) draw upon extensive literature in their reviews, aiming to establish a definitive answer to how deficiency or insufficiency should be classified, and propose that values between studies results differ too

widely. In turn as levels of vitamin D naturally fluctuate during pregnancy, with the peak in early pregnancy due to the fundamental need for calcium absorption to bone growth, the accurate assessment of plasma vitamin D levels is even more challenging.

The physiological impact of vitamin D in cell metabolism elsewhere in the body is already established and it would seem sensible that as the placenta is a mass of cells a lack of vitamin D could inhibited its development (Shin et al 2010). Combining this with the theory that the aetiology of pre-eclampsia is due to altered implantation of placental villi (Cowan, Redman and Walker 2017), there is possibly an argument to connect the two. As identified earlier the prescription of Healthy Start vitamins from first trimester would contain vitamin D and there is a possibility this could be an ideal route to alter levels of deficiency. The supplements other key ingredients, folic acid and vitamin C, will be considered later in this literature review leaving the current focus on vitamin D at this stage.

Studies into the role of vitamin D in pregnancy proffer differing conclusions whereby some such as Gidlöf et al (2015), Bomba-Opon et al (2014) and Yu et al (2013) through case control studies did not identify any links between low serum 1,25(OH) $_2$D in the first trimester and the development of pre-eclampsia; whilst others offer alternative views. Two such examples are cohort studies, conducted by Burris et al 2014 and Wei et al (2012) using similar methods of data collection and testing within a combined total of 2281 women across the two studies. Both studies demonstrated statistically significant results for an increased risk of pre-eclampsia in women with low levels of serum vitamin D, detected in the third trimester and not the first or mid trimester. Though a relatively small sample overall compared to the UK birth rate, it has the potential to be generalisable to the wider population, as across the studies representation from a breadth of ethnicities, seasons, age groups and other demographics occurred. In contrast, a case control with a much larger sample conducted in 2010 with matched participants for ethnicity alone, drew alternative conclusions of an association between low levels in the mid trimester and an increase in the condition (Baker et al 2010). A conclusion not supported by the findings of Wetta et al (2015), although these findings were based on a much smaller cohort than that used by Baker et al (2010).

These conflicting findings do not provide a clear foundation on which to base clinical practice and arguably further investigation on a much larger scale, is required. However, current focus seems to be in favour of consideration of first trimester serum levels and more specifically placental implantation/function and its impact on pregnancy outcome. Kiely et al in 2016 conducted a prospective cohort study of 1768 low risk women and clearly demonstrated that a serum concentration of >75nmols/L provided protection from utero-placental dysfunction. Notably within the group of women with low concentrations, a higher percentage of pre-eclampsia was evident, ascertained through using accredited liquid chromatography tandem mass spectrometry, ensuring internal validity. The conclusion reached suggest a serum concentration of >75nmol/L provided a protective association for pre-eclampsia. Their options for inclusion and exclusion criteria also proved beneficial when establishing those for this doctorate. Coinciding with the work by Kiely et al (2016), research directly on placental cells known as syncitiotrophoblasts, conducted by Diaz et al (2002), identified that regulation and metabolism of $1,25(OH)_2D_3$ occurred within these placental cells and that low maternal serum levels could impact on this physiology, leading to pre-eclampsia.

A number of authors have described the physiology underpinning this. Lui and Hewison (2011) for example, describe the immunomodulatory properties of vitamin D and theorise how immunity is maintained through effective implantation of the trophoblast into the decidual (lining) of the uterus. Pre-eclampsia is considered to develop from an immune response hence the original name toxaemia. Most recently, Ganguly et al (2018) provided an extensive review of the available literature on this topic, though several are based on laboratory studies and a number consider other outcomes for maternal morbidities, not just pregnancy outcomes. Despite this, their deduction is that vitamin D plays an important role in placental physiology, in particular on the action of the trophoblast cells as figure1.1 presents. Again, the issue of timing of supplementation is raised and in light of the findings they recommend early pregnancy rather than later. Evidently, more research is needed to try to ascertain more conclusively what would be the best approach, and this study aims to add to the greater understanding.

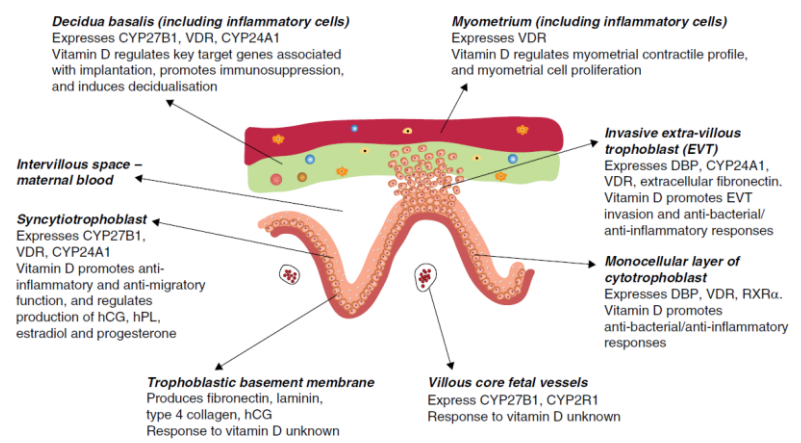

Figure 1
Vitamin D pathway components at the maternal–fetal interface associated with implantation. Schematic showing key cell types involved in implantation and associated expression of components of the vitamin D system: CYP2R1, vitamin D-25-hydroxylase; CYP24A1, vitamin D-24-hydroxylase; CYP27B1, 25-hydroxyvitamin D-1α-hydroxylase; DBP, vitamin D binding protein; hCG, human chorionic gonadotropin; hPL, human prolactin; RXR, retinoid X receptor; VDR, vitamin D receptor.

Figure 1. 1 Vitamin D and implantation. ©Ganguly et al (2013 pR95)

1.5.3 Correction of vitamin D deficiency and associated risks

From the literature discussed thus far it can be determined that should there be a positive correlation between improving levels of vitamin D and reducing pre-eclampsia risk, then how this is managed requires investigation.

Before taking such an approach however, it is important to recognise any risks involved in supplementing with vitamins especially for women with normal levels of serum 25-hydroxyvitamin D [25(OH) D]. Indeed, is there a case for measuring levels in all women and correcting those who are deficient, especially if there is as some studies indicate a link between deficiency and pre-eclampsia? A question for later studies perhaps, but from searching the literature, some studies including that by Harvey et al (2014) have considered the potential risks with a supplementation approach.

The available literature is again inconclusive regarding what would be an appropriate and effective way of correcting any deficiency. Indeed, whilst the majority of women remain untested in pregnancy, then it would be difficult to ascertain those who would benefit best from supplementation. The RCOG guidance for pregnant women on healthy eating and

supplementation (RCOG 2014) alongside the cost effectiveness study undertake by Filby, Lewis and Taylor (2014), recommend universal supplementation and conclude that this would provide greater benefit than risk. In contrast, however, Roth (2011) strongly recommends more randomised control trials before taking any action.

Both Vieth (1999) and Harvey et al (2014) write that total-body sun exposure would provide all the required vitamin D to maintain adequate serum levels. However, this is unlikely to happen in the general population especially in colder climates such as in the United Kingdom. Alternatively, as in some cultures, an expectation that women remain fully covered or can be inhibited from leaving the confines of the home exists, further limiting sun exposure. This perhaps gives the case for supplementation. Though now dated the review of the literature by Vieth (1999) included work from 10 countries from across the world including the UK, which provides generalisability. A limitation, however, arises from the low numbers of participants of childbearing age. Despite this, from a physiological stance the correlation between the effectiveness of managing deficiency through different methods is still relevant.

What is evident from the literature is that to enable a serum level of >100mmols/L a supply of 100micrograms (4000 IU/dl) is required. The same studies by Vieth (1999) and Harvey et al (2014) though conducted several years apart, indicate that regular extreme doses of ≥1000 micrograms can lead to toxicity. With a known half-life of two weeks and a dose of 10micrograms daily Healthy Start vitamins are unlikely to cause harm. In reality, the dose is arguably too low. However, if universal uptake is to be encouraged then the risks of overdosing women even with normal levels of serum vitamin D is minimal.

Hollis et al (2011) provide some clarity on dosage from their double-blinded RCT comparing varying doses of supplement and serum levels throughout pregnancy. Though a smaller sample than that used by Harvey et al (2014) in their meta-analysis, Hollis et al's findings significantly indicate with a p value of <0.0001 and a risk ratio (RR) of 1.60 that those in the group taking more than the current recommended dose of 4000IU had a significant improvement in serum levels, without any adverse effects. Indeed, whilst the heterogeneity of the studies included by Harvey et al (2014) brings challenges with interpretation of results, the overall picture from both sets of authors could suggests a rethink is required on

current recommendations. However, at present it is only possible to base this study on the dose provided by Healthy Start vitamins currently in use.

1.6 Seasonal impact on vitamin D and pre-eclampsia

As vitamin D is metabolised mainly through sun exposure it can lead to significant insufficiency and deficiency within the populous, when sunlight hours are reduced. Having established the importance of vitamin D for maintaining physiological process and placental implantation, then season and degrees of sun exposure are relevant for inclusion as a variable. In turn, the potential for improving levels of vitamin D through supplementation needs further exploration.

From searching the literature there was a predominance of studies undertaken in countries outside of the UK. Extensive numbers of studies for example originated from Scandinavia, considering the seasonal impact and vitamin D absorption. Geographically this region has a reduced level of sun exposure and subsequently is an area where vitamin D deficiency is common. Evidence from, Haugen et al's (2009) study included over 23,000 subjects and presented statistically significant results relating to vitamin D deficiency, although the choice of questionnaires regarding diet and supplementation could raise questions of potential bias and misinterpretation due to its structure and completion in the presence of the staff. His work, however, has a basis on other work by Magnus and Eskild (2001) with support for the methods used and results generated.

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In addition, several previously discussed papers including TePoel, Saftlas and Wallis (2011) begin to report seasonal variations in vitamin deficiency and deviations in levels of pre-eclampsia.

The conclusions drawn from two systematic reviews in 2012 by Hovdenak and Haram and Thorne-Lyman and Fawzi (2012) contradict each other, most likely due to the studies included. The former Scandinavian review supports an association between the seasonal impact on vitamin D synthesis and pre-eclampsia, especially in dark skinned women. The information is difficult to interpret however, due to a lack of detail about the papers selected for inclusion. There also appears to be a poorly structured search, principally due to the use of a non-systematic approach. The latter United States review in contrast, provides more detail of the selected studies, but those chosen are very different in their

construction. The overall analysis did though conclude that there is unlikely to be an association between vitamin D and pre-eclampsia, though the heterogeneity of the studies brings the conclusions into doubt. Both reviews recommended further research.

Indeed, the following year a study by Tabesh et al (2013) reached similar conclusions to Thorne-Lyman and Fawzi (2012) whereby there appeared to be an association from studies in the United States however, from testing for the heterogeneity between the studies selected, as with other reviews their marked differences in approaches prevented concrete conclusions.

Studies conducted in relation to season and skin colour have a tendency to be in countries outside of the UK, with Algert et al (2010) in Australia, Ali, Adam and Abdallah (2014) in Sudan and Makhseed et al (1999) in Kuwait for example. Interestingly, the latter two authors contradict each other's findings, with one finding no seasonal affect and the other an increase in hypertensive disorders during winter. A potential reason as to why the results differed so greatly was the former study conducted in Australia is less likely to have reduced sunlight hours and included a higher proportion of women with lighter skins. The study by Algert et al (2010) considered vitamin D levels specifically within the study of seasonal impact. Their findings suggested a decrease in raised blood pressure when there was greater sun exposure and resulting vitamin D at the time before delivery (though not at conception). There was a notable increase in pregnancy hypertension in spring compared to the autumn. The substantial sample of 424,732 pregnancies adds further credence to the findings.

However, Madhu et al (2015) in a nested case control trial based in India, using blood samples from 500 women, also identified a much higher percentage of vitamin deficiency in the women who developed pre-eclampsia. However, India has arguably more sunny months than the UK reducing generalisability of the findings to the British population as a whole although the results pertaining to dark skin is of value to this thesis as approximately 42% of the city are from Black, Asian and Minority Ethnic groups (BAME) Birmingham City Council 2018). Despite this, their findings demonstrated a direct association with reduced vitamin D metabolism in winter especially in darker skinned women, though for many of the participants the impact of reduced activity outside also played a part.

The specific designs of the remaining studies sourced aimed to look for associations between at pre-eclampsia and season. Three papers were discovered, one prospective cohort study and two retrospective studies, by Immink et al (2008) Gostine et al (2013) and Bodnar et al (2007a). All of which provided statistically robust findings, linking higher incidences of pre-eclampsia during winter months. Bodnar, Catov and Roberts(2007b) in particular, designed a cohort study to consider equal numbers of white and black skinned women to limit the impact of skin colour as a variable, making the study more reliable, valid and potentially more generalizable to the UK due to its multi-ethnic society.

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The final paper identified in contrast was a cross-sectional study conducted by Soroori, Sharami and Faraji (2007) whose results failed to demonstrate any association between seasons and pre-eclampsia. However, they do acknowledge the similarities between the seasons in Iran, something we cannot relate to the United Kingdom.

Though many of the studies were conducted outside of the UK and with mixed conclusions, it is suggested that season, vitamin D levels and pre-eclampsia could be linked. The UK has unpredictable degrees of sunlight and a very multi-ethnic society with groups of women with darker skins, who also may remain indoors or fully veiled. This carries forward into the local population used for inclusion as the catchment area for the regional unit includes a large multi-ethnic society.

Furthermore, as the literature discussed in section 1.6 implies, ethnicity and deficient vitamin D levels are often associated. With the combination seasonal shifts in sun exposure combined with a higher risk local population the impact of season was deemed relevant. Consequently, season would be considered as a variable of interest within this doctoral study, not least due to its potential as a confounding variable, as this may well lead to an issue with internal validity of the results (Stewart 2016). In addition, collection of this data would enable further review of the impact on the development of pre-eclampsia and provide additional understanding in this area by adding to the current pool of knowledge, but more specifically to a UK population.

1.7 Ethnicity, vitamin D levels and the risk of pre-eclampsia

The topic of ethnicity has been touched on throughout this literature review as it proved challenging to separate out ethnicity as an independent variable with in the available evidence. Specific papers considering ethnicity as a risk factor for pre-eclampsia were few in nature and for three authors; abstracts only were located despite use of library support and various databases. More commonly, vitamin D deficiency and ethnicity proved to be the primary focus of the majority of the literature. Various studies by Holmes et al (2009); Dror and Allen (2010), Yu et al (2008); Brooke et al (1980); Kazemi et al (2009), and an article by Prentice (2008) all presented credible cases for supplementation of vitamins for women of ethnic minority populations. A recommendation based on the majority of the participants when tested, being found to be either deficient or insufficient in vitamin D much like the UK population (Purswani et al 2017). To add to this body of knowledge Sahu et al (2009) ascertained the same conclusions but took it a step further by considering the impact of season and sun exposure levels. Though conducted in Northern India where sun exposure is higher and economic status may differ from the UK, therefore perhaps generalizable to only a proportion of the UK, accurate testing of serum levels identified rural, low socioeconomic communities had a high prevalence of deficiency especially during pregnancy and in the winter months. These findings are supported by a now dated small study conducted in Saudi Arabia by Serenius, Elidrissy and Dandona (1984), though here the risk of deficiency was raised when veiled clothing was worn. Additional research by authors including Islam, Akhtaruzaman and Lamberg-Allardt (2006); Hossain et al (2011), both demonstrate an association between skin colour and deficiency though with a statistically significant P value of 0.02.

Much of the scientific explorations have considered women living in hot climates and who have dark skin. However, a number of papers were found involving women from maritime climates more similar to the United Kingdom. Though in several cases dark skinned women remained the key focus as in studies by Madar, Stene and Meyer (2009); Alfaham et al (1995) and Datta et al (2002); other studies compared Caucasian and different ethnic groups. Bodnar et al (2014) and Van de Meer et al (2006) found for example, a significant difference in the serum levels between the ethnicities. Though Caucasian women still suffered insufficiency, black women in particular are at a greater risk. Anderson et al (2013) add that even a tanned Caucasian skin can also lead to deficiency.

Not all of these studies consider the impact of pregnancy, unlike in a systematic review and meta-analysis by Nassar et al (2011) and a prospective cohort study by Shand et al. (2010). Both sets of authors agreed with the previously discussed studies regarding deficiency and ethnicity but failed to prove any association with adverse pregnancy outcome including pre-eclampsia. Nassar et al (2011) presents a comprehensive forest plot, providing an overview of picture of the evidence in a diagrammatic form as recommended in Stewart (2016), detailing the mean vitamin D levels for different ethnic groups and showing much lower values in dark skinned ethnicities. However, as only two databases were searched it is appropriate to avoid taking their findings at face value despite the approach being systematic as it only resulted in five papers being worthy of inclusion, potentially omitting key research.

Papers by Reeves et al (2014), Ghazal-Aswad et al (2013), and Bener, Al-Hamaq and Selah (2013) plus Abedi et al (2014) provide additional evidence. Though one case control method was utilised (Reeves et al 2014) the remaining studies (Ghazal-Aswad et al (2013), Bener, Al-Hamaq and Selah (2013), Abedi et al (2014) used a cohort methodology and identified a direct association between pre-eclampsia and ethnicity though not all considered vitamin D deficiency alongside. As with several other studies Reeves et al (2014), for example, utilised serum level testing for greater accuracy, but took umbilical cord blood as opposed to blood taken from the women. Single blinding of the researchers to the owner of blood samples added greater validity (Stewart 2016). For the minority groups involved there was a significant proportion of women with vitamin deficiency.

Those papers sourced were found to be reliable and robust in their undertaking and do provide logical approaches to grouping ethnicities, which could be of benefit for coding data within this study. These studies also build on evidence resulting from the findings of Goodwin and Mercer (2005), Caughey et al (2005) and Abubakar et al (2009) whose interest lay in comparing and contrasting different subgroups of ethnicities, the ethnicity of both parents and different tribes respectively. Contradicting the work by Sibai et al (1995), Goodwin et al's (2005) work, found higher incidences of haemolysis, elevated liver enzymes and low platelets (HELLP syndrome), which is a condition directly associated with pre-eclampsia. Whereas African American women were at much greater risk of severe hypertension.

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Caughey et al (2005) not only revealed a higher inclination for raised blood pressure in African American women but also a much lower rate in Asian women, a finding reflected in the paternal ethnicity. Where parents were of different ethnicity, the results suggested a 13% increase in pre-eclampsia. The association with paternal ethnicity may, however, be explained physiologically by Cowen, Redman and Walker 2017 p34) in that the genetic makeup of the father's semen can increase the likelihood of pre-eclampsia due to the immune response to the foreign cells forming part of the trophoblast cells at the point of placental implantation. A point further explored in the gravida and parity section. Contrastingly, Sibai et al (1995) found no connection between black race and increased risk of pre-eclampsia or hypertension, though these results were secondary findings from data in a study into Aspirin and pre-eclampsia, so its relevance could be debated.

Overall, the varied quality of studies and their findings fail to enable clarification of how ethnicity could affect pre-eclampsia. It is difficult to ascertain whether there is any association between ethnicity, vitamin deficiency and pre-eclampsia exists as a result, which leaves room for further exploration within this study. With the potential for different ethnicities to be affected by vitamin deficiency and having a higher rate of pre-eclampsia, alongside the acknowledgement that the centre for the study is based in a multi-ethnic city; the desire for this study will be to encapsulate as ethnically diverse a cohort as possible and aim for a representative population of women. In addition, it will explore the possibility that supplementation could benefit these groups of women especially.

This section of the literature review so far has considered aspects relating to Vitamin D however, this is only one component of Healthy Start vitamins. Currently, few women are advised to take this as a supplement independently, meaning it would not be possible to research this individually within this study. However, with the principle aim of this research being to consider the use of combined vitamins, the evidence purporting the benefit of vitamin D in cellular development and blood pressure control, could support the prescription of a supplement containing this vitamin.

Moving forward it is necessary to consider the remaining components of Healthy Start vitamins, that is, folic acid and vitamin C too. Establishing what evidence is available regarding these two and pre-eclampsia will aid understanding and if any correlation is

identified, serve to support the need for the research study under consideration. Consequently, the initial systematic search led to retrieval and critique of the literature pertaining to these vitamins and their relationship in the development of pre-eclampsia.

1.8 Folic acid and pre-eclampsia

For several years health professionals have been advocating the use of pre-conception folic acid to prevent neural tube defects at a dose of 400 micrograms per day, currently the same dose as that found in Healthy Start vitamins (NICE 2008, Duckworth, Mistry and Chappell 2012 and Scholl and Johnson 2000). Some studies collated as early as 1999 in a systematic review by Ray and Laskin, found a degree of connection between reduced folic acid intake and pre-eclampsia, though studies of the time were limited in their data amount. The lack of folic acid in the maternal diet despite fortification of some foods Garratt (2009) and Garrett and Bailey (2018) does little to aid this. Whilst there is some suggestion that an excess of folic acid could increase the risk of cancer, this was based on significantly higher doses than that given in pregnancy (Greenberg, Bell and Guan et al (2011) and is unlikely to be an issue. Despite the aforementioned literature providing conflicting viewpoints, the benefits of taking folic acid still seem to outweigh any risks. Guo et al (2012) in a comprehensive article regarding folate metabolism and pre-eclampsia, acknowledge the work to date on this area and conclude there is a protective benefit of adequate or improved folic acid levels. They continue to recommend the need for further studies into folic acid and its benefits, not least to establish exact mechanisms of folate deficiency and pre-eclampsia.

Folic acid (folate or B_9) is a water-soluble vitamin, which aids the synthesis of deoxyribonucleic acid (DNA) and from a number of studies, has been shown to impact on reducing systolic and diastolic blood pressure (a key symptom of pre-eclampsia). This may be through lowering plasma homocysteine, reduction of blood pressure and decreased effect of thrombosis (Das Singh 2015).

To support the theory of the changes to homocysteine levels a case –control study by Makedos et al (2007) using a small cohort, identified an association between high homocysteine levels and raised incidence of pre-eclampsia. Indeed a significantly larger cohort study undertaken in the following year by Wu Wen et al (2008) and later studies built

on from in the FACT trial (Folic acid clinical trial) led by Wu Wen in 2013, resulted in findings which clearly provide support for this theory. At the time of completing the literature review, the randomised control trial was unpublished, but preliminary findings presented in the 2013 paper showed a notable physiological difference in women taking folic acid and those taking a placebo. More specifically results from the Wu Wen et al 2008 study, witnessed double the risk of pre-eclampsia in women who took the standard dose of folic acid when compared to a group taking a much higher dose of 1000micrograms. This study also involved women from a wide range of demographics lending external validity and generalisability to the study, but their inclusion criteria focussed primarily on women who were of higher risk of pre-eclampsia unlike the cohort to be used for this thesis.

Moving forwards a number of further studies have had similar findings. Williams, Mistry and Morgan (2012) for example, undertook pathological studies on the placental folate transporters within placentae from cases and healthy women. There was a marked reduction within the case's placentas and the supposition made was that there was a potential for reduced cell proliferation (angiogenesis) in addition, to antioxidant protection. There are certain challenges with this study however, primarily with the use of placentas from termination cases. Arguably as pre-eclampsia would normally show up much later in pregnancy, the accuracy of linking a potential for the condition to an underdeveloped placenta could be questioned. In addition, consideration of the ethics of using these tissues needed to occur, though it was not clear from the article how the researchers addressed this. However, the testing was to consider the placental tissue and not embryonic cells and mothers consented before use. Furthermore, the study required investigation at a cellular level to enable close studies of the physiological impact of folate metabolism, perhaps justifying the methods used. Employment of blinding of the researcher meant they would have no knowledge of whether the placental tissue came from a case or a control, adding more validity, reliability and support to the findings.

A Danish report by Catov et al (2009) also recommended the uptake of folic acid for pre-eclampsia and suggested that continued doses after pre-conception and the first trimester may have a greater benefit, with post conception having the most effect. These recommendations tie in with the question raised for this study, whether supplementation such as Healthy Start are, if given during pregnancy as currently, could affect the outcome of

pre-eclampsia. As does the study by Wang et al (2015) who through following a cohort of over 10,000 women propose that supplemented folic acid alongside a healthy intake from their diet during pregnancy can improve on the severity of the condition. Underpinning their findings of OR 0.61 (CI 0.43 – 0.87) they also identified the impact on homocysteine levels raised by Williams et al (2012). A preceding cohort study by Li et al (2013) adds the impact is greatest when folic acid is taken early on in pregnancy, though they acknowledge that there were several other confounding variables including different dosages and maternal characteristics such as raised BMI, which may have affected their results.

Further credence towards a beneficial effect of supplementation with folic acid, was given in a meta- analysis prepared by Yang et al (2016), which draws together a number of leading studies including a triple blinded RCT by Hashemi et al (2016) and an Australian study by Vanderlelie (2014). From the pooled evidence, there was a 67% reduction in pre-eclampsia after supplementation in the first trimester. Moreover, Hashemi et al (2016) recommends a higher dose of 5micrograms per day, currently only prescribed for women with a raised a BMI.

The available evidence is generally of high quality with findings suggesting benefits arising from folic acid. The timing of supplementation is less clear, and at the time of writing this thesis, current guidance recommended pre-conception and first trimester as optimal rather than through the pregnancy or from the second trimester onwards. This is however, to prevent neural tube defects.

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1.9 Vitamin C and pre-eclampsia

The third component of Healthy Start vitamins is 70milligrams of vitamin C (ascorbic acid), an antioxidant found mainly within fruit and vegetables, and which meets the recommended daily amount (RDA) for adults. Yet to be established is whether this amount meets the needs of pregnant women and her fetus but as a supplement, it would be in addition to any dietary intake, assuming this is already a healthy one. There is evidence that vitamin C aids tissue strength and can aid the development of the amniotic membrane (Hart 2014). However, as Symonds and Symonds (2004) identify in pre-eclamptic women, the decrease in antioxidant activity leads to further cell damage. Furthermore, Visioli et al (2002) identified the positive effect vitamin C had on nitric oxide synthesis production,

which leads to vasodilation. It could therefore be logical to conclude that as vasoconstriction of blood vessels leads to elevated blood pressure in pre-eclampsia, which in turn leads to damage to cell structures, a deficiency of vitamin C would have a negative impact. This established physiology has subsequently led to a number of trials aiming to support the hypothesis that vitamin C can reduce the risk of raised blood pressure in pre-eclampsia.

The trials to date have predominantly been randomised control trials (RCT) of differing sample sizes, using a placebo and a higher dose of 1000mgs per day. Two studies were identified that presented findings suggestive of a positive impact of vitamin C. A relatively small but robustly structured case control study by Zhang et al (2002) accompanied by a 3-armed RCT by Chappell et al (2002) found clear evidence for a doubling of pre-eclampsia risk with low vitamin C uptake. The latter study used placebos and compared outcomes for low and high-risk women. The specific findings related to the positive effect of ascorbic acid on aiding placental development, which would support the theory of supplementation affecting the aetiology of pre-eclampsia. Whilst both of these studies covered a range of population characteristics, before application of the findings to a much wider and diverse population, such as that found in the trust used for this thesis, a degree of caution is needed. Unlike the local region used for this study, in the studies by Zhang et al (2002) and Chappell et al (2002) there was limited representation from certain groups and variables, including a range of age groups and different ethnicities. What the previously mentioned studies do provide however, are the useful classifications of ethnicities, selected for use in this study.

Only one study identified within the literature search was conducted in the UK and of the remaining sourced articles regarding this topic, all tested a combination of vitamins C and E supplementation as opposed to purely vitamin C. However, none of these studies found evidence to link either vitamin to a reduction in hypertensive disorders. One multi-centred RCT by Villar et al (2009) on behalf of the World health Organisation (WHO), and a study entitled the VIP trial conducted by Rumbold et al (2007); found no beneficial effect of vitamin C but considered only high risk women and across third world countries. In contrast, despite being halted due to lack of funding, but using a similar approach in their study, Beazley et al (2005) from the results generated before ending the trial, was beginning to suggest a small benefit was being demonstrated. In contrast to these trials, this study will

consider the vitamin C as part of the multi vitamin and in a lower risk population as it is not usual practice to prescribe vitamin C supplements routinely.

Regarding risks associated with vitamin C supplementation some of the literature sourced failed to identify any. One editorial by Lindheimer and Sabai, (2006) however, did discuss extracted results from the VIP trial by Villar et al (2009) and proposed that vitamin C supplementation may lead to complications of earlier onset of the symptoms, exacerbated symptoms amongst other complications, though this is not as succinct or explicit in the article presenting the research itself. Likewise, Xu et al (2010) using similar methodology suggest there is a higher incidence of preterm labour and no obvious benefit. What could be argued is that these along with trials by Poston, Briley and Seed (2006) who presented similar conclusions to the VIP trial is that the dosage of supplement administered far outweighs that of the RDA and subsequently skewed the findings and conclusions. Intriguingly though a study in Uganda by Kiondo et al (2014), ascertained no benefit from vitamin C they found that there were no adverse effects even at the higher dose. A conclusion supported by McCance et al (2010), would endorse the research by Kiondo et al (2014). Despite no evident positive benefits of supplementation affecting their sample of women with diabetes, they recommend continued research into women with low antioxidant status as does the Cochrane review of 2015 also conducted by Rumbold et al (2015). This review considered 29 trials with low levels of bias and various degrees of quality, which overall concluded that vitamin C supplementation has little effect on pregnancy outcome. It is possible that a degree of selection bias influenced these findings however, as two of the authors undertook both the Cochrane report and the VIP study.

The fact that vitamin C is one of the main components of Healthy Start vitamins, albeit in a smaller dose than used in the aforementioned trials, it was not possible to draw specific conclusions based on vitamin C independently. However, when considering the mixed results from previous trials it was deemed pertinent and important to consider the supplements as a whole for this thesis and relate findings to all three elements of the Healthy Start vitamins.

To conclude, overall, with mixed results and differing quality of evidence surrounding vitamin D, folic acid alongside vitamin C supplementation and their role of improving rates

of pre-eclampsia, further investigation would aid clarity. With a potential for each vitamin to improve either the blood pressure or pre-eclampsia when administered independently as suggested in a series of trials including Wang et al (2015) and Purswani et al (2017), it is reasonable to ask whether providing a multicomponent approach to supplementation as found in Healthy Start vitamins would be of greater benefit. Consequently, this research will consider any associations between triple supplementation and the outcome of pre-eclampsia. As previously mentioned, though this study aims to consider women of low risk of pre-eclampsia, it is impossible to eliminate all confounding variables. It is possible however, to consider several key ones within the data collection and analysis, which may aid recognition of the impact of any confounding, as well as identify where supplementation may be of benefit. Having an appreciation of the available literature pertaining to these variables is therefore fundamental.

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1.10 BMI and pre-eclampsia

A further consideration for maternal characteristics, which may act as a confounding variable is maternal BMI. NICE (2014) recommendation is to prescribe women with a high BMI 10mcgs of vitamin D and a higher dose of folic acid. The rationale for this, is due to the reduced metabolism of vitamin D, which stays within the adipose tissue rather than entering the blood stream for distribution to cells leading to deficiency (Robson and Waugh 2013). This issue subsequently led to consideration of what the effect BMI may have on pre-eclampsia and the impact of Healthy Start supplementation within this, along with a review of vitamin deficiency and BMI. In addition, further investigation and understanding regarding the potential effect it may have on development of pre-eclampsia was needed.

Several of the papers generated from the search demonstrated an association between pre-eclampsia and altered BMI, but with varying degrees of success. The earliest located study by Stone et al (1994) used a case control approach to compare a small number of women with pre-eclampsia with a considerably larger group without pre-eclampsia. Here the researchers aimed to consider only incidences of severe pre-eclampsia, which would account for the small number of cases. However, what is not clear in the study is how the degree of severity of pre-eclampsia was determined, nor why women with only certain BMIs were included. These considerations potentially lead to a degree of bias (Hicks 2009) and

could impact on the validity of what would seem to be statistically significant results. A cross sectional study in 2007 by Stone et al does however, provide a much clearer positive correlation and demonstrates a more stringent methodology in the method chosen for retrieval of data.

Two retrospective studies conducted in 2001 by Sebire et al (2001a and b) provide further evidence to support Stone et al's (2007) findings. Within both analyses, the risk of data retrieval and recording bias is higher than for Stone et al (2007), as the records accessed may not have been accurately completed (Peat et al 2002). However, in this instance, because validation of the source of information occurred, and as the researchers reviewed 287,213 women's records, there was room for omission of cases where data was not available. Despite no record of how the management of these cases took place, the remaining sample was substantial in size. Sebire et al (2001) also indicate that they included late bookers (booked after the recommended 10 weeks of pregnancy), and that the accuracy of their BMI reading may alter the results. However, the low numbers of late bookers included is unlikely to have a large impact on the statistically significant findings overall. This UK study also considered women from a wide range of ethnic backgrounds allowing for greater generalisability for the current multi-ethnic population in the UK.

The second Sebire et al study (2001b) in contrast, considers the impact of a low BMI on the incidence of pre-eclampsia. Data gathered was from large numbers of cases and controls and multiple logistical regression was used to eliminate other confounding factors. Through using a 99% confidence interval (CI), as opposed to a more commonly used 95%, greater reliability and validity was achieved. The resulting narrow ranges in CI and P value of <0.0001 and odds ratio of 0.76 supports the conclusion that a lower BMI reduces the risk of pre-eclampsia. In addition, a relatively small case control study in 2013 took the concept of obesity and pre-eclampsia further by exploring how significant weight gain in pregnancy could raise the risk of developing the condition, not just the effect of pre pregnancy weight (Aksornphusitaphong and Phupong (2013). As current practice no longer includes routine weight measurement, the impact of the findings of this study are less generalizable to maternity care in the UK.

In 2003, O'Brien, Ray and Chan, reviewed 13 cohort studies comprising of 1.4 million women and used extracted data from these studies to calculate risk ratios. Whilst the findings support the theory that the risk of pre-eclampsia increases with increasing BMIs, a degree of caution is needed with interpretation of their conclusions, as not all of the studies used demonstrated a positive correlation between BMI and pre-eclampsia. Therefore, a review of additional research findings could prove beneficial.

Indeed, other papers continue to question the relationship between obesity and PE. Catov et al (2007) for example, conducted a large cohort study in Denmark along similar lines to O'Brien et al (2003). Records were utilised to screen for cases of severe PE and chi square tests used to compare characteristics. Their results endorse the findings of the other studies whereby nulliparous women with a raised BMI are shown to be at greater risk of developing severe pre-eclampsia during their pregnancy, a conclusion supported by narrow ranges of confidence intervals and a higher risk ratio. However, when considering the data presented for multigravida women (those experiencing a second or subsequent pregnancy), whilst an association is demonstrated for these women developing pre-eclampsia, the results cannot be considered significant when subdivided into early onset or severe pre-eclampsia as the authors suggest, due to the fact that the confidence intervals include zero or one (Harris and Taylor 2004). Arguably, the validity and reliability (Polit and Beck 2014) of the study is weakened because the women were recruited by individual general practitioners (GPs), then interviewed at various stages by different people and unexplained exclusions and loss of participants occurred. Alongside this, there were several other possible confounding factors to account for, not least, that women with untreated but known hypertension were included.

The study by Mbah et al (2010) appears in contrast to be more robust, having less risk of bias. Although the authors chose an alternative methodology in their case control study, the data gathered was from a validated source and for a much larger cohort than those by Catov et al (2007) were. In addition, the results clearly demonstrate, as others have, a statistically significant positive association between BMI and pre-eclampsia. In addition, the study by Catov et al (2007) considered ethnicity as a separate issue, which demonstrated a further increased risk with non-white populations.

Though relevant, the studies to this point and indeed much of the literature sourced, described predominantly cohort or case control studies, considered by many scientists, to sit lower down the on hierarchy of evidence, as they are less trustworthy (Greenhalgh 2010). However, no randomised control trials (RCTs) were available, but this is not surprising when considering weight and development of a condition, as there is no controllable intervention as weight maintenance is challenging in pregnancy. One meta-analysis by Cnossen et al (2007) was identified relating to this topic but considered BMI and pre-eclampsia from a different perspective, that is, rather than look for an association between the two factors during pregnancy, they aimed to determine whether BMI at booking could be used as a predictor of pre-eclampsia. Their selection process is described in detail and appears appropriate, as does their inclusion and exclusion criteria, although there was only one UK study and there is little discussion regarding the possibility of heterogeneity (Greenhalgh 2010). In fact, the 36 studies reviewed were of mixed quality and methodology with wide differences in numbers of participants. Following pooling of the evidence the overall conclusion was that BMI is a weak predictor of pre-eclampsia although interpretation of the results would have been clearer if the findings had been presented for example, in a forest plot.

Having reviewed the above studies alongside smaller projects including an Iranian study by Aghamohammadi (2011), and articles by Walsh (2007), there is sufficient evidence despite issues discussed around some of the studies to support an association with BMI and PE and subsequently a potentially key confounding variable worthy of inclusion in the data collection and analysis.

1.10.1Vitamin deficiency and obesity.

With enough evidence available to suggest that there is a likely association between pre-eclampsia and obesity, it is necessary to consider any connection with vitamin status, considering the possibility of an association between deficiency and pre-eclampsia. With the aim of his study to establish any impact of vitamin supplementation and the reduction of the severity of the disease, it was important to explore any literature linking obesity and vitamin deficiency.

Scientists have understood the unique property of vitamin D being metabolised through UVB rays of light, for some time. Karlsson (2013) and Karlsson et al (2015) provide a clear outline of the physiological process beyond this. They explain how following metabolism it is stored primarily in adipose tissue and then muscle. She evidences different opinions on how this alters in women with higher BMI and some studies found levels in the only the adipose tissue rather than in the blood serum. It was not clear however, what the impact or significance of this was, especially as other studies found little differences in women of normal weight to those with raised BMI. However, with recognised negative associations with obesity and poor diet, along with an increase in the risk of pre-eclampsia, it could be surmised, that there is a possible triangulation with vitamin D.

Despite searching extensively and sourcing several papers regarding folic acid and vitamin C, none related specifically to obesity. Discussion of those papers discovered, which were relating to pregnancy, pre-eclampsia and these vitamins will take place elsewhere. The search did, however, uncover a number of studies, which related to the remaining content of Healthy Start vitamins, that is, vitamin D; which warranted review and add clarification of thought towards the work by Karlsson and her colleagues (2013 and 2015) findings.

A variety of studies were retrieved, many with a focus on vitamin deficiency in obesity. Foss (2009) provided a detailed discussion regarding the physiological association between vitamin D deficiency and obesity and generated some useful references, particularly as database searches were sometimes unproductive. The sum of evidence specifically focussed at obese subjects, found in one key paper from Vimaleswaran et al (2013). The study looked at the genetic link between individuals from a range of countries and their metabolism of vitamin D. Vimaleswaran et al (2013) discovered much lower serum vitamin D levels in the participants with a higher BMI, most especially those from North America where obesity rates are high. Extensive and strictly controlled laboratory testing of the serum samples from a large cohort of over 42,000 individuals, leads to a robust, reliable and valid study. It is therefore possible to propose that there is a case for supplementation and correction of vitamin D levels in women with raised BMI.

In addition, a wealth of literature was available supporting a high incidence of vitamin D deficiency in global populations including in the United Kingdom (UK). However, as the focus

of this review concerns obese women, consideration of specifically related literature only will be undertaken, with reference made to other studies if appropriate.

A systematic review by Renzaho, Halliday and Nowson (2011) perhaps provides the best comprehensive study of this issue, although even here there are limitations. This Australian review considered 20 papers, predominantly from the USA and all but one within the last ten years. As the title mentions just ethnic minorities, this suggests the aim was to focus on ethnic minorities only, although white individuals were involved in all the studies selected for inclusion. In addition, the intention to undertake a meta-analysis was hindered by the heterogeneity of the available studies and the inconsistency of the categorisation of ethnicity within the research, inhibiting validity of any findings. A further criticism is that the authors chose to consider other chronic diseases alongside obesity, which may have confused the findings and introduced too many confounding factors. What this paper does do though, is demonstrate the complexity of answering of what at first appears to be a simple question of whether vitamin D deficiency is associated with obesity. Furthermore, it is helpful that the studies selected for analysis used the same serum levels to define deficiency as with many other studies there remains confusion as to what figure signifies deficiency.

Despite the criticism directed at the studies in question Renzaho et al (2011), conclude that there is a link between vitamin D deficiency and obesity and a need for improving serum levels. Indeed, considering current clinical guidance in the UK (NICE 2014) of recommending supplementation for obese women in pregnancy, there must be some merit in the conclusions drawn. What is not fully evident is the benefit of doing so as available research proves inconclusive according to recent findings in De-Regil et al's (2016) Cochrane review. Significantly, UK guidelines and Cochrane results only use data primarily from the 1980s and refer to only a handful of the studies found during this search, suggesting a need for further investigation.

Other than those identified by Renzaho et al (2011) there were few other specific citations sourced. Wortsman et al's (2000) randomised control trial, where subgroups of obese and normal weight individuals were exposed to either ultra violet (UV) light or oral supplementation of vitamin D, did demonstrate a marked reduction of serum vitamin D

levels in the obese individuals prior to treatment with UV rays. However, the lack of detail regarding the degree of exposure to UV light raises ethical issues. As Parahoo (2014), writes no harm should come to the participants within a study, this is especially relevant with Wortsman et al's study in 2000, in light of a known skin cancer risk. Other studies considering body fat and vitamin D deficiency by for example, Arunabh et al (2003) also demonstrate a positive correlation, but several of them used small numbers of participants and some involved males and or age ranges beyond childbearing years, so are less generalisable to pregnant women. Few studies including a very dated one by Scragg et al (1995), disagreed with the aforementioned authors including Renzaho et al (2011) about reduced vitamin D and obesity, with the majority supporting an association.

1.11 Other considerations

It was acknowledged that a pregnant woman cannot be stereotyped into one characteristic, and this raises the issue of the number of variables, which could impact on not just the findings of this study, but also how best to collect data to enable a more reliable and valid study. To this end, it was important to consider other factors including maternal age, gravida (number of pregnancies), parity (number of births), blood type and lifestyle habits such as smoking and alcohol consumption; alongside any other confounding variables such as ethnicity. The rationale for choosing these characteristics especially, revolves around current thinking amongst health professionals, that these are risk factors for developing pre-eclampsia.

Consequently, it was necessary to search for literature to support or contest these factors in order to not only undertake an appropriate method of data collection, but also to account for these possible influences on results obtained and complete the researchers understanding of this context.

1.11.1 Maternal age and pre-eclampsia

Despite extensive searching, few papers surfaced which specifically regarding maternal age and pre-eclampsia. The majority including a systematic review by Duckitt and Harrington (2005) considered pre- eclampsia among many other co-morbidities. Their findings do suggest some association and it is common for health professionals to identify older and teenage mothers as more likely to develop the condition. The impact of older maternal age

in turn is borne out by the findings of Ogawa et al in 2017) Mostello et al (2008) and Sibai et al (1997). Indeed, as the average age of women have their first baby is increasing and more women are having babies in their 40's (as demonstrated in table 1.2), advancing maternal age is an area worthy of further research and subsequently were considered as part of this study, not least as a potential confounding variable.

Maternities by age of mother, 1938 to 2015
England and Wales

Year	Age group							
	All ages	Under 20	20 to 24	25 to 29	30 to 34	35 to 39	40 to 44	45 and over
1940	622,376	27,909	148,281	200,926	140,820	77,263	24,943	2,234
1950	704,102	31,256	192,282	230,484	138,823	84,291	25,125	1,841
1960	791,584	52,238	241,950	244,293	151,832	78,579	21,213	1,479
1970	786,587	81,583	289,959	238,340	114,166	48,584	13,014	941
1980	654,501	60,914	201,342	222,547	129,211	33,741	6,108	638
1990	701,030	55,535	179,317	250,670	154,553	51,259	9,193	503
2000	598,580	45,827	107,336	169,319	177,635	83,571	14,248	644
2010	715,467	40,580	136,754	197,532	199,727	113,759	25,491	1,624
2014	687,346	25,931	112,086	195,116	212,864	113,113	26,425	1,811
2015	689,751	23,925	107,603	196,363	214,870	118,524	26,474	1,992
2016	663.335	20,965	96.641	186,007	210.782	120,371	28,669	

Table 1. 2 Birth rate per age group per decade and preceding few years to 2016

Commented [AE32]: data updated but only available up to 2016 on ONS

Likewise, are lifestyle habits, predominantly smoking, recreational drug habits and alcohol intake. Routine enquiries are made by midwives about these behaviours at a woman's first antenatal appointment, and for many women, pregnancy is a time where habits are changed. These lifestyle choices are well publicised as being harmful leading to poor health outcomes, including hypertension and several cancers in the general population (Venkatarman et al (2013) and Haldorsen et al (2015). With the risk of hypertensive disorders especially, arguably these harmful lifestyle choices could develop into a risk factor for pre-eclampsia.

1.11.2 Smoking and pre-eclampsia

Smoking in pregnancy has long been considered to have a negative impact on health in pregnancy for both mother and fetus. It is evident from a meta-analysis by Lu, Mackay and Pell (2014) that smoking can have a detrimental effect on health through the narrowing of major blood vessels leading to raised blood pressure. Lu et al's (2014) study drew on findings from over 56 studies, which were selected using a clear and structured inclusion criterion. Subsequently, interest lay in the exploration into the negative effects of smoking on the development of pre-eclampsia through impeded blood flow alongside the addition of another risk factor or confounding variable for women. If the available literature suggested any such correlation, then investigation into the introduction of vitamins as a way of reducing this risk would be beneficial.

Despite thorough searching of the relevant databases however, very little evidence was found directly relating smoking in pregnancy and pre-eclampsia only several studies relating to fetal outcome. The habit of smoking is seeing a decline with the increasing switch to E cigarettes but remains a leading cause of premature death and in pregnancy can lead to fetal growth restriction, placental abruption and stillbirth (Moran and Findlay 2013). Only one paper was discovered, which specifically related to smoking and its effect on pre-eclampsia. This research undertaken in 2007 by Stone et al however, did include a substantial cohort study of 129,674 women. The authors found little difference in risk for women who smoked, irrespective of their BMI. The results require a degree of caution in their interpretation however, as several other confounding variables such as diet, may have influenced the findings. Likewise, Sibai et al much earlier on in 1997 from an initial univariate analysis of several variables, demonstrated no link between smoking and negative outcomes in pregnancy, but had no specific reference to pre-eclampsia. In contrast, Dekker and Sibai (2001) following on from Sibai et al's study in 1997, reconsidered this area and identified smoking as a risk factor, but did not quantify this in the published article.

Such limited evidence fails to provide conclusive findings and leaves a gap in knowledge. It became relevant to investigate further into smoking in pregnancy. Despite a reduction in smoking generally, women are continuing to smoke, and this subsequently raises a potential

risk. If such a risk exists, then investigation into reducing this risk through supplementation may be worthwhile. This provided justification for further research within the context of this study.

1.11.3 Alcohol intake and pre-eclampsia

Frequently alcohol consumption and smoking are habitually undertaken in unison (Vink, Hottenga, De Jeus et al 2014. Consequently, the researcher felt it was important to include this topic as a variable and the need to review the available literature became necessary. However, despite the use of various synonyms and all available databases, there were limited hits. Of these only two studies regarded alcohol consumption and the possible impact on pregnancy.

In one by Salihu et al (2011), their findings provided evidence of associated smoking alongside drinking and the negative impact it could have on placental function. Much earlier in an old paper from 1996, Klonoff-Cohen and Edelstein conducted a case-control study and found little correlation. Whilst it is difficult to draw comparisons between the two, as they utilised different methods of data collection and analysis, and society has changed greatly in the years between them, these conflicting opinions leave a gap in the available field of knowledge enabling investigation of this variable with this study.

However, Harrison and Sidebottom (2008) confirmed that trends exist between smoking habits, alcohol consumption and drug use. Using interviews and diary entries, it was ascertained that women in lower socio-economic environments were at greater risk of these habits, but equally a high proportion ceased or reduced their habit during pregnancy. The researcher's do provide clear descriptions of how the data was collected and classified but there still remains the possibility of recall bias as the data was collected for 30 day periods and also women deliberately under reporting information (Harvey and Land 2017).

1.11.4 Recreational drug use and pre-eclampsia

Alongside alcohol and smoking habits there has been an increase of uptake of recreational drugs as found by Vink et al (2014). The meta- analysis collated results from large number of pooled results generated from genotyping studies mapping habits across a wide age range from 17yrs to adults. This type of study is considered of high quality due to its high

standards of reliability and validity and was also the most recent of a few studies found looking into polysubstance use and is therefore more generalizable to a modern culture.

As it appeared from the literature that the three lifestyle habits of smoking, alcohol consumption and drug use often coincided it was concluded that inclusion of the three elements was important when considering potential confounding variables.

Interest here lay with the possible impact on recreational drug use as opposed to prescription medication on development of pre-eclampsia as polysubstance misuse has become an increasing problem in the UK and the area of focus (Birmingham City Council 2018). However, despite extensive searching using a wide range of databases, as well as using several possible synonyms for drug use, only one article arose directly relating to this aspect. Even here, there were only vague references to the impact of cocaine, opiates and other substances. Principally class A drugs, the respective authors suggested such drugs can impact on the cardiovascular system (Cohen, Osorio and Page 2017), on fetal development, Scott-Goodwin, Puerto and Moreno (2016) and have the potential to lead to placental separation (Rosenak et al 1990); although these are already a potential outcome from pre-eclampsia without the introduction of harmful substances.

What is evident from some of the older literature, such as that by Bishai and Koren (1999), is the correlation of lower social economic status and polysubstance misuse, a finding supported in later mortality reports such as Knight et al (2016). Though Bell and Harvey-Dodds (2008) surmise that women in their study stopped using substances when they were pregnant, they often did not fully declare their habits which provides a challenge when trying to ascertain facts (Peat et al 2002). With such limited and somewhat dated findings, aligned with the increasing numbers of women using recreational drugs in the UK (NHS Digital National statistics (2018), the relationship between drug use and pregnancy outcome was included for consideration as part of this doctorate. Information collected by midwives at the booking interview aided the collection of this information though this relied on the honesty of disclosure from the women involved.

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1.11.5 Blood type and pre-eclampsia

Reference was made in some of the studies found through the literature review to a possible association between blood type and pre-eclampsia, but this was also limited in

number and quality. As blood group could potentially be a further confounding variable, it was included within the study. Collection of data for this would be relatively easy due to it being recorded in the pregnancy notes, with analysis undertaken to eliminate or confirm it as such.

Blood groups in humans consist of either A, B, AB or O with a rhesus factor of positive or negative. The distribution of blood type within different countries globally can differ considerably. In the UK, there is a predominance of O and A blood groups for example, as portrayed in figure 1.2 (Wicklin 2014). However, due to the diversity of ethnicities within the local population, this needs to be considered within the cohort. There were also no UK studies found exploring this concept leaving a gap in knowledge.

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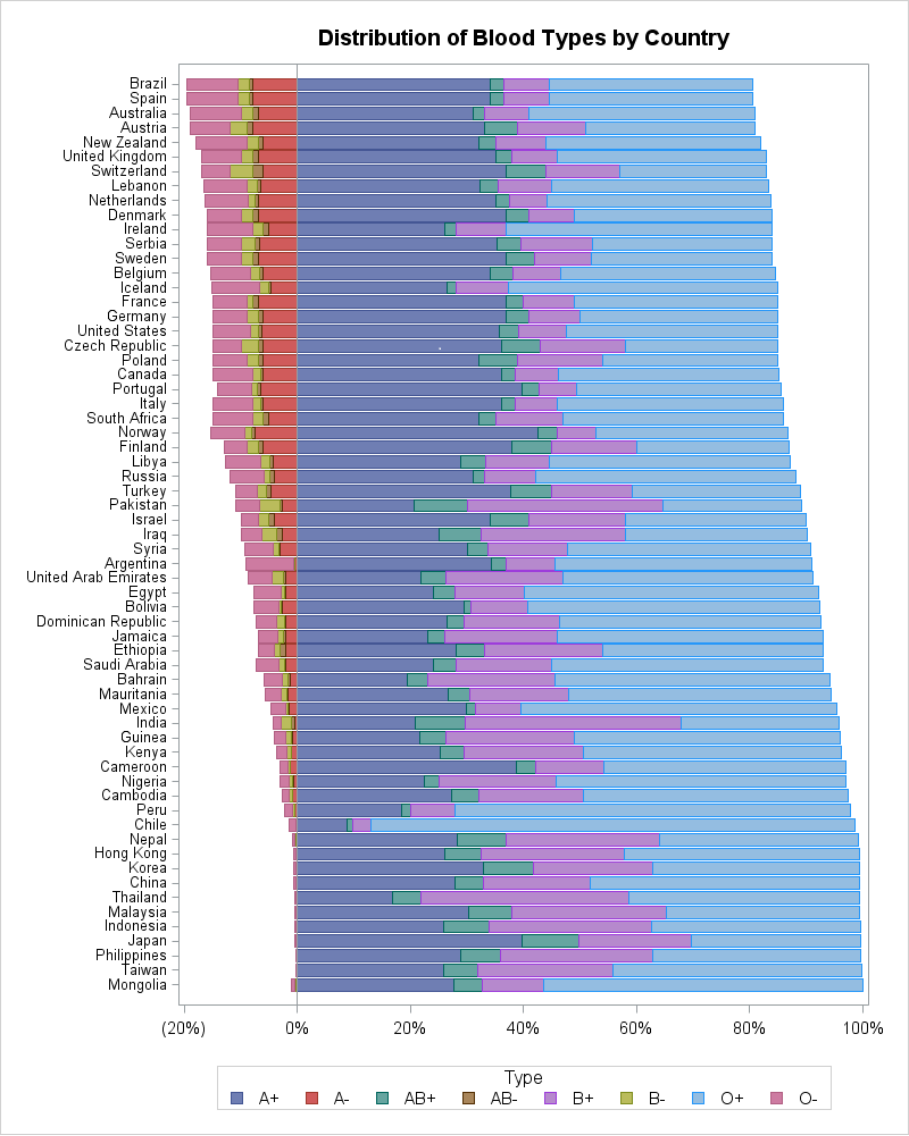

Figure 1. 2 Global distribution of Blood group ©Statsblogs 2014

A woman's blood group could affect a variety of conditions, which has led researchers to consider whether there could be an impact on the development of pre-eclampsia. From the evidence found during the literature search for this study, there does not appear to be any

consistent findings leading to consideration of blood group as a potential confounding factor within the data collection for this study.

A pooled result from a systematic review by Clark (2008) found no correlation between blood type and the condition with a CI of 0.86-1.22. There was no heterogeneity within the 17 eligible studies collected. Likewise, two papers by Hentscke et al (2014) and Alpiom et al (2011) both using a case control approach, though with significantly different sample sizes, found no statistical difference between blood group for controls and cases and the impact on pre-eclampsia. This is in stark contrast however, to the work found in other studies.

Two case-control studies by Hiltunen et al (2008) and Spinillo et al (1995) alongside two cohort studies by Lee et al (2012) and Phaloprakarn and Tangjitgamol (2013) all identify that some association is more likely with an AB blood group. All the studies took place outside the UK where AB blood types form the lowest percentage of the population. What some of these studies do, however, is try to identify the underpinning physiology and the link between blood factors such as Lewis antigens (Minas et al 2007) or the Von-Willebrand factor (Aliom et al 2011), which naturally affect coagulation in the body and can lead to hypertensive disorders, of which pre –eclampsia is a form.

To this end, this doctoral study will consider blood type as an independent variable, with the aim of adding to the body of knowledge in this area and potentially drawing a more conclusive finding.

1.11.6 Gravida, parity and pre-eclampsia

Health professionals have long held the belief that women in their first pregnancy (nulliparous/primiparous) have a much higher risk of developing pre-eclampsia, which in turn led to an increased risk in the second pregnancy should they experience the condition in the first. To this day health professionals continue to use gravida and pregnancy as part of their assessment for assessing risk of pre-eclampsia. Finding specific literature to underpin this concept however, proved unexpectedly difficult. The evidence accessed proved predominantly and perhaps logically to utilise cohort or case-control methods. Trogstad, Magnua and Stoltenberg (2011) provide a comprehensive overview of such evidence and add further support to the impact abnormal trophoblast invasion and later development of

symptoms. Interestingly they refer to scientists who identified primiparity as a risk factor as early as 1694.

Probably the most reliable paper extrapolated from the annual searching of numerous databases, was a systematic review and meta-analysis conducted by Bartsch, Medcalf and Park et al in 2016. Considered to be of a gold standard of evidence, this paper does encapsulate an extensive number of cohort studies and a pooled cohort of over 25 million women from across Europe and the United States. Though not specifically considering nulliparity, there was enough evidence to support the current thinking and a confidence interval range of 27.4% – 37%. They also identified a high-risk ratio for women undergoing fertility treatment to conceive and a marked increase in risk factors should a woman have a raised BMI. It is worthy of note that in order to reduce the possibility of any further confounding variables, women having conceived by assisted conception were excluded from this doctorate in light of this potential. This combination of factors were also conclusions drawn in a literature review conducted by Myers (2017), Chang, Muglia and Macones (2010), Mostello et al (2008). Previously mentioned authors, including Sibai et al (1995), Gordon et al (1995) alongside Dekker and Sibai (2001) similarly provide evidence to support this theory.

To add to this Duckitt and Harrington (2005), identified from a combination of case control and cohort studies, that women in their first pregnancy were at triple the risk of developing pre-eclampsia. An additional conclusion from this review, however, contradicts a long held belief that a change in partner increases this risk. Along with Steegers et al's (2010) work, there is a greater propensity to the disease not just with primigravid women, but also those who have an interval of around 10 years between pregnancies.

Hernández-Díaz, Toh and Cnattingius (2009) similarly conducted a large study from Sweden, covering a comparable demographic to the UK. Their results provided no correlation between women opting to avoid further pregnancies after experiencing pre-eclampsia, but again support the concept that primigravidas are of higher risk. Finally, a somewhat dated study by Lie et al (1998) and later Li et al (2014) both add support to the body of knowledge in this area but also highlight a possible association between paternal factors and pre-

eclampsia. Here they propose that maternal DNA and shared fetal DNA from the father can lead to the development of pre-eclampsia.

Cowan, Redman and Walker (2017), explain the underpinning physiology, behind the aforementioned work, whereby some specialist trophoblast cells in the placenta send out signals, known as HLA-C, which help prepare the lining of the uterus to not reject foreign cells especially fetal cells. In fact, the uterine cells aid the trophoblast in its work of breaking down the spiral arteries in the uterus. A mechanism of "tolerisation" takes place which starts at preconception as the maternal immune cells become exposed to the male's semen. Dependant on the period between conceptions, this process may not complete and so the immune system becomes intolerant to the trophoblast cells, resulting in miscarriage or pre-eclampsia. If the placenta finishes the "tolerisation" process, this may well reduce the risk of recurrence of pre-eclampsia in a subsequent pregnancy.

With such evidence, recognition of the impact that gravida and parity could have on any results found for this doctorate and it is highly likely that a range of gravida /parity status will naturally arise from random sampling. Subsequently data for this will form a key variable for consideration.

1.12 Conclusion

The literature provided an extensive but inconclusive picture of several key variables, which could significantly influence a woman's risk of developing pre-eclampsia and potentially the severity of the condition. Also ascertained was that a number of maternal characteristics including age, BMI and lifestyle choices could be of key relevance and should be included within any data collected due to their potential for confounding. Researchers throughout the literature used several different strategies and methodologies, and this heterogeneity can lead to further confusion when selecting an appropriate method for this doctorate. However, consideration of techniques used within this literature provided some basis for the choices made with this study, for example, the categorisation of ethnicities for coding purposes.

Regarding vitamins and their association with pre-eclampsia, the literature suggested there are benefits from taking vitamin supplementation particularly vitamin D and potentially folic

acid, though none could be found considering multivitamin supplementation. The optimal timing for supplementation during pregnancy is also unclear, particularly as the two vitamins are at present given separately if at all and not consistently across the population. It is also evident that vitamin insufficiency can be a problem, but one that has the potential to be addressed quite easily.

The potential for multivitamin supplementation (as found in Healthy Start vitamins) and its impact on pre-eclampsia, that is, using the potential for positive benefits of giving vitamin D and folic acid plus vitamin C concurrently to reduce risk has yet to be tested. Furthermore, if there is confirmation of the possible reduction in development alongside greater clarity on which women represent with higher risk factors, such as raised BMI, it may be possible to encourage a more targeted approach to management of their pregnancies.

The study outlined in the subsequent chapters will therefore aim to draw clarity on what maternal characteristics and lifestyle choices may increase her risk of developing pre-eclampsia, but also seek any associations between combined supplementation of vitamins and a reduction in pre-eclampsia.

Through necessity, as the Healthy Start vitamins are prescribed mainly later in pregnancy, this study will consider the impact of the combined vitamins at this time of pregnancy. However, further study may be useful in the future to investigate multivitamin supplementation at pre-conception and into the first trimester as folic acid is, considering the evidence around the individual vitamins and their effect on cell physiology. However, the potential for the multivitamins during pregnancy to change the incidence of pre-eclampsia is worthy of exploration, which this study aimed to do. It was evident from the literature that other pertinent variables were worthy of investigation including, the relationship between maternal characteristics, such as age and BMI, and pre-eclampsia along with how vitamin supplementation may influence pre-eclampsia. The design of this research will therefore aim using appropriate methodology to investigate these further.

Methodology

2 Methodology

2.1 Introduction

This study intended to explore potential correlations between vitamin supplementation and a reduction of the incidence of pre-eclampsia alongside consideration of a series of possible confounding variables such as BMI and lifestyle choices. This therefore necessitated the use of a methodological approach, which would enable the collection of statistical data. Thus, a quantitative approach was taken, taking a positivist stance.

The positivist paradigm of investigation using quantitative methods is used frequently within the field of health and the findings from such studies underpins much evidence based clinical practice. This contrasts with the interpretivist paradigm using qualitative methods, which seeks understanding, but considers the world to be not as clear-cut as the positivist approach (Grix 2004). The positivist principle lies with the acceptance that it is possible to measure phenomena statistically through quantitative methodology, and its quality and significance relies on validity and reliability of process alongside the generalisability to the wider population. O'Leary (2017) adds that positivist studies are not influenced by emotions, personal prejudices and are less subjective that alternative approaches. Both methodologies, however, have a key place in modern health care provision and an increase of the use of mixed methods is evident. Importantly, there always needs to be a defined and clear strategy using appropriate methods that enable a specific question to be answered. When selecting an appropriate methodology for a study it is therefore pertinent to consider whether a positivist, interpretivist or a mix of both approach is appropriate.

Subsequently, consideration of the concepts underpinning both the quantitative and qualitative stances took place when deciding on the most appropriate paradigm for this research. The aim was to ascertain the most valid and reliable design for this study, and to use robust and systematic processes to consider the provision of the supplement in managing a condition leading to the selection of a quantitative methodology. Due to the nature of the preliminary aim of determining if there were any correlations between maternal characteristics, supplementation of vitamins and the development of pre-eclampsia., the study required statistical and numerical data to test the intervention,

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I don't think you need to include as much information here on positivism, as we outlined in our feedback

One problem here is that you have a lot of text talking about the research approach but have not described your research questions yet!

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therefore a quantitative approach and positivist paradigm were seen as entirely appropriate.

2.2 Quantitative research

As Bowling (2014) evaluates, positivist scientific research based on quantitative methods rely on rigorous and meticulous gathering and analysis of data. There is a need for valid and unbiased processes resulting in clear conclusions. However, she suggests that scientific approaches are rarely so rigid when dealing with anything other than inanimate objects and that whilst deductive processes are applied, induction can be used to construct "a better theory" (Bowling 2014 p106), when interpretation of findings is reached. It could be argued however, that the systematic steps used to undertake a scientific piece of research, provides a valid and reliable approach from which conclusions can be surmised regarding cause and effect. The flexibility of interpretation reflects the imperfect nature of human beings and the complexities of clinical practice. A quantitative study aims to be reliable in that similar results would be obtained on the same group of individuals but in different conditions, and valid in that the methods used measure what was intended, and conclusions are warranted (Everitt 2009).

When considering research within clinical practice, especially within midwifery, quantitative methods continue to dominate (Harvey and Land 2017), especially with the desire for evidence-based practice. Indeed the 'medical' model predominantly applied to midwifery and obstetric care presently perpetuates the undertaking of quantitative research. Furthermore, with many women now able through advances in medicine, to become pregnant despite pre-existing medical conditions such as cardiac disease; there is a clear need for trials and research to find the best ways of managing their pregnancies. However, as Harvey and Land (2017) write, over recent years there has been a significant shift towards an increase in the use of qualitative methodology coinciding with quantitative studies as care becomes more holistic rather than just physical. Subsequently qualitative methods are increasingly selected and are especially beneficial when focusing on experiences and behaviors. However, whilst both methodologies are helpful in clinical practice in order to develop more holistic care, qualitative approaches are not useful when investigating the

aetiology of a physical disease or ways of treating them and were therefore not selected for use for this study.

Using a mix of methods was conceived as a viable option initially, which would have included asking women about compliance with taking supplements. However, the retrospective nature of the method finally chosen and the widespread geographical locations of the participants, in addition to the number of participants required, meant this was not feasible. In addition, the potential for the Hawthorne effect of women responding with what the researcher wishes to hear may have generated unreliable data (Bowling 2014).

Fundamentally, quantitative research depends on the development of variables and concepts, which are measured and examined statistically. The researcher looks for patterns and relationships to test questions and hypotheses (Grix 2004) meeting the need for this study. However, even with well-conducted studies, establishing direct cause and correlation with absolute certainty, when working with complex human subjects, can be challenging is important to acknowledge (Stewart2016). Equally the identification of one specific variable as the sole cause of a condition is difficult, if not impossible. Due to the complexity of the subjects in this study, it was also impossible to consider only one variable such as the vitamin supplement alone, therefore a variety of variables were explored for example, BMI, age and parity. Despite this argument, the continued exploration of conditions such as pre-eclampsia and its continuing detrimental effects on pregnancy and mortality and as in this case consideration of possible ways of reducing risk, through quantitative methodology is vital.

2.3 A conceptual framework and the research question

Any research project requires clarity regarding the reason for the study along with the ultimate goal the researcher wishes to achieve (O'Leary 2017). Though predominantly used within a qualitative methodology (Grant and Osanloo 2014), a conceptual framework can be helpful as it can put the study into context or as McGaghie, Bordage and Shea (2001) expressed it, 'setting the stage'. As Regoniel (2015) proposed, a framework provides the explanation of a phenomenon based on the 'synthesis of the literature', that is a review of current knowledge providing a basis from which to build a study. Likewise, it demonstrates

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the researcher's interpretation of the way the variables connect. Such a framework proved useful when establishing the focus of this research.

As Regoniel (2015) suggests, the origins of a topic often generate from experience or interest alongside a knowledge of current literature. Indeed, the selection of the topic of pre-eclampsia and supplementation arose not just from the researcher's existing interest in the condition, but also from reading the available literature around the topic. It was evident from the available literature reviewed for this thesis that the studies to date provide conflicting mix of conclusions and are yet to reach a complete picture, not least regarding which women may benefit from provision of vitamin D or folic acid and any impact on the risk of pre-eclampsia. What became evident was that if scientific studies provide enough evidence that these vitamins can improve outcome independently, then providing them together could have a much greater positive impact. As there was no current evidence base to support this, it was important that research be conducted to establish best practice in regard to managing pre-eclampsia.

In addition, following discussions with midwives and other clinicians it also became clear anecdotally that they provided only a small percentage of women with 'Healthy Start' supplementation, though specific numbers were not available at the time. The same clinicians stated that women on average would regularly take folic acid prior to conception and in the first few weeks of pregnancy. Only those deemed high risk due to raised BMI, were taking vitamin D supplements as recommended by NICE (2014). The proportion of women provided with Healthy Start vitamins seemed inconsistent, with some being offered them and others not, and they were usually provided towards the end of the first trimester and into the second trimester, rather than pre-conception.

What was also noticeable from the literature reviewed was that despite investigations into several different characteristics, results were inconclusive for example with age. These characteristics, however, cannot be ignored as a possible confounding variable as they are inherent in women, and so as many as logistically possible were identified for inclusion within this study.

Having therefore reviewed the available literature the final concept for the study was generated and is presented in figure 2.1.

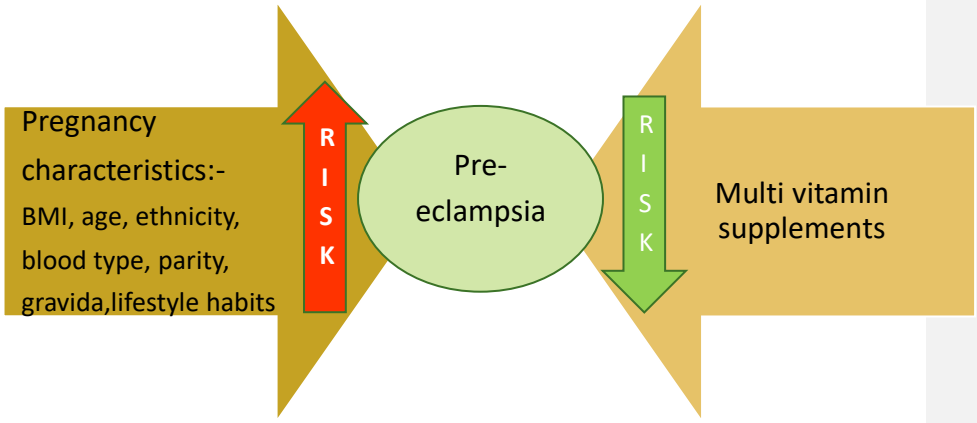

Figure 2. 1 Conceptual framework indicating risk factors and possible impact of Healthy Start vitamins.

The concept that some of the characteristics and lifestyle choices of pregnant women could increase their risk of pre-eclampsia, generates questions as to whether supplementing these particular women with Healthy Start vitamins could counteract some of these risk factors and reduce the risk of development of pre-eclampsia. Indeed, as Sandberg and Alvesson (2011) document, a perceived gap in the literature such as this is a prevalent way used by researchers to generate a research question. Though critical of this method in some ways, their recommendation is to apply some "problematization" as this leads to a more robust research question as it challenges assumptions.

With this in mind, the overarching research question and objectives culminating from this area of interest, needed to reflect the scope of the study, within the limitations of time and resources available to the researcher as recommended by Harvey and Land (2017). The aims of the research, need to clarify the desire to establish any potential benefit from vitamin supplementation, whilst questioning existing assumptions made by current evidence Alternatively, as Farrugia et al (2010) write, a research question should specify the population of interest, have clinical relevance and be of interest to both the scientific community and potentially the general public. Not only this, any findings should add to clinical relevance and knowledge. Furthermore, Grix (2004) stated that being quantitative in

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nature the question needs also to be measurable. Using these principles, the primary research question for this thesis ultimately became

"Does vitamin supplementation in pregnant women within a regional maternity unit, reduce the risk of developing pre-eclampsia?"

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Or a null hypothesis of - Vitamin supplementation has no impact on the reduction of risk of pre-eclampsia.

Secondary questions developed from considering the literature

1. What effect if any does maternal and paternal ethnicity have on development of pre-eclampsia
2. Do correlations exist between maternal characteristics and/or lifestyle choices, and the development of pre-eclampsia?
3. Does supplementation with vitamins alter the effect of these possible confounding variables on the development of pre-eclampsia and its severity?

2.4 Research aims and objectives.

Research aims and objectives provide statements as to how the study will answer the research question and consequently help with the development of the design of the study (Farrugia et al 2010).

The overarching aim of the study was identified as

- To determine if there were any correlations between maternal characteristics, supplementation of vitamins and the development of pre-eclampsia.

The resulting objectives were

- To use a retrospective cross sectional study method to gather data on vitamin supplementation uptake and compare outcomes for pre-eclampsia.
- To collect data from medical records concerning maternal characteristics such as age, BMI and parental ethnicity, lifestyle choices and behaviours and season of birth to aid assessment of risk factors and acknowledge confounding variables.
- To analyse the data collected to establish whether correlations exist between various factors and the development and severity of pre-eclampsia.

Commented [BD45]: behaviours is probably better than 'choices'

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- To disseminate the findings of the research to inform future clinical practice and provide recommendations for best practice.

2.5 Study Design

Prior to the decision to adopt a retrospective cohort format, alternative quantitative research methods were evaluated. A randomised control trial (RCT) for example, for many scientists, would be considered optimal to establish the true effectiveness of supplementation (Stewart 2016). However, to enable this, women would be randomly selected and allocated to groups either be prescribed a placebo or the Healthy Start vitamins, potentially alongside a group experiencing conventional care of single vitamin supplementation. This approach would not be achievable for this study due to limited resources available and the fact that some women are already taking supplements, which may inhibit accurate data gathering. Whilst this type of study is considered by many scientists and medical professionals to be the "gold standard" for research, which remains high ranking in the hierarchy of evidence (Stewart 2016, p161), they can require significant resources to undertake and can be costly. There may also be a need for a substantial sample size and access to the vitamins and a placebo for supplementation, which is beyond the scope of a lone researcher. Furthermore, tracking the participants could prove difficult as the researcher is always not in the Trust and even in this scenario there is no guarantee of compliance with uptake of the medication (Brown and Bussell 2011). Due also to limited funds and time, in addition to issues relating to access to enough women with a sole researcher, and the difficulties with the logistics of managing medication, this method was considered unfeasible at this point. However, should the findings of this investigation prove statistically significant there could be justification for further investigation through a RCT in partnership with the respective trust or further afield.

An alternative, quicker and less costly approach would have been a case-control study. This method requires data to be collected for cases with pre-eclampsia and an equal number of matched controls of women who did not develop the condition. Consideration would then be given to whether they took vitamins or not. Again, this method has benefits and weaknesses, but case controls studies are deemed to be of lesser quality when compared with other methods such as a cohort or controlled trial, not least due to selection bias when attempting to match participants (Bowling 2014). Matching can also lead to challenges

when trying to effectively pair from the many different characteristics found within the diverse nature of the women in the chosen catchment area, even when for example age ranges are used. In addition, to enable such matching there is often a need for much greater numbers of participants to draw from which would be beyond the scope of this study. There is also the potential consequence that constraining the selection of participants from the wider pool, in order to meet and match defined characteristics at the exclusion of others, may remove the chance of representation and generalisability to the wider population; For these reasons, a case-control method was not selected and ultimately a cohort method was chosen.

A cohort study can be either prospective or retrospective in nature, of which the latter was adopted for this study. This method is described as “an observational study in which a defined group of people (the cohort) is followed over a period of time” (Harvey and Land 2017. P22). Mann (2003) adds that longitudinal cohort studies can be useful for studying incidence, causes and measure events in a chronological order to distinguish cause and effect; or alternatively for example a cross sectional study which considers a particular period in time only.

Ultimately, a retrospective approach was chosen for a variety of reasons. When using such a retrospective approach, data is collected from medical records or through direct contact with individuals. Information is gathered to create a data set for a cohort of participants who have or have not experienced a condition (in this case pre-eclampsia) at the beginning of the episode of time being studied. Whilst similar in nature to the case- control method, there is no requirement to match the participants. The researcher is therefore more able to provide a representative sample through a wider random selection from a larger pool (Stewart 2016). This in turn should aid content validity, as there should be sufficient data to encompass a full range of variables (Harvey and Land (2017) and generalisability. Euser et al (2009 c214), also define cohort studies when undertaken retrospectively as an “efficient and elegant” way of answering questions of existing data enabling the study of multiple exposures and outcomes.

It is acknowledged, that with many such studies it is not possible to draw specific conclusions regarding actual cause and effect. However, it is possible to consider the impact

of more than one variable on the outcome and consider correlations, an aim of epidemiological studies (Euser et al 2009). Indeed, much like Euser et al (2009), Mann (2003) purports additional benefits of retrospective cohort studies being considerably quicker and should quality reliable data be collected the effect of each variable on an outcome, or the relative risk, can be readily ascertained. Mann (2013) goes on to add that whilst there is a risk of loss of participants with prospective studies, and of recall bias these are not likely if conducted retrospectively, due to the data being already in existence. In addition, as Euser et al (2009) discuss, whilst prospective studies are considered by some researchers to be superior to retrospective ones, if conducted well the latter can be equally as good. Both Mann (2003) and Euser et al (2009) add that when compared to a randomised control trial cohort study, due to less restriction for exclusion and broader inclusion criteria, the results are often more generalisable to clinical practice, adding further justification for the selection of this method.

When applying Stewart's (2016) model of a retrospective cohort method to this study of supplementation and pre-eclampsia the following figure 2.2, is established.

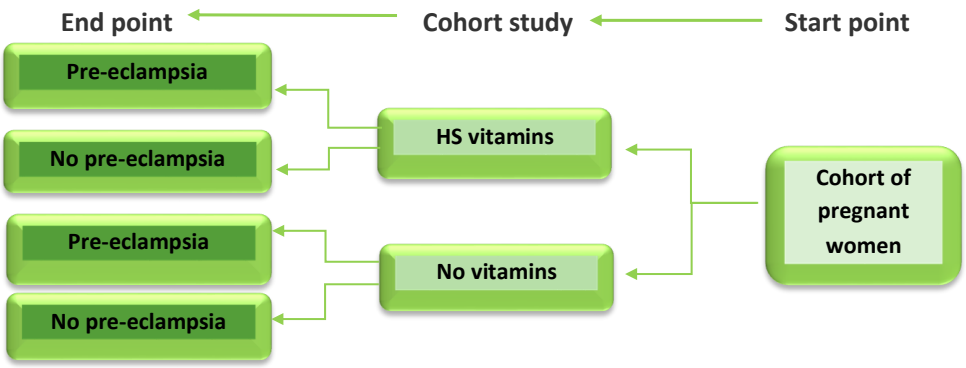

Figure 2. 2 Study design represented using Stewart's model (2016, p138)

Further justification for the choice of a retrospective cohort method is that the nine months of pregnancy provides a clearly defined period in which to follow the progress of the cohort and all of the women begin with the same baseline regarding pre-eclampsia.

In addition, much like Mann (2013) and Euser et al (2009), other research authors such as Thadhani and Tonelli (2006) write, a well conducted cohort study can be as effective as a randomised control trial in enabling the testing of multiple hypotheses, though the potential for bias relating to multiple comparisons needs to be acknowledged. They go on to add much like Stewart (2016), that external validity (that is generalisability) may be stronger due to the non-randomisation of the sample. In addition, Stewart (2016) suggests that a retrospective approach can reduce the chance of follow up bias as there is no need to follow up the cohort due to the period in question being represented within the required time frame. Furthermore, as the collection of data will not require the involvement of other staff other than the researcher and with already busy midwives, it makes the management of resources more straightforward. Not involving other staff also reduces the potential for recording bias (Stewart 2016), and the impact of the aforementioned Hawthorne effect, whereby the staff or the researcher collecting the data may influence responses and behavior (McCambridge, Witton and Elbourne (2014); Bracht and Glass 1968). Subsequently improving internal validity by reducing impact of any external influences other than those intended for study on the results (Harvey and Land 2017).

It is evident that the chosen method for this study carries many advantages to alternative options if conducted well and when the processes are transposed onto the topic of interest, the application of method to the study becomes clearer. Ultimately this research into Healthy Start vitamins would identify a group of women, consider a range of characteristics and lifestyle choices and follow their pregnancy at regular intervals retrospectively, identifying whether vitamins were taken or not and then whether they were still taking them later in pregnancy as an acknowledgement of compliance. The women would all have started at the same point of conception and will have notes from the point of booking, without pre-eclampsia. The end point for data collection would be the date of the baby's birth and the outcome of any development of pre-eclampsia. Choosing a retrospective approach enabled the tracking of these factors relatively easily. The women's medical records provided a single point for information gathering and enabled the tracking of their progress at specific points during the pregnancy. It was possible to check vitamin uptake through the notes made at each meeting with the midwife, characteristics and risk factors at the point of booking and progress of any disease.

2.6 Ethics

Any study, especially those including human participants requires ethical consideration in order to maintain the safety of the participants, especially significant in health fields where often those involved can be vulnerable (Harvey and Land 2016; Denscombe 2012). In addition, Denscombe (2012) goes on to add that dependent on the type of methods used, the type and level of ethical approval will differ. Furthermore, both Harvey and Land 2016 and Rees 2011) acknowledge, that the ethical principles do not purely relate to the method used but also to the way data is managed and how the findings are disseminated.

Adherence to ethical principles and codes is paramount and there are a number of parties involved in the approval process for a study. These can include the permission from the Trust involved, consent from the local research and development departments, participant consent if required, alongside approval from national ethics committees. The researcher also had to provide evidence of completion of the (Post graduate certificate (PGCert) in Research and have presented their study proposal with the then Faculty Research Degrees committee. Actions raised from the committee had to be addressed before any further progress could be made and Indemnity Insurance was granted.

Any study involving research with people requires indemnity insurance and sponsorship. This was agreed through the University supporting the researcher's PhD. Indemnity insurance provides security that should any harm occur to an individual because of the study, compensation will be available. It is necessary even if no potential for harm is evident (Harvey and Land 2017).

Once achieved the starting point for this study itself lay with discussions with the Head of Midwifery and lead consultant at the chosen Trust. This involved outlining the proposed methods and the desire to access medical records only.

The Director of Nursing and Midwifery is also the Caldicott Guardian for the Trust, and their written permission was required before proceeding (appendix 2). Their role is to ensure that the six principles of protection listed below and outlined by the UK Caldicott Guardian Council (GOV.UK 2018b, accessed August 2018) are adhered too.

1. Justify the purpose of the use of data

2. Do not use identifiable personal information unless absolutely necessary
3. Use the minimum amount of personal identification information
4. Access should be on a need-to-know basis
5. Everyone is aware of their responsibility
6. Understand and comply with the law in this case the data protection laws previously outlined.

The work of the Caldicott guardian is further supported by the completion of the National Information Governance Board for Health and Social care (NIGB) application. This group at the commencement of the project was responsible for section 251 of the National Health Service act (NHS Act 2006) though their jurisdiction has now been transferred to the Health Research Authority (HRA). The remit of relevance here refers to where identifiable patient information is needed, but where consent is not practical. The NHS Act (NHS 2006 accessed August 2018) permits patient information to be used when in the public interest and in the interest of improving care. The desire to seek association between supplementation and pre-eclampsia was considered to fall under this remit.

The Research Governance Framework (v3 updated in 2017 Department of Health. Gov.uk 2018a) also provides a set of standards to which researchers must work. As an example, it identifies the need for assigning a Chief Investigator who takes overall responsibility for the conduct of the research study. In this instance the researcher was the only individual involved therefore this responsibility lay with them.

The researcher should also always maintain professional practice. As a registrant on the Nursing and Midwifery Council, adherence to the Code (NMC 2015 and NMC 2018) must be ensured, the guiding principles of which are to prioritise people, practice effectively, preserve safety and promote professionalism and trust.

The Head of Midwifery provided written agreement to proceed in 2013 (appendix 1), which led to the next step of speaking to the Research and Development team at the same Trust. Their joint agreement enabled access to medical records under the honorary contract with the trust held by the researcher and in addition, to do this without the need for direct consent from every participant. This application was aided further by the Trust being a

leading centre for teaching and research and women frequently give permission to be included in projects.

Further advice from the research department on the requirements from a Trust perspective, led to an Integrated Research Application System (IRAS) account being set up which, enabled easy management of required paperwork and also enabled documents to be shared between supervisors, the research and development team as well as links to the National Research Ethics Service (NRES). This ethics service provide literature on the level of ethical approval needed in addition to aiding submission to the National Research Ethics Committee (NREC). This level of ethical approval was required for this study as data from human participants would be needed (National Health Service (NHS) National Patient Safety Agency GOV.UK 2009). From studying the literature provided by NRES, it became evident that NREC approval would be required, which is within the jurisdiction of the Health Research Authority. A list of information was therefore needed prior to submission to the ethics panel, including the evidence of undertaking training in data protection as discussed later on page 94.

The application for ethical approval was submitted to the East Midlands and Nottingham committee (Ref: 14/EM/1312). The meeting finally took place in February 2015 and a small number of adjustments were requested following the panel's consideration. Ethical approval to proceed was granted following resubmission of the updated information, without the need for another panel meeting (appendix 3).

2.7 Access to data and confidentiality

Access to pregnant women's information relied upon having access to the medical records department at the selected maternity unit within the Trust. As discussed in section 4 an honorary contract was already in place with the Trust, which enabled access to the records department, and key permissions had been provided. Along with permission from the medical records manager, these permissions meant that the researcher could work within the records library, collect data and screen the notes. As long as any information was securely managed and anonymised, all parties were happy for data collection to commence. In addition, it removed the need for consent to be obtained directly from every participant. Should consent from every woman be required, this could have impacted markedly on the

progress of the research and would have been logistically unachievable. Both verbal and written permission was provided from the trust staff and was essential to support the ethics application.

With permissions in place, the researcher was the able to contact the Informatics department at the Trust. The requested information was then shared via encrypted, and password protected emails, on a monthly basis, via the researcher's university laptop with firewall protection.

To further ensure confidentiality and protection of personal data, as per guidance within the Data Protection Act (Great Britain 1998) it was necessary to avoid removing any lists of ID numbers from the trust. To aid tracing of records however, women's identification numbers (IDs) were needed, therefore it was agreed with the records department manager that the lists could remain within the medical records department, in an unmarked folder in an agreed secure place. The records department is also locked when unmanned and screening of records took place during office hours only when the staff were present. Only the date of the baby's birth and severity or absence of pre-eclampsia were sent alongside the ID number which would be the only trackable detail sent through from the Informatics department. When transferred to the electronic data file for analysis the ID numbers were changed, and each woman was allocated a number between 1 and 952. This meant that only the researcher could trace specific individuals. The electronic data file was only accessible to the researcher, via a password and was securely locked away when not in use. Supervisors and statisticians were able to view only the anonymised data and whilst the chief investigator was present.

Adherence to the data protection guidance, outlined in the Data Protection Act of 1998, was ensured throughout and was assured at the time of completing this thesis. Now the General Data Protection Regulation included within the updated Data Protection Act has superseded this Act (GOV.UK 2018a accessed August 2018), requiring reconsideration of the data handling processes. To comply with these new rules regarding the safe storage of data the lists of ID numbers has been stored in a safe central location within the University campus, in a sealed confidential envelope and in locked storage facilities. In addition, the researcher undertook the online training packages regarding data protection and its regulation.

2.8 Sampling

Epidemiological studies using a cohort approach entail the identification of a sample from which development of a disease and associated risk factors within a population are studied. With a concept identified and a method in place, a number of other factors, including having access to the sample to collect data, the size of the sample required, and the choice of sampling technique require consideration.

Research authors such as Bowling (2014) and Burns (2000) provide some insight into the complexities of establishing the most appropriate sample size for a study. As Burns (2000) writes, the larger the sample the better as there is likely to be less error. However, there is little point in collecting masses of data if significant results may emerge from a much smaller sample. Watson, Atkinson and Egerton (2006) support this principle and acknowledge that limited availability of resources can constrain sample selection, so the aim is to provide as representative a sample as possible instead. Careful management of samples is also required when conducting studies that are potentially at risk of high attrition, particularly when follow up of participants is required, as would occur in a prospective cohort study. Using a retrospective approach ultimately and essentially removed this latter possibility, but there was still a desire to have a representative sample.

Representativeness for this study aimed to provide a range of women of different age ranges, having different BMI and from a cross section of the many ethnicities and socio-economic backgrounds found within the city where the chosen Trust was located. Success at this depended on availability of a wide enough sampling frame and effective sampling (Abbott and Sapsford 1999). Should the study participants demonstrate a proven representative sample population then it provides a stronger platform to generalise the findings to the rest of the pregnant population (Harvey and Land 2017). Conversely should the random sampling be deemed not representative, then the conclusions drawn may be limited to the women selected for inclusion only, limiting any clinical application. Lucas (2003) does apply caution however, when linking external validity and generalisability unless every influencing factor is considered and argues that external validity and representative samples do not always equate. Lucas (2003) does though consider this limitation to apply more specifically to non-probability sampling and when controlled experimental studies are conducted, but even here, caution when drawing conclusions is still advised. In contrast,

Onwuegbuzie (2000), in a comprehensive opinion paper presented in Florida, determines that despite efforts to manipulate at least one variable in an attempt to manage confounding variables, these types of limitations are a normal part of research. Openness regarding possible limitations to studies as a result, places studies in their "proper context" and in turn aids the development of further research. Despite these conflicting opinions, for this study establishing a representative sample remained an ideal and aim.

As indicated by Hicks (2009) establishing such an appropriate sample, however, can be complex, not least when deciding on the optimal size of the cohort to generate significant findings. Whilst authors Etikan and Bala (2017) suggest that at least 50 units may be adequate, other research texts including those by Hicks (2009) and Bowling 2014) provided mixed opinions as to what would be optimal targets for sample size for quantitative research. However, what is commonly recommended by for example Hicks (2009) and Stewart (2016), is that to establish what is optimal a power calculation is beneficial. Hicks (2009 p114) and Bowling (2014 p149) identified four pieces of information required to undertake such a power calculation

1. The level of power – or the probability that the null hypothesis will be rejected, and which avoids type I and type II error. Hicks (2009) adds that for clinicians the 80% power level is acceptable.
2. The clinical importance (development or not of the condition) of the independent variables effect (for example in this study Healthy Start vitamins or BMI) on the dependent variable (pre-eclampsia). The national percentage of pre-eclampsia for the United Kingdom is suggested to be 2-7% (Webster, Dodd and Waugh 2013)
3. The standard deviation of the samples
4. The significance levels.

Due to the complexities of the logarithms involved in this calculation, assistance was gained from a statistician to help calculate the sample size for this study and create a range of relative risk samples sizes. It is worth noting that these calculations can be conducted pre study as in this case, but also post study once data has been collected (Pallant 2013).

When contacted by the researcher, Public Health West Midlands provided an initial estimate of 23% uptake of vitamin supplements taken from a recent internal audit. Though

this was not based on a published study, it was the only figure available to work with for undertaking a power calculation initially. However, a later paper by McGee (2013) published prior to data collection, but after the initial power calculation, provided a more accurate but much lower figure of 10%. Considering this updated information, the power calculation was reconfigured using this new figure. Using 80% as the power the calculations presented in tables 5.1 and 5.2, aimed to establish optimal sample sizes for a range of effect sizes.

Relative risk according to Watson, Atkinson and Egerton (2006) is the probability of an event occurring in two groups who receive different treatments. When applied to this study these two groups would be the supplement group and the non-supplement group or the pre-eclampsia versus no pre-eclampsia groups. In principle as Bowling (2014) writes, it is created by dividing the population exposed to a phenomenon by the non-exposed population. An example, as provided by Scott and Mazhindu (2005, p205), should a relative risk be 0.72 of a hundred participants, 72 would have a positive outcome or develop a disease if exposed to a particular risk.

Relative risk (RR) figures, though similar in nature and principle to odds ratios, are viewed to be more accurate and useful for cohort studies (Viera 2008), so these were calculated to generate the sample size. However, where the outcome (in this case pre-eclampsia) occurs in less than 10% of the unexposed outcome the odds ratio can provide equitable values. With the frequency of pre-eclampsia cases booked at the unit used being unknown for the year to be used, initial power calculations were based on relative risk values initially (Viera 2008, Schmidt and Kohlmann 2008). Stewart (2016) further explains that a relative risk of one identifies there is no association between the risk factor and the disease. If the figure was greater than one, then there is an increased risk of developing the disease or exposure to a risk may cause the disease. Conversely, a figure of less than one demonstrates a decreased risk through exposure. Ultimately, the figures presented for the 10% uptake were accepted by the researcher to be more accurate to start the study and were therefore used when collecting data and for analysis.

Relative risk	Sample size
0.5	1047
0.33r	565
0.25	437
0.20	379
0.1	291

Table 2. 1 Relative risk and estimated sample size for vitamin uptake at 23%

Relative risk	Sample size
0.5	2200
0.33r	1217
0.25	954
0.20	834
0.1	653

Table 2. 2 Relative Risk and estimated sample size for vitamin uptake at 10%

2.8.1 Sample selection and the Sample Framework

Knapp (2017) provides a comprehensive description of the sampling framework, as the part of a population that is potentially accessible to the researcher. From this framework the required sample is drawn.

When applied to this study the sampling frame was in the form of the total number of women who give birth on an annual basis at the selected maternity unit within the West Midlands. The unit in question has around 8000 births per year and is based in a large multiethnic city. Being a tertiary unit means it also takes referrals of high-risk women from around the UK as well as from its direct catchment area. The number of referrals, however, was not available. Though a centre of excellence for high-risk care, women who are low risk are also provided for, meaning a large enough sample frame is available to draw from, for example, around 700 births occur in the low-risk birth unit alone. The childbearing population in the city represent a wide range of ages and ethnicities, with the highest rates

of fertility between the ages of 25 and 39 years (Birmingham.gov.uk 2019. Accessed November 2019) and a higher fertility rate compared to England as a whole. Figures from Birmingham City Council (2018) also indicate that 42% of the city's population are of Black, Asian, Indian and Minority ethnic groups alongside a decline in unhealthy behaviours, such as smoking. In addition, there are higher percentages of lower socio-economic status women than much of the UK, alongside a higher incidence of obesity than the UK average. Within the total number of births at 8000 and a possible 10% uptake of vitamins, there was the potential for access to 800 women who have taken Healthy Start vitamins at the maternity unit of choice.

2.8.2 Inclusion criteria

The inclusion criteria, or the characteristics which enable participants to take part in the study were in part dictated by the ethical standards set at the ethics approval, for example age and the exclusion of women 17 or under, and also by the need to encapsulate the most appropriate sample frame to draw from (Harvey and Land 2017). The sampling frame as defined by O'Leary (2017) is a list of every member of the population and for this study the population was the 8000 women who gave birth at the regional unit selected over a twelve-month period between April 2015 and March 2016. The resulting inclusion criteria and rationale are presented in table 2.3

Inclusion criteria	Rationale for choice
Women aged 18yrs and over with the mental capacity to consent (Mental Capacity Act. GOV 2005)	Though individual consent was not directly required for this study, adherence to these standards was viewed as good practice by the researcher and was a stipulation from the ethics committee
Pregnant women - Primigravid and multigravid women who had not conceived using assisted methods of conception and were seen in their first trimester by the midwife	All pregnant women have the potential to develop pre-eclampsia and including both would enable comparisons of these two groups. The use of assisted conception raises the risk of pre-eclampsia so was seen as a further confounding variable.
Singleton pregnancy (1 fetus)	Women having multiple pregnancies are at increased risk of pre-eclampsia. Not including these women provided fewer confounding variables plus, it enabled the participants to start on a similar level of risk.
Women not included in other studies, which may include supplementation or treatments, which may influence the development of pre-eclampsia.	Ethical principles limit the number of studies women can be involved in. At the trust used, the research and development department specified no more than two studies at one time. This prevented any harm and maintained the safety of the women.

Table 2. 3 Inclusion criteria with supporting rationale

2.8.3 Exclusion criteria

In contrast to inclusion criteria, there is a need to identify those who are not eligible to take part. There is also a need to protect any participants who may be subject to harm should

they be included and to minimise the impact of confounding variables on the results. The resulting exclusion criteria and rationale are presented in table 2.4

Exclusion criteria	**Rationale for criteria**
Women aged 17yrs and under and not meeting the standards for mental capacity to consent.	Women less than 18 years old are ethically underage to participate in the study
Women with an existing medical condition such as raised blood pressure, renal disease a previous history of pre-eclampsia; or those who have used assisted conception methods	The pre-existing condition or hypertension could affect the development of pre-eclampsia and add a further confounding variable, limiting clarity of analysis.
Multiple pregnancy (2 or more fetus)	Multiple pregnancies are also at greater risk of developing pre-eclampsia. Excluding this group removes a further variable
Women included in other studies, using supplementation or treatments, which may alter the risk of developing pre-eclampsia.	To protect women from over exposure to too many studies and to meet ethical standards.
Women experiencing their second pregnancy within the same year.	To avoid repetition of data and enable accurate analysis using the correct tests and meeting the assumptions applied to these tests
Women who have booked later than the recommended 10 weeks or have their first appointment after the first trimester.	These women will not have experienced the same level of care as those who booked early and may have an additional risk factor towards developing pre-eclampsia. As the intention was to include low risk women at the commencement of pregnancy these women would therefore be excluded.

Table 2. 4 Exclusion criteria with supporting rationale

2.8.4 Sample selection and screening of records.

Aiming for external validity by using a representative sample is a key aspect of quality research. Knapp (2017) and Hicks (2009) both justify the need for this and state that randomly selecting the sample can go some way towards this, particularly when there is a large population to extract from. Both authors go on to add however, that random sampling is not always possible due to practical problems or insufficient numbers though caution is advised here regarding generalising results to a wider population if randomization is not achieved. The researcher made the decision to not pre audit the medical notes as this would potentially encourage sampling bias through deliberately searching through extra records to find enough women taking vitamin supplementation. In addition, even at 10% uptake (McGee and Shaw 2013) there was the opportunity to capture a percentage of 800 births within the chosen sample size of 954.

With consideration of the power calculation and the need for a sample size of at least 954, which needed to include both cases and healthy women, a stratified random sampling technique was used. This method enables selection from two identified strata: in this case woman with pre-eclampsia and those who did not develop pre-eclampsia. This division of the groups is helpful when there is a low prevalence in the overall population and a need to establish representation (Bowling 2014, Knapp 2017). An advantage further expressed by Kandola et al (2014) in that it can be better than simple random sampling in providing a greater potential for wider representation Additionally as Cochran (1997) writes, this method can provide a smaller variance in the estimated mean than simple random sampling, important when establishing effect size during analysis. Though Hicks (2017) agrees with the quality of this method she also suggests that stratified sampling can be costly, difficult and time-consuming. However, it was possible to screen around fifteen to twenty sets of notes per day, depending on availability and location of the notes within the substantial records department. In addition, spreading the data collection days across the months and year proved an effective and worthwhile approach due to spreading the workload and providing access to a wider range of women. An additional bonus was that taking this approach meant that cases for the entire year would be included allowing for any seasonal variations in cases of pre-eclampsia which was included within the group of variables under consideration

Once permission was secured to access records, and the lists of births and identification (ID) numbers were available from the Informatics department (aspects discussed in more detail in section 4 and 4.1, the researcher aimed to spend on average two to three days per month within the medical records department. A period of a year was set aside for data collection as this fitted within the timeframe for completion of the doctorate alongside the time limitations of placed on the researcher. This was a pragmatic decision based on the timeframe for completion of the doctorate and the availability of the researcher. It also provided a complete period of time to enable cover of the seasons of the year. Accessing the records department two or three days per month was not always possible across the whole year however, due to researcher annual leave and workload. The intention was to search for sufficient records within a year period between April and March inclusively, with the aim of reaching the figures achieved in the power calculation for at least a risk ratio of 0.25. This timeframe enabled the capture of data across all seasons, which could be significant when considering metabolism of vitamin D through sun exposure, alongside fitting within the available time available for completing this study. The intention was to collect data for each season in order to consider as representative sample and season as a confounding variable. In addition, the aim was to use the recommended probability sampling associated with quantitative research (Harvey and Land 2017).

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The medical records were picked by hand by the researcher to remove the cost of having this done by the medical records team. Access to records at times was dependent on whether they were on site as secure external storage was used by the Trust, located some distance away. The medical records team were willing to have some notes transferred within the unit itself as this incurred no cost, but to have notes transferred from external storage incurred a cost. Access was also limited when less time was available to spend within the department, due to other commitments, or when records were transported to alternative locations during these times of absence.

The intention was to acquire as random a sample as possible and to reduce the potential for bias. As Bowling (2014) writes, random probability sampling can potentially provide an equal chance of each member of the population being selected and can subsequently

enhance the representativeness of the study population. Choosing the optimal method can however, depend on what and how data is needed. For this study, the requested lists sent through from the Informatics department were formatted in order of time of birth and in monthly blocks, which also ensured accuracy with the identification of season. This meant that names and identification numbers were mixed, as opposed to being alphabetical or numerical. This shuffling increased the likelihood of all women having a chance of selection rather than those for example, who come last alphabetically, being excluded because data collection had been completed to the required sample size, before reaching their part of the alphabet.

During the search to enable effective tracking of the notes within the sampling frame, symbols were used to indicate notes, which were included, not found, or excluded. It was observed that some of the notes initially marked as 'not found' were sometimes being returned to the department at a later date, so those highlighted as not found, were later reviewed enabling potential inclusion.

As the study was retrospective in nature and because a substantial number of notes were accessible the risk of selection bias through loss of participants is greatly reduced as noted by Stewart (2016). Systematically working through the lists as described in this section and strict adherence to the inclusion and exclusion criteria throughout, further minimised the potential for selection bias through specifically targeting certain groups of women. Furthermore, those records where there was any key data or relevant information missing were not included, as this would prevent the need to work around this during analysis or the need to return to the records at a later stage, which as Stewart (2016) writes, in turn reducing the risk of internal validity. The researcher's health and safety requirements were also paramount as notes were often heavy and on high shelves, a point discussed further in section 7.

Due to the potential scarcity of women, even in a tertiary centre having a diagnosis of pre-eclampsia, given the highest average national figure of 7% suggested by Webster, Dodd and Waugh (2013), potentially only 560 women would be available to sample from across the whole year. The application of the exclusion criteria further adds to a reduction of potential cases. Indeed figures from previous years at the Trust showed a much lower percentage of

potential candidates for example, in the 12 months between April 2010 and March 2011 only 2% (134) of the total 7331 women who gave birth developed the condition, and 2% (150) out of 7997 total live births between April 2012 and March 2013. Though the birth rate has steadily increased over the years, there remained a potential for limited numbers to aid establishing any correlations. Should there have been a small number of this total selected overall then potentially the sample would be unrepresentative, which in turn could have significantly affected the analysis, interpretation of findings and application of them.

Consequently, although the same principles were applied to selection of records for screening the non-pre-eclampsia women, as anticipated the list of pre-eclampsia cases provided much smaller numbers to draw from. In order to enable effective analysis and to detect a powerful enough result draw conclusions from, a higher proportion of women from the pre-eclampsia strata were included. An action supported by Mann (2013), who encourages the inclusion of as many women at risk of developing the outcome of interest to ensure the data is of value. The final search, therefore, resulted in a large proportion of the pre-eclampsia cases relative to the number of controls and the resulting data collected led to 37% of the cohort being pre-eclamptic women.

In contrast, the number of women without pre-eclampsia provided a substantial but logistically unmanageable pool to draw from. It was not possible to collect data for all the non-pre-eclampsia women at this time due to limited time and resources, so the aim was to work towards an overall sample size relative to the power calculation. Indeed, as Burns (2000) indicates that to collect over this number may actually be unnecessary (Burns 2000); even though higher sample sizes could improve representation and generalisability of findings. In order to provide some logic and consistency to the search, as the women were ranked by baby's birth date on the lists supplied, the top ten from each page of said lists were searched for, then screened using the inclusion and exclusion criteria for the study, outlined on pages 81 and 82. Once the final page of the lists had been reached, the search continued from the eleventh identification number listed commencing back in page 1. The rolling approach enabled a spread of women across the month to be included. This process continued until a large enough sample in relation to the power calculation for the relative risk of 0.25 was established.

Using the same inclusion and exclusion criteria for both pre-eclampsia and non-pre-eclampsia aided consistency when considering whose data to extract. This search resulted in a final sample figure of 353 women (approximately 4% of the total births in the unit with pre-eclampsia) and 599 without pre-eclampsia.

Within the pre-eclampsia group, the cases were sub divided into severity of mild, moderate and severe using the definition applied by the Trust and NICE (2010). The definition used is founded on the range of blood pressure and can be associated with varying degrees of protein in the urine and other symptoms though as Tranquilli et al (2013) write there is no consensus of what constitutes severity by other symptoms than just blood pressure. Generally, however, it is currently accepted by clinicians that the higher the blood pressure the greater the protein excreted in the urine. The ranges of blood pressure used by the Trust and within this study were: -

- **Mild hypertension** diastolic blood pressure 90–99 mmHg, systolic blood pressure 140–149 mmHg.
- **Moderate hypertension** diastolic blood pressure 100–109 mmHg, systolic blood pressure 150–159 mmHg.
- **Severe hypertension** diastolic blood pressure 110 mmHg or greater, systolic blood pressure 160 mmHg or greater.

The Informatics department had already identified on the list provided the level of pre-eclampsia experienced, which was beneficial in grouping the severity for coding purposes, but also provided potential to reduce bias in selection, as the researcher did not have to classify each case. The risk of selection bias was further reduced due to the majority of women experiencing pre-eclampsia being included and those who were excluded had not meet the specified inclusion and exclusion criteria or their notes were not available. It was evident however, when viewing the dataset as a whole, that a notable proportion of those excluded who had developed pre-eclampsia also had pre-existing conditions such as diabetes. There was also, though not perhaps unexpectedly, a much higher percentage of mild cases of pre-eclampsia presenting in the total number of births, of which 119 were excluded as they failed to meet the inclusion criteria. Perhaps significant for analysis, with

only a small number of moderate and severe cases available in total from the years data alongside those excluded (10 in total due to existing medical conditions) this places limitations on data analysis, as cell values will be subsequently low. Further cases for these categories were deliberately not sought, for as Stewart (2016) points out this minimises issues of sampling bias. Ultimately, over the twelve months period 1255 women's records in total were screened for inclusion. In total 303 set s of records were excluded.

As a consequence of using the specified inclusion and exclusion criteria, 129 of the entire list of 482 pre-eclampsia women were not included, as they predominantly had pre-existing conditions, one was in her second pregnancy within the same year and for others their notes were not accessible. This left 353 cases of re-eclampsia within the cohort suitable for analysis. The final number of non-pre-eclampsia women in the cohort subsequently came to 599.

With a higher representation of pre-eclampsia women in the overall cohort sample, there was a need to balance out the representation within the whole cohort. Therefore, a much larger proportion of non-pre-eclamptic women were selected for inclusion within the time available. An action that Peat et al (2002) suggest can be appropriate when seeking possible advantages and disadvantages of a treatment. They suggest that the small loss to statistical power of a 2:1 ratio is offset by the information gained during analysis. The overall final sample size also lay as close as possible to a relative risk of 0.25 but it is acknowledged, that a higher number of healthy women or data collection of the entire number of births available (7946 in the year in question) may have been useful but logistically this was not feasible.

2.8.5 Screening of records

The retrospective nature of the study relied on screening each record at the same points (weeks gestation, at booking, 28, 35, term and point of birth) for consistency. To enable this the data was drawn from the handheld records completed during the pregnancy, which had been inserted back into the main hospital notes following the woman's discharge by the community midwife. These handheld records, produced by the Perinatal Institute (2017), are designed to be carried by the woman throughout her pregnancy and every episode of care is documented in them to enable effective communication. The initial pages contain

the demographic details required including lifestyle habits of smoking and alcohol uptake alongside information regarding ethnicity, blood group and any medical history.

The subsequent pages provide details of previous medical and pregnancy history that enabled the application of the inclusion and exclusion criteria and was the starting point for screening. Should these pages show any indication of not meeting the set criteria then the notes were returned to the shelves without further review.

The provision of Healthy Start vitamins and folic acid (if given) is recorded in the medications section and the page entitled 'Plans for Pregnancy' under the healthy eating section as shown in figure 2.3. The researcher then reviewed the entries made at the pregnancy appointments in particular around booking, 28 and 36 weeks of pregnancy. For allocation into the Healthy Start supplementation group, there had to be clearly documented evidence of provision of vitamins at the beginning of pregnancy and reference to uptake at these subsequent appointments. Though there was the chance records may not have included other types of vitamin supplementation, those not demonstrating specific reference to Healthy Start vitamins were assigned to the non-supplementation group.

Figure 2. 3 Extract from maternity pregnancy records V17.2 (Perinatal Institute October 2017)

2.8.6 Bias

Bias at this stage of a research project is often unavoidable in observational studies and can take a variety of forms principally selection, information and confounder bias (Healy and Devane 2011). Firstly, information bias can occur if the researcher does not maintain an objective, consistent and accurate approach to selection and documentation. A standardised data collection tool is therefore advised, as well as consideration of both groups equally. Healy and Devane 2011) consider that retrospective methods lesson this chance of such bias, as in contrast to prospective studies there is no risk of losing continuity

and consistency when follow up of participants is not required. Healy and Devane's (2011) comments add further support for the choice of sampling method used within this study. In addition, due to the consistent approach used for collection of data applied to all participants irrespective of outcome, this choice reduced the potential for information bias.

Bias can also occur when records are used and complete information not being included, known as recording bias (Stewart 2016). It was evident that on a small number of occasions, documentation was not always fully completed, so where occasional missing pieces of information were identified, in either stratum, these women were not included in the cohort. Advantageously due to the number of available notes most especially in the non-pre-eclamptic group, there were sufficient to extract data from the next batch of notes screened, which were complete and met the inclusion criteria. As information was extracted directly as recorded in the notes however, it was not possible to account for any entries misrepresenting the facts. It is possible for example, that women may declare they smoke or drink less than is documented, as evidenced in a study by Aurrekoetxea et al (2013) who identify discrepancies. It was only possible to work with the actual data available and to acknowledge this as a potential. It is unlikely however, that midwives would document anything other than information they are presented with, as stipulated by the Nursing and Midwifery Code (NMC 2015 and 2018). In addition, a later study by Tennekoon and Rosenman (2015) suggests that the inaccuracies may only be minimal.

Secondly, consideration was needed to limit any selection bias and confounder bias. Again, as both Eusar et al (2009) and Healy and Devane (2011) write, these types of bias can be a risk associated with cohort studies, but can be managed. Selection or sampling bias can arise from the over representation of some groups or when the sample selected does not accurately reflect the population (Nohr et al 2006). Attempts to reduce this possibility was through consistent selection from the lists provided from Informatics and not over recruiting to the non-pre-eclampsia members or searching for women with more severe degrees of the condition. Furthermore, the limited details on the Informatics lists prevented the deliberate targeting of women with specific characteristics. Stringent application of the exclusion and inclusion criteria was applied. As the cohort study was retrospective this also removed the potential for selection bias through loss of participants during follow up.

Finally, confounder bias as Healy and Devane (2011) outline, applies when the effect of two different confounding variables are confused or as Harvey and Land (2017) write when spurious associations are made between an intervention and a measurement. As previously purported, it is not possible to identify specific cause and effect with a cohort study, but it is possible to establish correlations. When doing so it is acknowledged that careful analysis was needed to reduce the risk of drawing incorrect conclusions and as Crombie (1996) recommends consideration of a third factor driving the observed relationship between two seemingly related factors. Stewart (2016) also recommends that ensuring appropriate selection of samples and the stratified analysis used here can help prevent confounding bias occurring.

2.8.7 Data collected.

With a cohort involving the complexities of human existence, it can be impossible to account for every possible confounding variable. However, as Mann (2013) indicates for a study to be valid, as many as possible should be considered and having a representative sample can go some way to enabling this. Mann (2013) goes on to say is that all the participants must have the potential to develop the outcome of interest, which, all of the women included in this study demonstrated. Indeed, none of the women included would have had the condition at the point of booking and all potentially carried a degree of risk of developing pre-eclampsia due to them being pregnant. Mann's (2013) views regarding studying not just the variable of interest, but the consideration of other available variables, are supported in an earlier paper by Szklo (1998), who adds that a cohort study is an effective way of doing this. To aid this however, Bracht and Glass (1968), provide a logical stance that the researcher requires a thorough knowledge of the population used as this aids identification of what is appropriate to consider which the researcher has. Furthermore, Martinez-Mesa et al (2016) support the use of effective sampling through understanding the appropriate sample frame to select from

Consideration was subsequently applied collection of data of those potential confounding variables available within the records and key ones identified regularly within the sourced literature. Firstly, information was gathered regarding uptake of Healthy Start vitamins and folic acid uptake, as this would meet the primary aim of the study.

The final additional variables selected for collection were:

- **Maternal age.**
- **Maternal BMI at booking.**
- **Gravida and parity status,**
- **Ethnicity of both parents.**
- **Lifestyle habits of smoking, alcohol, and recreational drug use, recorded at booking**
- **Blood group.**

Notably **maternal occupation** was initially recorded but was not used in the final analysis. This was because there were too many different occupations to recode the information adequately for effective analysis. This same issue could have arisen when using alternative possible variables such as postcode, as women from around the country attended the unit not just the local catchment area.

The identification of which type of data is collected is significant when coding and analysis takes place, as the appropriate tests require the appropriate data to be used. For this dataset, the final data collected consisted of ordinal and nominal data only, examples of which are presented in appendix 5. There was no ratio or interval data. The alignment of data to type relied on definitions provided by Watson, Atkinson and Egerton (2006) and Hicks (2009), whereby ordinal data is a level of measurement where the values are ranked as in BMI or age and nominal data is where the values are differentiated by name or value and not by a number scale such as ethnicity.

It was not possible to use the researcher's laptop in the records department primarily due to security reasons, as it would have had to remain at a desk in another room, whilst notes were being collected. Therefore, the data was transcribed by hand onto a template (please see appendix 5) kept with the researcher whilst data collection took place, and otherwise stored in the department in a secure location. This data was later uploaded onto a password protected computer for safe storage. This process ensured the information stayed with the researcher was always stored securely. When not in use the hard copy data was stored securely adhering to the Data Protection Act (Great Britain 1998) and separately from the lists of Identification numbers.

When undertaking the transfer of written data to electronic it was important to ensure accuracy in the transcription. In turn, ensuring accuracy is fundamental when trying to maintain validity and rigour (Rees 2011). The use of the data sheet demonstrated in appendix 5, provided an easy layout to follow when transcribing and enable regular cross checking of information entered. The transfer of data took place on a monthly basis rather than all at the end of the data collection period. This enabled the researcher to revisit any unclear data entries by revisiting any records. Importantly the tool devised required no changes during its use improving instrumentation validity (Rees 2011). Once transferred into an Excel spreadsheet the data could then be easily transposed into the Statistical Package for the Social Sciences programme version 26 (SPSS) for data analysis. The spreadsheet required careful completion as the package is designed to copy data from previous cells to help reduce repetition of typing, but this may go unnoticed by the user. The researcher then selected to cross check all entries to avoid this. In addition, to further aid validity, there were no missing entries.

2.9 Data Analysis & Coding variables

SPSS was selected as the programme of choice for analysis of the data, as it quickly and efficiently enables the construction of nominal and ordinal data and provides an extensive range of parametric and nonparametric tests (Pallant 2013). In addition, SPSS was chosen for pragmatic reasons as it is accessible to the researcher who had prior training in its use. In order for effective analysis of the variables collected, several were identified as nominal data for example, season and smoking, or ordinal where a range of entries were gathered as with the example of ethnicity or body mass index, to collate into manageable groupings. There were no categories providing ratio or interval data.

Using BMI as an example, the data collected ranged between BMI 19 and BMI 40. Utilising these as individual figures would lead to a wide distribution of results and possibly lead to ineffective analysis. To minimise the risk, the national ranges for BMI used in the UK from the World Health Organisation (WHO 2000 please see page 108), were used, and the sample was grouped accordingly.

Following this, to enable effective analysis, each group was assigned a number or value, for example, those in the underweight range were allocated a value of 1. Subsequent ranges

were 2 to 6. This was particularly relevant when measuring effect size for some of the non-parametric tests such as the Kruskal-Wallis as the results are defined by the degrees of freedom (the number of groups minus 1), (Field 2018).

Whilst most variables were easily assigned appropriate values, for example smoker (1) or non- smoker (0) and responses to vitamin uptake being yes 1 and no 0, the data for maternal and paternal ethnicity proved more challenging. As there were around thirty identified ethnic groups using the options specified within the medical records and with some of these having very few within the sample, there were too many variables to enable a valid logistical approach to analysis. Various options were tried including using fewer groups by combining those where only a few women were represented and keeping the ethnicities with larger representation as separate entities; alternatively European and others was considered. It was decided, however, that neither of these would be practical for analysis or interpretation of results as the former still left a substantial number of groups for coding purposes and the latter may have been too narrow to provide valid results.

It was also not possible to ascertain from the medical records what skin colour individuals had, which would be beneficial when considering vitamin D absorption. Therefore the geographical location of the continent associated with the documented ethnicity were used to create the final four groupings of Asian, European, African and others. The latter group, encapsulated individuals from for example, the Caribbean where only one member of the sample was identified. Likewise, coding of the ethnicities for the paternal data used the same four groupings as the maternal data to maintain consistency. The option selected within the records is by the women and partners therefore there is also room for individuals to place themselves in any category regardless of their actual place of birth. This may lead to inaccuracies in interpretations based only on skin colour however, as stated, it is not possible to ascertain this from the records, therefore, ethnic origin could only be seen as related to solely what individuals described themselves as.

Once coding had been completed for each set of variables, initial analysis took place using descriptive statistics, followed by crosstabulation against the dependant variable of the severity of pre-eclampsia. Non-parametric tests were then applied using the SPSS online software package. The findings are presented in the following chapters.

Using this as a basis for further analysis, correlation formulae were applied to create output data for each variable against pre-eclampsia and the vitamin uptake. Following this, a decision was made to pursue binary regression analysis for those variables showing a statistically significant result of a potential correlation.

2.10 Researcher training

To ensure permission to continue with the study and to meet the requirements for the Research and Development department within the Trust, it was necessary to undertake Good Clinical Practice (GCP) training every two years. This online package provides thirteen quality standards set by the International Conference on Harmonisation (UK policy Framework for Health and Social Care research, NHS health research Authority updated 2018, accessed via http://www.hra.nhs.uk August 2018). These standards originate from the Declaration of Helsinki and are intended to ensure subject's rights safety and well-being are adhered to and that data is valid complete and well documented (Harvey and Land 2017). The particular course required by the trust was produced by the National Institute for Health Research and was required and completed every three years from the initial undertaking prior to ethical approval for the study

2.11 Prevention of risk to the researcher.

When conducting any study particularly one involving any form of lifting, consideration for the researcher's wellbeing is essential. In this instance, the medical records were frequently bulky and heavy and could be filed on high shelves. In addition, desk space was limited within the actual medical records department. To prevent injury a number of health and safety rules were therefore adhered to :-

- It was agreed by the department manager that a desk space was identified on a daily basis. Though this differed on each data collection day, it ensured a work environment, which adhered to the Display, Screen, and Equipment (DSE) guidance for the Trust.
- A trolley was used for the movement of notes between the shelving and the desk reducing the need to carry heavy piles of notes.
- Steps and stools were already provided for the use of all of staff in the department, which meant that there was no reaching or excessive bending.

- Mandatory manual handling training was undertaken on an annual basis

2.12 Annual reports on progress

To ensure adequate progress was made and to ensure correct adherence to the policies surrounding conduct of the study was undertaken, the researcher was required to provide the following: -

- Monthly meetings with the allocated supervisors and written summaries stored within the Doctoral Research College of the University.
- Annual reports to the Research and Development Centre at the Trust.
- Annual updates to the NRES committee for approval for the research to be continued.
- The University supporting the researcher also required an annual review form completed by the Director of Studies, 2nd supervisor and an allocated reviewer.

2.13 Funding

Applications for funding for the PhD were submitted on an annual basis and were approved through the researcher's employer. There was no requirement for funding beyond this point, as any additional costs for materials were met by the researcher.

The only potential financial outlay was from travel to the trust for collection of data however, this was no further than the standard journey to work. No costs were incurred for access to the records or for assistance with data collection or analysis. In addition, the university maintained a license for the use of SPSS enabling access for data analysis.

RESULTS

Frequencies, Demographics,

Correlations &

Binary regression

3 Results

3.1 Cohort distributions

This chapter will present the findings from the breakdown of each variable collected and demonstrate the distribution of the sample in relation to said variable within the cohort of 952 women, commencing with the demographics of the participants. To continue the theme considering the impact of Healthy Start supplementation, data gathered were analysed using descriptive statistics and cross-tabulation conducted on the uptake of vitamins per variable. These are presented at the end of each section. Demographic data were collected and analysed. Age, maternal and paternal ethnicity as defined by the pregnancy records, BMI, lifestyle choices, gravida and parity status and blood group were included. In addition, season of birth was considered as a possible confounding variable due to the potential for deficiency in vitamins. Following collation of frequencies, correlation analysis took place to identify which variables were worthy of further analysis and demonstrated statistical significance.

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3.2 Demographics of sample

3.2.1 Age

The intention was to consider age as a possible confounding variable and seek any correlations between maternal age and pre-eclampsia initially, followed by any impact of vitamin supplementation. Successful achievement of this was enabled through the availability of data within the records and choice made about adopted age ranges. For coding, with the exception of a small number of outliers the women included in the study fell within the commonly accepted age range for childbearing of between 18 and 40, both biologically and statistically (ONS accessed 2018). For the ethical reasons set out by the ethics committee, along with the additional confounding variables this vulnerable group may present with, data for women under the age of 18 was not collected.

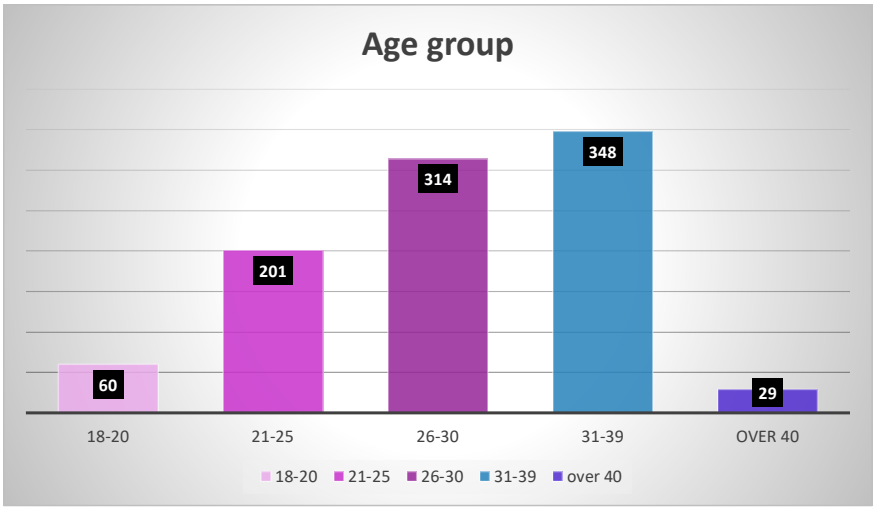

Figure 3. 1 Distribution of ages within the study group

Figure 3.1 presents the age ranges of the participants randomly selected from the records. It is evident from this that the largest proportion of the group captured were pregnancies occurring in the later childbearing years. Whilst this could be purely coincidental it could be argued that this also reflects the increase in women delaying having children nationally and locally, though statistically this does provide a skewed distribution curve. The mean age was 29.0 years and a standard deviation of 5.51. Data was collected for all 952 women so no missing data occurred.

3.2.1.1 Age and vitamin uptake

With a focus on the uptake of Healthy Start vitamins and pregnancy outcome, the number of women using the supplementation per age range was established as seen in table 3.2

Age range	No Healthy Start vitamins	Healthy Start vitamins uptake (%/ age range)	Total
18-20	47	13 (22%)	60
21-25	132	69 (34%)	201
26-30	201	113 (36%)	314
31-39	247	101 (29%)	348
Over 40	19	10 (34%)	29
Total	646	306 (32%)	952

Table 3. 1 Vitamin uptake associated with age range.

Evidently, for some age groups, particularly the 18 -20 and 31-39 ranges the uptake of vitamins versus no supplementation was noticeably different with less than half choosing supplementation. Overall, less than half (32%) of the cohort were reported as taking Healthy Start vitamins.

3.2.2 Body Mass Index (BMI)

BMI be especially meaningful as a variable when considering the rapid increase in the level of obesity and associated morbidities and mortalities found in the UK population, not least in childbearing women. To calculate the BMI the weight in kilograms is divided by the height in centimetres squared. With the aim of considering BMI as a key characteristic, data was collected and analysed. Data was again readily available within the records.

However, given the wide variety of weight differences between the women selected and the possibility of difficulties for coding and analysis, the standard categories used by the World Health Organisation (2000) and adopted by the Department of Health through Public Health England (GOV.UK and Public Health England 2018), were used to aid management of the data.

These categories used were: -

Underweight (<18 kg/m2)
Normal 18.5 – 24.9)
Overweight (25 – 29.9)
Obesity 1(30 – 34.9)
Obesity 2 (35 – 39.9)
Extreme (morbid) Obesity (40+)

The resulting groupings for weight /BMI within the 952 records provided a skewed distribution curve and placed the majority of the women in the normal to overweight ranges, with the next highest group having a BMI of between 30 and 34.9. The mean weight for this group was 26.8 and a standard deviation of 5.92 and there were no missing data.

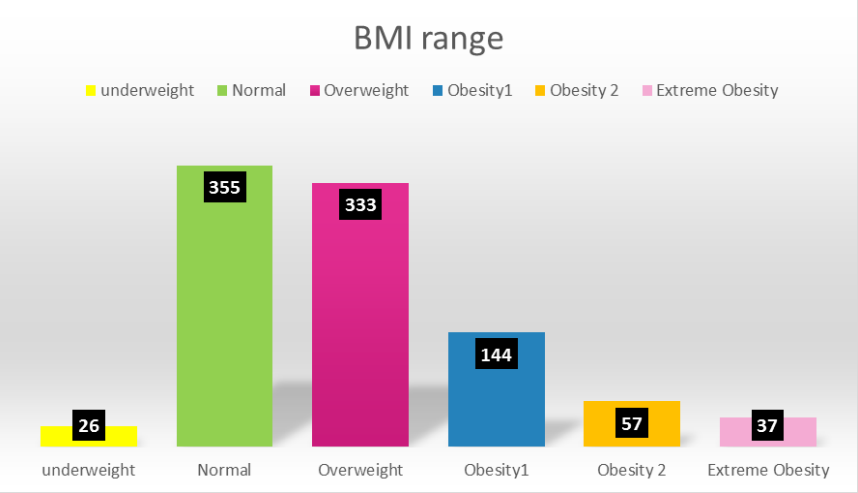

Figure 3. 2 Distribution of BMI ranges within the study group.

3.2.2.1 BMI and vitamin uptake

The uptake of vitamins relating to weight should provide some insight into the lifestyle choices made by women and as with age, it was of interest to establish any patterns within this cohort. From the data collected and processed using SPSS the resulting frequencies were generated (please see table 3.1)

BMI Range	Did not take vitamin	Vitamins taken (% = per BMI range)	Total in cohort
Underweight	18	8 (31%)	26
Normal	239	116 (33%)	355
Overweight	228	105 (32%)	333
Obesity1	93	51 (35%)	144
Obesity2	43	14 (25%)	57
Extreme Obesity	25	12 (32%)	37
Total	646	06	952

Table 3. 2 Uptake of vitamins as per weight range

It is clear that the number taking vitamins (306) is less than half those not taking supplements (646). The majority of BMI ranges have similar uptake of vitamins with the exception of those women with a BMI over 35. It would appear that in this group of 57 women only a quarter took vitamins in contrast to the other ranges where on average third selected to take them. There is a clear disproportion of vitamin takers and non- vitamin takers presented across the whole populous. This raises the supposition that should a case be proven for the benefits of supplementation in high-risk individuals alongside greater clarity regarding the higher the BMI the greater the risk of pre-eclampsia, there is a justification for greater promotion and education within society.

3.2.3 Gravida status of participants

Gravida status (the number of pregnancies including the one used for this study), and "parity" (the number of live births over 24 weeks gestation), are principal characteristics used for establishing risk not least for pre-eclampsia when planning a care pathway for women. Subsequently, they are worthy of consideration within the overall aims of the study.

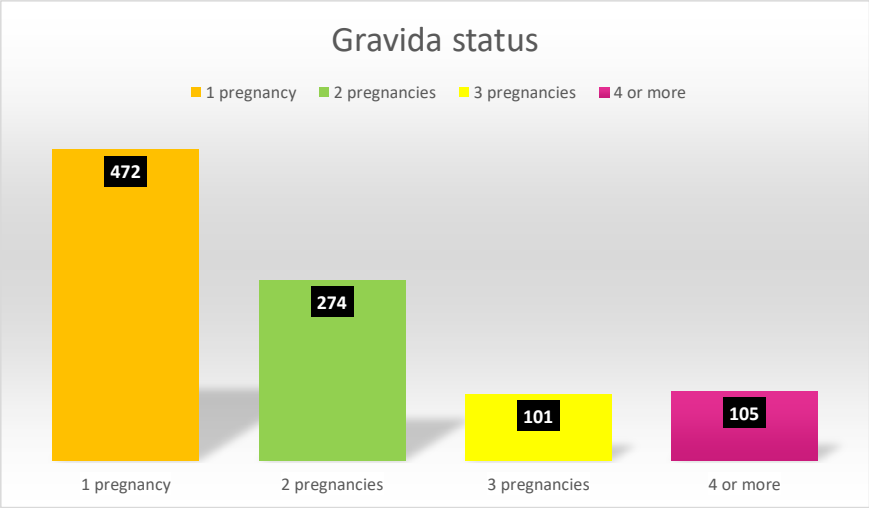

Figure 3. 3 Gravida status of study group

Figure 3.3 demonstrates that despite random sampling taking place the greater percentage of participants are primigravida women, though it could be argued that this also reflects a common pattern within the general childbearing population of the UK where many families have no more than two children (Office for National Statistics accessed 2018). There were a small number of women in the dataset, who had more than seven children, but were too few in number to allocate to a separate group and were at risk of being treated as outliers during analysis. Subsequently for coding purposes as there were minimal numbers of values within the higher pregnancy brackets, women experiencing 4 or more pregnancies were grouped together, providing a more balanced ratio for subsequent analysis.

3.2.3.1 Gravida status and vitamin uptake

Though data in table 9.6 intimates that on average less than half of women were taking Healthy Start vitamins within each gravida group, a greater percentage of women on their fifth pregnancy selected to do so. Most notable deficits, however, lie within the primigravida group where 68% of the group were not taking vitamins as opposed to 32% who did. In addition, within the third pregnancy group only 25% took supplementation.

With the theory that the risk associated with increasing numbers of pregnancies and the development of pre-eclampsia reduces Bartsch, Park et al (2016), the impact of reduction in uptake of supplementation could be seen as less relevant. However, the risk though possibly less for multigravida women, still exists as it does for all pregnant women. Subsequently should any statistical evidence be established for prescribing the combined supplementation provided by Healthy Start vitamins, then these figures suggest there is a large percentage of women who may benefit.

Gravida group (number of pregnancies)	Did not take vitamin	Vitamins taken (% of total in group)	Total
1	321	151 (32%)	472
2	178	96 (35%)	274
3	76	25 (25%)	101
4	42	18 (30%)	60
5	15	14 (48%)	29
6	10	0 (0%)	10
7	1	1 (50%)	2
8	0	1 (100%)	1
10	1	0 (0%)	1
12	2	0 (0%)	2
Total	646	306	952

Table 3. 3 Number of pregnancies and vitamin uptake

3.2.4 Parity

Due to a notable difference in uptake of vitamins identified from table 9.7, between those experiencing a first or subsequent pregnancy; parity was also considered for statistical analysis. As with gravida for coding purposes, the subgroups were adjusted accordingly.

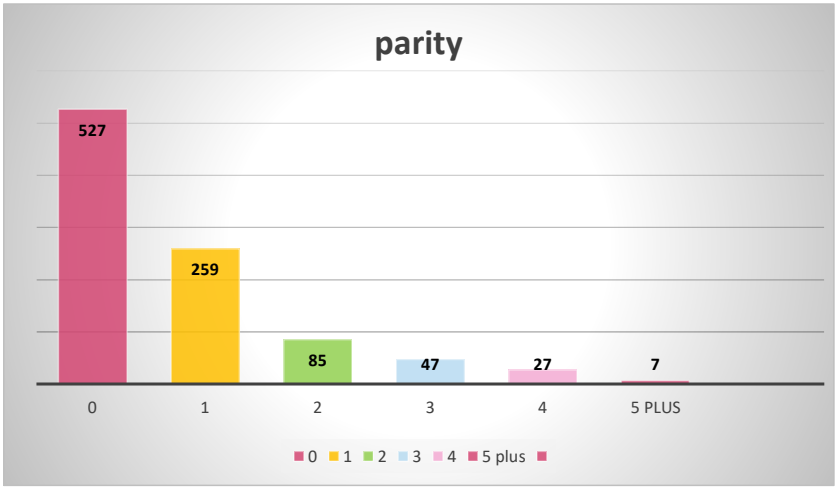

Figure 3. 4 Parity status of the study group

3.2.4.1 Parity status and vitamin uptake

When parity is considered, a degree of caution must be applied, as it is less straightforward than gravida. This is because not all women will go on to have a full-term live birth. Those included in this study all gave birth at term (37-42 weeks gestation), but for previous pregnancies they may not have completed a full pregnancy. Subsequently the table 3.3 below shows different figures to the gravida group. This is principally due to the effect of the inclusion of women who may be experiencing for example, their fifth pregnancy, but they may not have five live births resulting from miscarriages or terminations, which will not be included in the parity quota.

Unlike previous variables, there are marked differences between the groups regarding vitamin uptake when parity alone is considered. The balance is similar to the gravida status as the women with one child and those with four children groups demonstrated a higher percentage of women taking vitamin supplementation.

Parity	Did not take vitamin	Vitamins taken (% per parity)	Total
0	359	168 (32%)	527
1	168	91 (25%)	259
2	62	23 (27%)	85
3	39	8 (17%)	47
4	12	15 (56%)	27
5	3	0	3
7	0	1(100%)	1
8	1	0	1
9	1	0	1
10	1	0	1
Total	646	306	952

Table 3. 4 Vitamin uptake as per parity

3.2.5 Impact of lifestyle choices

In order to consider the impact of lifestyle choices on the development of pre-eclampsia and to accommodate for confounding variables. Data was collected regarding the number of women who adopted what would be considered harmful lifestyle choices including smoking, consuming alcohol and the use of any recreational drugs. This was taken directly from recorded information within the woman's notes (taken at the initial meeting for booking) and relies on accuracy of information being shared with the midwife and how it was expressed. Smoking was documented in the records as the number cigarettes per day and alcohol by units per week at the point of booking but to minimise potentials for negative effects on analysis, such as cell values being inadequate, the data collected for smoking and drug use were considered as yes /no. The following figures, however, provide a breakdown of characteristics for the cohort for alcohol intake, smoking and drug use alongside the associated frequencies of uptake of Healthy Start vitamins, prior to coding for analysis.

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3.2.5.1 Alcohol intake

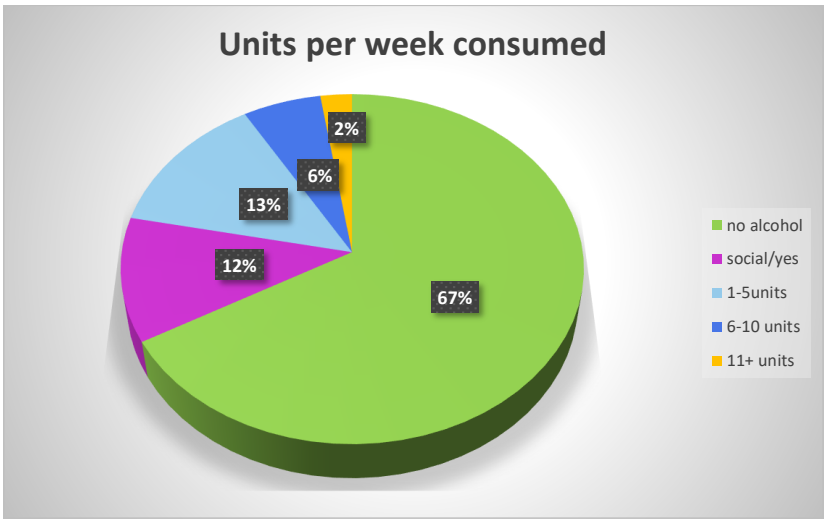

Figure 3. 5 Distribution of recorded alcohol consumption

3.2.5.2 Smoking

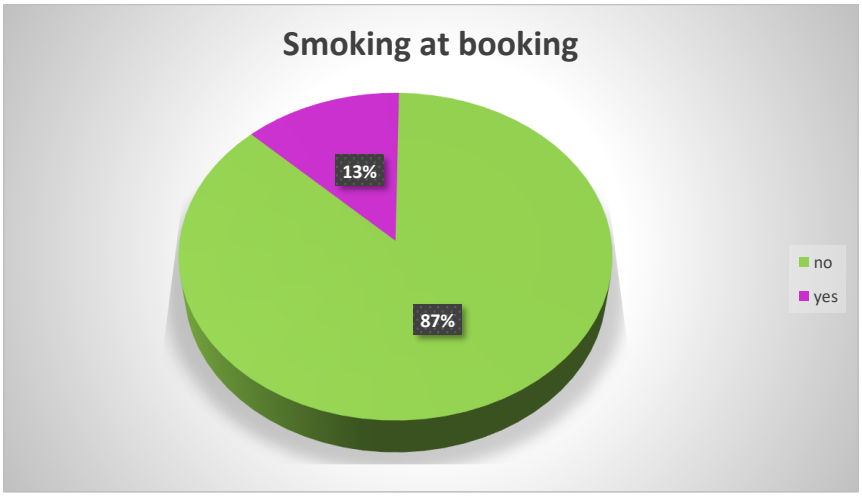

Figure 3. 6 Smoking status of study group

3.2.5.3 Recreational Drug Use

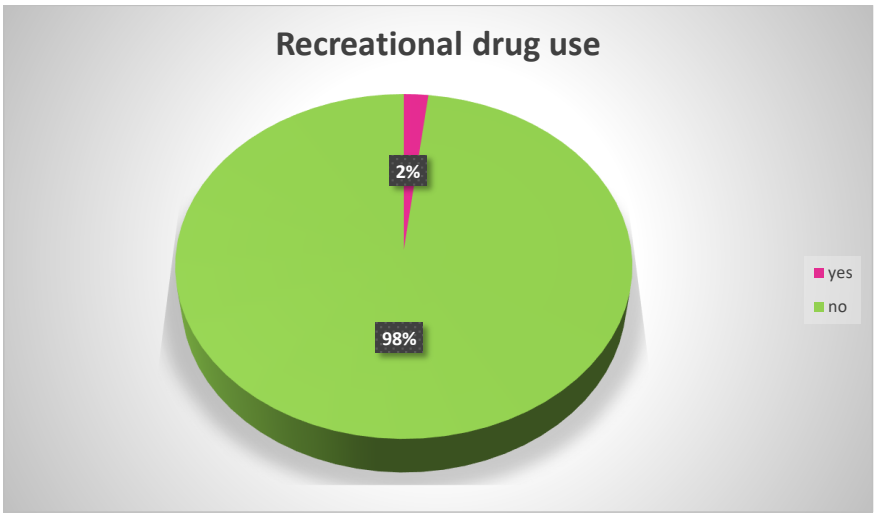

Figure 3. 7 Recreational drug use in the study group

3.2.5.4 Lifestyle habits and vitamin uptake

As with other variables, there is a marked difference in the number of women who took vitamins and those who did not. Indeed, less than 50% opted to take them. The percentages presented below in table 3.4, however, would suggest, that women opting to consume higher units of alcohol (at booking) were more likely to take the vitamins.

Alcohol intake by units per week	Did not take vitamin	Vitamin uptake (% per group)	Total
No	423	212 (33%)	635
Social/Yes	73	36 (33%)	109
1-5 units	95	32 (25%)	127
6-10 units	40	17 (30%)	57
11 + units	15	9 (39%)	23
Total	646	306	952

Table 3. 5 Uptake of vitamins associated with alcohol intake

The decline in smoking is perhaps reflected in the data presented in figures 3.5 and table 3.5, where there is a relatively small number (13%) of declared smokers in the whole cohort.

Smoking status	Did not take vitamin	Vitamin uptake	Total
Non smoker	555	274 (33%)	829
Smoker	91	32 (26%)	123
Total	646	306	952

Table 3. 6 Vitamin uptake and smoking status

Similar figures concerning smoking and alcohol intake are noted. It could be argued, this is due to lifestyle choices, whereby smoking and alcohol are both social activities that are closely affiliated. It is also possible that women who participate in less healthy options are less likely to consider taking vitamins. Therefore, should an association with pre-eclampsia and these lifestyle choices be evident there could be grounds for pursuing vitamin supplements for these women in particular and therefore these variables will be considered in more detail.

3.2.5.5 Recreational drug use and vitamin uptake

Data on the use of recreational drugs was initially collected as it was included in the initial research aims, and again demonstrates considerable differences between groups. However, the minimal number of drug users identified provides data too limited for further investigation as analysis could not provide valid results and it is therefore presented for information only.

Drug status	No vitamin uptake	Vitamin uptake (% per drug status)	Total
No	630	304 (33%)	934
Yes	16	2 (11%)	18
Total	646	306	952

Table 3. 7 Recreational drug use and vitamin uptake.

3.2.6 Season and preeclampsia

Combined with the impact of skin colour and cultural choices, principally the desire to cover the body; the effect of vitamin D levels could potentially have a significant impact on the outcome of pregnancy. In order to consider this concept in more detail and to support or refute the findings of previous studies, alongside considering the impact of season as a confounding variable, data was collected for women across all seasons as presented in figure 3.7 below.

To facilitate data collection, lists of ID numbers were forwarded to the researcher from the Informatics department, which were set out by each month of the year of data collection. It should therefore be acknowledged, that the pregnancy would have taken place over the preceding season. For example, for a birth taking place in September/Autumn, means the woman would have been pregnant over the summer season. Only term births (over 37 weeks gestation) providing accuracy in allocation to groups

Within the UK climate, spring and autumn are most likely to have reduced sun exposure, winter the least sun exposure and summer anticipated to have the greatest sun exposure. This in turn will alter the potential for metabolising adequate levels of vitamin D, with the lowest levels occurring in the darker months as modelled in table 3.8.

For the purposes of categorisation within this study, the following conventional meteorological breakdown was used: -

- Winter = December, January and February.
- Spring = March, April and May;
- Summer = June, July and August;
- Autumn = September, October and November;

Season of birth	Pregnant season	Most likely level of sun exposure in pregnancy	Suggested Impact on vitamin D metabolism
Winter	Summer /Autumn	High to moderate	Raised
Spring	Autumn/Winter	Low	Minimal
Summer	Winter/Spring	Low to moderate	Reduced
Autumn	Spring/Summer	Moderate to high	Raised

Table 3. 8 Vitamin D levels per season

The number of participants giving birth per season is presented in figure 3.8

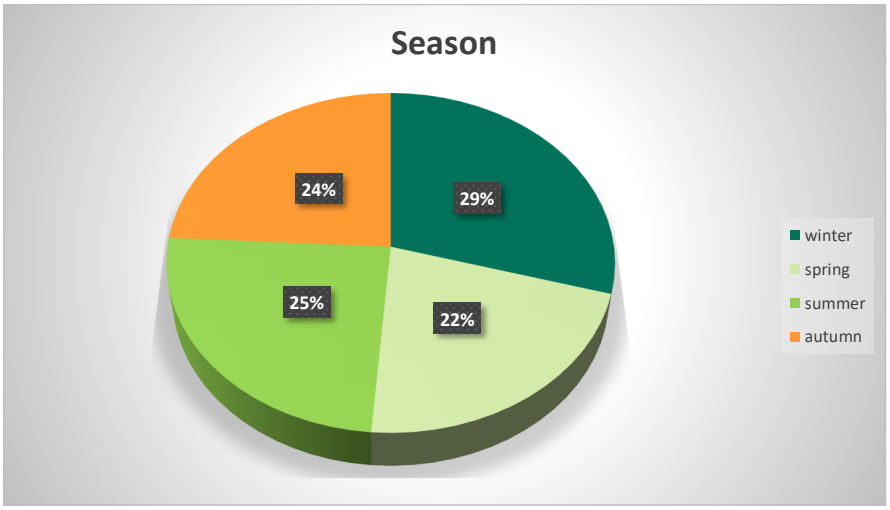

Figure 3. 8 Number of participants per season of birth.

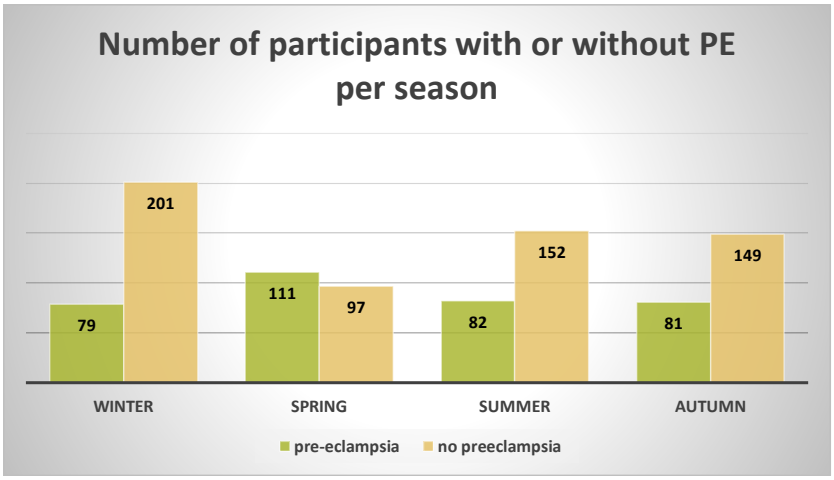

Figure 3. 9 Number of women with and without pre-eclampsia per season of birth.

It is noted that despite an aim of having equal numbers of participants within each season, due to availability of records, natural deviations in the number of births over the year and the timing of data collection, it was not possible to achieve this, and it is acknowledged that there was a higher number of women in the winter group. However, all pre-eclampsia cases for each season, eligible for inclusion, were included.

3.2.6.1 Season and Healthy Start vitamins

As with other variables, interest lay with the impact Health Start vitamins may have had on pre-eclampsia and in this case, whether supplementation may counteract any possible impact of low vitamin D due to the changes in levels of sun exposure.

The figures in table 3.8 suggest that similar percentages of women took take Healthy Start vitamins per season although a higher percentage of the winter subgroup chose not to. It is noted that there are less participants within the spring subgroup however, and there remains a possibility that season could impact on pre-eclampsia outcomes and is worthy of further analysis.

Ultimately, detection of a correlation between the seasons and pre-eclampsia, provides justification for further study into supplementation as a method of reducing risk.

Season	Did not take vitamin	Vitamin uptake (% per season)	Total
WINTER, (Dec, Jan, Feb.)	177	103 (38%)	280
SPRING, (March, April, May)	162	46 (22%)	208
SUMMER, (June, July, August)	160	74 (32%)	234
AUTUMN, (Sept, Oct, Nov)	147	83 (36%)	230
Total	646	306	952

Table 3. 9 Uptake of Healthy Start vitamins per season.

3.2.7 Blood type

Data regarding blood type was readily available in the medical records, and in order to consider this possible confounding variable, women's blood type was collected as part of the overall data collection. It was anticipated, that with the multi-ethnic groups living in the surrounding area, plus those referred to the regional centre from around the country, this would provide a representative sample of the range of blood types.

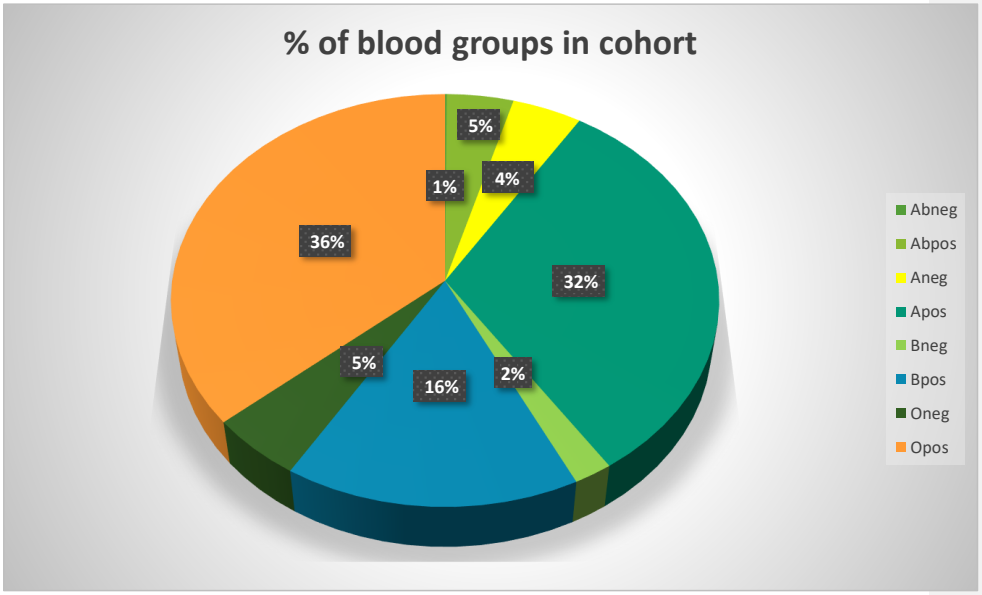

Figure 3. 10 Blood group distribution of study group.

It is important to recognise that despite the multi- ethnic makeup of the catchment area used in the study, this is an expected distribution for the population of the UK with a greater number having an O positive status (around 44%) followed by A positive (approximately 42%) (Blood Transfusion service accessed October 2012). Unlike countries such as Pakistan and Bangladesh where the more common types are B groups and AB, the least common blood group in the UK is AB negative. Despite the ethnic makeup of the population in the UK possibly fluctuating due to migration and possible shift in the breakdown of blood types, this has not impacted significantly on the overall picture. This in turn provides reliability and validity that the data collected as from a representative sample.

The wide range of values for each blood group means there is significant variation in the number of women from each group who take up vitamins. However, as shown in table 3.9 for the main UK blood groups of O Positive and A Positive, as in other variables, less than 50% took supplements, with only 31% of the A Positive group and 32% of the largest represented O Positive group.

Blood Group	Did not take vitamin	Vitamin uptake	Total
ABNEG	0	1 (100%)	1
ABPOS	25	16 (39%)	41
ANEG	37	6 (14%)	43
APOS	207	95 (31%)	302
BNEG	12	8 (40%)	20
BPOS	93	59 (39%)	152
ONEG	36	12 (25%)	48
OPOS	236	109 (32%)	345
Total	646	306	952

Table 3. 10 Blood group and Healthy Start vitamins uptake.

3.2.8 Ethnicity and country of origin

It was anticipated that with the multi-ethnic diversity within the surrounding catchment area to the study site, in addition to the potential inclusion of some women referred to the regional centre from around the country, this would provide a representative sample of a wide range of ethnicities. The ethnic group categorisation was lifted from the maternal

notes and was a direct transcription of what had been identified by the woman using the options listed. For this instance, the term ethnic origin was used to describe where the individual's family originated. It is noted that it was not possible to ascertain skin colour from this and that the categories utilised are not all categories of ethnicity commonly in use but was the available source of data.

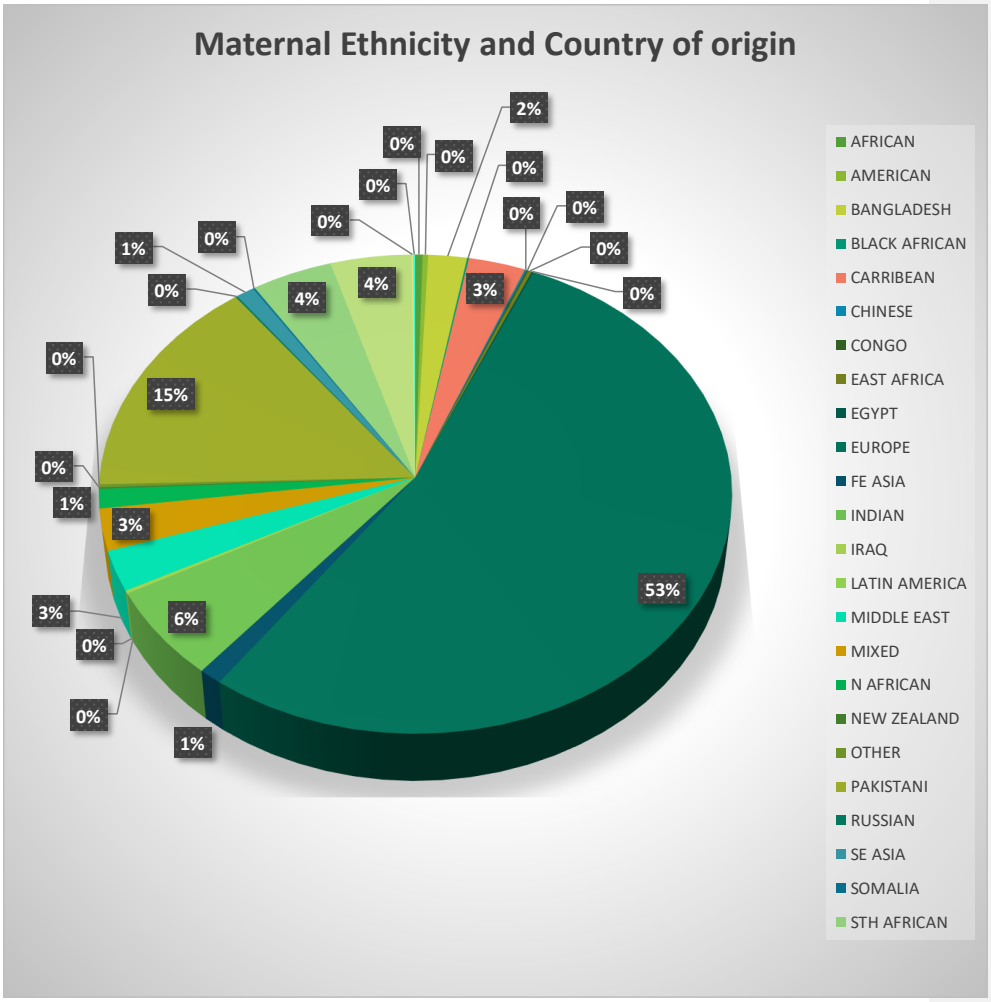

Figure 3. 11 Distribution of study group by percentage N.B Where % are below 0.5% these are been rounded down automatically to 0%)

Figure 3.10 clearly demonstrates that the largest proportion of the cohort was from the Europe, followed by women from Pakistan and/or of Pakistani background. However, in lesser numbers there was representation from a wide range of other groups.

Due to the limited numbers of individuals within some groups it was necessary for further analysis, to narrow down the wide number of categories into four groups, to ensure the statistical relevance of the tests undertaken and to create effective coding categories for SPSS analysis. To aid this, the model used by Reeves et al (2014) was selected, as this addressed the complexities of categorising extensive lists of variables into a more workable system.

The resulting groupings for coding therefore became as outlined in table 3.10.

Sub group	Number of participants
Asian	255
European	532
African	69
Others	96

Table 3. 11 Number of participants in sample subgroups following narrowing of categories.

3.2.8.1 Paternal ethnicity and country of origin

With the possibility of paternal DNA affecting placental and fetal development it was important to consider this as another potential confounding variable and it was therefore added to the list of variables for data collection. A summary of the range of paternal ethnicities is presented in figure 3.11. As with maternal ethnicity the same categories were used based on the place of birth and the data collected were obtained from the maternal handheld records. It is not possible to know whether this was decided by the mother or the father, which may be a consideration when undertaking analysis and interpretation.

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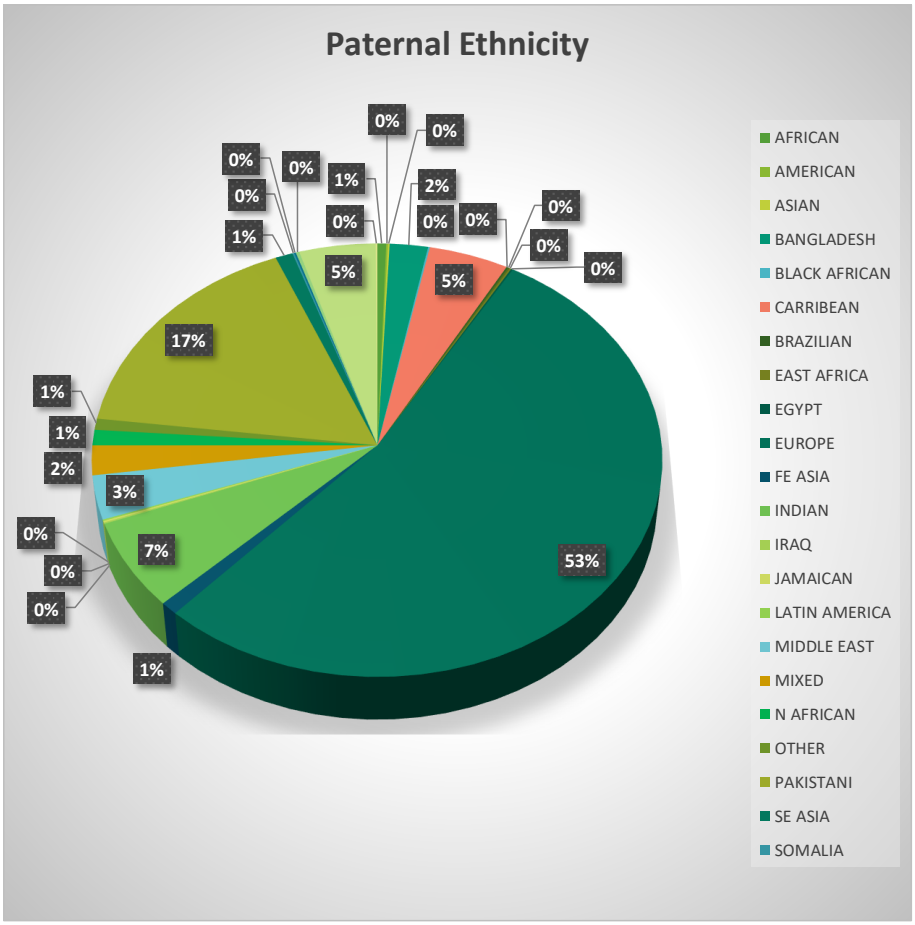

Figure 3. 12 Distribution of study group by percentage N.B Where % are below 1% these have been rounded down automatically to 0%)

3.2.8.2 Maternal Ethnicity and vitamin intake.

Maternal ethnicity and vitamin uptake continues to reflect the patterns detected in other variables. However, for the Bangladeshi group more had vitamins than not, though in too small a number to be of statistical significance and 47% of the Pakistani women had vitamins. Many of the remaining values within the cohort were too small to draw conclusions. However, a notably low percentage of Europeans within the sample (28%) had recorded vitamin uptake.

Ethnicity	Did not take vitamin	Vitamin uptake (% per ethnic group)	Total
AFRICAN	4	0	4
AMERICAN	2	0	2
BANGLADESH	10	11 (52%)	21
BLACK AFRICAN	1	0	1
CARRIBEAN	20	11 (35%)	31
CHINESE	0	1 (100%)	1
CONGO	1	0	1
EAST AFRICA	2	0	2
EGYPT	1	0	1
EUROPE	383	147 (28%)	530
FE ASIA	5	6 (55%)	11
INDIAN	36	25 (41%)	61
IRAQ	1	0	1
LATIN AMERICA	0	1 (100%)	1
MIDDLE EAST	20	7 (26%)	27
MIXED	18	10 (28%)	28
NORTH AFRICAN	11	2 (15%)	13
NEW ZEALAND	1	0	1
OTHER	2	1(50%)	3
PAKISTANI	80	71 (47%)	151
RUSSIAN	0	1 (100%)	1
SE ASIA	8	2 (20%)	10
SOMALIA	1	0	1
STH AFRICAN	2	0	2
SUB S AFRICA	34	10 (23%)	44
UKRAINE	1	0	1
USA	1	0	1
W AFRICAN	1	0	1
Total	646	306	952

Table 3. 12 Vitamin uptake associated with maternal ethnicity

3.2.9 Frequency and severity of pre-eclampsia

Data from 952 sets of records was collected and divided into pre- eclampsia present or absent, followed by subdivision into the severity of the condition between mild, moderate and severe, utilising the aforementioned definition used by the regional centre adopted for study (see page 94). There were no missing data sets, therefore the figures provide a complete overview of the entire group of participants and the range of women's demographic data are suggestive of what would be expected in a population of childbearing women in the United Kingdom. However, several women who developed this level of the condition could not be included due to the presence of other complicating factors such as an underlying medical condition. Despite this, the total number of cases identified in the cohort studied within the year of data collection, was higher than national averages. The rationale for this lies potentially with the fact that the women booked to deliver at the study site are more likely to be referred if they are considered to be of higher risk due to the unit being classed as a tertiary centre, that is, a centre of excellence for high-risk obstetric care. There was also a need to have a large enough representation of cases to provide a strong enough signal to enable analysis of any value.

Severity	Sample size (n)	Percentage
NIL	599	63%
MILD	317	33%
MODERATE	19	2%
SEVERE	17	2%
Total	952	100%

Table 3. 13 Frequency of pre-eclampsia by severity present in cohort

3.2.10 Vitamin supplementation

The key element to this study lay with the exploration of the uptake of Healthy Start vitamins and their potential impact on the severity of pre-eclampsia. To this end, data identifying those women who took the vitamins and those who did not was collected. This was taken directly from the maternal records and was recorded as yes (Y) or no (N). The breakdown of vitamin uptake per variable is presented in the subsequent sections. However, overall uptake of vitamin supplementation occurred in less than a third of the group, as presented below.

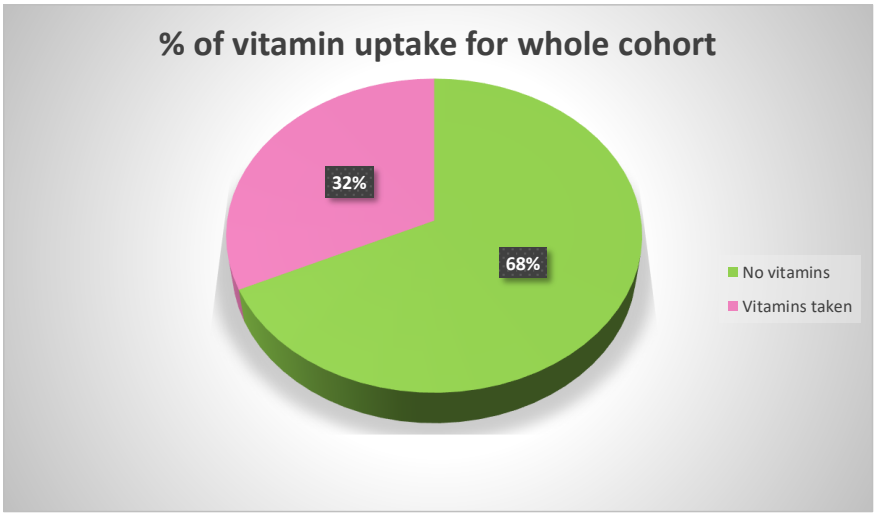

Figure 3. 13 Uptake of vitamins for overall cohort without variables.

Notably, the initial power calculation to establish sample size and relative risk utilised the figure provided by Public Health England of 23% uptake of Healthy Start vitamins. For this particular cohort this percentage is much higher and is considerably greater than the uptake of only 10% provided by McGee and Shaw (2013). No rationale was established for this difference and potentially this could be purely circumstantial, mainly because the women were selected randomly.

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Vitamin uptake	Sample (n)	Percent of cohort	Cumulative %
N	646	68%	68
Y	306	32%	100
Total	952	100%	

Table 3. 14 Vitamin uptake for cohort

3.2.11Uptake of Folic acid not as part of a multivitamin

Though it is not possible to identify women who had taken vitamin D as a separate entitity (as this was not recorded in the medical records), which would have aided comparison to other leading studies, it was possible to account for the women's uptake of folic acid. Generally this is taken prior to pregnancy and into the first few weeks of pregnancy, primarily to reduce the risk of fetal abnormalities. In contrast Healthy Start vitamins are taken most frequently later in pregnancy. As folic acid is also a main constituant of the

Healthy Start multi vitamin supplement, it needed to be considered within this study as a separate entity lest it impacted on the findings for the combined vitamin.

Despite wide spread health promotion of folic acid's benefits and its prevention of neural tube defects it is evident from the percentages in figure 3.13, that from this cohort of 952 not all women selected to use or possibly declared that they took folic acid during their pregnancy. In total, only 729 women took the standard dose and 16 the higher dose, leaving 207 in the no supplement group. As the higher dose of folic acid is prescribed for high risk women for example, those with a very high BMI, the small number of women taking the higher dose may be a reflection of the number of women in the selected sample who are considered to be this category.

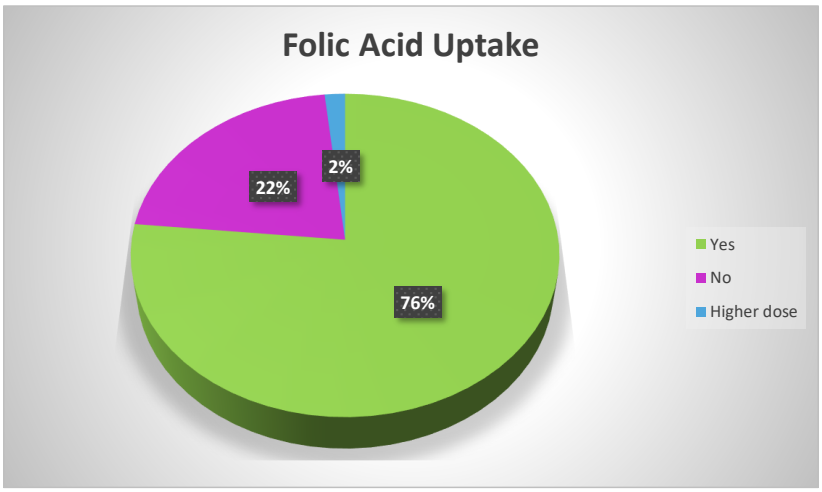

Figure 3. 14 Folic acid uptake within study cohort

3.2.12 Folic acid and Healthy Start uptake

It is possible that some women in the sample chose to either take double supplements of folic acid initially and later Healthy Start vitamins. From the figures presented in table 3.14. 78% of the cohort took folic acid with 23% opting to take both supplements. The remaining 22% of the overall cohort did not report that they had taken folic acid and 13% of the 952 participants chose not to take either type of vitamins. Only 9% were recorded to have Healthy Start vitamins.

Pre-eclampsia present or not	Healthy start uptake(% of cohort)	Single dose folic acid (% of cohort)	Double dose folic acid (% of cohort)
N (*n* 646)	121 (13%)	515 (55%)	10 (1%)
Y (*n* 306)	86 (9%)	214 (23%)	6 (0.6%)
Total (*n* 952)	207 (22%)	729 (78%)	16 (2%)

Table 3. 15 Folic acid uptake and Healthy Start vitamin uptake

3.3 First stage analysis

3.3.1 Descriptive statistics

With the nominal, ordinal and categorical nature of the different variables, two tests for analysis were utilised. Chi square analysis was considered however, this relies on there being two nominal dependant variables, which this data was not. It would have been possible to divide pre-eclampsia into just 'yes' or 'no' but this may have lost valuable insight into the impact of variables on the severity of the condition.

Subsequently the tests used were: -

Mann-Whitney U a non-parametric test used for ordinal variables including gravida and parity and Healthy Start vitamins, as this tests for differences between an independent variable with two categories (in this case yes and no) on a continuous measure and accommodates any shifts from standard deviation which categories such as these variables can present (Stewart 2016; Pallant 2013).

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Kruskal-Wallis a non-parametric test which is used to test a nominal variable with 2 or more categories including blood group and age, as this test enables analysis of scores where continuous variables are grouped or ranked such as ethnicity or blood type and then tested against the ordinal dependant variable; in this case pre-eclampsia (Stewart 2016; Pallant 2013).

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Usefully unlike Chi squared, these tests are less inclined to be affected by low cell counts and deviations from standard distribution (Field 2018).

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Analysis was conducted using the order of variables discussed in the preceding chapter and the tabulated results are presented as the number of participants and percentage of the overall sample size. Results demonstrating any statistical significance with a p-Value of

≤ 0.05 were then investigated further using logistical regression as outlined in the following chapters. Statistical significance being the low probability of the relationship between variables occurring if there was no relationship on the cohort (Muijs 2016) or the point where a null hypothesis can be rejected (Everitt 2009)

3.3.2 Primary analysis of pre-eclampsia and variables

For initial analysis, the categories used for pre-eclampsia were based on the definition used by the study site. For coding purposes 1= no pre-eclampsia, 2 = mild pre-eclampsia, 3 = moderate pre-eclampsia and severe pre-eclampsia = 4. However, once initial analysis had been performed for logistical regression it proved to be more effective to narrow this down to just pre-eclampsia - yes or no. This is principally because for some variables there are some very low cell values within the breakdown of the sample, mainly in the moderate to severe range. This can lead to empty cell values, which can affect valid regression to taking place.

An initial appraisal of the results found in table 3.15, considering Healthy Start vitamins as a key variable and as a way of reducing risk of developing pre-eclampsia, appear to provide an indication of an association between pre-eclampsia and vitamin supplementation within the cohort as a whole. There is a much lower incidence of pre-eclampsia in the group who selected to take vitamins, presented in the yes column. The intention therefore of the analysis was to test this observation alongside consideration of the possible effect of each variable on the severity of pre-eclampsia. The order of the analysis considered the combination of Healthy Start vitamins and folic acid alone initially, followed by the potential confounding variables identified from the literature and discussed in the literature review. These being, BMI, lifestyle choices and behaviours, ethnicity, folic acid, Healthy Start vitamins, gravid and arity status, blood group and age.

3.4 Correlations with pre-eclampsia

With a primary aim and objective of considering the impact of vitamin supplements, either as a multi vitamin or as folic acid alone, on the incidence of pre-eclampsia, analysis on these variables was undertaken first. This was then followed by analysis of each potential confounding variable using either the Kruskal Wallis or Mann-Whitney tests.

3.4.1 Healthy Start Vitamins and Folic Acid alone with pre-eclampsia

Currently data were available in the medical records for the Healthy Start combination given during pregnancy, in addition to folic acid pre-conception and in the first 12 weeks of pregnancy. This enabled collection of data of both for this cohort study, meeting the aim of establishing patterns of supplementation alongside, looking for any statistical correlation with outcome.

For coding purposes Healthy Start vitamins were considered as yes or no, but for folic acid several women had taken an increased dose; most likely due to a raised BMI as per NICE (2014) guidance. Therefore, uptake of folic acid was categorised as yes (Y) no (N) or (YY) for the higher dose. This meant coding for three values was applied. The cohort findings are shown in table 3.15 and 3.16

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Variable	n (952)	No PE (599)	Mild PE (317)	Moderate PE (19)	Severe PE (17)
Healthy Start vitamins					
No	646 (68%)	405 (68%)	209 (66%)	17 (90%)	15 (88%)
Yes	306 (32%)	194 (32%)	108 (34%)	2 (10%)	2 (12%)

Table 3. 16 Frequency correlations demonstrate the distribution of supplement uptake respective to the severity of PE

Variable	n (952)	No PE (599)	Mild PE (317)	Moderate PE (19)	Severe PE (17)
Folic acid only					
No (N)	207 (22%)	67 (11%)	125(39%)	9 (47%)	6(35%)
Yes (Y)	729 (76%)	526 (88%)	183 (58%)	9 (47%)	11(65%)
Higher dose (YY)	16 (2%)	6 (1%)	9 (3%)	1 (6%)	0 (0%)

Table 3. 17 Folic acid uptake associated with severity of pre-eclampsia.

Analysis for these two variables required different tests as the Healthy Start vitamins fitted the criteria and assumptions for the Man-Whitney U having two independent nominal variables, whereas the Kruskal-Wallis test was the best fit for the folic acid variable.

The result of the Mann Whitney test for Healthy Start vitamins - **Z value -0.599** $\mathrm{p-value} =$ **0.549** represents a non-statistically positive finding. This is reflected in the result of the calculation for effect size as previously referred to equation under the heading gravida and parity, and the mean rankings presented in table 3.17, demonstrating little difference between the groups.

Healthy Start supplementation	Mean ranks
No supplementation	477.58
Supplementation recorded	474.22

Table 3. 18 Mean rankings for Healthy Start vitamins and pre-eclampsia

The calculation for effect size revealed a result of

(Md (median) =1, n **(952) r = 0.2**

When measured on the Cohen D scale this would be considered as a lower effect size and therefore no regression analysis was undertaken on this variable.

However, in contrast to Healthy Start vitamins, the folic acid component of the supplement when run through the Kruskal- Wallis model, leads to a statistically significant result of

$\mathrm{H}(2)$ **= 112.222** $\mathrm{p-value} =$ **< 0.001.**

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Supported by the mean rankings presented in table 3.18, this made this variable worthy of further investigation.

Folic acid uptake	Mean rank
No folic acid	622.45
Standard dose folic acid	432.42
Higher dose of folic acid	596.75

Table 3. 19 Mean rankings for Folic acid

Sample 1 –sample 2	Test statistic	Std. error	Std. Test statistic	Sig.	Adj. Sig
Yes – higher dose	-164.333	58,718	-2.799	.005	.015
Yes--no	190.037	18.298	10.386	.000	.000
Higher dose - no	25.704	60.287	.426	.670	1.000

Table 3. 20 Pairwise test for folic acid and pre-eclampsia

The ranking for folic acid suggests a significant link between non supplementation and the development of pre-eclampsia which is borne out by the value of p **< 0.001.**
This is supported by the adjusted significance values for folic acid p **< 0.001, *r* = .010**, thus rejecting the null hypothesis that not taking folic acid has no effect on the risk of developing pre-eclampsia.

There does seem to be an anomaly in that the higher dose increases the risk of pre-eclampsia instead of a lower dose p **< 0.005, r = 0.10**. However, this may be because this group of individuals were more likely to present with a higher risk of developing pre-eclampsia overall. There was no significant difference seen between taking a double dose of folic acid as opposed to no supplement although again this may be skewed by the difference in sample size. The case for folic acid versus no folic acid, however, warrants more analysis.

3.4.2 Age and pre-eclampsia

In this study, women below the age of 18 were excluded for ethical reasons (p88 and 89) therefore data was collected for predominantly women aged between 18 and 40, which in a usual population would be a standard period for childbearing age. Randomly selecting records resulted in women of each age range being represented with higher percentages of women as expected to be higher in the 25 to 39 brackets.

Table 3.1 indicates the percentage of women within each age range and the frequency of each level of severity of preeclampsia.

Variable	n (952)	No PE (599)	Mild PE (317)	Moderate PE (19)	Severe PE (17)
Age					
18-20	60 (6%)	40 (7%)	17 (5%)	2 (2%)	1 (6%)
21-25	201 (21%)	115 (19%)	78 (32%)	5 (26%)	3 (18%)
26-30	314 (33%)	205 (34%)	100 (32%)	4 (21%)	5 (29%)
31-39	348 (37%)	222 (37%)	112 (35%)	7 (37%)	7 (41%)
Over 40	29 (3%)	17 (3%)	10 (3%)	1 (5%)	1 (6%)

Table 3. 21 Sample percentages per severity of pre-eclampsia

When considering undertaking any analysis it is important to consider the standard distribution of the sample and whether this is skewed. As the graph for age ranges in the frequencies section identifies (figure 3.1), this sample provides a negatively skewed standard distribution curve. Whilst this could be considered a normal representation of the childbearing population it affects the choice of tests undertaken and could affect the accuracy of results (Pallant 2013). However, Hylands-White (2013) stipulates there is no need to consider standard deviation with this type of ordinal data. In addition, as the sample used is unmatched and there are more than 3 groups, when using this model for selecting appropriate tests a Kruskal-Wallis H test was deemed appropriate. Despite the reassurance from Hylands-White (2013) however, prior to running any tests in SPSS a number of assumptions still needed to be met. For the Kruskal-Wallis test, these being: -

Assumption 1 – the dependant variable should be of ordinal scale – in this case pre-eclampsia is ordinal in nature.

Assumption 2 – The independent value, in this case age, should consist of two or more categorical groups, which it does.

Assumption 3 – There is no relationship between the groups. That is, no participants appear in more than one of the groups occurring in the sample. This was met as every data entry involved a different individual's information. (Field 2014)

Subsequently the data was run through SPSS and the results reviewed to consider if the resulting value represents a significant difference between the groups.

Though the mean rankings provided by this test were established for each age range, the overall the Kruskal- Wallis test showed there was no statistical difference between the age groups and the development of or severity of pre-eclampsia for this cohort, $H(4)$ **=4.172,** p **= 0.383.** The mean rankings also show only small differences within them, with the 21-25 age range and those aged over 40 bracket, having the highest mean average. The mean rankings shown in table 3.21, should however, be interpreted alongside the p value (Pallant 2013).

Age range	Mean rank
18 - 20	461.37
21-25	502.92
26-30	464.09
31-39	472.95
over 40	501.72

Table 3. 22 Rankings for age ranges

As the P- value was established as greater than p< 0.05 this suggested a non-statistically significant finding therefore further analysis on this variable was not conducted.

3.4.3 BMI and pre-eclampsia

With the aim of considering potential confounding variables within the analysis any association between BMI, vitamins and pre-eclampsia was considered along with the possibility of reducing risk through supplementation. The percentage distribution within each range for this cohort are presented in table 3.23

Variable	n (952)	No PE (599)	Mild PE (317)	Moderate PE (19)	Severe PE (17)
BMI					
< 18	26 (3%)	23 (4%)	3 (1%)	0	0
18.5-24.9	355 (37%)	256 (43%)	88 (28%)	6 (32%)	5 (29%)
25-29.9	333 (35%)	203 (34%)	116 (37%)	8 (42%)	6 (35%)
30 – 34.9	144 (15%)	72 (12%)	64 (20%)	4 (21%)	4 (24%)
35 -39.9	57 (6%)	33 (6%)	23 (7%)	1 (5%)	0
40+	37 (4%)	12 (2%)	23 (7%)	0	2 (12%)

Table 3. 23 Sample size for BMI and severity of pre-eclampsia

There are clearly some cells with values of zero, which leaves a potential for type I and II errors (Muijs 2016) so careful interpretation of findings is required. Despite this, the percentages alone suggest there is some relationship between weight and severity of pre-eclampsia.

As the data are ordinal in nature and there are more than three unmatched and unrelated groups, the Kruskal-Wallis tests was deemed appropriate to use for this variable as well. Likewise, the data met the required assumptions for this test as outlined previously. Unlike age, in this analysis, the Kruskal- Wallis test demonstrated there was a statistical difference in the BMI groups mean rankings, as shown in table 3.24, as well as the p value $H(5) = 45.061$ $p < 0.001$.

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BMI range	Mean rank
18 - 20	352.15
21-25	433.18
26-30	486.19
31-39	538.83
over 40	495.79

Table 3. 24 Mean rankings for BMI status

As the P- value was established at less than p <0.05 this suggests a statistically significant finding. Therefore, further analysis on this variable was conducted.

It is also important to note that an initial decision was made to not reduce the groups further at this stage of analysis. However, the possibility to recode for regression purposes remained an option, as any missing values could lead to inaccuracies in calculation and interpretation of any future findings.

When a result such as this is found there is a need to consider the power or magnitude of difference (effect size) (McCrum –Gardner (2010), and to look for trends as the probability value alone cannot provide information regarding the degree of association (Pallant 2013). The effect size can measure the difference in outcomes between the control and experimental groups but also the size of difference between mean rankings. The latter being of relevance for the analysis in this study (Everitt 2009) and forms an important part of any quantitative research.

However, as McCrum –Gardner (2010) state, these figures need to be considered in the context of clinical relevance as well as for statistical significance. Within these tests, the Bonferroni adjustment must be applied (Pallant 2013) to reduce the chance of type I errors. Type II errors or false positives are where the null hypothesis is falsely rejected. Likewise, a type II error occurs when the null hypothesis is falsely accepted (Everitt 2009). The SPSS package used for this analysis applied this automatically.

To establish effect size the data was analysed using a pairwise comparison test, to test for any differences in the medians in the groups. The Jonckheere-Terpstra test (Field 2016) was also utilised, providing more meaningful data for the order and ranking of the groups (Field 2016). The *r* value was then calculated for values rejecting the null hypothesis as highlighted in table 3.25.

Sample 1 –sample 2	Test statistic	Std. error	Std. Test statistic	Sig.	Adj. Sig
Underweight - normal	-80.337	47.204	-1.702	.089	1.000
Underweight to overweight	-133.340	47.310	-2.818	.005	.072
Underweight –obesity2	-142.943	54.983	-2.600	.009	.140
Underweight –obesity 1	-185.987	49.507	-3.757	.000	.003
Underweight –extreme obesity	-266.667	59.456	-4.485	.000	.000
Normal-overweight	-53.003	17.724	-2.990	.003	.042
Normal –obesity 2	-62.606	33.152	-1.888	.059	.884
Normal –obesity 1	-105.650	22.955	-4.603	.000	.000
Normal –extreme obesity	-186.330	40.137	-4.642	.000	.000
Overweight-obesity 2	-9.603	33.303	-.288	.773	1.000
Overweight – obesity 1	-52.647	23.172	-2.272	.023	.346
Overweight –extreme obesity	-133.327	40.262	-3.312	.001	.014
Obesity 2-obesity 1	43.044	36.357	1.184	.236	1.000
Obesity 2 – extreme obesity	-123.724	49.050	-2.522	.012	.175
Obesity 1 – extreme obesity	-80.680	42.822	-1.884	.060	.893

Table 3. 25 Pairwise comparisons for BMI and pre-eclampsia.

From this analysis, pairwise comparison using the adjusted p values it is evident that BMI influences the incidence of pre-eclampsia. Those with a higher BMI appear more likely to develop the condition. The results suggested

- Those who are considered to be extremely obese compared to normal weight demonstrate a significant difference **(**$p < 0.001$**,** ***r* = .15);**
- For underweight compared with extreme obesity **(**$p < 0.001$**,** ***r* = .15)**
- Normal weight compared with obesity 1 **(**$p < 0.001$**,** ***r* = .15).**
- Normal compared to overweight ($p < 0.042$**,** ***r* = .10);**
- **U**nderweight compared with obesity 1 **(**$p < 0.003$**,** ***r* = .12)**
- Overweight and extreme obesity **(**$p < 0.014$**,** ***r* = .11)** having a slightly less significant difference.
- No significance was seen between normal and underweight individuals nor those ranging between obesity and extreme obesity.

These significant findings provide justification for analysis through regression to support or reject the null hypothesis that BMI has no effect on pre-eclampsia and justifies the consideration of this characteristic within the study aims and objectives.

3.4.4 Gravida, Parity and Pre-eclampsia

When first meeting a pregnant woman one of the key questions asked by midwives would be to establish what number pregnancy this was (gravida) and how many live births there had been previously (parity), as this can not only alter future management but also identify any risk factors for future complications.

The analysis of both the gravida and parity variable data were considered worthy of investigation as potential confounding variables and were therefore imported into SPSS and analysed using the Mann- Whitney U test.

The percentage of women experiencing pre-eclampsia in these categories is presented in table 3.26, It is to be noted that for coding purposes, due to the wide range of variables and the low cell values within some of these; the range of options have been reduced to two groups only. This also meant that the sample sizes for each group were more evenly balanced leading to more reliability and validity of results as there is less impact from outliers and subsequently less risk of type I or II errors.

Variable	n (952)	No PE (599)	Mild PE (317)	Moderate PE (19)	Severe PE (17)
Gravida					
Primigravida 1st pregnancy	472 (49.6%)	271 (45%)	175 (55%)	15 (79%)	11 (65%)
Multiparous 2 onwards	480 (50.4%)	328 (55%)	142 (45%)	4 (21%)	6 (35%)
Variable	n (952)	**No PE (599)**	**Mild PE (317)**	**Moderate PE (19)**	**Severe PE (17)**
Parity					
1 birth	527 (55%)	294 (49%)	205(65%)	15 (79%)	13 (76%)
2+ births	425 (45%)	304 (51%)	113 (35%)	4 (21%)	4 (24%)

Table 3. 26 Sample size for gravida and parity status with severity of pre-eclampsia.

As with the Kruskal Wallis text, the data used for the Mann-Whitney test utilised to analyse this variable is required to meet a series of assumptions. For the Mann-Whitney these are:
Assumption 1 – The dependant variable, in this case pre-eclampsia, was ordinal in nature
Assumption 2 – The independent variable consists of two independent groups: these being gravida one or more and parity one or more.
Assumption 3 The Mann-Whitney test requires there to be no relationship between the participants within the group (Pallant 2013). This cohort met the assumption that there should be completely different participants within both the case group and the non-pre-eclampsia group.

The final **assumption** relates to the distribution of the independent variables. Usefully Mann- Whitney does not rely on standard deviation; though it can be beneficial to know

whether the groups have standard deviation curves, (Laerd statistics accessed 2018). The results of this will identify whether mean or median ranks can be calculated.
For this particular data, the groups presented with different shaped distribution curves. Therefore, in order to provide improved reliability, the legacy procedure was utilised within SSPS when performing analysis and the establishment of mean rankings

After undertaking the analysis, the Mann-Whitney U test revealed a statistically significant correlation between both gravida and parity and pre-eclampsia within this cohort. The tests indicated: -

Gravida = Z value of -3.715, p **< 0.001**

Parity = **Z value of -5.350,** p **< 0.001**

With a statistically significant result of p **< 0.001** for both variables, there is a justification for further analysis. A conclusion further supported by the results found following the calculation of size of effect.

3.4.5 Effect size

In this instance as recommended by Hicks (2009) and Pallant (2013), having completed the Mann-Whitney analysis it can be helpful to consider the size of effect. From the rankings in both categories, first pregnancy and first baby came highest in the rankings. When calculated within SPSS the median for each variable and grouping equalled 1.00
The formula below was used to generate the Cohen D value and subsequently the effect size (Rosenthal 1991, p.19)

r = z /square root of N (where N = total number of cases)

The results revealed an effect size of: -

Primigravida **(Md = 1**, n **472) r = 0.6** and multigravida **(Md =1,** n **480) r = 0.6**

1 birth (**md = 1,** n **527) r =0.6** and 2 or more births **(Md= 1,** n **484) r = 0.6**

Applying this to Cohen (1988) suggests a moderate to large effect can be concluded (Field 2018). Consequently, the significant *P* values and the result from effect size calculation make this variable warrant further investigation through regression analysis.

3.4.6 Lifestyle choices and pre-eclampsia

For the purposes of analysis, this section will consider smoking and alcohol intake only because the number of individuals disclosing the use of recreational drugs was too limited to enable any worthwhile analysis (see table 3.6). The percentage of women within this cohort who provided details of their lifestyle choices for smoking and alcohol intake at the point of booking is presented in tables 3.27. and 3.28

Smoker	n (952)	No PE (599)	Mild PE (317)	Moderate PE (19)	Severe PE (17)
No	829 (87%)	525 (88%)	272 (86%)	17 (90%)	15 (88%)
Yes	123 (13%)	74 (12%)	45 (14%)	2 (10%)	2 (12%)

Table 3. 27 Percentage of smokers and non-smokers with severity of preeclampsia.

Alcohol intake at booking	n (952)	No PE (599)	Mild PE (317)	Moderate PE (19)	Severe PE (17)
Nil	636 (67%)	379 (61%)	233 (74%)	12 (63%)	12 (70.5%)
Social/yes	109 (11%)	88 (15%)	20 (6%)	1 (5%)	0
1-5	127 (13%)	84 (14%)	35 (11%)	3 (16%)	5 (29.5%)
6-10	57 (6%)	38 (6%)	17 (5%)	2 (11%)	0
11+	23 (2%)	11 (2%)	11 (4%)	1 (5%)	0

Table 3. 28 Percentage of alcohol (units per week) intake with severity of preeclampsia

The percentages for drug use are also provided.

Drug use	n (952)	No PE (599)	Mild PE (317)	Moderate PE (19)	Severe PE (17)
No	934 (98%)	584 (98%)	314 (99%)	19 (100%)	17 (100%)
Yes	18 (2%)	15 (2%)	3 (1%)	0	0

Table 3. 29 Percentage of drug intake with severity of preeclampsia.

3.4.6.1 Smoking

As the dependant variable remains ordinal and there are only two groups 'yes' and 'no', the Mann- Whitney test was utilised. As with other categories, the assumptions for this test outlined on p140, were also met.

The findings from the analysis demonstrated a result of no statistical significance with a p **> 0.549.** With this finding, no further analysis was undertaken.

3.4.6.2 Alcohol intake and pre-eclampsia

Within table 3.30 regarding alcohol intake, there are a number of cells which contain 1 or 0 which limits the value of analysis in this form. Subsequently, to enable more accurate analysis and validity of interpretation of results and to reduce the risk of type I and II errors (Muijs 2016), the five groups were recoded into just three = No intake, social to 5 units per week and over 6 units per week.

The three groups in this variable presented in ordinal format and the data met the aforementioned assumptions for the Kruskal-Wallis test presented on pages 130-1 therefore this test was implemented.

The findings from the Kruskal- Wallis non-parametric analysis presented a statistically significant result of $H(2)$ **=** p **< 0.002.**

As this result is below the recommended $p < 0.05$ then this variable was deemed worthy of further investigation, particularly as little could be found relating to this area when conducting literature searches.

When pairwise comparisons were made between the three ranges for no alcohol to over six units of alcohol per week, the results were not as expected. It was anticipated that women drinking larger amounts of alcohol would be at greater risk however, as table 3.30 and 3.31 presents from the breakdown of the pairwise testing, this may not be the case.

Alcohol intake per week	Mean rank
No alcohol	492.47
Social to 5 units	430.99
6 or more units	483.77

Table 3. 30 Mean rankings for alcohol intake

Sample 1 –sample 2	Test statistic	Std. error	Std. Test statistic	Sig.	Adj. Sig
Social to 5-6 or more units	10,487.000	564.888	1.853	.032	.096
Social to 5-no alcohol	65,355.000	2,789.606	-3.475	.000	.001
6 or more units – no alcohol	24,975.000	1.498.760	-.310	.378	1.000

Table 3. 31 Pairwise comparisons of alcohol consumption and severity of pre-eclampsia.

When applying the calculation of *r* to ascertain the effect size the following calculation applies $r = z/\sqrt{N}$ **(where N = total number of cases) or as for this variable**

$$r = -3.48/\sqrt{872}$$ **(=30)**

These results suggest that women who drink up to five units of alcohol per week at the start of pregnancy are at greater risk of developing pre-eclampsia **(**$H(2)$ **=** p **= 0.001, *r* 0.12).** However, a degree of caution must be applied when considering the smaller numbers in the group with over six units. Regression analysis for this variable was conducted to explore this conclusion further.

3.4.7 Season and pre-eclampsia

Vitamin D also forms a significant component of Healthy Start vitamins, making seasons a worthy variable to explore due to increased levels on deficiency and insufficiency. Alongside a suspected increase in the number of cases of pre-eclampsia, it is a logical step to try to correlate the two. Indeed, as research remains conflicted on the impact of sun exposure, this possibly confounding variable required investigation.

Season	n (952)	No PE (599)	Mild PE (317)	Moderate PE (19)	Severe PE (17)
Winter (December to February	280 (29%)	201 (34%)	72 (23%)	5 (26%)	2 (12%)
Spring (March to May)	208 (22%)	97 (16%)	96 (30%)	7 (37%)	8 (47%)
Summer (June to August)	234 (25%)	152 (25%)	76 (24%)	0 (0%)	7 (35%)
Autumn (September to November)	230 (24%)	149 (25%)	73 (23%)	7 (37%)	1 (6%)

Table 3. 32 Percentages of cohort per season associated with severity of pre-eclampsia

From the data in table 3.32, with the exception of spring, similar numbers of participants were collected in the available timeframe for each season and were coded into the four groups. With codes of 1 to 4 applied and the assumptions met for the Kruskal-Wallis test, initial analysis took place.

The findings from initial analysis provided a result of $H(3)$ **= 35.591** p **< 0.001** indicating a statistically significant association between season and pre-eclampsia and a variable worthy of further exploration through regression. However, the reduced number of participants collected in the spring group could affect the results; therefore, a degree of caution is required in interpretation. Subsequent analysis continued as with BMI and alcohol using the Jonckheere-Terpstra test (Field 2016), to ascertain effect size through pairwise comparisons and calculation of the value of *r*.

Season	Mean rank
Winter	433.55
Spring	557.22
Summer	465.26
Autumn	467.22

Table 3. 33 Mean rankings for season

Sample 1 –sample 2	Test statistic	Std. error	Std. Test statistic	Sig.	Adj. Sig
Winter - summer	-31.715	20.578	-1.541	.123	.740
Winter - autumn	-33.667	20.676	-1.628	.103	.621
Winter -spring	-123.671	21.667	-5.815	.000	.000
Summer - autumn	-1.952	21.573	-.091	.928	1.000
Summer -spring	91.956	22.140	4.153	.000	.000
Autumn -spring	90.004	22.231	4.049	.000	.000

Table 3. 34 Pairwise comparisons for season

The results utilising the adjusted significance value suggest a significant difference in outcome between women giving birth during spring (therefore pregnant during the winter) and those giving birth in the winter (pregnant in late summer and autumn). The mean rankings indicate the spring births are at greater risk than the winter births. **(H (3) =** p **< 0.001, *r* = 0.19).** Other results suggest that women giving birth in spring are also more at risk than those in the autumn **H (3) =** p **< 0.001, *r* = 0.13** and births occurring in summer **H (3)** p **< 0.001, *r* = 0.13**. When comparing other seasons there appears to be no significant difference. These results suggest that the null hypothesis claiming season does not affect pre-eclampsia can be rejected.

3.4.8 Ethnicity and pre-eclampsia

Though gathering data on ethnicity was relatively straightforward, through using the classifications provided with the maternity records based on the woman's self- reported selection, analysis proved more challenging. As referred to on page 93, this was predominantly due the extensive range of ethnicities identified; with some groups containing only a single individual. Therefore, as previously intimated it was necessary to define the participants into more contained groups without narrowing them too much as

Muijs (2016) recommends. A variety of options were considered, for example, using only two groups 'European' and 'others'. However, further consideration led to the use of four subgroups being used, 'Asian', 'European', 'African' and 'others'; the latter contained for example, one woman from America and one from Jamaica. This decision was made as it permitted more effective analysis by avoiding cells with little or no values. In addition, this strategy was aimed at encapsulating the potential for differences in skin colour. It was not possible to ascertain skin colour directly from the records, but there remains a potential for the impact of reduced absorption of vitamin D with darker skins or through the full covering of skin. This in turn could impact on the development of pre-eclampsia, so it was considered important to not group the women into too few variables.

Despite this there remains some cell values of 1 as presented below and this needs consideration when interpreting findings from the analysis.

Variable	n (952)	No PE (599)	Mild PE (317)	Moderate PE (19)	Severe PE (17)
Ethnicity					
Asian	283 (30%)	189 (32%)	86 (27%)	7 (37%)	1 (6%)
European	532 (56%)	322 (54%)	193 (61%)	10 (53%)	7 (41%)
African	70 (7%)	43 (7%)	18 (6%)	1 (5%)	8 (47%)
Others	66 (7%)	44 (7%)	20 (6%)	1 (5%)	1 (6%)

Table 3. 35 Subgroups for maternal ethnicity and associated severity of pre-eclampsia

For this variable as there are more than two nominal independent variables with unrelated and unmatched grouping the Kruskal-Wallis test was used as the assumptions were met. The resulting mean rankings are found in table 3.36

Maternal ethnicity	Mean rank
Asian	456.27
European	485.70
African	499.62
Others	457.36

Table 3. 36 Mean rankings for ethnicity and pre-eclampsia

Following analysis, the mean rankings suggest a slightly higher correlation between African ethnicity and pre-eclampsia. Potentially it could be argued that this is due to a greater likelihood of having darker skins, but this is not supported by the p value of

$$H(3) = 4.122 \quad p = 0.249.$$

Therefore, it was concluded that ethnicity did not provide statistically significant results for this cohort and would not be further analysed.

3.4.8.1 Paternal ethnicity and pre-eclampsia

The same coding groups were used for paternal as for maternal ethnicity. However, it was noted that there were 6 missing entries within the data which was due to no record being entered in the medical notes. This is potentially due to the absence of the father or perhaps due to sperm donation and the use of Invitro fertilisation (IVF) however, as it was not possible to ascertain this information, these entries were excluded from the analysis.

When comparing paternal ethnicity and their partner's development of pre-eclampsia the following percentages in table 3.37 can be seen.

Variable	n (946)	No PE (594)	Mild PE (316)	Moderate PE (19)	Severe PE (17)
Paternal Ethnicity					
Asian	298 (32%)	203 (34%)	87 (28%)	6 (32%)	2 (12%)
European	508 (53%)	305 (51%)	186 (58%)	11 (58%)	6 (35%)
African	67 (7%)	39 (7%)	19 (6%)	1 (5%)	8 (47%)
Others	73 (8%)	47 (8%)	24 (8%)	1 (5%)	1 (6%)

Table 3. 37 Subgroups for paternal ethnicity and associated severity of pre-eclampsia

As this data was again nominal and had more that one value, the Krsukal- Wallis assumptions were met. After running the Kruskal- wallis test for this data unlike the maternal ethnicity, the initial results presented in tables 3.37, 3.38 and 3.39 suggest a correlation between Asian and European fathers and Asian and African fathers in relation to risk of pre-eclampsia. It is suggested that the null hypothesis be subsequently rejected. Despite this, when consideration of the adjusted p value is used, this significance is lost. The null hypothesis cannot therefore be rejected. As a consequence no further analysis was undertaken with this variable.

Paternal ethnicity	Mean rank
Asian	447.17
European	485.14
African	512.30
Others	464.39

Table 3. 38 Mean rankings for paternal ethnicity and pre-eclampsia

Sample 1 –sample 2	Test statistic	Std. error	Std. Test statistic	Sig.	Adj. Sig
Asian - other	11,276.500	670.793	.596	.276	1.000
Asian - European	81.811.500	2,689.972	2.275	.011	.369
Asian -African	11,311.000	645.407	2.058	.020	.119
Other - European	17,721.000	1,146.150	-.716	.237	1.000
Other - African	2,202.000	206.081	-1.182	.119	.712
European - African	18,046.000	1,099.924	.935	.175	1.000

Table 3. 39 Pairwise comparison for paternal ethnicity and pre-eclampsia.

3.4.9 Blood group and pre-eclampsia

With blood group identified as a potential confounding variable alongside the desire to explore such variables, the decision was made to do further analysis into this potentially blood grouping confounding variable.

As with other variables in this study, it was not considered appropriate to minimise the number of independent variables, predominantly in this case due to the unique properties of each blood type. Whilst it could be interesting to reduce them to blood group only, this may not provide a full picture as it may be the Rhesus factor (positive or negative) which is important. As a consequence, there were cells with no or low cell values as shown in table 3.40. Subsequently, although enabling a fuller picture of blood group and pre-eclampsia, the data for this variable, which met the assumptions for the Kruskal-Wallis analysis, should be interpreted with a degree of caution.

Variable	n (952)	No PE (599)	Mild PE (317)	Moderate PE (19)	Severe PE (17)
Blood group					
AB Neg	1 (0.1%)	0	1 (0.3%)	0	0
AB Pos	41 (4%)	28 (5%)	13 (4%)	0	0
A Neg	43 (5%)	28 (5%)	15 (47%)	0	0
A Pos	302 (32%)	187 (31%)	107 (34%)	7 (39%)	1 (6%)
B Neg	20 (2%)	13 (2%)	6 (2%)	1 (5%)	0
B Pos	152 (16%)	99 (10%)	46 (15%)	3 (16%)	4 (24%)
O Neg	48 (5%)	31 (5%)	15 (5%)	0	2 (11.8%)
O Pos	345 (36%)	213 (36%)	114 (36%)	8 (42%)	10 (59%)

Table 3. 40 Blood groups percentage associated with severity of pre-eclampsia

The results from the Kruskal-Wallis analysis indicated $H(7)$ **= 3.147** p **= 0.871.** This demonstrates a non- statistically significant result and will not be analysed any further. However, it cannot be concluded that this disputes the findings of other studies in this area.

3.4.10 Summary

This study's aims and objectives were intended to explore correlations and variables which may impact on the development of pre-eclampsia. Subsequent correlation analysis has provided a spectrum of findings across the range of variables. The frequencies provide a clearer overview of the cohort and demonstrate the diversity within the sample. For a number of variables analysed including blood group, age, Healthy Start vitamin supplementation and ethnicity, there were no statistically significant findings. However, results from the analysis of BMI, folic acid, season and alcohol uptake, in addition to gravida status, do demonstrate significant correlations between them and the development of pre-eclampsia. However, the strength of this analysis and the conclusions drawn requires further investigation to ensure validity and reliability. To enable this the variables were further analysed using logistic regression, the results of which are presented in the following section.

3.5 Binary logistic regression

There are a number of techniques for performing regression, the adoption of which depends on the structure of the dependant variable used for analysis. Where a continuous dependant variable is used, multiple regression is most effective (Muijs 2016). For categorical or nominal dependant variables, logistic or binary regression is more suitable as this would enable assessment of probability of developing the outcome being studied or not, in the presence of different independent variables. Logistic regression also provides odds ratios (OR) described by Muijs (2016) as the probability of the outcome occurring divided by the probability of the outcome not occurring. Alternatively, as Stewart (2016) expresses, the odds of a subject with a disease being exposed to the variable in question divided by the odds that a subject is without the disease. When applied to this study this is presented as

$$\frac{\text{Odds of subjects with pre-eclampsia being exposed to Healthy Start vitamins}}{\text{Odds of subjects without pre-eclampsia being exposed to Healthy Start Vitamins}}$$

Although risk ratios and odd ratios are seen as similar in nature, there is a possibility that the odds ratios may exaggerate the risk ratio. However, this potential was considered when undertaking analysis and interpretation of the results. The calculation of confidence intervals also provided a more holistic overview of statistical significance, subsequently reducing risk of over exaggeration (Viera 2008; Schmidt and Kahlmann 2008).

The choice of which type of regression to use also depends on a number of assumptions including sample size, the number of outliers and the collinearity of the variable (Pallant 2013). Collinearity is defined as the existence of a strong relationship between the dependant variable and the predictor variable, but not between predictor variables (Pallant 2013).

Within the frequencies and correlation sections 3.2 and 3.3, the levels of severity of pre-eclampsia experienced by the participants were analysed. However, following an initial analysis using ordinal regression for some variables, there were a significant number of cells containing zero or only small values, for example, gravida and parity status. This subsequently failed to meet the assumptions for multiple regression and risked invalid and

unreliable results being found through the inability to interpret the results effectively. Therefore, logistic binary regression was deemed appropriate to be used to assess the predictor or independent variables against the development of pre-eclampsia.

As binary logistic regression requires the outcome /dependant variable to be dichotomous (Knapp 2017), it was necessary to recode the dependant variable of pre-eclampsia into yes = 1 and no pre-eclampsia = 0, rather than degrees of severity. In addition, using the statistical findings from the initial analysis the findings suggest that BMI, folic acid, gravida status, season and alcohol intake showed a strong correlation between them and pre-eclampsia, but they remain independent variables from each other, thus meeting the third assumption. Using SPSS, the aforementioned independent variables against pre-eclampsia yes or no, the following variables were analysed further.

Folic acid
BMI
Gravida and parity status
Season
Alcohol intake

Initially the analysis was run using the variables individually, the results for which can be seen in table 3.41. For the independent variables of BMI, Folic acid, gravida and parity there are statistically significant results of p **< 0.001.** In contrast**,** unlike the results from the correlation analysis for alcohol uptake and season, undertaking regression provides no significant results of p **> 0.05**.

Variable	B		S.E.	Wald		df	p	Odds ratio	95% CI for odds ratio	
Folic Acid	-1.395		.161	75.278		1	.000	.248	.181	.340
BMI	-.384		.062	37.980		1	.000	1.468	1.299	1.659
Gravid status	-.470		.135	12.092		1	.001	.625	.479	.814
Season	.039		.058	.451		1	.502	1.040	.927	1.166
Alcohol intake	-.054		.062	.774		1	.379	.947	.839	1.069
Parity	-.722		.140	26.715		1	.000	.468	.369	.639

Table 3. 41 Results from initial binary regression

Though useful, these results do not provide a complete picture. At this point predictor variables have only been analysed independently and as single entities, but this fails to consider the complexity of humans. It cannot for example, be established how particular ranges of BMI such as overweight or extreme obesity affect pre-eclampsia development. Nor whether a standard or higher dose of folic acid has a more beneficial impact on the development of pre-eclampsia.

To enable further investigation, Muijs (2016) suggests creating *dummy* variables for variables such as these, as this enables the comparison of categories within the variable to one another. This required the recoding of data again but proved to be relatively easy using the SPSS package.

It is important to acknowledge any outliers or missing values as these can affect the results when conducting analysis, as they can lead to type I or type II errors, subsequently affecting validity. The influence of outliers or missing values as Muijs (2016) writes is affected by the overall sample size. A larger sample reduces the impact of a small number of outliers. Returning to the initial data as advised by Stewart (2016), showed no inaccuracies in recording of the data or missing values with this dataset.
The findings resulting from this further analysis subsequently provided a more detailed and reliable set of results as presented below.

3.5.1 Folic acid and pre-eclampsia

Findings so far has continued to demonstrate that folic acid has a strong correlation between its consumption and the development of pre-eclampsia with a p value of **<0.001 (CI (95%), 0.181 and 0.340, OR 0.248)**. The initial data recorded whether women had taken folic acid. A small proportion of women were prescribed a higher dose if deemed at risk of developing a fetus with a neural tube defect, for example if they had a high BMI. To reflect these categories, dummy variables were generated as follows: -

Folic acid = no (N), Folic acid = yes (Y) and a higher dose indicated by YY within the initial data collected.

The results from the regression are presented in table 3.42. No outliers were identified therefore the full sample of 952 participants were included in the analysis.

Commented [RC67]: Is this between 0.181 and 0.340 or between 0.181 and -0.340? I'd generally typeset as [lower, upper] to avoid confusion.

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	B	S.E	Wald	df	Sig	Exp(B) Odds ratio	95% CI for Exp (B) Lower	95% CI for Exp (B) Upper
Folic acid N	.226	.537	.177	1	.674	1.254	.437	3.594
Folic acid Y	-1.463	.523	7.825	1	.005	.232	.083	.645
Constant	.511	.516	.979	1	.323	1.667		

Table 3. 42 Regression results for Folic acid uptake and pre-eclampsia.

With any analysis, it is essential to ensure that the test used is the best fit for purpose. The SPSS package provides a range of tests, which enable researchers to establish the effectiveness of their choices. The results drawn from the omnibus testing of model coefficients table generated by SPSS, provides an indication of how well a model performed (Pallant 2013). The model here containing all predictors provided a result of χ^2 **(2, N = 952) = 111.23,** p **< 0.001.**

The statistically significant results suggest that with 2 degrees of freedom, the model has performed well in this instance. Alongside this, the Hosmer-Lemeshow Goodness of Fit test result of 1.000 further supports the choice of model. For the Hosmer- Lemeshow, a value less than 0.05 suggests a poor fit, which is not the case here. In addition, the model explained between 11% (Cox and Snell R squared) and 15% (Nagelkerke R squared) of vitamin uptake and classified 71% of the cases accurately. Field (2016) explains that as the Cox and Snell statistic is the less accurate of the two measurements of R squared the value of Nagelkerke is more useful. The nearer the value to one the more accurately the model is predicting the outcome variable. It is also noted that the higher dose folic acid group have been replaced by the constant.

The results within the B value column show a negative value for folic acid uptake and a positive value for no supplementation. As Pallant (2013) explains, the B value is used to calculate the probability of a case (in this situation, a woman with pre-eclampsia) forming part of a specific category. The positive and negative values indicate which direction the

relationship between them exists. That is, whether a variable increases the likelihood (positive value) or decreases it (negative value). For this analysis the results suggest that taking folic acid will reduce the likelihood of pre-eclampsia with a significance value of p **< 0.005, OR .252**, **CI (95%).083 - .645).** For the group containing no folic acid the value is positive suggesting an increase in pre-eclampsia cases.

The 95% confidence interval (CI) provides the range of values in which it can be 95% confident that the true odd ratio values are encompassed (Pallant 2013). From these results as the CI for taking folic acid falls between .083 and .645 and does not include the value of one, it indicates that the odds ratio is true. Furthermore, with an odds ratio of 1.254 the odds of a woman not taking folic acid and having pre-eclampsia is 1.25 times higher with all other factors being equal. The confidence interval for not taking folic acid does, however, contain the value one, suggesting the odds ration may not be statistically significant and a degree of caution is needed with interpretation.

3.5.2 BMI and pre-eclampsia

The findings in section 1.10, indicate that a woman's BMI could have an impact on her risk of developing pre-eclampsia. In addition, the initial regression figures provide results of

p **< 0.001, OR 1.468. CI (95%) 1.299-1.659.**

These outputs strongly suggest that BMI and pre-eclampsia could be correlated in some way, although it is not possible to ascertain specifically if one group of women present with a higher risk factor than others. It was therefore appropriate to create dummy variables for BMI.

To aid this, the BMI ranges already utilised in the previous analysis where recoded. The resulting dummy variables were therefore: -

Normal, Overweight, Obesity 1, Obesity 2 and extreme obesity.

When analysed, the underweight group were not included in the results generated and were replaced by the constant. However, all 952 participants were included in the model and there were no outliers.

The model containing all predictors provided a result of χ^2 **(5, N = 952) = 47.28,** p **< 0.001.** Alongside the Hosmer and Lemeshow test value of 1.000**,** both tests support the effective use of the model. The model explained between 48% (Cox and Snell R squared) and 66% (Nagelkerke R squared) of the variability in BMI ranges and classified 64% of the cases accurately. The regression analysis generated the results in table 3.43

	B	S.E	Wald	df	Sig	Exp(B) Odds ratio	95% CI for Exp (B) Lower	95% CI for Exp (B) Upper
Normal	1.087	.625	3.022	1	.082	2.965	0.871	10.096
Overweight	1.591	.624	6.502	1	.011	4.910	1.445	16.682
Obesity 1	2.037	.636	10.255	1	.001	7.667	2.204	26.671
Obesity 2	1.718	.670	6.580	1	.010	5.576	1.500	20.727
Extreme Obesity	2.771	.707	15.351	1	.000	15.972	3.994	63.877
Constant	-2.037	.614	11.011	1	.001	.130		

Table 3. 43 Results from regression analysis of BMI ranges

The predictor variables of overweight through to extreme obesity providing statistically significance values of p **< 0.05** as well as positive B values. This suggests that increasing weight including extreme obesity, which is represented by the constant, can have a negative impact on the risk of developing pre-eclampsia. In turn, then this suggests that lowering weight could have a positive benefit with a value of p **< 0.001** and negative B value of **-2.037**.

The strongest signals arise from the obesity 1 and extreme obesity groups with the odds ratio of 15.97 representing a 15 times increased risk of having pre-eclampsia for the extreme group. The confidence intervals for both obesity 1 and extreme obesity also do not cross the line of no effect and lie to the right of 1, suggesting a negative impact on development of the condition with increasing weight. The ranges between the upper and

lower figures are quite broad however, reducing significance. Regardless of this, overall, the findings bring some clarity for risk factors between BMI and pre-eclampsia.

3.5.3 Gravida and Parity with pre-eclampsia

From undertaking descriptive and correlation statistics, gravida and parity status could be associated with the development of pre-eclampsia. Binary regression for these variables was therefore, deemed appropriate.

Both gravida and parity status from the initial regression produced statistically significant values below 0.05 with negative *B* values. This would suggest that these variables could correlate with pre-eclampsia. A more detailed analysis using dummy variables was undertaken.

For gravida, 952 participants were included in the model with 63% of cases accurately defined. The model was seen to be of good fit with a Hosmer and Lemeshow test value of 1.000, 18% (Cox and Snell R squared) and 25% (Nagelkerke R squared) and χ^2 **(3, N = 952) = 17.61,** p **< 0.001.** There were no outliers

Table 3.44 provides the results from the regression using the dummy variables of: -

1 pregnancy, 2 pregnancies, 3 pregnancies and 4 or more pregnancies was replaced by the constant.

	B	S.E	Wald	df	Sig	Exp(B) Odds ratio	95% CI for Exp (B) Lower	95% CI for Exp (B) Upper
1 pregnancy	.227	.222	1.044	1	.307	1.255	.812	1.941
2 pregnancies	-.450	.243	3.419	1	.064	.638	.396	1.027
3 pregnancies	.021	.288	.005	1	.943	1.021	.580	1.795
Constant	-.526	.202	6.785	1	.009	.591		

Table 3. 44 Results from binary regression of gravida status and pre-eclampsia

From the *B* values alone, there is a suggestion that the number of pregnancies could influence the development of pre-eclampsia; however, this is not borne out by the significance values of $p > 0.05$ and all of the confidence intervals crossing the line of no effect, which, as Field (2017) writes, makes the odds ratio not significant. From these findings, it cannot be concluded that pregnancy number correlates significantly with pre-eclampsia.

For parity however, the picture is quite different. All 952 participants in the sample were included and there were no outliers or missing values. Initial regression using the dummy variables of parity 1 for first baby and parity 2, for women having subsequent babies, provided a result of χ^2 **(1, N = 952) = 27.473,** p **<0.001.** No Hosmer and Lemeshow test results were provided, but between 29% (Cox and Snell) and 39%, (Nagelkerke R square) of variance was explained and 63% of cases were correctly classified.

Compared to gravida status the results from the regression analysis on parity data provided more conclusive support for a correlation between the number of births experienced and pre-eclampsia development, as indicated in table 3.45.

	B	S.E	Wald	df	Sig	Exp(B) Odds ratio	95% CI for Exp (B) Lower	95% CI for Exp (B) Upper
Parity	-.700	.139	25.402	1	.000	.496	.378	.652
Constant	-.468	.206	5.162	1	.023	1.596		

Table 3. 45 Results from regression of parity status and pre-eclampsia

From this data, the statistical significance continues to provide evidence of a correlation between the number of babies born to a mother and her development of pre-eclampsia. The constant here represents women having their second or subsequent baby and the figures indicate a 1.5 times reduced risk of pre-eclampsia with an increase in the number of babies born.

3.5.4 Season and pre-eclampsia

Season of giving birth from initial cross tabulation analysis also suggested statistically significant results, most especially for those mothers giving birth in spring and winter where the pregnancies had been through the darker months. The relevance of the season therefore was analysed using logistic regression. When tested independently as just season without dummy variables there was, however, no results of any significance. In contrast when retested using dummy variables results were quite different. The omnibus test for coefficients showed χ^2 **(3, N = 952) = 33.303,** p **<0.001.** The test showed a good fit through a Hosmer and Lemeshow result of 1.000, with 34% (Cox and Snell R) to 47% (Naglekerke R) and there were no outliers or missing values. Accuracy of classification was at a moderate level of 64%. The constant replaced autumn in the calculation.

	B	S.E	Wald	df	Sig	Exp(B) Odds ratio	95% CI for Exp (B) Lower	95% CI for Exp (B) Upper
Winter	-.324	.192	2.867	1	.090	.723	.497	1.052
Spring	.744	.196	14.437	1	.000	2.105	1.434	3.090
Summer	-.008	.195	.002	1	.969	.992	.678	1.453
Constant	-.609	.138	19.493	1	.000	.544		

Table 3. 46 Results from regression of season and pre-eclampsia.

The findings in table 3.46, suggest there is no statistical significance overall with regards to summer and winter season although the negative B value suggests a possible reduction in the number of births with pre-eclampsia. This is not however, supported with the odds ratio, p value or the confidence interval containing the value of one. In contrast from the results for spring, p **<0.001, OR 2.105, CI (95%) 1.434 – 3.090,** these suggest that the odds of having pre-eclampsia are 2 times greater if giving birth in the spring.

3.5.5 Alcohol and pre-eclampsia

When analysed initially, with alcohol consumption and pre-eclampsia there was evidence of some correlation between the two variables with a p **<0.002.** Following, regression analysis this significance was lost. With both alcohol intake compared to no intake (p **<0.379)** and using dummy variables, the p **>1.000** for all categories no longer support a correlation between alcohol intake and pre-eclampsia. The 'goodness of fit' tests for binary regression demonstrated poor application of the model overall.

3.5.6 Summary for initial binary regression

From the logistical analysis, it has become evident that some of the variables under consideration, principally folic acid, BMI, parity and season continue to demonstrate statistical significance. Whilst gravida and alcohol, though both potential confounding variables, show little correlation between them and the development of pre-eclampsia. Women, however, are complex and many factors make up a person. It is important to acknowledge that each predictor variable is unlikely to present as a single characteristic in an individual. Consequently, what has yet to be established is whether there is any association between these predictor variables and pre-eclampsia and whether their impact is altered by the introduction of another factor. To consider this, the variables demonstrating significant results only were analysed using regression in combinations. Throughout each analysis, dummy variables were utilised.

3.6 Binary regression with combinations of variables

3.6.1 BMI and folic acid

	B (β)	S.E	Wald	df	Sig	Exp(B) Odds ratio	95% CI for Exp (B) Lower	95% CI for Exp (B) Upper
Underweight	-2.534	.735	11.877	1	.001	.079	.019	.335
Normal	-1.520	.393	14.939	1	.000	.219	.101	.473
Overweight	-.979	.392	6.245	1	.012	.376	.174	.810
Obesity 1	-.671	.412	2.643	1	.104	.511	.228	1.148
Obesity 2	-1.122	.471	5.667	1	.017	.326	.129	.820
Folic acid N	.473	.559	.717	1	.397	1.605	.537	4.799
Folic acid Y	-1.177	.545	4.660	1	.031	.308	.106	.897
Constant	1.362	.618	4.850	1	.028	3.904		

Table 3. 47 Results of regression for BMI and folic acid uptake on the dependant variable pre-eclampsia

The omnibus test for coefficients showed χ^2 **(7, N = 952) = 145.875,** p **<0.001.** The test showed a good fit through a Hosmer and Lemeshow result of .981, though with 14% (Cox and Snell R) to 19% (Naglekerke R). There were no outliers or missing values. Accuracy of classification was at an improved 71.6% on the initial testing. From these findings, there is no evident collinearity as there are no matching results between predictor variables. Results for extreme obesity and increased dose of folic acid are represented by the constant, as data of results for this category were not included.

The results in table 3.47, suggest that there is a decrease in pre-eclampsia for women of all weight ranges p **<0.001** when folic acid is introduced to the equation. With the exception of obesity 1, the confidence intervals fall below a value of one, and all show a reduced incidence of pre-eclampsia evidenced by a negative *B* value. It cannot be concluded that

folic acid is the cause of the reduction, but there is evidence of a correlation between the two variables

The odds ratio also suggests that women not taking folic acid are 1.6 times more likely to have developed pre-eclampsia. However, this cannot be classed as a statistically significant result overall p **<0.308, OR 1.605, CI (95%) .537 – 4.799** with a value greater than p **>0.05.** In contrast however, taking folic acid remains significant, though to a slightly lesser extent than when considered independently p **<0.005** to p **<0.031, OR .308, CI (95%) 0.106 - 0.897.**

3.6.2 BMI and Season

	B	S.E	Wald	df	Sig	Exp(B) Odds ratio	95% CI for Exp (B) Lower	95% CI for Exp (B) Upper
Underweight	-2.540	.711	12.745	1	.000	.079	.020	.318
Normal	-1.673	.376	19.827	1	.000	.188	.090	.392
Overweight	-1.193	.374	10.176	1	.001	.303	.146	.631
Obesity 1	-.699	.394	3.151	1	.076	.497	.230	1.076
Obesity 2	-1.095	.449	5.942	1	.015	.335	.139	.807
Winter	-.255	.196	1.694	1	.193	.775	.527	1.138
Spring	.762	.201	14.401	1	.000	2.143	1.446	3.177
Summer	.025	.200	.016	1	.901	1.025	.693	1.517
Constant	.615	.374	2.697	1	.101	1.849		

Table 3. 48 Results of regression for BMI and season on the dependant variable Pre-eclampsia

The omnibus test for coefficients showed χ^2 **(8, N = 952) = 76.706,** p **<0.001.** The test showed a moderate to good fit through a Hosmer and Lemeshow result of .878, but with a low 7% (Cox and Snell R) to 11% (Naglekerke R) and there were no outliers or missing values. Accuracy of classification was at an improved 67% on the initial testing. From these findings, there is no evident collinearity as there are no matching results between the predictor variables. Results for extreme obesity and autumn were used as the constant variables.

Table 3.48 indicates that winter is no longer significant p **<0.193,** whereas, a correlation between spring and pre-eclampsia remains. These results suggest that the odds of developing pre-eclampsia when giving birth in spring is 2.1 times higher (p **<0.001, OR 2.143, CI (95%) 1.446 – 3.177).**

For this scenario, considering BMI and season together as the predictor variables the significance of BMI has become stronger for all ranges, most notably with the overweight and obesity 1 ranges. This suggests that despite the two variables being independent of each other there may be some correlation between size and the time of birth.

3.6.3 BMI and Parity

	B	S.E	Wald	df	Sig	Exp(B) Odds ratio	95% CI for Exp (B) Lower	95% CI for Exp (B) Upper
Underweight	-2998	.717	17.487	1	.000	.050	.012	.203
Normal	-1.925	.381	25.485	1	.000	.146	.069	.308
Overweight	-1.318	.377	12.203	1	.000	.268	.128	.561
Obesity 1	-.820	.397	4.267	1	.039	.440	.202	.959
Obesity 2	-1.136	.452	6.331	1	.012	.321	.132	.778
First birth	-.866	.146	35.037	1	.000	.420	.316	.560
Constant	2.126	.32	24.270	1	.000	8.380		

Table 3. 49 Results of regression for BMI and parity on the dependant variable pre-eclampsia

The omnibus test for coefficients showed χ^2 **(6, N = 952) = 83.890,** p **<0.001.** The test showed a moderate to good fit through a Hosmer and Lemeshow result of .836, but with 8% (Cox and Snell R) to 12% (Naglekerke R). There were no outliers or missing values. Accuracy of classification was at an improved 66% on the initial testing of 62%. From these findings, there is no evident collinearity, as there are no matching results. Extreme obesity and parity greater than one were not included in the results.

The results in table 3.48, show that women experiencing their first birth are 0.420 times more likely to experience pre-eclampsia (p **<0.001, OR .420, CI (95%).316 – .560),** which is

similar to the results when tested independently**.** Women's BMI also remains significant with the greater reduction in risk by being within a normal weight range. With the exception of obesity 1 the risk of pre-eclampsia increases. This would suggest that both a high BMI and experiencing a first birth increases the likelihood of pre-eclampsia.

3.6.4 Folic acid and season

	B	S.E	Wald	df	Sig	Exp(B) Odds ratio	95% CI for Exp (B) Lower	95% CI for Exp (B) Upper
Folic acid N	.151	.544	.077	1	.781	1.163	.400	3.379
Folic acid Y	-1.495	.530	7.968	1	.005	.224	.079	.633
Winter	-.458	.203	5.107	1	.024	.632	.425	.941
Spring	.508	.208	5.960	1	.015	1.663	1.105	2.501
Summer	-.147	.206	.510	1	.475	.863	.576	1.293
Constant	.599	.540	1.231	1	.267	1.821		

Table 3. 50 Results from binary regression for folic acid and season with pre-eclampsia

The omnibus test for coefficients showed χ^2 **(5, N = 952) = 134.58,** p **<0.001.** The test showed a low to moderate fit through a Hosmer and Lemeshow result of 0.470, with 13% (Cox and Snell R) to 18% (Naglekerke R) and there were no outliers or missing values. This suggests a limited fit for the model. However, accuracy of classification was at an improved 71% on the initial testing. Autumn was omitted from the result presented and is represented by the constant.

The results in table 3.50, suggest that women taking folic acid may be less likely to experience pre-eclampsia (p **<0.001, OR .224, CI (95%).079 – .688),** though the impact is limited when considering the odds ratio. The confidence interval does; however, support the use of folic acid and a reduction in the incidence of pre-eclampsia. The significance of season also reduces. The uptake of folic acid though reducing the incidence of pre-

eclampsia remains conclusive and there could be some association between the season and uptake of folic acid. Taking folic acid in winter and spring may therefore be beneficial in reducing the number of cases of pre-eclampsia, although the impact of not taking folic acid cannot be established.

3.6.5 Folic acid and Parity

The regression analysis for folic acid and parity as a combination of confounding variables provides the results outlined in table 3.51. With this model, 952 participants were included. There were no outliers or missing values. Goodness of fit was suggested by the omnibus test for coefficients χ^2 **(3, N = 952) = 140.724,** p **<0.001.** The model explained between 14% (Cox and Snell R squared) and 19% (Nagelkerke R squared) of the variability and classified 71% of the cases accurately. The former figures alongside a moderate Hosmer and Lemeshaw outcome of 0.396 suggest a low to moderate goodness of fit overall. From this set of results parity remains significant (p **<0.001, OR .335, CI (95%).335 –.603).** The uptake of folic acid also demonstrates a statistically reduced incidence of pre-eclampsia (p **<0.006, OR .233, CI (95%).082–.661)**. Not taking folic acid in this scenario provides a positive result for the *B* value suggesting an increase of pre-eclampsia of 1.3 times higher than those taking folic acid. This however, is not borne out by a result of p **<0.589,** therefore**,** cannot be concluded to be statistically significant overall.

	B	**S.E**	**Wald**	**df**	**Sig**	**Exp(B)** **Odds ratio**	**95% CI for Exp (B)** **Lower**	**95% CI for Exp (B)** **Upper**
Folic acid N	.296	.547	.293	1	.589	1.345	.60	3.931
Folic acid Y	-1.458	.533	7.491	1	.006	.233	.082	.661
Parity 1	-.799	.150	28.384	1	.000	.450	.335	.603
Constant	1.626	.567	8.225	1	.004	5.085		

Table 3. 51 Results from regression analysis of folic acid and parity against pre-eclampsia.

3.6.6 Parity and Season

The final combination of two predictor variables contained parity and season. It was undertaken to ascertain whether there were any correlations between having a first baby during a particular season and the development of pre-eclampsia. Whilst the omnibus test demonstrated a goodness of fit at χ^2 **(4, N = 952) = 67.882,** p **<0.001,** a low Hosmer and Lemeshow test result of .234 and a 7% (Cox and Snell) and 9% (Nagelkerke R) of accuracy suggests a less accurate model. There were no missing values or outliers, and 67% participants were accurately accounted for. Table 3.52 provides the regression analysis results.

	B	S.E	Wald	df			Sig	Exp(B) Odds ratio	95% CI for Exp (B) Lower	95% CI for Exp (B) Upper
Parity	-.848	.147	33.104	1			.000	.428	.321	.572
Winter	-.308	.195	2.491	1			.114	.735	.501	1.077
Spring	.877	.202	18.824	1			.000	2.404	1.617	3.573
Summer	-.146	.199	.535	1			.464	.864	.585	1.277
Constant	.595	.248	5.744	1			0.17	1.812		

Table 3. 52 Results from regression of parity and season against pre-eclampsia

Parity and spring continue to be key variables demonstrating statistical correlation with pre-eclampsia with (p **<0.001, OR .428, CI (95%).321 –.572)** and (p **<0.001, OR 2.404, CI (95%) 1.617 –3.573)** respectively. This could suggest that women having their first baby in spring may be twice as likely to develop pre-eclampsia.

3.6.7 Summary of combined variables and binary regression

Throughout each level of analysis, a theme emerges whereby supplementation with folic acid shows a reduction in the incidence of pre-eclampsia. Alongside this, a woman's BMI at booking can also have negative or positive impact as well as the number of children she chooses to have. The effect of season again provides a possibility of altering the outcome of

pregnancy when considering pre-eclampsia. These variables thus far, have been investigated in isolation of each other or in pairs. To encapsulate the wider picture of a woman's experiences, it is important to take the analysis a step further and considered the potential impact of multiple variables on the incidence of pre-eclampsia.

3.7 Combining all variables demonstrating statistically significant results.

The variables, which demonstrated significance were analysed as a whole, again using the dummy variables and logistic regression. As women are multi-faceted, each variable could form part of her make-up and may subsequently affect each other as well as influence the development of pre-eclampsia. Therefore, testing of BMI, parity, season and folic acid together as predictor variables, against pre-eclampsia 'yes' or 'no', occurred. Table 3.53 presents the results of this analysis.

	B	S.E	Wald	df	Sig	Exp(B) Odds ratio	95% CI for Exp (B) Lower	95% CI for Exp (B) Upper
Parity 1	-1.101	.165	44.672	1	.000	.332	.241	.459
Winter	-.378	.211	3.208	1	.073	.685	.453	1.036
Spring	.716	.222	10.377	1	.001	2.046	1.324	3.163
Summer	-.269	.215	1.566	1	211	.764	.501	1.165
Folic acid N	.455	.590	.596	1	.440	1.577	.496	5.013
Folic acid Y	-1.2211	.576	4.492	1	.034	.295	.095	.912
Underweight	-2.650	.761	12.118	1	.000	.071	.016	.314
Normal	-1.849	.415	19.848	1	.000	.157	.070	.355
Overweight	-1.211	.411	8.680	1	.003	.298	.133	.667
Obesity 1	-.782	.432	3.281	1	.070	.458	.196	1.066
Obesity 2	-1.322	.495	7.131	1	.008	.267	.101	.703
Constant	3.208	.730	19.292	1	.000	24.739		

Table 3. 53 Regression analysis results for parity, season, BMI and folic acid against pre-eclampsia

The model used provided an omnibus test for coefficients of χ^2 **(11, N = 952) = 215.302,** p **<0.001**, seen as a good fit with the Hosmer and Lemeshow result of 0.861 being high. The overall model provided values of between 20% (Cox and Snell R) and 28% (Naglekerke R), there were no missing values or outliers and 73.5% of the participants were correctly classified.

For BMI as with previous analysis, all the weight ranges up to obesity two demonstrate statistical significance. The trend being a decrease in the BMI leading to a decrease in the incidence of pre-eclampsia. However, there is less significant impact for obesity one compared to all other BMI ranges though why this is the case, is unclear. Extreme obesity is likely to be contained within the constant along with the increased dose of folic acid and autumn. Though a p **<0.001** is indicated it is not possible to draw any conclusions regarding which of the three variables drives this impact. As an example, for those women considered to be of a normal BMI **(**p **<0.001, OR .157, CI (95%).070 – .355)** compared to obesity two (p **<0.008, OR .267, CI (95%).101– .703)** there is evidence of improved odds of reducing the development of pre-eclampsia the lower the BMI.

Furthermore, folic acid and season again demonstrate statistical significance with taking folic acid (p **<0.034, OR .295, CI (95%).095 – .912)** leading to a marked reduction in incidence **(*B* = -1.221).** However, when compared to previous results showing p **<0.001,** a degree of significance is lost. Likewise, spring's results of (p **<0.001, OR 2.046, CI (95%) 1.324 – 3.163)** suggest women giving birth in spring are twice as likely to be at increased risk of developing pre-eclampsia.

Parity continues to remain a statistically key predictor variable for pre-eclampsia (p **<0.001, OR .332, CI (95%).241 – .459)**

3.7.1 Summary of analysis all variables and binary regression

Grouping of the predictor variable enabled the establishment of any confounding factors. Women present with any number of the variables considered and all have the potential to confound each other, influencing the results from the analysis and the development of pre-eclampsia. From consideration of the correlation matrix generated at the same time as regression analysis, there was no evidence of any direct correlations between the different variables. Consequently, conclusions should be drawn that the risk of confounding is limited.

What is also evident from the analysis is that there were four variables continually demonstrating a statistical significance whatever scenario was tested: BMI, parity, season and the uptake of folic acid.

Having completed the analysis, the following chapters will discuss these findings and how they should be applied to practice.

DISCUSSION

4 Discussion

4.1 Overview

Following analysis, it is important to consider the findings in a wider context. Discussion regarding how the findings from this study sit within the current knowledge alongside where new knowledge is discovered is fundamental. Furthermore, how this new knowledge can be used to influence future practice and support further investigation is key. This following discussion will therefore consider such critical elements of the research process, beginning with an assessment of the effectiveness of the methods adopted.

The principle aims and objectives of this research (please refer to page 75), were to explore the potential correlation between supplementation of individual and multi vitamins and the development of pre-eclampsia. Healthy Start vitamins, a combination of vitamin D, folic acid and vitamin C are prescribed, mainly from during the pregnancy. From reviewing the analysis of collected data and the application of these to the initial goals of this study, the aims and objectives have been met. Data were collected for a sample of low-risk women, followed by consideration of a number of confounding variables, alongside the impact of vitamin supplementation on the incidence of pre-eclampsia has been undertaken and the results applied to practice. The methods chosen for the study have proven effective and dissemination of the findings will follow.

Pre-eclampsia is a condition affecting only pregnant women and although its aetiology begins at placental implantation, its symptoms manifest much later in pregnancy. With pre-eclampsia remaining in the top five causes of direct maternal deaths (Knight et al 2018) there remains a strong desire to further limits its impact.

Current available literature presents many theories as to the possible confounding or predictor variables, which may increase or decrease a woman's likelihood of having the condition. Amongst these are ethnicity (Reeves et al 2014), obesity (Cnossen et al 2007), and maternal age (Ogawa et al 2017). Alongside these theories, a focus has been placed on the effect of reduced vitamin D levels and folic acid. Bodnar et al (2014) keen advocates of vitamin D and its health benefits, highlight the positive impact of this vitamin on many functions of the human body, including the potential to improve placenta cell implantation. Others including Wu Wen et al (2013) promote the benefits of folic acid, most especially on

cell production and the control of blood pressure (a main symptom of pre-eclampsia). From all of the literature found to date however, the impact of these two vitamins combined, alongside the individual characteristics and lifestyle choices made by women had not yet been considered. To explore this potential correlation, this study was designed to explore key predictor variables alongside dual vitamin supplementation and to add to the body of knowledge regarding their use.

4.2 Study Design

Selecting an appropriate methodology to undertake the study was determined partly down to logistics and the optimal way of answering the questions without the opportunity to conduct an RCT. As the aim was to measure the impact of an intervention, a qualitative method would have not been appropriate. Subsequently a quantitative methodology was used under a positivist paradigm. The majority of the studies already undertaken utilised cohort methods or case control methods with few using randomised control trials. The latter, though seen as gold standard with the potential to establish cause and effect (Rees 2011) and ideal for testing a medication, can cost a significant amount and can require extensive resources (Thadhani and Tonelli 2006). Not only this they can present moral and ethical challenges (Rees 2011). In order to test effectively exposing women to a drug versus a placebo or no drug could have ethically been inappropriate. Alongside this, there is a sound argument for establishing a justification for undertaking an RCT. It would not be sensible to run a resource heavy RCT, should no statistically significant effect be engendered from the study undertaken here. In addition, as in this situation, some women were already being prescribed the vitamins and others were not. For the researcher this was also the first significant study of this size she had undertaken and indeed the first quantitative study. Having to undertake the study independently, working full time and without funding an RCT would have proved unrealistic. Though holding an honorary contract with the trust, an RCT would have needed the researcher to be actively involved and present on site as much as possible to monitor the conduct of the trial to ensure validity (Rees 2011).

Stewart (2016) supports the use of a cohort method as an effective way of gathering and testing data, indicating that it can be as valid as an RCT if conducted well. Though not able to demonstrate cause and effect, cohort studies can provide evidence to support the undertaking of further study using an RCT method (Rees 2011). Unlike case control studies,

there is no requirement to pair participants in a cohort study, which can enable larger samples, providing a wider representation of the population in question. As a first time and independent researcher, the retrospective cohort method adopted suited both time availability and access to data without the need to follow up women reducing the risk of recall bias. It also enabled the measurement of incidence rates for pre-eclampsia (Peat et al 2002). In addition, all the required data for each participant could be collated on a single occasion. Workload did constrain access to data collection towards the end of the data collection year but despite this, sufficient participants were identified to create a sample of 952 meeting the 0.25 relative risk of 954. As Hicks (2009) writes, it is important to have an adequately sized cohort to increase the likelihood of generalisability to the wider populous. Utilising and working towards the risk ratio created from the power calculation goes some way to achieving this aim. Gathering further data for a much larger cohort may have enhanced this but was not feasible at this time and there is a potential for collecting additional data, which adds no extra information, or deliberately risking selection bias to add to the sample (Hicks 2009). However, whilst the size of a sample can be significant it is equally important to aim for representation within the sample.

Within this sample, there were a range of age groups, BMI ranges, ethnicities and women experiencing different numbers of pregnancies extracted from the sample frame. Furthermore, the aim was to collect data on the occupation of the women and analyse its impact on pre-eclampsia. Unfortunately, when collected despite adding to the representative nature of the cohort, there was too much diversity amongst the occupations to enable effective and meaningful coding. There were a notable number of 'at home mothers', carers and nursery nurses listed, but it cannot be concluded that this is of any significance.

To ensure validity and reliability, strict inclusion and exclusion criteria were adhered to following a predefined method of selecting the participants. Rees (2011) promotes the consistent use of a tool to aid data collection also increasing the reliability or repeatability of the study. Appendix 5 shows an example of the tool used for this study. It is also important to note that the sole researcher undertaking the data collection addressed consistency. The tool also reduced the potential for researcher bias, which can be a challenge for cohort studies (Stewart 2016).

The exclusion of women with pre-existing medical conditions, such as diabetes and late bookers, also provided a sample whereby the participants had the same level of risk regarding any predisposition to pre-eclampsia; other than the characteristics being studied. Overall, the sample provided a representative picture of the population of childbearing women within the catchment area of the Trust. Though generally generating what appear to be skewed distribution curves for characteristics such as age and BMI in mathematical terms (Rees 2011) they do represent the populous within the catchment area of choice. It is important to recognise that when analysed however, the mean and median for the group encapsulated at least one standard deviation, with narrow confidence intervals for those demonstrating statistical significance overall, suggesting 95% of the participants were included within the calculation.

Ethical approval was obtained through all required parties, along with permission for access to data, and no women were put at risk during the study. Adjustments were needed to the initial application for approval resulting in it taking almost six months to complete, due mainly to the researcher's workload and timing of panels meeting.

The collection method selected worked well overall, though as Thadhani and Tonelli (2006) write the risk with retrospective collections of data, is the potential for missing data, as the information gathered was not originally intended for research purposes. However, they also promote the benefits of this retrospective method on time and efficiency, as long as accurate data collection is achieved. To ensure such data integrity, any possible candidates with missing values identified from the medical records were not included within the sample. In addition, notably there were a few potential outliers for example, one woman experiencing her eleventh pregnancy. Acknowledging outliers is important when conducting analysis as their presence can lead to type I or type II errors and subsequently affect validity. The influence of outliers is as Muijs (2016) states, affected by the overall sample size. A larger sample, as this study had, reduces the impact of a small number of outliers. From scanning the initial data to check for any inaccuracies in recording, as recommended by Stewart (2016), there were however, four potential outliers. These became evident when initial regression took place as the totals for classification failed to meet the 952 of the overall total. There were four entries missing each time parity was used. A review of the

data for parity was subsequently undertaken and a recoding of this variable ensured their inclusion in the subsequent analysis.

The data were analysed using the SPSS package and with support from a variety of sources including statisticians. Initially the suggestions made from different parties felt conflicting and at times confusing. In hindsight however, these discussions promoted independent decision making, a characteristic vital for a researcher moving forward. This in turn, has significantly influenced the researcher's understanding of the research processes required as well as interpretation and application of the findings. This, alongside reading and independent study has taken the researcher from a relative novice to someone with a working knowledge of how to undertake research of this kind and able to conduct effective analysis. The data collected influenced the choice of testing and work by authors including Parab and Bhalerao (2010) and Field (2018), provided useful guidance on test selection from a seemingly baffling number of options. There was a predominance of ordinal or nominal data for the independent variables and ordinal for the dependant variable. Subsequently, Chi square formulae would have proved less helpful, as this relies on 2 x 2 testing and can be affected by low cell values resulting from for example, a small number of underweight participants (Field 2018). Instead, Kruskal-Wallis and Mann Whitney analysis were applied, as these tests enabled the assessment of the severity of pre-eclampsia, rather than just pre-eclampsia as a single entity, and were able to account for multiple layers of variables. Any of the available tests require assumptions to be met, to ensure greater validity. For this study, the coding choices proved effective, and assumptions were all met. Though at initial stages of analysis multi-vitamin update and some of confounding variables demonstrated no statistical significance for any correlation, folic acid alone, BMI, season and to some extent paternal ethnicity/country of origin did. Therefore, these variables were analysed with the next stage of analysis of regression. This also demonstrates rigour as the researcher avoided manipulating data to seek alternative responses to the preliminary findings.

Overall, a logical and systematic approach was taken to undertake the study, which demonstrates rigour (Rees 2011). A consistent approach to data collection using an effective tool, strict adherence to the inclusion and exclusion criteria and the screening of records to minimise any risk of bias, alongside the selection of appropriate tests led to the final stage of analysis.

Logistic regression was ultimately used for the final stage of analysis. There are a number of options available including multivariate or multi-nominal regression, but these required much larger samples to avoid cells with missing values. In addition, as Balnaves and Caputi (2001) highlight there needs to be establishment of 'goodness of fit' and to establish any outliers as these residuals can affect the accuracy of the model used and can lead to poor fit. Initial plans were to apply multivariate regression to the data within this cohort of women, as this would have enabled seeking correlations between the independent variables and the severity of pre-eclampsia. When initial analysis took place using this method however, there were a notable number of cells with low values including zero and the 'goodness of fit' was questionable. If these results had been taken as the final analysis, their interpretation could well have been invalid and fundamentally flawed. It was evident therefore, that a change of approach was required, and the researcher accepted that it was more advantageous to use binary logistical regression with just the presence or absence of pre-eclampsia. Various authors including Hicks (2009) and Stewart (2016) support this approach, as logistic regression enables correlation between two dichotomous variables to be tested. The binary regression for each predictor variable in this study generated a mixed set of results, which will be considered in turn.

4.3 Healthy Start vitamins

Vitamin supplementation was a key confounding variable for this study, most especially Healthy Start vitamins and folic acid. Although Mcgee and Shaw (2013) identified around 10% of the general Midlands population took supplements in a combined form, for the women used in this study 32% of the sample were recorded as taking them, which equates more to a previously provided anecdotal figure from Public Health England of 23%. This higher figure could be seen as occurring due to sample selection. However, the notes were screened adhering strictly to the inclusion and exclusion criteria and vitamin uptake was last to be identified, therefore the higher percentage taking the vitamins was coincidental rather than a deliberate intention. There was also the potential for 800 women of the 8000 births to be taking them if the 10% figure is applied. Despite this higher number however, when cross tabulation analysis took place, there was no statistically significant findings for Healthy Start vitamins given from booking and no further analysis was conducted with this variable.

This subsequently means a null hypothesis cannot be rejected. In contrast, folic acid alone warranted further analysis.

There may be a number of possible reasons for this including despite the higher percentage of those taking the vitamin in the cohort this was still not large enough when mapped against pre- eclampsia to provide a big enough signal. Alternatively, it is possible that despite evidence suggesting that the components can have a beneficial impact on the condition, it is the timing of them that is not effective. Indeed, the findings from a newly published randomised control study testing folic acid alone in the third trimester, by Wu Wen et al (2018) found no affect for 2464 women deemed high risk of pre-eclampsia on the actual occurrence of pre-eclampsia. Seemingly, though different in method and choice of participants, both studies have found similar results for both low and high-risk women. When applying this to the aetiology and physiology of pre-eclampsia however, these findings are logical. It is appropriate to suggest there is a benefit to be had for vitamin supplementation at or preconception not least when several scientists have agreed that the disease begins at the point of implantation (Roberts and Gammill 2005; Ganguly et al 2018. With prolific writers including Bodnar et al (2014) and Purswani et al (2017), purporting the potential for vitamin D improving implantation, others including Wang et al (2015), advocate the anti-oxidising properties of folic acid in cell development. Studies conducted after the searches for literature were ceased, add further to this argument. Vitamin D levels and serology were tested in studies by Hutabarat et al (2018) and Pashapour et al (2019). Both of these demonstrated a clear association between low vitamin D levels and a rise in pre-eclampsia. A meta-analysis of other evidence conducted by Aguilr-Cordero et al (2020) provides further evidence of this correlation. Though in an additional meta-analysis by Bialy et al (2020) pooled data would argue against such a correlation, the studies selected for inclusion were all considered of low quality and subsequently diminish the impact of the findings. There remains a potential, therefore for prescribing a combination of two vitamins at the beginning or before pregnancy and impacting on the incidence of pre-eclampsia. To answer this question however, further studies are required, preferably a randomised control trial.

4.4 Folic acid

Having considered folic acid as an independent supplement the data from this study has shown a statistically significant correlation between folic acid and the incidence of pre-eclampsia in comparison to results from previous studies which have been inconclusive. Early studies outlined by Ray and Laskin (1999) considered an association between folic acid and pre-eclampsia, a concept supported by others including Das Singh et al (2015). In Wu Wen et al's paper published in 2013, the clinical trial findings proposed a marked reduction in pre-eclampsia cases in women who took folic acid. What the majority of studies do however, including the RCT by Wu Wen (2013) is trial higher doses of folic acid than the standard 400mcg. Indeed, the participants in the 2013 trial showed no alteration in the development of pre-eclampsia, unlike those taking a significantly higher dose. The later completed trial by Wu Wen (2018) did however, conclude that folic acid could prove beneficial and may have led to the addition of a statement regarding folic acid to the updated NICE hypertension guidelines (2019). Though the literature review for this thesis was completed in early 2018 further studies have since provided further evidence to support the use of individual vitamin supplements and their impact on pre-eclampsia. To date no studies considering combining these supplements have been conducted.

Lui, Lui, Wang and Zhang for example completed a meta-analysis in 2018 including over 30 studies. Pooled data suggested that folic acid impacts significantly on the reduction of pre-eclampsia and thus supports the findings of this study.

The findings from the analysis of folic acid uptake for the 952 women in this cohort study also showed a notable statistical difference in the incidence of pre-eclampsia with women taking the standard dose of folic acid. The higher dose used for higher risk women also showed some significance but here even this dose is well below that used by Wu Wen et al (2013). Through every level of analysis, folic acid demonstrated a positive impact on reducing the incidence of pre-eclampsia, even when adjustments are made for other predictor or potential confounding variables. The results suggest an increased risk of pre-eclampsia for those who choose not to take the supplement. Whilst the NICE 2019, hypertension guidelines, published after the completion of this study, stipulate that folic acid should not be given solely to reduce pre-eclampsia, study findings may provide further

evidence to promote its use for that purpose. Further investigation into the early administration of folic acid within a multi vitamin is still needed, however.

The models used throughout showed 'goodness of fit'. This evidence therefore adds support for the benefits of supplementation and adds credence to the possibility of dual supplementation at the beginning of pregnancy and pre-conception especially when the findings for folic acid are combined with the theory supported by the work of Ganguly et al (2018), who advocate the positive effect of vitamin D on trophoblastic function. It is possible to suggest that with both vitamin D and folic acid showing clear impact on cell development in the placenta and findings from this thesis demonstrating a reduction in outcome of pre-eclampsia; then further investigation into a combined approach would be appropriate.

It is not possible to ascertain the role of the final component of Healthy Start vitamins, vitamin C, within this study, however. The literature to date provides mixed views regarding the effect of vitamin C, although it is known that it can aid cell development (Hart 2014). Some authors, including Xu et al (2010), express concern over perceived risks of higher doses, but others acclaim (Visioli et al 2002) the potential for improved hypertension and nitrous oxide synthesis. It could be argued, that with only the recommended daily intake being included in the Healthy Start, there is unlikely to be a risk of overdose, and that vitamin C could be of benefit. Overall, with no statistical evidence found from this data supporting the use of Healthy Start vitamins in later pregnancy, it can only be concluded that the null hypothesis remains true. However, the results for folic acid in this study, alongside the findings of authors such as Bodnar et al (2014) and Hart (2014) regarding vitamins D and C respectively, provide a case for further testing of combined supplementation earlier on in pregnancy.

With this potential in mind, questions about which women should be encouraged to take such supplements could be asked. Should it be all women as currently with folic acid or those women of higher risk, such as high BMI women and vitamin D plus a higher dose folic acid? Reasoning promotes that all women would benefit from taking them. The fact that folic acid reduces the potential for neural tube defects and that a large majority of women in the UK are vitamin D deficient alone, provides enough support for mass supplementation. The possibility that an unknown 2-8% of women have the chance to develop a potentially

life-threatening condition adds further argument. Because of their increased potential to develop the condition however, targeting higher risk women would be a logical starting point. Establishing who these women are, however, is not necessarily straightforward. Many theories as discussed in the literature section (page 21), provide some indication as to who is at increased risk and some of these are born out in the findings of this study. The findings of other theories surrounding pre-eclampsia, such as women with sedentary lifestyles increasing risk (Genest et al 2012) remains subjective, mainly as they have limited evidence to support a conclusive finding. Subsequently, they are not perceived to have any bearing on the findings within this dataset and were not included in the assessment of risk.

4.5 Maternal age

Maternal age has long been seen as a risk for pre-eclampsia. Work by Ogawa et al (2017) provides evidence for an association, but this was not borne out in the findings from analysis of the data in this cohort. Despite a distribution of ages seen within the participants and a similar pattern to that of a childbearing populous there was no statistically significant finding. This leads to the conclusion that age has no impact on risk for this group of women, but as other studies such as that by Ogawa et al (2017), suggest otherwise, practitioners should continue to use age as a possible risk factor until further investigation is undertaken. What was notable however, was the limited uptake of supplementation in the 18 – 20 and 31-39-year age groups. Why this may be is not obvious, but these two groups are potentially of greater risk than the 21-30 age group. This may be purely a coincidence but is an aspect perhaps worthy of further exploration. If this picture reflects that of a wider population, there is a need for more extensive education about supplements for these women, should a beneficial effect on pre-eclampsia be determined.

4.6 Season of baby's birth

The season a woman gives birth is one example of where the findings for this cohort adds more clarity to previous theories. Globally, research has taken place over several decades, considering the association with sun exposure, skin colour and pre-eclampsia, with several linked with vitamin D deficiency. Tabesha et al (2013), Ali, Adam and Abdallah (2014) and Bodnar et al (2007a, 2007b) in particular, highlight a much greater incidence of pre-eclampsia in the winter months. In addition, Algert et al (2010) noted a marked reduction in blood pressure with increased sun exposure. Alternatively, Soroori, Sharami and Faraji

(2007), failed to find any association between sunlight and pre-eclampsia risk. The findings from this retrospective study, however, provides clear evidence that the incidence of pre-eclampsia is affected by the season, though in this case there were higher incidences in both winter and spring; with spring showing the highest impact. This corroborates the findings of several of the conclusions from other research, though it is not possible to ascertain any physiological effect of vitamin D deficiency. The initial subdivision of season for this cohort showed a notable reduction in the number of women taking Healthy Start vitamins in spring when compared to other months. Less than half of the cohort took vitamins across the year when compared to those who did not. Caution should be applied, however, as it is not possible to draw conclusions as to cause and effect as this may be circumstantial as there are several other potential confounding variables, not least skin colour.

However, when additional analysis was undertaken, spring and winter continued to demonstrate statistical significance when compared to summer and autumn. This significance remained even with the introduction of other predictor variables including parity, BMI and folic acid, during binary regression. Subsequently these findings should add to the argument supporting early pregnancy vitamin supplementation for pregnancies occurring during darker months. However, it was identified during the analysis, that there was a reduced number of participants within the spring strata, which could have affected the findings from analysis.

Following investigation, this discrepancy between seasons was mainly due to the curtailment of data collection in March and April at the end of the data collection year. The timing came at a peak of workload for the researcher. Therefore, in order to be able to accept or not the findings from this analysis, the researcher contacted the informatics department at the Trust involved, to have total monthly figures for all pre-eclampsia cases and healthy births resent. Potentially weakening internal validity by revisiting the records department to collect additional data for the spring months only was a risk, as this may have led to selection bias. However, the Informatics department identified that since the year of data collection for this study, the coding method for classification of pre-eclampsia had been adjusted. Extracting new data from this new sample frame could further affect both reliability and validity, as there is risk of repetition of individuals and the aforementioned sample bias. Therefore, the figures provided by the Trust were analysed using the same

technique for cross tabulations as the original collected data, using the totals for each season. The pattern of cases and controls in the new data did, however, follow a similar pattern to that of the cohort investigated.

There was a notable increase of cases of pre-eclampsia in the winter and darker months and a statistically significant result, though the level of significance dropped to a p >0.040. Consequently, it is difficult to draw strong conclusions regarding the impact between season and risk. The dataset for the cohort demonstrates a strong correlation, even when other variables including BMI and parity are considered, although further research would be required to ascertain a full picture considering the skewed distribution of participants. Should this additional research provide further clarification then there could be a rationale for increasing vitamin supplementation in pregnancies occurring in these seasons.

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4.7 BMI

Recommendations for vitamin supplementation of both a higher dose of folic acid and vitamin D have been encouraged for women with raised BMI for a few years (NICE 2014), although uptake of these are started at different times during the pregnancy. Early dual supplementation could be an option for this group of women. Research to date for example, by Aksornphusitaphong and Phupong (2013), identify links between obesity and pre-eclampsia and NICE (2014) base their guidance for vitamin supplementation on literature identifying the poor absorption of vitamins in these women. Clinically women with a BMI over 35 in particular are identified to be at greatest risk. Therefore, BMI was included as one of the key predictor variables and potential confounding factor. From the data collected, there was a notable number of women in the normal to obesity 1 ranges. For all BMI ranges, the number of participants taking vitamins was under half the total for each group. Perhaps coincidentally, there were cases of severe pre-eclampsia in the BMI 18 to 35 ranges and the highest number of moderate cases. Correlation analysis and binary regression analysis provides support for previous authors' such as Mbah et al (2010) findings whereby generally the increase in BMI raises the incidence of pre-eclampsia with extreme obesity demonstrating a 15 times greater risk. Obesity 1 however, showed a higher risk than obesity 2 and became non-significant during some binary analysis for example with season included. Why this has happened is not clear. The number of participants in this group is higher than obesity 2, which may explain this, or it may be due to the reduced number of cases of pre-

eclampsia in the obesity 2 group, but this remains inconclusive. The significance of BMI on the incidence of pre-eclampsia continues despite inclusion of other variables including parity and season, which provides reassurance that using BMI, as a risk factor is fundamental. Furthermore, introducing folic acid appears to reduce the impact weight has on the development of pre-eclampsia. It could also be suggested that this impact could be due to the physiological changes on blood pressure control as explored by Das Singh et al (2015) adding further support for additional investigation into continued supplementation.

4.8 Blood type

Regarding blood type, the findings from the analysis of data from this cohort shows no correlation between blood groups nor the Rhesus factor and pre-eclampsia. This compares favourably with the work of other authors such as Clark (2008) and Hentscke et al (2014). Those authors who suggest a correlation including Hiltunen et al (2008) linked their findings primarily to AB blood types, whereby women with this had a higher incidence of pre-eclampsia. When compared with global populations and the pattern of blood types as seen in figure 1.2, however, the data gathered for this cohort proved compatible with that of the UK, whereby AB blood types are relatively rare. Consequently, there were too few women in this study with AB blood groups to provide support for this conclusion.

4.9 Gravida and Parity

A further potential confounding factor took the form of the gravida and parity status of the participants and was therefore included as a key characteristic within this study. Work by Bartsch et al (2016) for example, identifies women having their first child as being of the highest risk for pre-eclampsia. This long held belief is that of many clinicians, and consequently forms a basis for more frequent antenatal checks. What the data from the 952 participants in this cohort supports an increase in risk the fewer births a woman has. Though gravida status showed no correlation, those who had given birth to their first baby had a much higher incidence. The range of women involved reduced the possibility of bias in these results, but it is noted that there is a higher frequency of first and second pregnancies in the cohort. However, arguably, this reflects the norm for childbearing women and the findings in this study reflect those of authors invested in this topic, including Bartsch et al (2016).

4.10 Lifestyle choices

Many women within the cohort also presented with a range of lifestyle options. Interest lay in the impact of smoking, alcohol and recreational drug habits particularly as potentially these could affect pregnancy. Little evidence could be found regarding these when the literature was searched which provides little for health professionals to base their practice on. Inconclusive findings are unhelpful when encouraging women to change their lifestyle. The alcohol uptake was recorded at the point of booking so may have altered through pregnancy and there is a potential for under reporting. However, the greater percentage of women indicated they did not consume alcohol at that point in their pregnancy. The results from analysis are somewhat confusing. There was evidence of a correlation between alcohol consumption on initial analysis however, when binary regression was undertaken, the results suggested that some alcohol could have a positive impact on pre-eclampsia development. As alcohol has been shown to be detrimental to fetal development (NHS 2018 accessed January 2019), potentially leading to fetal alcohol spectrum disorder, this result could be seen as concerning at face value. However, on closer consideration, the model used demonstrated a poor 'goodness of fit'; meaning caution should be applied to these findings.

Analysis of smoking habits provided no statistical evidence to support previous literature. Potentially this could be due to the limited number of participants involved who declared that they smoked. In contrast, when considering the uptake of recreational drugs, initial analysis suggested an association with pre-eclampsia. Physiologically it is logical to suggest that the impact of these drugs on the circulatory system could predispose someone to raised blood pressure (Ferdinand 2000), which is the most frequently seen symptom of pre-eclampsia. Indeed, the analysis suggested that the number of women in the cohort taking drugs aligned with that of the 1-2% of women taking them in the wider population of the catchment area used. (Cohen, Osorio and Page 2017). There is therefore a potential for greater exploration of this area, particularly with the suggested increase in use of recreational drugs, and the recognition that these women need closer monitoring for signs of pre-eclampsia. In turn, as a high-risk group, they may benefit from the benefits provided by vitamin supplementation.

4.11 Maternal and paternal ethnicity

The final characteristic considered involved maternal and paternal ethnicity. Though providing a representative sample containing over 30 different ethnicities, this led to challenges in coding the data. Various permutations were tested but none encapsulated the diverse nature of the group, which could affect generalisability to a wider population. Furthermore, the categories used within the maternity notes are arguably not true ethnic groupings although recorded as such. As this was the data available however, it was adopted for use within the data collection and analysis. Inspiration for the final selection of coding groups came from the available literature (Reeves et al 2014), as this had been previously tested, providing a degree of internal validity. A limitation being that it was impossible to consider the colour of individual's skin tones, which would be beneficial to know when looking at vitamin D metabolism especially. In contrast, even with similar choices of subgrouping to other authors such as Abedi et al (2014), this cohort provided no supporting evidence to correlate maternal ethnicity and pre-eclampsia. However, much of the available literature highlighted the increased risk of pre-eclampsia in different ethnic groups, alongside vitamin deficiency in darker skinned or tanned women. A lack of vitamin D especially is seen to be a factor for reduced placental cell development. It could be suggested therefore, that with further study these groups of women could benefit from supplementation of combined vitamins and potentially a reduction in pre-eclampsia.

In contrast, there were significant results for women who had partners of Asian and African descent. The results demonstrating a higher incidence of pre-eclampsia in women having an Asian or African father of the baby, irrespective of their own ethnicity. Again, the same challenges of coding existed and ultimately the same subgroups were used for analysis. A degree of caution is needed also as it is impossible to ascertain whether the group identified as the ethnicity came directly from the mother or the father. The latter would be seen as the more accurate recording. However, these results add knowledge to a small pool of existing literature in this area and provides a basis for further study. What is not clear is whether supplementation for the mother would bring any benefits. When applied to the work of Cowan et al (2017) regarding the immune response to the father's semen alongside the effect of vitamins D and C plus folic acid to the immune system, there are compelling arguments for multiple supplementation.

Overall, this study has provided a raft of additional information adding to the current body of evidence surrounding this fascinating but complex condition. The analysis provides several bases from which to build other studies, but also provides conclusions applicable to current practice.

5 Conclusion

5.1 Summary of findings

In summary the key messages and conclusions are:-

- **Folic acid taken in the first trimester and pre-conception correlates to a lower incidence of pre-eclampsia**
- **Combined with vitamin D and C, folic acid may have the potential to aid placental cell development and reduce the incidence of pre-eclampsia. This warrants further research.**
- **Healthy Start vitamins prescribed for women with raised BMI, giving birth to their first baby in winter or spring could reduce their risk of pre-eclampsia. This warrants further investigation.**
- **The combination of vitamins in the form of Health Start vitamins, given later in pregnancy, does not demonstrate any correlation with the development of pre-eclampsia.**
- **Women who are pregnant and give birth during darker months are potentially more at risk of developing pre-eclampsia.**
- **Women with a raised BMI are at significantly greater risk of developing pre-eclampsia as well as being insufficient in folate and 1,25- dihydroxyvitamin D.**
- **Women whose baby is fathered by an Asian or African male are more likely to develop pre-eclampsia.**
- **Blood type, age, smoking and alcohol consumption show no increase in the rate of pre-eclampsia for this cohort.**

5.2 Strengths of this study

The strengths of the study lie primarily with the straightforward processes undertaken. This in turn provides more robust reliability and validity. The tool used to collect data and the

stringent attention to the inclusion and exclusion criteria provided a solid foundation on which to work. As Everitt (2009) states reliability entails being able to measure the same measurements from individuals and yield similar results in a different situation. The approaches taken by the researcher would enable this.

Furthermore, the stratified selection of participants provided a wide range of different characteristics and demographics enabling representation of a broader group of individuals. In turn, this permits the results to be generalised more securely to the wider population used. With 8000 possible births occurring within the selected maternity unit annually, if national averages are considered, up to 7% (Cowen, Redman and Walker (2017) will have the potential to develop some level of pre-eclampsia (560). In this cohort, the sample of women with pre-eclampsia equates to 4% of the 8000 births in the unit. Of these a smaller proportion, approximately 1:2000, would go on to develop the more severe degree of the condition that is, eclampsia. The sample of moderate and severe categories represented in table 8.1, make up only 2% of each group. With figures similar to national averages then it can be suggested that the group can be representative of the wider populous in the local area.

From a logistical position the methods selected also allowed for data collection to be undertaken at a time more suited to the researcher's other commitments, although this was challenged towards the latter part of the year.

However, with any study no matter how well constructed and performed, there can be limitations.

5.3 Limitations of this study

The question of compliance of taking the medication was raised by the ethics committee at the first panel meeting and required consideration by the researcher. It was suggested that some way of measuring compliance would be beneficial however, how this could be achieved was difficult. Indeed, several papers including those by Claxton, Cramer and Pierce (2001) and a meta-analysis by Cramer et al (2008) question whether complete compliance is achievable. Compliance of taking medication in any study including an RCT is often a challenge and participants can provide inaccurate answers when asked about taking anything (Brown and Bussell 2011).

It is acknowledged, that whatever method to collect information regarding compliance can lead to possible errors. Everitt (2009) points out that even with pill counting used, as a method in RCTs, there are likely to be challenges. In addition, there is the potential for response bias or even the Hawthorne effect (Bowling 2014) where women may say what they wish the midwife to hear or change their behavior to suit the research. Models developed to address the potential impact on statistical findings where compliance is important have been tested, as outlined by Mark and Robins (1993), but even with these models, there remains no guarantee.

It is also possible that some women will have taken an alternative form of vitamins or Healthy Start, but not recorded this when asked by a health professional, or alternatively have not taken any prescribed vitamins. Interest for this study lay specifically with the medication prescribed by Public Health England that is, the Healthy Start vitamins and it was not possible to test these possibilities. This issue had the potential to limit application of the findings whatever methodology was adopted; however, it is also accepted that women during pregnancy are more likely to be motivated to undertake healthier options and many are keen to take folic acid in particular and vitamins if advised to do so for their baby. A recent qualitative study by Forbes et al (2018) monitored women's habits regarding positive changes in diet and health during pregnancy with many of the women being motivated to change due to a desire to prevent harm to their baby. These findings are supported by the cross-sectional study of Lindqvist et al (2017), whose research identifies the positive impact of pregnancy on motivation. These studies conducted in Canada and Sweden respectively, both developed countries like the United Kingdom; provide some reassurance as to the possibility of improved compliance with vitamin supplements within this cohort. Strict adherence to the requirement of clear documentation of uptake of vitamins will also have gone some way to address any negative implications.

The potential for using a questionnaire was considered to ask additional women about compliance, via the community midwives. However, when reading the medical records, the required information regarding vitamin uptake relating to the women being included in the study, was clearly documented. Asking additional women outside of the cohort, whose data was not used for the assessment of correlations, would not have provided a valid representation of the cohort and would have been logistically difficult. There would also be

the potential for women to tell the midwife what they felt t wanted to hear, bringing in a degree of unwanted bias. Consequently, those records showing recorded evidence of vitamin uptake were included and identified as such for coding and analysis purposes. Therefore, the possibility of asking women regarding their vitamin uptake in the community was logistically impractical to do and in the final undertaking of the study, the researcher did not approach women directly. However, initial application to the ethics committee required pertinent documents to be available, to ensure safe conduct should the researcher pursue this. These included information sheets and consent forms.

In relation to records there was also the potential for inaccurate data being recorded particularly when a potentially harmful habit such as smoking, alcohol uptake and recreational drug use is discussed. It was not possible to know how accurate the information was. Therefore, a decision to restrict smoking and drug use to yes or no and to group the alcohol intake into five categories was made.

In addition, when undertaking coding and analysis for some variables such as with pre – eclampsia and parity, there were occasionally cells containing little or no values. This can affect the quality and validity of the results generated from the analysis. To counteract this potential the coding system adopted took account of these lower values and reconfigured the groupings to include all entries and avoid empty or low value cells. This was particularly relevant when considering the severity of pre-eclampsia. Overall, there were only a few severe cases, especially when other variables were tested against this dependent variable. An initial aim was to consider the impact of variables on the severity of pre-eclampsia and to use multivariate regression. Descriptive level of analysis was feasible with the subdivision into no pre-eclampsia, mild, moderate and severe, but this was lost when regression took place as the break down created many cells with little or no value. These would be difficult to interpret with any confidence, therefore pre-eclampsia for regression was coded into having the condition or not and binary regression was used. The generation of odds ratios could reduce the statistical power of the findings, when compared to risk ratios, however the sample size was substantial at 952 and the generation confidence intervals balances out a degree of inaccuracy caused by using odd ratios.

5.4 Implications for practice

With pre-eclampsia status in the causes of maternal deaths and morbidity, any form of treatment or prevention is welcomed. This study not only provides greater clarification regarding which women are of increased risk of developing the condition, but also a potential for prevention following further investigation. Clinicians worldwide are creating risk assessment tools for many conditions in pregnancy with a commonly used example being the venous thromboembolism (VTE) assessment tools (NICE NG89 2018 and Knight et al 2018). Having specific details about who is likely to develop pre-eclampsia can go some way to the creation of a similar tool. Data from this study intimates that increased risk is associated with weight gain and extreme obesity raises the level of risk considerably. Alongside this, there was clear increased risk for those having their first baby. These are characteristics already monitored in pregnancy and can readily be added to a risk assessment tool and can readily be applied to care on a global scale.

Pre-eclampsia is not just a condition affecting women in developed countries. Up to 60,000 women globally, most especially in developing countries or third world countries, die annually from pre-eclampsia (Knight et al 2016 and 2018). Unlike in the UK, where improvements in antenatal care have reduced the number of fatalities significantly, the same cannot be said for other countries around the world. Healthy Start or other forms of multi vitamin can be purchased very cheaply and are easily accessible. For some women they are provided free of charge already. The findings of this study therefore, alongside the desire for further research into vitamin supplementation, has the potential to provide a relatively cheap way of helping women around the world as well as in the UK. It is already established, that vitamin D helps bone development, folic acid reduces risk of neural tube defect whilst aiding cell development and that vitamin C can aid cell development and general health. These reasons alone are justification for supplementation. With ever increasing numbers of studies including this one, providing additional evidence of the potential benefits of risk reduction for pre-eclampsia it could be argued that supplementation could be a key to further reductions in this risk. If continued research into the timing of dual supplementation in particular at pre-conception and in the first trimester shows a positive impact on reducing the severity or incidence of pre-eclampsia too, then this should be acted on.

Whilst it is easy to access vitamins in the UK and developed counties, this is not so easy within lower income societies and developing countries. However, there are ways to provide supplements to those in need through World Health Organisation programmes or charities such as the Red Cross for example. There would also be the need to improve uptake and motivation as seen in the sample used for this study, not all women chose to take even folic acid. There may be several reasons for this not least a lack of understanding of the possible benefits or a perceived cost implication.

Though ethnicity did not demonstrate any statistical significance in this study, other evidence has found ethnic minorities, especially dark-skinned individuals, can have an increased risk of pre-eclampsia (often associated with low vitamin D in particular), along with those from lower socio-economic backgrounds. These particular groups may need specialist education programmes or one to one conversations with health professionals and provision of supplements. Indeed, in some areas of the country, these groups are already targeted to receive free vitamins, but currently these are provided during pregnancy rather than the beginning. Conclusions drawn from this study would suggest that supplementation at this stage could be too late to have any impact. Targeting higher risk groups early on may be optimal instead. There will continue to be issues with compliance as discussed in the limitations section but using evidence to underpin practice is a fundamental principle for midwives and medical practitioners and they have a duty of care to promote health and encourage compliance.

Clearly, this study alone does not provide all the answers, however, when in combination with findings from the work of others, there is a clear indication for additional exploration into the possible benefits for women from early supplementation to aid placental development and minimise risk of developing pre-eclampsia later on. Implementing any positive findings has the potential to improve lives across the globe for a relatively small cost.

Moving forward there is a need to disseminate the findings from this study and pursue possibilities for trials introducing early multivitamins and the potential for prevention of such a challenging condition. Such trials should perhaps be multi-centred and include as large a sample as possible. For the researcher such a large study is impractical therefore

there is a need to take the findings from this study and collaborate with trusts to expand the trial. An RCT is justified into testing Healthy Start vitamins or an alternative multi vitamin, and the outcome of pre-eclampsia. Though Healthy Start vitamins during pregnancy do not demonstrate a correlation, the evidence suggests that the benefit may come when taken at preconception and during the early stages of pregnancy. To minimise issues relating to ethics a comparison with folic acid alone would reduce the risk of women developing any complications for the fetus. An RCT would also provide a controlled environment where regular screening of serum vitamin D and folate could be undertaken. Furthermore, such a study would enable comparisons between standard doses of vitamins and a higher dose of vitamins, providing further clarity on which provides the most benefit.

5.5 Overall conclusions

From the outset this study set out to reject the null hypothesis that vitamin supplementation had no impact on the development of pre-eclampsia. It is possible from the findings resulting from regression analysis to conclude that whilst Healthy Start vitamins have no impact when administered during the pregnancy; there is enough persuasive evidence to suggest that folic acid taken early on does have an impact, irrespective of a number of possible confounding variables such as raised BMI. Consequently, though it is not possible to establish cause and effect, it is possible to demonstrate a strong correlation between pre-eclampsia and folic acid supplementation. Furthermore, when read in the context of research into other vitamins, principally vitamin D, there is further compelling support for combining the two vitamins at the point of conception, with the potential to reduce risk of developing the condition. There is a strong case for continued investigation into Healthy Start vitamins, with a recommendation for more research into the optimal timing of supplementation. Though no correlation was detected the study has achieved the learning objective exploring the potential of Healthy Start.

In addition, the overall aim of the study was to determine correlations between confounding or predictor variables including maternal characteristics and supplementation, with the development of pre-eclampsia. Following consideration of a variety of confounding or predictor variables in the data analysis this aim was achieved. Indeed, it has been possible to add further knowledge regarding the maternal characteristics and lifestyle choices, which may alter women's risk status for pre-eclampsia as well as seeking any

changes following the introduction of supplements. Although findings for some variables found no evidence to support existing research, for example regarding age or ethnicity, blood group and smoking this does not rule them out as potential risk factors. In contrast, analysis of other variables provided credible statistical evidence to justify inclusion in any risk assessment conducted with pregnant women. An increase in BMI has a noticeable effect on the incidence of pre-eclampsia, as does a lower parity. Alongside this, there is support for the growing body of evidence into the impact of season. What has yet to be established is whether this is a physiological impact through vitamin D exposure, but this would warrant more investigation. There is also some suggestion that alcohol intake could have a bearing on pre-eclampsia though not conclusively.

In conclusion, overall, the study has achieved what it set out to do. It intended to consider correlations between women's characteristics, lifestyle choices, vitamin supplementation and their impact on pre-eclampsia. The methodology suited the collection of data and the options used for analysis proved effective. Despite finding no effect from Healthy Start vitamins in particular the findings from folic acid intake alone provides justification for further investigation. With the final objective being to disseminate these findings and provide recommendations for practice there are clear messages emanating from the analysis to initiate further investigation and add to the evidence health professionals can use in their practice both locally and nationally.

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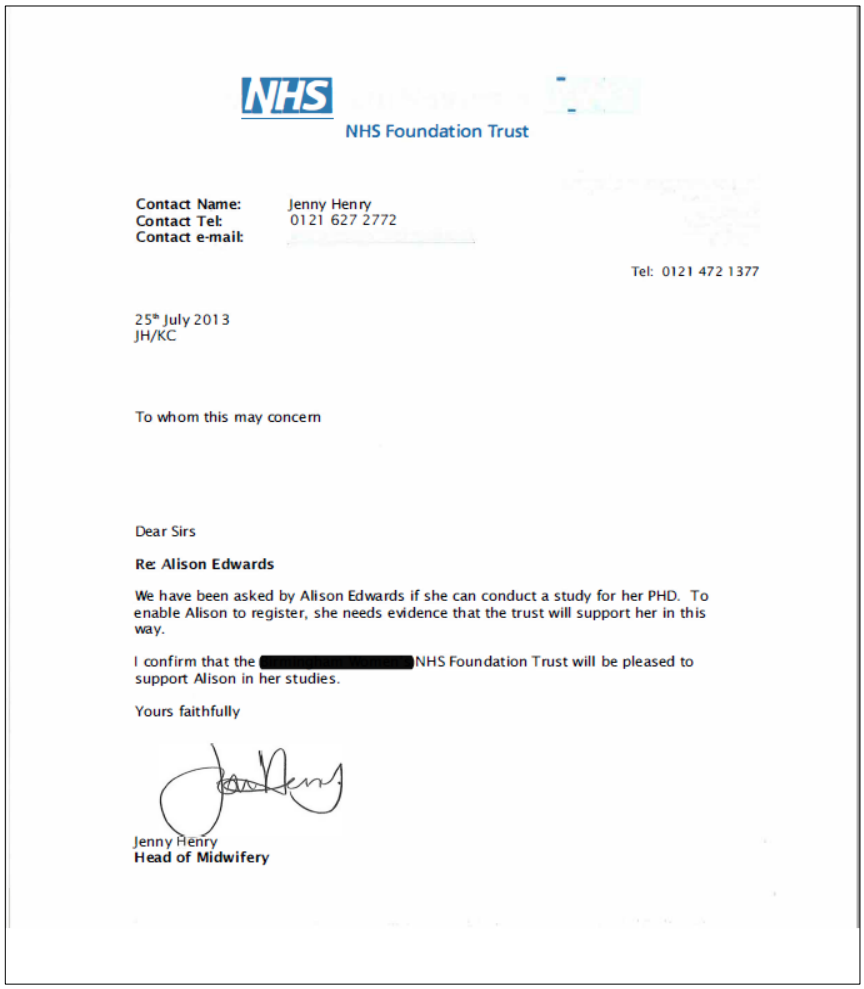
Appendices

7 Appendices.

7.1 Appendix 1. Letter of permission from the Heads of Midwifery



NHS Foundation Trust

Contact Name: Jenny Henry
Contact Tel: 0121 627 2772
Contact e-mail:

Tel: 0121 472 1377

25th July 2013
JH/KC

To whom this may concern

Dear Sirs

Re: Alison Edwards

We have been asked by Alison Edwards if she can conduct a study for her PHD. To enable Alison to register, she needs evidence that the trust will support her in this way.

I confirm that the [redacted] NHS Foundation Trust will be pleased to support Alison in her studies.

Yours faithfully

Jenny Henry
Head of Midwifery

7.2 Appendix 2. Permission from Caldicott guardian.

local care: global impact

NHS Foundation Trust

15 January 2015

To whom it may concern

Dear Sirs

Re: Alison Edwards

We have been asked by Alison Edwards if she can conduct a study for her PhD and to this end she needs evidence that the Trust will support her in this way.

As the Trust Caldicott Guardian, I am writing to confirm that Birmingham Women's NHS Foundation Trust will be happy to support Alison Edwards in conducting a study for her PhD.

If you require any further information please do not hesitate to contact me.

Yours sincerely

Helen Young
Director of Nursing & Midwifery and
Trust Caldicott Guardian

Chief Executive: Professor Ros Keeton *Chairman: Elisabeth Buggins CBE DL*

7.3 Appendix 3. Ethical approval.

NHS
Health Research Authority

05 March 2015

Miss Alison Edwards Senior Midwifery lecturer
Dear Miss Edwards

NRES CommitteeT

Study title:	**Does supplementation of Healthy Start vitamins in pregnancy impact on the risk of developing pre-eclampsia in women booked at a regional maternity unit?**
REC reference:	**15/WM/0013**
Protocol number:	**N/A**
IRAS project ID:	**147829**

Thank you for your correspondence of 25 February 2015, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair and one other member.

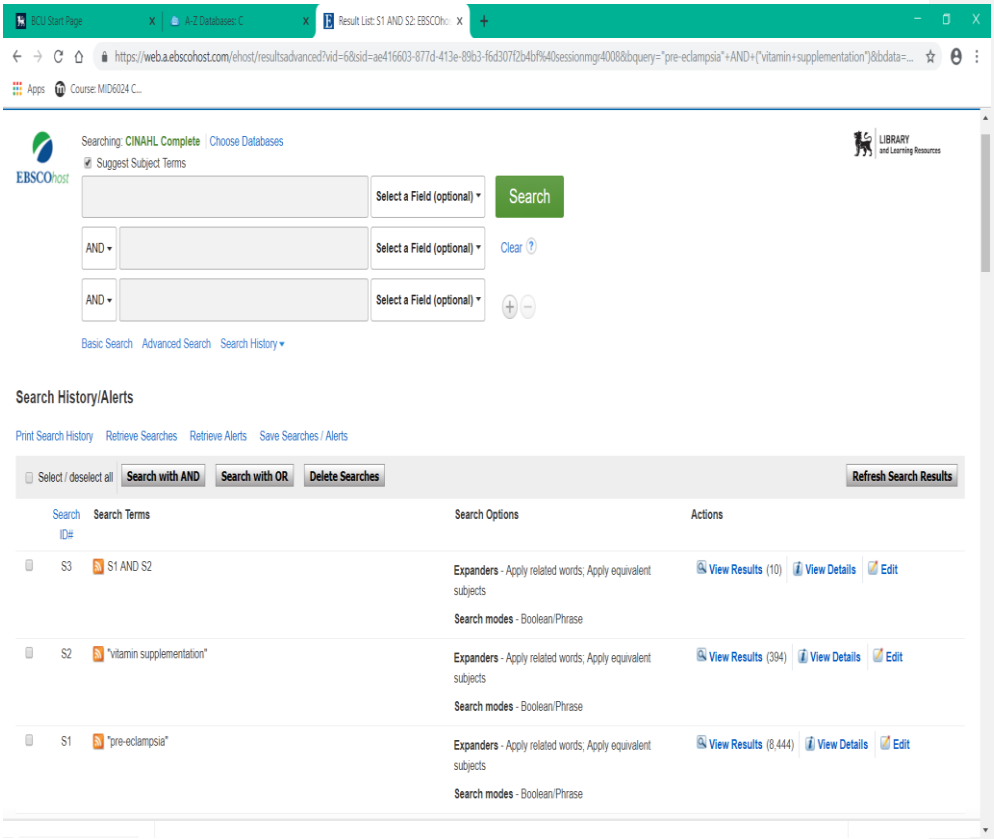
We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point, wish to make a request to defer, or require further information, please contact the REC Manager, Ms Helen Poole, at

Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised.

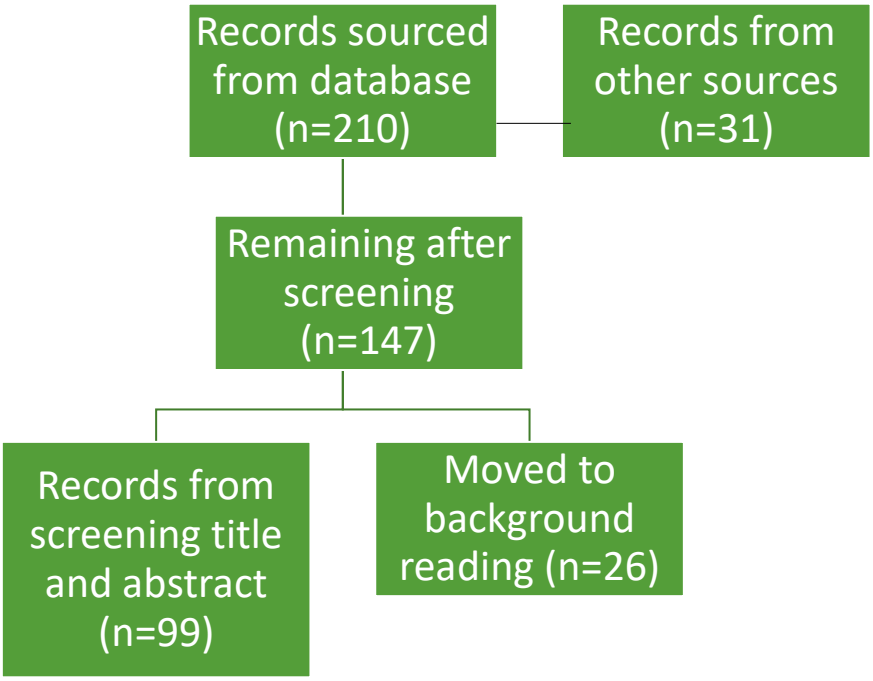
7.4 Appendix 4. Example of search strategy



Examples of hits from initial search for pre-eclampsia, vitamin D and vitamin supplements

Mother and Infant care - 11 hits
CINHAL - 19 hits
Cochrane 2 reviews
Ethos - 0 hits
Assia – 2 hits
Google – 1 non-UK study
Science Direct – 6 hits
RCOG – 2 guidence notes
NICE – 2 guidelines relating to folic acid and vitamin D
Medline – 210 hits
Summon – several hits non- accessible in full. All but one found through other sources.

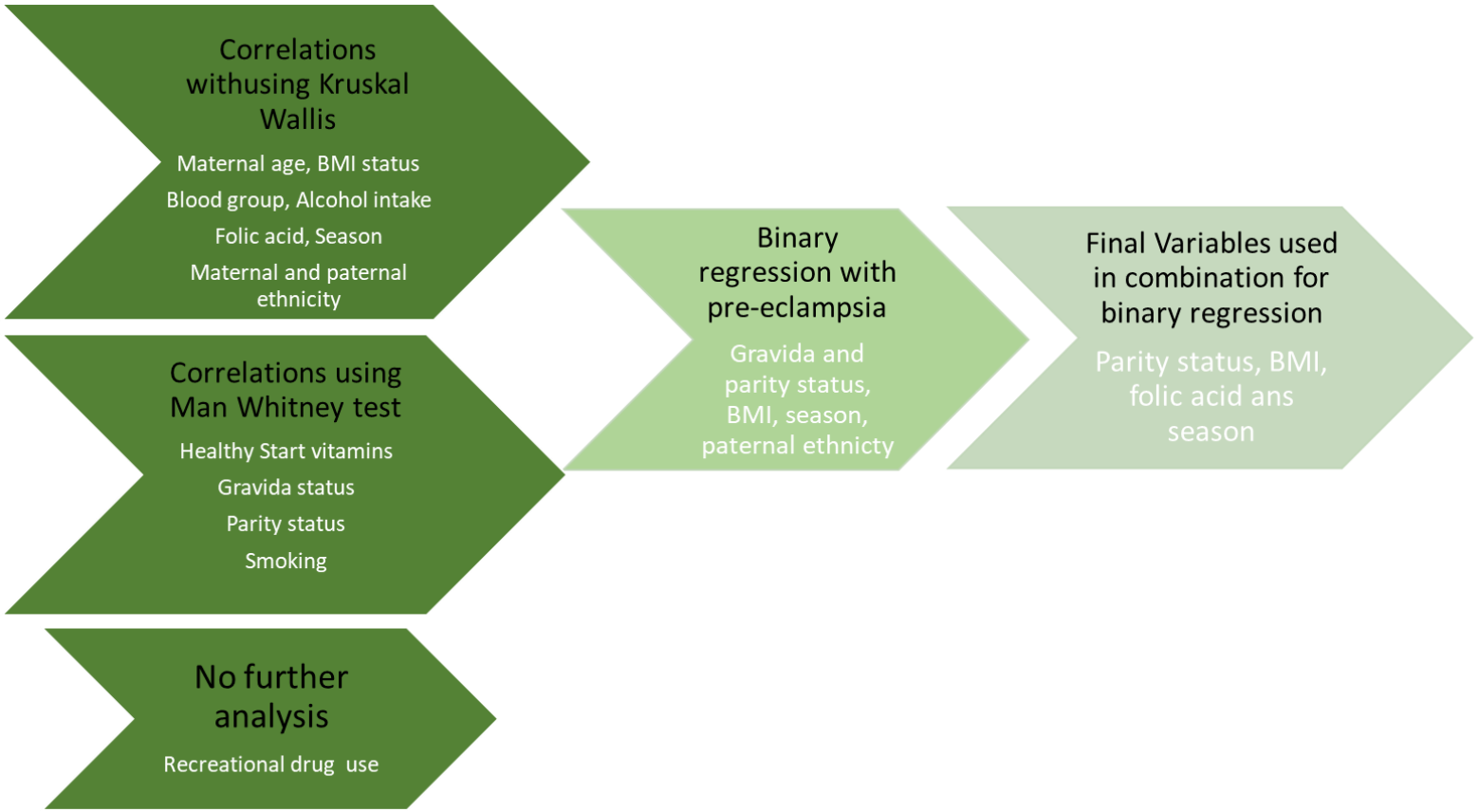
Number if papers sourced and processed.



7.5 Appendix 5 -Example of data collection sheet.

Number	Age	Gravida	Parity	BMI	Mother ethnicity	Father ethnicity	Blood group	Occupation	Smoke	Alcohol	Drugs	Medical condition	Folic acid	Healthy Start Vits
1	22	1	1	28	Pakistani	Pakistani	Opos	Secretary	Y	N	N	N	YY	Y
2	20	1	1	34	British	Caribbean	Apos	Nursery nurse	N	5units	Y	Asthma	N	N
3	38	3	2	27	SE Asia	SE Asia	ABneg	Solicitor	N	1-2Units	N	N	N	N
4	30	3	3	40	British	British	Oneg	Housewife	Y	11units	N	Depression	Y	N
5	25	2	1	30	Polish	Polish	Aneg	Retail	Y	Social	N	N	Y	Y
6	31	2	1	35	Bangladesh	Bangladesh	Bpos	Housewife	N	N	N	Asthma	Y	N
7	26	4	4	26	Sub S Africa	British	Oneg	Doctor	N	6-8units	N	N	Y	N
8	45	11	9	20	British	British	Oneg	Housewife	N	social	N	N	Y	Y

7.6 Appendix 6 – Flow chart of statistical analysis of variables



7.7 Appendix 7- Conference poster prepared for conference submission.

Does supplementation of vitamins in pregnancy reduce the risk of developing pre eclampsia in a regional maternity unit?

Alison Edwards RGN. RM. MSc. PGcert. Birmingham City University

Pre eclampsia

Pre eclampsia is a condition specific only to pregnancy. It can affect between 2- 7 % of the pregnant population and can lead to significant maternal and fetal morbidity and mortality. Dependent on it's severity women can experience a range of symptoms including headaches, oedema, affected vision, epigastric pain and proteinuria. Most significantly a woman's blood pressure can rise leading to organ damage if left untreated. Numerous studies have looked into the causes, methods of prevention and cures but as yet without much success. What has occurred however, is a drop in the number of mortalities from the condition MBRRACE (2014)

Fig. 1. healthy start vitamins. Current recommendations from public health Birmingham are for women to receive healthy start vitamins in pregnancy Current uptake runs at only 23%. Contains Vitamin D, Folic acid and vitamin C

Background to study

In 2008 supplementation during the first 12 weeks of pregnancy with healthy start vitamins (containing folic acid, vitamin C and vitamin D); was recommended as part of routine antenatal care (National Institute for Clinical Excellence (NICE) 2008). The primary rationale for this was to reduce the risk of rickets in infants, particularly as a large proportion of women in the UK population were found to have insufficient levels of plasma Vitamin D and the incidence of rickets was increasing (Duckworth et al 2012).

The literature also suggests however, that low vitamin D and folic acid levels may be linked to a higher risk of pre-eclampsia. Subsequently with current practice now involving supplementation, there is the potential to explore the benefit of reducing the risk of pre-eclampsia with vitamin supplementation, a rationale supported in recommendations from several published studies, including that by Thorne-Lyman and Fawzi (2012).

Sampling

From conducting power calculations using a known 10% uptake of Healthy Start vitamins in the year 2012 to 2013 (McGee and Shaw 2013) and a range of risk ratios, suggested sample sizes are outlined below.

Relative risk	Sample size
0.5	2200
0.33r	1217
0.25	954
0.20	834
0.1	653

Currently the trust has over 8000 births per annum and around 250 cases of Pre eclampsia per year.
Id numbers are sent through encrypted mail in date of birth order to maintain confidentiality and meet ethical standards. Records are screened to meet inclusion and exclusion criteria.

Aims

To determine any correlations between maternal characteristics, supplementation of vitamins and the development of pre-eclampsia.

Objectives

- To use a retrospective cohort study method to gather data on Healthy Start vitamin uptake and compare outcomes for pre-eclampsia.
- To collect data from medical records concerning maternal characteristics such as age, BMI and parental ethnicity, lifestyle choices and season to aid assessment of risk factors.
- To analyse the data collected to establish whether correlations exist between them and the development and severity of pre-eclampsia.
- To disseminate the findings of the research to inform future clinical practice and provide recommendations based on the findings.

Methodology

A retrospective cohort method is being used. This will enable the researcher to look for correlations between two groups of women i.e. those who develop pre-eclampsia and those who haven't and their uptake of vitamins. Consideration is being given to other possible variables which could impact on the development of preeclampsia. As a consequence data on BMI, ethnicity, age, season, occupation and social factors, including alcohol or smoking habits is being collected.

Once a large enough sample has been collected cases and controls will be matched by Gravida (number of pregnancies), age and BMI. If feasible ethnicity will also be used.

Achievements to date

- Ethical approval received
- Six months spent collecting cases which span births across each season. Data for 350 cases collected which match the inclusion criteria.
- Lists of id numbers for women without pre eclampsia have been obtained to begin data collection of controls.

Future plans

- Collect data for controls
- Undertake initial analysis in April 2017
- Continue collecting cases and controls until a large enough matched sample is obtained.
- Repeat literature reviews annually.

Emerging patterns

- Issues arise with some omissions with documentation, meaning some cases cannot be used.
- There is a significantly larger percentage of women with mild pre eclampsia than moderate or severe pre eclampsia.
- The vast majority of cases are in their first pregnancy, which matches current evidence of the association of risk and pregnancy number.
- A small minority of the cases took no supplementation at all . With the majority taking only Folic acid

Literature cited

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For further information

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local care: global impact

BIRMINGHAM CITY University

7.8 Appendix 8 Presentation for Family Health Research cluster event at BCU 2015

My PhD journey
Alison Edwards

Research question

- **Does supplementation of healthy start vitamins reduce the risk of preeclampsia in a regional referral centre?**

Gap in the market!!

- **Ongoing discoveries of possible causative factors for Pre eclampsia or risk factors. What is known is that faulty placental implantation has a marked effect.**
- **Several papers suggest an association between vitamin D deficiency and the development of pre eclampsia.**
- **Others have identified an association with folic acid deficiency and a suspicion that vitamin C plays a part.**
- **What hasn't been considered is whether a 3 pronged attack will have any effect.**

My methodology

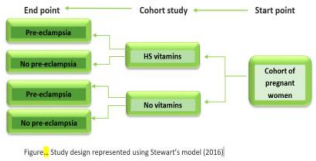

Figure 1 Study design represented using Stewart's model (2016)

Inclusion/ exclusion criteria

Inclusion criteria:-

- Women aged 18 and over.
- Primigravid women (first pregnancy)
- Multigravid(women in subsequent pregnancies) women with no history of raised blood pressure (hypertensive disease). It may be of value however, to consider their previous pregnancy as opposed to the current one.
- Singleton pregnancy
- Women not included in other studies involving vitamin supplementation or treatments which may affect the development of pre-clampsia

Exclusion criteria:-

- Women under the age of 18yrs old
- Any women with a medical or obstetric condition such as diabetes, existing hypertension or renal (kidney) disease, which may increase the likelihood of developing pre-eclampsia
- Multiple pregnancy- as these women have a higher risk of getting pre-eclampsia
- Women included in other studies involving vitamin supplementation or treatments which may affect the risk of pre-clampsia

The journey so far

- **PGCert in research methods completed (2013)**
- **9R undertaken – study registered (2014)**
- **Proportional review applied for – referred to NHS NREC panel (2014/5)**
- **Indemnity obtained (2015)**
- **Ethical approval finally in (2015)**
- **Meeting with trust colleagues to establish access to data March (2015)**

Current situation

- Data collection days in diary
- First batch of ID numbers received
- Spreadsheet set up
  (a steep learning curve in itself)

7.9 Appendix 9. Presentation for University research conference.

PHD Presentation
Alison Edwards

Background/findings

- There is a known relationship between raised BMI and risk of pre-eclampsia
- Research findings favour an association between vitamin D deficiency and raised risk of PE although quality of evidence is mixed.
- There is a higher incidence of vitamin D deficiency in obese women.

Rationale

- Over 50% of the female population are vitamin D deficient. 1:5 pregnant women are obese
- NICE guidelines have recommended supplementation of vitamin D/healthy start vitamins since 2008 but to prevent rickets(Lawler 2013 findings found no benefits) Practice may change as a result

So

- PE affects 2-7% of the pregnant population and remains a leading cause of mortality.
- If women are no longer encouraged to take supplements and remain deficient could we loose an additional benefit of reducing the risk of PE?
- To continue with current practice we would have to prove that vitamin D is making a difference.

The research question

- Does Vitamin supplementation reduce the risk of Pre-eclampsia?

Where am I at

- A decision needs making:
- Option 1
  - A prospective cohort study comparing supplemented groups against non-supplemented groups and outcome for pregnancy

Option 2

- A retrospective case-control comparing records prior to the introduction of supplements with those after.

Progress to date

- Permission to pursue PHD within trust from Head of Midwifery obtained
- Access to records/ data explored
- Continued review of the literature
- Meeting with statistician regarding options
- Discussions with trust R and D department regarding feasibility of study and current studies occurring within the trust

7.10 Appendix 10. My PHD experience blog by Alison Edwards (2nd year in)

My journey towards commencing a PhD at BCU was filled with trepidation and questions. Amongst many others I questioned whether would I cope doing a full time job too?, what exactly was expected of me? and would I look stupid because my knowledge of research I felt was pretty limited?

I am not sure that two years in I can answer any of these questions but what I do know is that I have been caught up in the desire to make a difference, no matter how minor. To have come up with an answer to a question, which adds to the body of current knowledge is no small thing and the journey to that point is in itself both challenging and daunting but at the same time exciting.

Life as a PhD applicant began with the somewhat bewildering concept of choosing what to study. I had some vague ideas around my choice of topic, but what I have learnt through the previous months is that you need not stress about it, as the vagueness actually is not a problem. If anything, it's an advantage, as the study I am now embarking on bears little resemblance to where I started initially. It's actually beneficial to be able to toss ideas around with others and be able to adapt.

It's also proved invaluable to access the knowledge of those around you. Apart from the basics within my master's degree and various modules, my in depth knowledge of research was I would say lacking. You soon however, begin to develop your skills in this arena, not least from reading the copious amount of literature already out there. The main driving forces supporting me through this however, have been my supervisors. Without their knowledge and support, I would not have got to the point of registration to undertake the study for real. Access to an expert and very approachable statistician was also incredibly helpful if not essential and especially important to my chosen methodology.

Working a full time job alongside study has been the most difficult aspect. It has not been possible to fully engage with the activities that go along with doctorate study, let's face it getting even free time to read can occasionally be impossible, and so you can expect to feel a bit isolated and lost at times. You need to look for alternative routes of support and keep the goal in sight. Having a picture of myself in a borrowed graduation out stuck in my folder just gives me the right kind of reminder why I am embarking on this adventure.

My next steps are into the foggy world of ethical approval. Yet another complex and tangled web of forms and new terminology. It's yet another process which scares me, but I know once I've got over the hurdle I've made the next step towards my ultimate goal and it's that thought I'm holding onto.