The association of parity with cardiovascular disease: systematic review and cohort study

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Declaration

The outline of this MPhil research was proposed by my supervisor Dr Pensée Wu. The final research question and focus of the systematic review and cohort study included in this research was decided through discussion, between myself and my supervisors: Dr Pensée Wu and Professor Kelvin Jordan.

I took the role of lead reviewer for the systematic review and created the protocol and search strategy, with help from Dr Pensée Wu and the systematic review team at the Research Institute for Primary Care and Health Sciences at Keele University. Dr Pensée Wu acted as the second reviewer and screened the search results by abstract and full text, alongside myself, as part of dual screening. I completed all of the data extraction and quality assessment of the studies included in the systematic review, and Dr Pensée Wu checked 30% of the studies to quality assess my work. All of the statistical analysis for the systematic review was completed by myself.

The methods used to conduct the cohort study were decided through discussion between myself and my supervisors. I compiled the list of Read codes myself, with some codes originating from previous published work. The electronic health data used to complete the cohort study was taken from the Consultations in Primary Care Archive (CiPCA). I was not involved in the collection or compilation of this data. The CiPCA data custodian, Mr James Bailey, collated the necessary data from the CiPCA, using the Read codes I supplied. Professor Kelvin Jordan cleaned the data and presented it in a useable dataset. I then recoded some of the data to form new variables and completed all of the statistical analysis, which was checked for quality by Professor Kelvin Jordan.

All of the findings, interpretations and conclusions within this research are my own.

Abstract

Background and Aims: A woman's cardiovascular and metabolic systems undergo considerable adaptations during pregnancy, which can affect a woman's physiology long term. This research aimed to investigate whether parity increases the risk of future cardiovascular disease (CVD).

Methods: The systematic review identified cohort and case-control studies assessing the relationship between parity and morbidity and/or mortality from coronary heart disease (CHD) and stroke. Two separate meta-analyses for the outcomes of CHD and stroke were performed. The cohort study was conducted using data from general practices across North Staffordshire, contained within the Consultations in Primary Care Archive (CiPCA). Due to the short follow up time available from the database, the study was conducted as a feasibility study, to test the potential methods for future research using electronic health records.

Results: The systematic review included 18 studies (2,869,391 participants), comprised of 13 cohort and 5 case-control studies. The adjusted ever parous versus nulliparous risk estimate from the cohort studies showed no association between parity and risk of CHD or stroke. However, cohort studies with a longer follow up and the case-control studies were more likely to find an increased risk in ever parous women. In the parity level analysis, the risk of CHD and stroke was not equivalent for each parity level, with para 5+ women having a statistically significant increased risk of stroke (risk ratio 1.21 (95% CI 1.06 – 1.39), after adjustment for several CVD risk factors. The risk of CHD was also increased in para 5+ women, however, after adjustment this estimate did not reach statistical significance. The CiPCA cohort study comprised 20,513 women, aged 15-45 years at baseline, with a median follow up length of 3.8 years. No association between parity and risk of future composite CVD was found.

Conclusion: Parity may be considered as a risk factor for CVD during CVD risk assessment by healthcare professionals. Grand multiparous women should be informed of their increased cardiovascular risk to encourage healthy lifestyle behaviours. Further research is needed to assess the association of parity with CVD with a longer follow up of participants.

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List of Abbreviations

ACS	Acute Coronary Syndrome
AF	Atrial Fibrillation
AMSTAR	A MeaSurement Tool to Assess systematic Reviews
AW	Ashleigh Woodland
BHF	British Heart Foundation
BMI	Body Mass Index
BP	Blood Pressure
CHD	Coronary Heart Disease
CIMT	Carotid Intima-Media Thickness
CINAHL	Cumulative Index to Nursing and Allied Literature
CIPCA	Consultations in Primary Care Archive
CPRD	Clinical Practice Research Datalink
CVA	Cerebrovascular Accident
CVD	Cardiovascular Disease
DALY	Disability Adjusted Life Year
DM	Diabetes Mellitus
EHR	Electronic Health Records
GBD	Global Burden of Disease
GDM	Gestational Diabetes Mellitus
GP	General Practice
HDL	High Density Lipoprotein
HR	Hazard Ratio
HRT	Hormone Replacement Therapy
IMD	Index of Multiple Deprivation
KJ	Professor Kelvin Jordan
LDL	Low Density Lipoprotein
MI	Myocardial Infarction
MOOSE	Meta-analysis of Observational Studies in Epidemiology

NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NOS	Newcastle-Ottawa Scale
ONS	Office of National Statistics
OR	Odds Ratio
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PVD	Peripheral Vascular Disease
PW	Dr Pensée Wu
QOF	Quality and Outcomes Framework
RevMan5	Review Manager Version 5.3
RR	Risk Ratio
SD	Standard Deviation
T2DM	Type 2 Diabetes Mellitus
TIA	Transient Ischaemic Attack
WHO	World Health Organisation
UK	United Kingdom
US	United States

1 Introduction

This research explores the association of parity with cardiovascular disease (CVD) and was conducted as part of an MPhil degree during an intercalated year of the author's undergraduate medical training, at Keele University. This chapter gives a brief rationale for this research on the association of parity with CVD, presents the aims and objectives for the research and provides a summary of the thesis.

1.1 Rationale

CVD is the leading cause of mortality worldwide and coronary heart disease (CHD) and stroke cause 85% of these CVD deaths (GBD 2016 Mortality Contributors, 2017). These diseases pose a substantial burden not only on individuals but also on society due to the cost of caring for patients and working days lost. Substantial research has focussed on the risk factors for CVD, which can be used to reduce an individual's risk of disease. This research has found that the established risk factors for CVD, such as smoking and obesity are not responsible for all of the CVD incidence (Newton *et al.*, 2015). Further research is now concentrating on other potential risk factors for CVD, including life experiences which may be attributed with the unexplained incidence in CVD. Pregnancy causes considerable changes in a woman's physiology, especially the cardiovascular and metabolic systems (Blackburn, 2017). These changes have been shown to affect the cardiovascular system long term, which may increase the risk of CVD (Aggarwal *et al.*, 2017; Harris *et al.*, 2018). As 82% of women in the United Kingdom (UK) have at least one child (Office for National Statistics, 2017b), it would be beneficial to determine the relationship between parity and CVD. As highlighted in chapter 2, a systematic review by Lv *et al.* (2015) attempted to synthesise the published research on this relationship, with conflicting results from individual studies. However, this review did not include morbidity from CVD (only mortality) and only investigated composite CVD. This did not account for the large proportion of CVD burden caused by CHD and stroke specifically and their potentially different relationship with parity. It is therefore necessary to evaluate the risk of parity

for CHD and stroke morbidity and mortality to reflect the true incidence of these diseases. Knowledge of any association would allow clinicians and women to evaluate their risk of CVD and act on this accordingly through lifestyle changes or medical intervention. This would in turn lower the incidence rate of these conditions.

1.2 Aims and Objectives

The aim of this thesis was to explore the relationship between parity and future CVD and the specific research question addressed was: is parity a risk factor for CVD? To work towards this overall aim, several research objectives, set out at the beginning of the research, were achieved. These objectives were as follows:

1) Evaluate, through a systematic review, the current evidence on the relationship between parity and future morbidity and mortality from CHD and stroke.

2) Assess how this relationship varies between CHD and stroke.

3) Explore the relationship between parity and cardiovascular disease in the local population of North Staffordshire using routinely recorded primary care data and assess the feasibility of using such data to investigate this relationship.

As is explained in the background chapter (see chapter 2), CHD and stroke are responsible for the largest burden on society out of all the CVDs. Therefore, objectives 1 and 2, which related to the systematic review, focussed on the risk of CHD and stroke with parity, rather than the broader term of CVD. As the cohort study, set within a local primary care database, was likely to have a small number of outcomes, objective 3 addressed a primary outcome of composite CVD risk as well as secondary outcomes of: myocardial infarction (MI), CHD, stroke and type 2 diabetes mellitus (T2DM).

1.3 Summary of Thesis

This thesis consists of seven chapters, the first of which is this introductory chapter.

Chapter 2 gives an in-depth background into both parity as an exposure and CVD and its known risk factors. The chapter also summarises the current research which has attempted to ascertain the links between parity and CVD. The previous systematic reviews (Rich-Edwards *et al.*, 2014, Lv *et al.*, 2015) assessing the relationship between parity and CVD risk are critically appraised. Chapter 3 explains the benefits of systematic reviews within research and describes the methods used to conduct the systematic review and meta-analysis of the current literature investigating the relationship between parity and CVD risk. Chapter 4 presents the results of the systematic review and discusses the notable findings. Chapter 5 explains the advantages of using primary care data for research and describes the methods used to conduct the cohort study outlined in objective 3. Chapter 6 displays the results of the cohort study and discusses the notable findings. Chapter 7 combines the results of the three objectives and compares them to the current published literature and guidelines for the prevention of CVD. The potential mechanisms for the link between parity and CVD risk are explored and a conclusion is drawn as to whether parity is a risk factor for CVD. Finally, the implications of this research on future research and clinical practice are considered.

2 Background

This chapter will expand upon the brief synopsis of the association of parity with cardiovascular disease (CVD) given in the introduction chapter. First the prevalence of the exposure of parity will be described as well as the outcome of CVD. The potential biological mechanisms behind the relationship will be described alongside justification for conducting research on this relationship. The previous research on this topic will be explored and the resultant overall question of this research explained.

2.1 Trends in Fertility

Since the introduction of the contraceptive pill in 1961 in the United Kingdom (UK), under the National Health Service (NHS), women have benefited from increasing control over their reproductive habits (NHS Choices, 2018). Women are now able to approximately plan when they become pregnant and how many children they have in total. This has led to changing fertility trends and societal ideals throughout the past half century, with women often postponing their childbearing in favour of further education and career progression. This is visible in the birth statistics for the UK, with the total fertility rate, in 2016 decreasing to 1.81 children per woman, compared to 2.93 in 1964 (Office for National Statistics, 2017a). The total fertility rate is defined as the average number of live births every woman in the population would have if she was exposed to the same age specific fertility rate, of the year in question, in this example 2016, for all of her childbearing years (Office for National Statistics, 2017a). Also, since 2005, most babies are born each year to women aged 30-34 years, whereas previously the most common age for giving birth was 25-29 years (Office for National Statistics, 2017a). Furthermore, even with advances in fertility treatment under the NHS, the number of women who do not reproduce is increasing, meaning more women are choosing to not have children. In 2016, the 1971 birth cohort turned 45 years old and have mostly completed their reproductive years. Of these women, 18% were childless,

compared to only 11% of their mothers in the previous generation (Office for National Statistics, 2017b).

Advances in both contraception and gender equality within British culture, have resulted in the growing attitude that pregnancy is not inevitable during a woman's lifetime. This development of choice for woman is facilitated by research into the effects of pregnancy long term. This research can be explained to women during family planning, to inform them of the potential effects childbearing may have on their future health. Furthermore, this knowledge can also be used to monitor a woman's health for primary prevention of diseases following pregnancy, throughout life.

2.2 Parity Definition

When a woman is pregnant it is necessary to know how many times she has been pregnant previously and what happened in these prior pregnancies, as the management of subsequent pregnancies may vary based on this information. For example, it is expected that a woman's first labour will be slower than the subsequent pregnancies. Therefore, if the first stage of labour is delayed in a subsequent pregnancy, a full assessment must be made by an obstetrician, as this can indicate an obstructed labour (National Institute for Health and Care Excellence, 2014). It is important to be able to convey this knowledge consistently and clearly to other healthcare professionals. Therefore, specific terms for example; parity, nulliparity and multiparity are used to explain a woman's obstetric history.

Parity describes the number of times a woman has delivered a live birth or stillbirth, after 24 weeks gestation (Creinin and Simhan, 2009; Symonds and Arulkumaran, 2013; Impey and Child, 2017). For example, a woman who has been pregnant once and delivered one live baby will have a parity of one. A woman who has been pregnant once and delivered twins would have a parity of two, while a woman who has been pregnant twice and delivered one live birth after each pregnancy would also have a parity of two. The description of parity level is often shortened into 'para' followed by

the number of potential live births, which in this case is para 2 (Creinin and Simhan, 2009; Symonds and Arulkumaran, 2013; Impey and Child, 2017).

The term nulliparity or nulliparous is used to describe a woman who has never delivered a live birth or stillbirth (Impey and Child, 2017). This includes women who have had a miscarriage before 24 weeks gestation and no live birth or stillbirth from another pregnancy. In this case a woman's parity would be zero or para 0. Conversely, multiparity or multiparous describes a woman who has delivered two or more live births or stillbirths, after 24 weeks gestation (Impey and Child, 2017). This is represented by para 2 or more. Finally, grand multiparity or grand multiparous describes a woman who has delivered five or more times, recorded as para 5+. These definitions were all discussed with and approved by a consultant obstetrician, Dr Pensée Wu (PW).

2.3 Parity as a Risk Factor

It is necessary to research the consequences of normal pregnancy on a woman's physiology and long-term health, as the majority of women in the UK, 82%, deliver at least one child in their lifetime (Office for National Statistics, 2017b), therefore a large number of women are exposed to the potential risks or benefits of pregnancy. Pregnancy can be considered as a stress test on a woman's body and any complications which occur can be indicators of future ill health. For example, the occurrence of several obstetric conditions including, preterm birth, low birthweight, gestational diabetes mellitus (GDM) and pre-eclampsia have been shown to increase the risk of type 2 diabetes mellitus (T2DM) and CVD in the future (Hauspurg *et al.*, 2018). For example, a systematic review found that a history of pre-eclampsia, doubled the risk of coronary heart disease (CHD) and stroke (Wu et al., 2017). Both GDM, which is glucose intolerance due to insulin resistance beginning in pregnancy, and pre-eclampsia which is high blood pressure alongside multiorgan dysfunction during pregnancy, share risk profiles similar to that of CVD (Blackburn, 2017; Hauspurg *et al.,* 2018). However, these conditions do not affect all pregnancies, with the prevalence for each being up to 8% of pregnancies (Hauspurg *et al.,* 2018). The association of parity with future diseases has

therefore been investigated, as this exposure reflects all pregnancies and not just those with adverse outcomes.

The effect of parity on all-cause mortality has been researched with inconsistent results. A reoccurring trend in results is however seen across multiple systematic reviews and individual studies (Barclay *et al.*, 2016; Zeng *et al.*, 2016). This trend is a 'J' shaped or 'U' shaped non-linear association between parity and mortality, suggesting that nulliparous women are at a higher risk of early mortality from varying causes compared to women of lower parities, for example para 1 and 2. This association then reverses with increasing parity as women with higher parities of para 5+ have greater risk of early mortality than the women of lower parities. These results are seen for all cause and cause specific mortality including circulatory diseases (Barclay *et al.*, 2016). A systematic review of parity and future T2DM also demonstrated an increased risk of diabetes with increasing parity level (Li *et al.*, 2016)

Parity as well as other reproductive factors is associated with female cancers, with nulliparity increasing the risk of breast and endometrial cancer, while high parity is protective (Kelsey, Gammon and John, 1993; Ali, 2014). As oestrogen and progesterone are linked to the development of female cancers, this effect of parity on risk is likely to be due to a woman's lifetime exposure to these hormones. This is dependent on the number of menstrual cycles a woman has and is therefore reduced by pregnancy (Kelsey, Gammon and John, 1993; Barclay *et al.*, 2016). On the other hand, high parity has been shown to increase the risk of cervical cancer (Grundy and Kravdal, 2010)

2.4 Cardiovascular Disease

CVD is an umbrella term for a plethora of conditions which affect the heart and blood vessels and is the largest cause of mortality worldwide (GBD 2016 Mortality Contributors, 2017). Although each of the individual diseases have specific development pathways, symptoms and treatment, the dominating pathophysiological cause is atherosclerosis.

Atherosclerosis is a progressive condition which begins in infancy and develops for decades before the onset of associated symptoms, which will be discussed later in this section (Mallika, Goswami and Rajappa, 2007). The process is initiated by damage to the inside of blood vessels, the endothelium, which could be from high pressure flow of blood, known as hypertension, or chemical irritants or toxins, for example; tobacco smoke (Mallika, Goswami and Rajappa, 2007). In response to the damage, there is an influx of inflammatory cells through the endothelium, into the intima and media layers of the blood vessel wall. This results in the deposition of collagen and lipids, predominantly cholesterol, into these layers which over time develops into a fatty plaque with a fibrous cap (Grech, 2003; Mallika, Goswami and Rajappa, 2007).

Atherosclerotic plaques cause CVD by reducing blood flow to the downstream tissues, which become deprived of oxygen and undergo ischaemia. Ischaemia is the process of cells converting to anaerobic metabolism due to the lack of oxygen, which results in the accumulation of metabolic waste products (Rhee, Sabatine and Lilly, 2011). If the oxygen supply is not reinstated, an area of irreversible cell death, called infarction, will develop. This will inhibit the optimal function of the organ long term (Rhee, Sabatine and Lilly, 2011).

Strom and Libby (2011) present five mechanisms of atherosclerosis consequences which can lead to CVD. Firstly, the plaques narrow the inside of the blood vessel where the blood flows, called the lumen, and subsequently impede blood flow. As well as this fixed vessel narrowing, the damaged endothelium surrounding the plaque is unable to maintain normal physiological control. This endothelial dysfunction results in vasoconstriction or spasm of the arteries, which further reduces blood flow to the tissues. Secondly, the plaques may rupture leading to blood clot formation (thrombosis) which significantly reduces the vessel lumen, if not occluding it entirely. Thirdly, the microvessels inside the plaque can burst causing intraplaque haemorrhage which rapidly expands the size of the plaque and therefore impedes adjacent blood flow. Fourthly, small fragments of the plaque (emboli) can break off into the circulation and become lodged in smaller blood vessels

downstream, therefore occluding the vessel and preventing blood flow. Finally, the inflammatory process of atherosclerosis weakens the blood vessel walls which leads to aneurysm development, where the vessel walls balloon out into pockets, which are susceptible to rupture and clot formation. Figure 2.1 displays the five consequences of atherosclerosis described by Strom and Libby (2011).

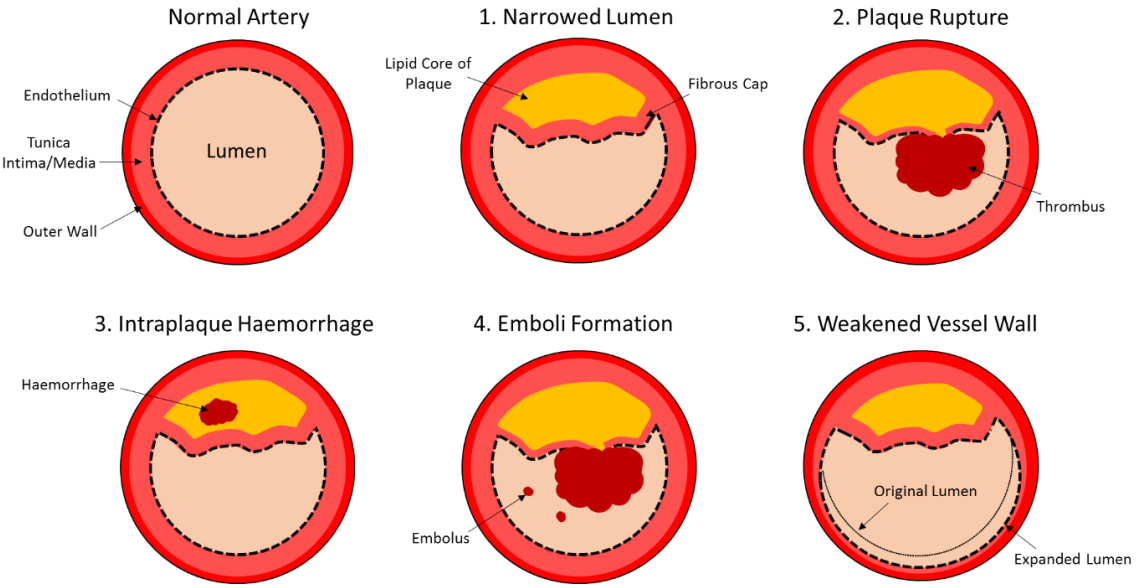

Figure 2.1 Consequences of atherosclerosis within an artery.

These mechanisms occur at different sites of the cardiovascular system, meaning the diseases contained within the umbrella term CVD have diverse disease processes (Strom and Libby, 2011) These atherosclerotic CVDs are; Coronary Heart Disease (CHD), Cerebrovascular Disease (CeVD) and Peripheral Vascular Disease (PVD) (Strom and Libby, 2011).

CHD, also known as ischaemic heart disease, is a condition characterised by atherosclerosis in the coronary arteries, which are the blood vessels surrounding the heart (Grech, 2003; Libby and Theroux, 2005). The plaques can impede the blood flow to the heart muscle (myocardium). The subsequent ischaemia or infarction of the myocardium causes severe pain in the chest and results in an impaired cardiac output (Libby and Theroux, 2005). CHD has two distinct manifestations; angina, which is the repeated onset of reversible pain from myocardial ischaemia during physical

exertion, and myocardial infarction (MI) which is an acute life-threatening event characterised by irreversible pain due to the occlusion of a coronary artery by a thrombus (Grech, 2003; Libby and Theroux, 2005). As illustrated in figure 2.1, the atherosclerosis consequence 1 (narrowed lumen) correlates to symptoms of angina and consequences 2 and 3 (plaque rupture and intraplaque haemorrhage) are causes of MI.

CeVD encompasses all conditions relating to the vasculature of the brain, the most prominent being stroke. Stroke is defined by the World Health Organisation (WHO) as an acute onset focal (or global) neurological deficit of vascular origin, lasting more than 24 hours (Hatano, 1976). 85% of strokes are ischaemic, meaning a reduction in the blood supply to an area of the brain, due to the occlusion of a cerebral vessel. This occurs from either an embolus from a distant atherosclerotic plaque, or a thrombus forming over a ruptured plaque in situ (Deb, Sharma and Hassan, 2010). The remaining 15% of strokes are haemorrhagic in nature, and the majority of which, 60%, are accredited to hypertension which causes an inflammatory process similar to atherosclerosis, termed hyperplastic arteriolosclerosis (Testai and Aiyagari, 2008). The hypertension induced damage instigates smooth muscle proliferation, which is replaced by collagen deposition, leading to weak vessel walls which are susceptible to rupture (Testai and Aiyagari, 2008). A transient ischaemic attack (TIA) is a condition causing the same symptoms as a stroke, however these only persist for up to 24 hours (Coupland *et al.*, 2017).

PVD is a broad term including many different pathologies which affect the arteries and veins throughout the body excluding the coronary and cerebral vessels (Ouriel, 2001; Liang and Creager, 2011). Venous disease, for example varicose veins, is not due to atherosclerosis, instead being caused by structural damage to the venous system, leading to venous hypertension (Eberhardt and Raffetto, 2014). The main atherosclerotic diseases affecting the peripheral vasculature are aneurysms, which were discussed earlier in section 2.4, and peripheral arterial disease. Peripheral arterial disease is characterised by plaques narrowing the lumen of any artery supplying the limbs

(Liang and Creager, 2011). The subsequent impaired blood flow to the limb leads to ischaemia of the downstream tissues upon physical exertion, causing pain in the limb which resolves with rest (Ouriel, 2001). This clinical picture is termed limb claudication and is similar in pathology to angina, which is also caused by consequence 1 (narrowed lumen) of atherosclerosis (Liang and Creager, 2011). The chronic vascular insufficiency also manifests in the skin, causing ulcer formation and death of skin tissue. Acute limb ischaemia occurs when an artery is completely occluded either by an embolus from a proximal plaque or due to thrombus formation over a ruptured plaque in situ. This presents with sudden onset severe pain in the limb which cannot be resolved without medical intervention (Ouriel, 2001; Liang and Creager, 2011).

Table 2.1 summarises the pathophysiology of the different types of CHD, Stroke and PVD using the five consequences of atherosclerosis described by Strom and Libby (2011).

Table 2.1 Pathophysiology of coronary heart disease (CHD), stroke and peripheral vascular disease (PVD) according to the consequences of atherosclerosis proposed by Strom and Libby (2011).

	Narrowed vessel lumen (1)	Plaque rupture (2)	Intraplaque haemorrhage (3)	Peripheral emboli (4)	Weakened vessel wall (5)
CHD					
Angina	*				
Myocardial Infarction		*	*		
Stroke					
Embolic				*	
Thrombotic		*	*		
Haemorrhagic					*
PVD					
Limb claudication	*				
Limb Ischaemia	*	*		*	
Aneurysm					*

2.4.1 Cardiovascular Disease Burden

Data from the Global Burden of Disease (GBD) study, which collects health data worldwide, demonstrate that CVD mortality rates have been decreasing since 1990 (Newton *et al.*, 2015; Bhatnagar *et al.*, 2016). However, CVD still poses a large burden on both individuals and society worldwide. CVD as a whole is the leading cause of mortality worldwide, with CHD and stroke

accounting for 85% of these CVD deaths (GBD 2016 Mortality Contributors, 2017). Therefore, atherosclerosis poses a significant threat to health. CVD causes 45% of all European deaths, with just under half of these due to CHD (Wilkins *et al.*, 2017). In the UK, CVD accounts for 26% of female deaths, of these 10% are due to CHD, 8% are from stroke and the remaining 8% are caused by all other CVD (Townsend *et al.*, 2015). CHD alone is the biggest individual cause of years of life lost in the UK (Townsend *et al.*, 2014; Newton *et al.*, 2015).

When examining CVD morbidity in the UK by gender, using the British Heart Foundation (BHF) CVD Statistics from 2013/14 (Townsend *et al.,* 2015), the incidence is greater in men compared to women, with CVD accounting for 10% of hospital admissions in men and 6.2% in women. The prevalence of stroke and CHD in women in the UK is 2.1% and 3.5%, respectively. CeVD and CHD were also in the top 3 causes of disability adjusted life years (DALYs) in the UK in 2013 (Newton *et al.,* 2015). A DALY is defined as a year of healthy life lost and is the sum of the number of years of life lost due to premature death from a condition and years lived with a disability due to a condition (Newton *et al.,* 2015). Therefore, of all CVD, CHD and stroke present the greatest burden on health.

As well as CVD affecting the lives of individuals, there is a large economic cost imposed on society, both from working days lost due to CVD morbidity and the cost of caring for patients with CVD. In the UK, the NHS spent £8.8 billion on treating CVD in 2015, with the majority of expenditure in secondary care. This represented 5% of the total healthcare expenditure for that year (Wilkins *et al.,* 2017). Alongside this, a further £3.2 billion was spent on informal care for people with CHD and stroke in the UK in 2015. The UK also suffered production losses amounting to £6 billion in 2015, due to death and illness from CHD and stroke in those of working age (Wilkins *et al.,* 2017).

2.4.2 Cardiovascular Risk Factors

Several risk factors have been identified for CVD (Newton *et al.,* 2015) which relate to the development of atherosclerosis and are therefore consistent for both CHD and stroke (Hankey, 2006). The WHO defines a risk factor as “any factor which increases the probability of an adverse

health outcome" (World Health Organisation, 2009). The GBD study 2015 (GBD 2015 Risk Factors Collaborators, 2016) categorises these characteristics into metabolic, behavioural and environmental factors. Metabolic factors are those which are physiological, for example hypertension and high cholesterol. Behavioural factors are those relating to lifestyle, including tobacco smoking and dietary intake. The environmental factors also incorporate occupational risks and include air pollution and unsafe drinking water (GBD 2015 Risk Factors Collaborators, 2016). In high income countries, where poor sanitation is uncommon, environmental risk factors attribute only a small proportion of DALYs (Newton *et al.,* 2015). The GBD 2015 report does not include the risks of increasing age, and family history of CVD which are key risk factors but cannot be modified. These non-modifiable risks must be considered when evaluating an individual's risk of CVD. However, as they cannot be incorporated into public health promotion they are not further discussed in detail.

The outcomes of the GBD Study 2015 (GBD 2015 Risk Factors Collaborators, 2016) demonstrate that the total number of DALYs from all-cause morbidity and mortality in the UK are attributable to the following top five risk factors: smoking, hypertension, high body mass index (BMI), total cholesterol and fasting plasma glucose. As hypertension, BMI, cholesterol and fasting plasma glucose level are partly determined by diet, dietary risks are the biggest cause of DALYs in the UK. Furthermore, most DALYS are attributable to behavioural risk factors. (GBD 2015 Risk Factors Collaborators, 2016). Looking specifically at modifiable CVD risk factors in women in the UK, the biggest attributable causes of CVD are dietary risks, hypertension, high BMI, high cholesterol and smoking (Newton *et al.,* 2015). However, there are multiple interactions between these risk factors with some proven to be within the causal pathway of atherosclerosis, such as smoking, while others are indicators of increased risk ((Yusuf *et al.*, 2001; GBD 2015 Risk Factors Collaborators, 2016)). For example, poor diet, meaning a diet high in fat and salt and low in fruits and vegetables, is a predisposing factor to high cholesterol and hypertension (Wilkins *et al.,* 2017; Townsend *et al.,* 2014; GBD 2015 Risk Factors Collaborators, 2016). Poor diet is estimated to account for half of all

hypertension cases and is therefore a predisposing factor to CVD by inducing hypertension (Townsend *et al.,* 2014; Yusuf *et al.,* 2001).

Another predisposing risk factor for CVD is socioeconomic status (Mackenbach *et al.*, 2000; Yusuf *et al.*, 2001; Cubbin *et al.*, 2006). There are clear trends in health inequality based on socioeconomic status which are presented by Public Health England in the 2017 report into inequality in health (Public Health England, 2017a). This research revealed that women in the most deprived areas in England endure a life expectancy 9 years shorter than in the least deprived areas, with 24% of this gap attributable to CVD (Public Health England, 2017a). The prevalences of behavioural risk factors, such as smoking and dietary risks, also increase proportionally to the amount of deprivation. Low socioeconomic status is therefore a risk factor for CVD due to its presence indicating an increased likelihood of behavioural risk factors. Furthermore, the rate of premature mortality from CVD is 3.5 times higher in the most deprived compared to the least deprived (Public Health England, 2017a).

Table 2.2 lists the traditional modifiable risk factors for CVD categorised into behavioural and metabolic factors.

Table 2.2 List of modifiable risk factors for cardiovascular disease grouped into behavioural and metabolic factors.

Behavioural Risk Factors	Metabolic Risk Factors
Tobacco Smoking	Total Cholesterol
Low Physical Activity	High Low Density Lipoproteins
High Body Mass Index	Low High Density Lipoproteins
Diet	Hypertension
	Elevated Fasting Plasma Glucose
	Carotid Stenosis

As well as these traditional risk factors, there are other proposed factors. These are termed novel risk markers and are based on an extensive list of genetic, inflammatory, and haematological markers, among others (Hankey, 2006; Strom and Libby, 2011). Research is being conducted to determine if these biomarkers are risk factors for CVD or if they present new methods of measuring atherosclerosis progression and targets for future risk reducing therapies (Strom and Libby, 2011).

However, there is limited evidence to confirm that these biomarkers are risk factors for CVD (Hankey, 2006; Strom and Libby, 2011) and will therefore not be discussed in this thesis.

There is a dynamic interplay between all of the risk factors mentioned above (GBD 2015 Risk Factors Collaborators, 2016) and it is beyond the scope of this thesis to determine the exact causal pathway between each risk factor and CVD. The following paragraphs give a brief synopsis of the modifiable risk factors most pertinent to the research within this thesis.

2.4.2.1 Dietary Risks

A systematic review by Mente *et al.* (2009) evaluated the evidence of certain dietary components increasing the risk of CVD. Strong evidence was found indicating that high dietary intake of fat increases the risk of atherosclerosis, while high salt intake increases the risk of hypertension. Conversely, high intake of vegetables and a 'Mediterranean' style diet are protective against CVD (Mente *et al.*, 2009). The Mediterranean diet consists of olive oil, high levels of plant based foods including nuts and vegetables, with low to moderate levels of animal products including dairy and meat (Salas-Salvadó *et al.*, 2018). A narrative synthesis of several meta-analyses identified a reduced risk of both CHD and stroke in those who adhered to the Mediterranean diet (Salas-Salvado *et al.,* 2018). This may be due to the reduction in low density lipoproteins (LDLs) and body weight which was associated with adherence to this diet (Salas-Salvado *et al.,* 2018). A recent review of the evidence relating to dietary management of high blood pressure, found that a reduction in salt intake and adherence to a Mediterranean style diet reduced blood pressure in a dose response progression (Ozemek *et al.*, 2018). Elements of the Mediterranean diet have therefore been incorporated into national guidelines, including those for the UK (Khanji *et al.*, 2018). Multiple guidelines recommend that a healthy diet includes five portions of fruit or vegetables a day and less than 6g of salt. However, according to data collated by the BHF 2014 report, less than a third of adults in the UK meet the advised quota of five fruit and vegetable portions a day and 70% of the population exceeded the recommended level of salt intake. Therefore, dietary risks are still highly

prevalent in the population and contribute the most to CVD DALYs (GBD 2015 Risk Factors Collaborators, 2016).

2.4.2.2 Hypertension

Hypertension is defined by the National Institute for Health and Care Excellence (NICE) Guidelines as a blood pressure over 140/90mmHg (NICE, 2016). It is a common condition affecting 14.5% of women in the UK (Townsend *et al.,* 2014) with estimates of lifetime prevalence reaching 90% for people over 55 years (Lee, Williams and Lilly, 2011). The cause of hypertension is unknown in approximately 90% of cases, however targeting obesity and poor diet, as discussed above, has been shown to reduce blood pressure (Geleijnse, Grobbee and Kok, 2005). These risk factors must therefore contribute to the development of hypertension. Hypertension has many detrimental effects on the cardiovascular system among other organs, for example, kidneys, by weakening blood vessel walls and accelerating atherosclerosis progression (Lee, Williams and Lilly, 2011). This damage leads to CVD as previously explained in section 2.4 and contributes to hypertension being a major risk factor for both CHD and stroke (Wilkins *et al.,* 2017).

2.4.2.3 High Body Mass Index

BMI is a measure of adiposity and is calculated by dividing a person's weight in kilograms (kg) by their height squared in meters (m^2) (World Health Organisation, 2018) The scores of BMI are categorised into: underweight <18.5 kg/m^2, ideal 18.5-24.9 kg/m^2, overweight 25-29.9 kg/m^2 and obese >30 kg/m^2 (WHO, 2018). A high BMI describes those falling within the overweight and obese categories. Overweight and obesity are caused by an energy intake which exceeds the energy expenditure, causing energy to be stored as fat (Wilkins *et al.,* 2017). A high BMI is therefore associated with poor diet, high cholesterol and low physical activity, which are also risk factors for CVD (Wilkins *et al.,* 2017). Research beginning with the Framingham Heart Study (Hubert *et al.*, 1983) has proven that obesity is an independent risk factor for CVD, irrespective of the associated risk factors. While the prevalence of most CVD risk factors is declining worldwide due to health

promotion and preventative healthcare, there is a pandemic of obesity across western countries (Wilkins *et al.,* 2017). The prevalence of overweight and obesity in the UK has steadily increased, with the national average BMI of 23.4 kg/m^2 in 1975 increasing to 27 kg/m^2 in 2016, with 28% of females being obese in 2016 (WHO, 2017).

2.4.2.4 High Cholesterol

Cholesterol is a molecule produced by the liver from dietary fat, which is transported in the blood by lipoproteins. The two types of lipoproteins commonly recorded in medical practice are: low density lipoproteins (LDLs), which can build up in the intima of blood vessels, perpetuating atherosclerosis development and high density lipoproteins (HDLs) which transport cholesterol away from the peripheries back to the liver for disposal (Strom and Libby, 2011). Due to these distinct differences in function, LDLs are termed 'bad cholesterol' and the levels of this should be low, while HDLs are termed 'good cholesterol' and should be at higher levels (Strom and Libby, 2011; Wilkins *et al.*, 2017). Due to the increasing prevalence of obesity alongside a diet with increasing fat content and low physical activity levels, 57% of women in the UK in 2011 had elevated total cholesterol levels (Townsend *et al.,* 2014).

2.4.2.5 Tobacco Smoking

Cigarette smoking is a major cause of mortality worldwide, with 14% of CVD deaths attributable to smoking in the UK in 2013 (Townsend *et al.,* 2014). Smoking accelerates atherosclerosis at all stages, by causing inflammation in the intima of blood vessels, increasing the oxidation of LDLs and promoting a prothrombotic state in the blood (Ambrose and Barua, 2004; Strom and Libby, 2011). Many studies including the large Framingham Heart Study (Mamun *et al.*, 2004) and the INTERHEART Study (Teo *et al.*, 2006) have reported statistically significantly increased risks of CHD and stroke in smokers compared to non-smokers. The INTERHEART Study found that the increased risk in smokers reduced from an odds ratio (OR) of 2.95 (95% Confidence Interval (CI) 2.77-3.14) to 1.87 (95% CI 1.55-2.24) after three years of smoking cessation. Following this, public health

campaigns have succeeded in reducing the prevalence of smoking by half, with 18% of women in the UK smoking regularly in 2011 compared to 41% in 1974 (Townsend *et al.,* 2014).

2.4.2.6 Elevated Fasting Plasma Glucose

T2DM is a condition characterised by high plasma glucose due to insufficient insulin production from β islet cells in the pancreas, which causes the uptake of glucose into cells (Kahn, Cooper and Del Prato, 2014). Insulin resistance in these tissues also occurs and is caused in part by hormones released from adipocytes, which store fat (Kahn, Cooper and Del Prato, 2014). Therefore, there has been an increase in T2DM incidence alongside the increasing prevalence of obesity (Wilkins *et al.*, 2017). As well as being associated with obesity, T2DM is an independent risk factor for CVD. This is because raised plasma glucose levels irritate the endothelium of blood vessels, thereby catalysing atherosclerosis (Strom and Libby, 2011). It is estimated that elevated plasma glucose causes 15% of CVD deaths in Europe (Wilkins *et al.,* 2017). In 2014, the UK female age standardised prevalence of T2DM was 4.9% of the population (Wilkins *et al.,* 2017).

2.4.2.7 Carotid Stenosis

Carotid stenosis occurs due to the development of atherosclerotic plaques in the carotid artery which is the main artery supplying the head and neck. A common cause of ischaemic stroke is from embolization of small plaque fragments within the carotid artery which occlude cerebral vessels downstream (Deb, Sharma and Hassan, 2010). The risk of both CHD and stroke increases proportionally to the thickness of the intima media layers in the carotid artery, known as the carotid intima-media thickness (CIMT) (O'Leary *et al.*, 1999). After adjustment for other CVD risk factors the increased risk of a high CIMT remained, meaning that carotid stenosis is an independent risk factor for CVD (O'Leary *et al.*, 1999).

2.4.2.8 Oestrogen Exposure

The female sex hormone oestrogen has been shown to elevate HDL and lower LDL levels, improve endothelial function and reduce insulin resistance (Gerval and Stevenson, 2017). Therefore, the

incidence of CVD is much lower in premenopausal women compared to men of the same age, however after menopause the incidence is similar for both sexes (Strom and Libby, 2011; Gerval and Stevenson, 2017). This suggests that the oestrogen deficiency which occurs after the menopause is a risk factor for CVD. Giving low dose oestrogen replacement to postmenopausal women has been shown to be beneficial in reducing the risk of CVD in these women (Gerval and Stevenson, 2017).

2.4.2.9 Attributable Risk

Despite in-depth research into the traditional risk factors and novel biomarkers for CVD, the GBD 2013 Study found that the attributable burden of these known risk factors only accounted for 83.9% of CVD DALYs (Newton *et al.,* 2015). Therefore, there is a requirement for ongoing research into exposures such as parity, to identify any further risk factors for CVD, the knowledge of which can inform public health initiatives to reduce the risk of CVD at both global and individual levels.

The increased risk of CVD associated with obstetric complications, such as pre-eclampsia is not accounted for in the GBD studies, and therefore will represent some of these unexplained CVD DALYs. It is also therefore difficult to compare the attributable risk of the obstetric complications and the traditional risk factors. However, growing evidence demonstrates that these obstetric complications increase the risk of CVD, and should therefore be considered as major risk factors, particularly GDM and pre-eclampsia which lead to hypertension and a high fasting plasma glucose (Wu *et al.,* 2017; Hauspurg *et al.*, 2018). Although, this research aims to determine if parity is a risk factor for CVD, the associated I ncreased risk, if any, is likely to be small compared to that of the known traditional risk factors and obstetric complications.

2.5 How pregnancy might lead to CVD

There are several biological mechanisms which underpin the theory of parity being a risk factor for CVD. These focus on the drastic changes to the anatomy and physiology of the cardiovascular and metabolic systems during pregnancy. These adaptations are necessary to accommodate the

developing foetus and prepare the mother and baby for labour. However, some studies have shown that these adaptations during pregnancy progress into CVD risk factors later in life. These risk factors are: increased blood pressure, reduced insulin sensitivity, and increased lipid levels during pregnancy. All of which are mediated by the sex hormones oestrogen and progesterone.

2.5.1 Cardiovascular System

The major maternal adaptation in the cardiovascular system is the steady increase in cardiac output throughout pregnancy which reaches a peak increase of 40-50% in the third trimester. This is to ensure the placenta receives enough blood to nourish the fetus. Cardiac output is determined by the stroke volume, which is the volume of blood forced out of the left ventricle per cardiac cycle, and the heart rate, which is the number of heart beats per minute (Thornburg *et al.*, 2000; Tan and Tan, 2013; Sanghavi and Rutherford, 2014).

The equation is as follows: Cardiac output = stroke volume x heart rate

In order for the cardiac output to increase so drastically, there are vast changes throughout the cardiovascular system as pregnancy progresses.

Firstly, the pregnancy hormones oestrogen and progesterone cause the release of renin from the kidneys which activates the Renin-Angiotensin-Aldosterone System. This pathway ultimately leads to the retention of salt and water in the kidneys, which increases the amount of fluid in the blood (Heidemann and McClure, 2003; Sanghavi and Rutherford, 2014). The fluid component of blood is called plasma, and therefore this physiological process results in an approximate 45% increase in plasma volume. With the increased plasma volume there is more blood reaching the heart from the systemic circulation. To accommodate this, the maternal heart undergoes remodelling during pregnancy. The heart is lifted up and rotated forwards, the chambers are dilated, and the muscular walls double in thickness (Tan and Tan, 2013; Sanghavi and Rutherford, 2014). These anatomical changes allow the heart to hold a greater volume of blood at the end of the filling phase of the cardiac cycle, a term called end-diastolic volume. As well as the increased end-diastolic volume the

heart increases its muscle mass to force the blood into the circulation (Tan and Tan, 2013). These two adaptations lead to an increase in stroke volume.

Also, oestrogen and progesterone relax and dilate the systemic blood vessels, causing a decrease in peripheral vascular resistance. The resistance decreases by approximately 40% of the baseline, with the majority of the change occurring in the first trimester (Sanghavi and Rutherford 2014; Tan and Tan, 2013). Due to the reduced resistance the heart is able to pump more blood out into the aorta, therefore increasing the stroke volume further. Overall, the stroke volume increases by 30% during the first and second trimester of pregnancy and is the main factor which contributes to the increased cardiac output (Heidemann and McClure, 2003).

The systemic vascular dilatation leads to a drop in blood pressure of up to 10 mmHg which reaches its lowest point in the second trimester. The reduced blood pressure causes a reflex tachycardia, which means an increased heart rate, which contributes to the increasing cardiac output. Oestrogen itself also acts directly on the heart to increase the heart rate. The heart rate increases by 25% of the baseline rate which equates to an extra 10-20 beats per minute. The heart rate progressively increases throughout pregnancy reaching its maximum in the third trimester (Heidemann and McClure, 2003; Sanghavi and Rutherford, 2014).

These cardiovascular adaptations are exaggerated in women with a multiple pregnancy, meaning a pregnancy with more than one fetus, for example twins (Tan and Tan, 2013). The cardiac output of women with twins is 15-20% higher than women with a singleton pregnancy due to a higher stroke volume and heart rate. The cardiac remodelling is also more pronounced (Tan and Tan, 2013; Sanghavi and Rutherford, 2014).

The cardiovascular changes which occur are summarised in figure 2.2 below:

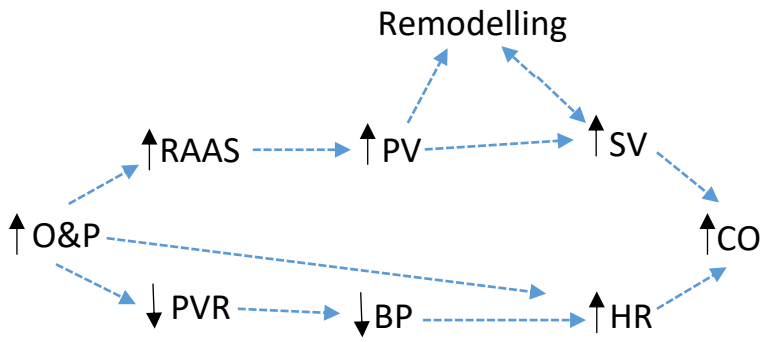

Figure 2.2 Cardiovascular adaptations during pregnancy which achieve an increased cardiac output.

Key: CO; Cardiac Output, SV; Stroke Volume, HR; Heart Rate, PV; Plasma Volume, PVR; Peripheral Vascular Resistance, BP; Blood Pressure, RAAS; Renin-Angiotensin-Aldosterone System, O&P; Oestrogen & Progesterone.

The evidence suggests that these adaptations have long lasting effects on the cardiovascular system and that these effects become more prominent with increasing parity. Firstly, Clapp and Capeless (1997) found that the increase in stroke volume and end diastolic volume during pregnancy is greater in a woman's second pregnancy compared to her first. Demonstrating that a woman's cardiovascular system will undergo more strain with each subsequent pregnancy. Most recently, Harris *et al.* (2018) measured a number of anatomical and physiological parameters within the hearts of 3,019 women and found significant associations between parity and longer cardiac cycles, increased left ventricular end volumes and left ventricular mass. These findings demonstrate that the heart is affected in numerous ways by repeated pregnancies. More specifically Keskin *et al.* (2017) and Aggarwal *et al.* (2017) assessed left ventricular diastolic dysfunction in women using echocardiography. It is hypothesised that cardiofibrosis occurs in the cardiac remodelling process of pregnancy, which can cause ventricular dysfunction in the future. Both studies discovered that grand multiparous women had significantly higher rates of ventricular dysfunction than nulliparous women, with 81% of grand multiparous women showing ventricular dysfunction compared to 46% of nulliparous women (p <0.01) (Aggarwal *et al.,* 2017). Although, slightly higher rates of ventricular dysfunction were found in women from lower parity levels, these were not statistically significant,

suggesting that with each repeated pregnancy the long-term effects on the cardiovascular system are increased. However, both studies were small, with less than 2000 participants combined and both study samples were not generalisable. The study by Keskin *et al.* (2017) only included women with a clinical indication for echocardiogram, and therefore the study sample does not represent the normal, healthy population. Whereas Aggarwal *et al.* only included Hispanic and Latino women. Although Keskin *et al.* explain that women with certain previous medical conditions, including pre-eclampsia were excluded, as well as those with structural heart changes, a clear description of the exclusion criteria was not presented. Aggarwal et al. did not exclude women with pre-eclampsia or other previous medical conditions but did adjust for the main CVD risk factors: age, BMI, hypertension, diabetes mellitus and pre-diabetes, smoking status, cholesterol level, education and income. Keskin *et al.* also adjusted for age, BMI, diabetes mellitus, smoking, hypertension and hyperlipidaemia. However, neither of the studies adjusted for breastfeeding, which is known to be protective against CVD, or the duration of inter-pregnancy intervals or age at first-birth, which are both associated with an increased CVD risk. Considering these limitations, there is more research needed to examine the cardiac function in women of all parity levels to determine if multiparty increases the risk of CVD. Preferably future studies will take repeated measurements from the same women, after recurrent pregnancies, to give a clearer picture of the reversibility of the cardiac adaptations during pregnancy as well as in the effect on the cardiovascular system long term.

As well as the studies assessing pregnancy cardiovascular changes, studies have shown that an increased heart rate in the general population is associated with higher blood pressure and therefore a greater risk of CVD (Palatini and Julius, 1997).

As well as the heart being affected by the maternal adaptations of pregnancy, it is thought that the blood vessels are damaged due to oxidative stress, caused by increased LDLs and increased oestrogen levels (Dhawan, Brookes and Kaufman, 2004). A study by Dhawan, Brookes and Kaufman, (2004) using rats found that due to this damage, repeatedly bred rats had a reduced arterial

compliance when compared to virgin rats (p <0.05). This study suggests that the arteries of multiparous rats were stiffer and therefore more likely to lead to high blood pressure in the future, and in turn CVD. However, due to the invasive nature of the experiment these results have not been replicated in humans.

2.5.2 Metabolic System

The metabolic system undergoes physiological changes during pregnancy to balance the energy demand of both the mother and fetus. Pregnancy is characterised by two distinct metabolic phases: the anabolic phase which occurs in the first two trimesters and the catabolic phase which occurs in the third trimester (Zeng, Liu and Li, 2017). The aim of the anabolic phase is to increase maternal lipogenesis, meaning that energy is stored as fat until it is needed later in pregnancy. This is achieved by an increase in hepatic glucose production through glycogenolysis which is mirrored by an increase in insulin secretion from the pancreatic beta islet cells (Blackburn, 2017). It is thought that the increase in maternal insulin levels is the cause of the increased fat storage (Zeng, Liu and Li, 2017). The maternal adipocytes, fat cells, are more sensitive to insulin than other tissues, allowing a shift of circulating glucose into adipose tissue. This means that although there is an increase in maternal glucose supply during pregnancy the serum glucose levels are lower than pre-pregnancy values (Blackburn, 2017).

As pregnancy progresses the increase in placental hormones; specifically, oestrogen, progesterone, and human chorionic somatomammotrophin cause maternal tissues to become less sensitive to insulin (Zeng, Liu and Li, 2017). The maternal sensitivity to insulin decreases by 50-70% in pregnancy, causing a relative insulin resistance and diabetic state (Blackburn, 2017). This leads to a net flux of glucose from the mother to the fetus, to fulfil the high metabolic demands throughout the third trimester. It is the insulin resistance which is thought to be the cause of the switch to a maternal catabolic state.

The catabolic state is dominated by breakdown of lipids which were stored in adipose tissue during the anabolic stage. This lipolysis results in high levels of serum triglycerides, which are broken down into fatty acids and glycerol, as well as LDLs (Zeng, Liu and Li, 2017). These lipoproteins increase throughout gestation and the overall cholesterol levels increase by 50% by late pregnancy. As the maternal tissues are unable to uptake adequate glucose, the energy is created by fatty acid oxidation, which highlights the need for the lipid production and storage during the anabolic phase (Blackburn, 2017).

Overall pregnancy leads to weight gain due to fat storage and a diabetogenic state with hyperinsulinaemia. To counteract this lack of available glucose for the maternal tissues, lipid breakdown occurs leading to hyperlipidaemia and hypercholesterolaemia, with triglycerides increasing by 250% at term (Blackburn, 2017). As diabetes, hypercholesterolaemia and obesity are risk factors for CVD, there has been extensive research to determine if these maternal adaptations resolve completely after pregnancy or if they have long term effects on the mother's physiology.

According to Trikudanathan *et al.* (2013) there is no statistically significant association between parity and adiposity measures when adjusted for age and lifestyle behaviours, such as smoking and exercise status. The study involved 868 women who participated in the Framingham Heart Study and underwent computerised tomography to measure body composition. However, the Stockholm Pregnancy and Women's Nutrition Study (Linné *et al.*, 2003) found that increased weight gain during pregnancy, and failure to lose that weight after 1 year post-partum was an indicator of being overweight 15 years after pregnancy. Gunderson *et al.* (2004) also found that weight gain relating to pregnancy is greatest in women who were overweight before pregnancy. Therefore, even though there is no clear association of parity with adiposity, the physiological changes which occur during pregnancy appear to affect women's weight gain throughout life on an individual basis.

Studies have also shown statistically significant results in the relationship between parity and the development of atherosclerosis (Skilton *et al.*, 2010; Eren *et al.*, 2013). The CIMT of women, which

is the layer of the blood vessel where lipids are deposited, increases with each parity, irrespective of age. In contrast to this, the CIMT of men was inversely proportional to increasing number of children, suggesting that the biological mechanisms of pregnancy for women is directly linked to the atherosclerosis development (Skilton *et al.,* 2010). The cumulative effect of lipoprotein alterations, insulin resistance and oxidative stress which occurs during pregnancy, causes progressive insults on the blood vessels with each successive pregnancy, contributing to atherosclerosis development (Eren *et al.,* 2013).

The relationship between parity, in the absence of GDM, and type 2 diabetes mellitus (T2DM) has also been investigated. A large Australian study (Liu, Jorm and Banks, 2010) of 52,731 women reported that compared to nulliparous women, parous women who did not breastfeed are at an increased risk of T2DM in the future (Odds Ratio OR 1.48, 95% CI 1.26–1.73, p<0.001), however this risk is reduced by breastfeeding. Further to this, another large study by Carr *et al.* (2008) found that among women who did not reach the criteria for a GDM diagnosis, increasing glucose intolerance led to an increased risk of developing T2DM in the future. This suggests that even the normal physiological changes in insulin sensitivity during pregnancy, which do not reach pathological limits, can increase the risk of future T2DM compared to nulliparity. It could be postulated that with subsequent insults on the metabolic system from increasing parity, this risk would be increased in multiparous women when compared to nulliparous women.

2.6 Parity and Cardiovascular Disease Literature

Through a search of the database MEDLINE, two published systematic reviews synthesising the literature on the association of parity with CVD were identified (Rich-Edwards *et al.*, 2014; Lv *et al.*, 2015). The author Ashleigh Woodland (AW) evaluated these reviews using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist (Moher *et al.*, 2009) and an assessment of their quality was made using the AMSTAR 2 tool (Shea *et al.*, 2017). The PRISMA checklist (Moher *et al.*, 2009) was developed to guide professionals in appraising systematic reviews

but also to devise a standardised approach for researchers to report a systematic review and meta-analysis. The checklist consists of 27 items related to the methods, results and discussion of a systematic review which should be reported in a publication. This enables readers to draw appropriate conclusions from the research, having learned the specific characteristics of the review, for example the study population or the risk of bias from the included studies.

The AMSTAR 2 (A MeaSurement Tool to Assess systematic Reviews 2) (Shea *et al.,* 2017) is used to critically assess the quality of systematic reviews of both randomised and non-randomised studies. It has recently been updated from the original AMSTAR tool (Shea *et al.*, 2007), which has been validated for use in critically appraising systematic reviews (Shea *et al.*, 2009). The suggestions for improvement received for the original tool have been implemented in the AMSTAR 2 update. For example, more domains have been introduced related to the risk of bias from included studies and the heterogeneity of individual study results. The authors of AMSTAR 2 (Shea *et al.*, 2017) explain that the tool does not produce an overall score of quality, as this can overlook critical weaknesses in specific areas of the study methods. Instead the AMSTAR 2 generates an overall rating of confidence, which is dependent on the review's performance in seven critical domains. These domains are related to: the risk of bias from the included studies, publication bias, the comprehensiveness of the literature search and screening process, the appropriateness of the statistical analysis and whether the protocol was registered before the review began. The authors have identified these domains as those most likely to affect the validity of a review. The rating of confidence generated by the tool is on a scale from high to critically low confidence (Shea *et al.,* 2017).

The first review by Rich-Edwards *et al.* (2014) collated studies assessing the association between a number of pregnancy complications as well as parity with CVD mortality and morbidity. Because multiple exposures were assessed in this review, there was not an in-depth review of the literature focussing on parity and CVD, as more emphasis was given to pregnancy complications. Only four

studies were identified which addressed the exposure of parity and a meta-analysis was not performed. The short narrative synthesis focussed mainly on a study by Parikh *et al.* (2010). The Parikh study is the largest to date and followed up 1,332,062 Swedish women for 9.5 years starting from their 50th birthday. The large sample size allowed an in-depth analysis of CVD risk per parity level, which found a statistically significant 'J shaped' association between parity and CVD. The risk of cardiovascular mortality decreased initially inversely to parity level, with the lowest risk at two pregnancies, after which the risk increased with each subsequent pregnancy. The review (Rich-Edwards *et al.,* 2014) was not fully reported (Moher *et al.,* 2009) with only 7/27 items from the PRISMA checklist completed. There was no explanation of the selection of included studies and there was no quality appraisal. Also, three out of the four parity studies cited within the review were published before the year 2000, meaning many newer studies had not been included, which had been identified in the second systematic review published a year later (Lv *et al.,* 2015). This may be because only one database was searched for relevant studies. According to the quality assessment using the AMSTAR 2 (Shea *et al.,* 2017), the review was of critically low quality, with flaws appearing in all of the critical domains. For example, an adequate literature search was not completed, there was no assessment of the risk of bias from studies and no justification for including or excluding studies. Due to these limitations, an accurate conclusion on the relationship between parity and CVD cannot be drawn from this systematic review (Rich-Edwards *et al.,* 2014).

The second review by Lv *et al.* (2015) was more comprehensive than the Rich-Edwards *et al.* (2014) paper and completed 22/27 items on the PRISMA checklist (Moher *et al.,* 2009). The quality was also better, and was graded as a high quality review using the AMSTAR 2 tool (Shea *et al.,* 2017) with all seven critical domains satisfied. This was mainly due to the incorporation of a risk of bias assessment in the study methods, the results of which were considered when drawing conclusions from the meta-analysis output. The review included ten cohort studies evaluating the risk of cardiovascular mortality by parity level. A meta-analysis including six of these studies reporting ever parity results found a reduced risk, albeit non-statistically significant, of mortality from CVD in ever

parous women when compared to nulliparous women (Risk Ratio 0.79, 95% CI: 0.59-1.06). However, there was significant heterogeneity of results (I^2 = 90.9%; p <0.001) which the authors suggest could be due to the inconsistent adjustment for smoking among the studies. Following from this, a dose-response meta-analysis revealed a non-linear 'J shaped' association between parity and CVD mortality with the minimum risk at four pregnancies, with the risk increasing thereafter until para 10. This conflicts with the large Swedish study (Parikh *et al.*, 2010) which found the lowest risk was at two pregnancies. This may be due to the homogenous sample population in the Swedish study compared to the varied study populations used across the studies identified by Lv *et al.* (2015). Also, the cardiovascular outcomes used by Parikh *et al.* (2010) included hospitalisation as well as death from CHD and stroke whereas Lv *et al.* (2015) only evaluated studies reporting mortality as outcomes. Hence the study by Parikh *et al.* (2010) was not included in the review by Lv *et al.* (2015).

Although this systematic review was of high quality, there are several limitations to the research due to the methods and criteria utilised in the review process. Firstly, whilst cardiovascular mortality is an important outcome, as 49% of all deaths in females in Europe are due to CVD (Wilkins *et al.*, 2017), cardiovascular morbidity should not be ignored as a long term outcome of pregnancy. The GBD Study for 2013 (Newton *et al.*, 2015) rates CHD and cerebrovascular disease in the top three diseases causing most disability adjusted life years (DALYs). It is therefore also important to assess parity as a risk factor for cardiovascular morbidity as well as mortality.

Another limitation of the systematic review by Lv *et al.* (2015) is that the authors only conducted a composite meta-analysis of the included studies, without stratifying by type of CVD. As the two CVDs used as outcomes for the review; CHD and stroke, have different pathogeneses, parity may influence the risk of developing each disease differently. Performing meta-analyses for CHD and stroke separately may therefore clarify the relationship between parity and these two diseases.

Finally, the reviews by Lv *et al.* (2015) and Rich-Edwards *et al.* (2014) did not include case-control studies which also provide observational evidence and can be used to evaluate the association between parity and CVD. By only including cohort studies the authors may have excluded papers which would have contributed information to the review, given the low number of cohort studies identified.

The aforementioned limitations of both systematic reviews (Rich-Edwards *et al.*, 2014, Lv *et al.*, 2015) highlight the need to produce an updated systematic review. This review would include both cohort and case-control studies which reported outcomes of morbidity and mortality from CHD and stroke. The data from these studies could be used to conduct separate meta-analyses for the two CVDs.

2.7 Conclusion

In conclusion, there are several theories linking parity with an increased risk of future CVD. The potential biological mechanisms behind this association focus on the maternal adaptations of pregnancy and the long term effect on a woman's physiology. There has been no comprehensive synthesis of the published research evidence on the link between parity and CVD morbidity and mortality, focussing specifically on CHD and stroke, which cause the most DALYs of all CVD. As the majority of women are parous by the end of their reproductive years, it is important to establish the effect of parity on the risk of these two diseases. Research has shown that the current known risk factors for CVD are not responsible for all of the CVD DALYs. This gap in attributable risk of CVD may be in part due to parity. To address this, the aim of this research is to determine if parity is a risk factor for CVD, specifically CHD and stroke. Chapter 3 will explain the methods used to conduct the systematic review of the association between parity and CHD and stroke for this MPhil research.

3 Systematic Review on the association of parity with CVD: Methods

The previous chapter discussed the importance of conducting research on parity and cardiovascular disease (CVD) and summarised the biological mechanisms of pregnancy which are theorised to increase the risk of future CVD. The current literature was also briefly evaluated, which focussed on the two published systematic reviews addressing this relationship (Lv *et al.*, 2015 and Rich-Edwards *et al.*, 2014). Due to the limitations of these reviews that were highlighted in Chapter 2, it was necessary to complete another systematic review with separate meta-analyses for coronary heart disease (CHD) and stroke. By synthesising the relevant published research, this review will aim to determine if parity is a risk factor for CHD and stroke.

This chapter will begin by outlining the principles of a systematic review and explain the value of systematic reviews to health research. Following this, the methods used to conduct this systematic review and meta-analysis are reported and the justifications for using certain resources or methods discussed. The results of the review are reported in Chapter 4.

3.1 What is a systematic review?

Throughout the 1990's there was a shift in medical training to encourage the integration of health research into everyday clinical practice, rather than the previous reliance on the experiences of senior professionals (Rosenburg and Donald, 1995). This style of medical practice, termed 'evidence based medicine', has progressed throughout the past three decades to underpin the extensive clinical guidelines which direct modern day medicine. To enable this, the quantity of influential research being published has risen dramatically during this time. Records from the United States (US) National Library of Medicine (US National Library of Medicine, 2016) indicate that compared to the 392,354 citations added to MEDLINE in 1995, there were 869,666 new citations incorporated into the database in 2016 alone. It is therefore difficult for healthcare professionals to keep up to

date with the relevant literature. This overload of available research has fuelled the increasing requirement for systematic reviews, which summarise and appraise several studies into one article.

The Cochrane Collaboration, which was established in 1992, conducts systematic reviews of randomised controlled trials and other clinical research within health care. These Cochrane Reviews compile high quality research to guide evidence-based medical practice, thus improving the treatment and management of medical conditions (The Cochrane Collaboration, no date). Systematic reviews are also widely used within epidemiological and public health research, synthesising observational studies as well as randomised controlled trials.

The overall aim of a systematic review is to collate and summarise the available literature on a certain topic and to draw conclusions from the combined studies to answer a specific research question (Boland, Cherry and Dickson, 2014). A systematic review differs from a literature review because of the requirement to follow a predetermined method of identifying and critically appraising studies addressing a specific question. This planned method is explained in-depth within a protocol which is developed before the systematic review begins. The protocol is then adhered to by all researchers involved, throughout the progression of the review. This ensures the best quality evidence is included and reduces the risk of introducing reviewer bias during the research process.

Bias is introduced into a review when the selection criteria are ambiguous, meaning different reviewers could include or exclude studies based on their own opinions or knowledge (McDonagh *et al.*, 2013). This may inherently alter the results and conclusions of a review. For example, if a review protocol assessing the effectiveness of treatment for pneumonia, defined the population of interest as patients with pneumonia, a reviewer could include studies of patients which required hospital treatment or those who were treated in the community. As the severity of pneumonia would be greater in hospitalised patients, the outcomes of the review may suggest that the treatment was ineffective. However, if the study population was community-based patients, the

results may demonstrate an effective treatment. This process of introducing bias can occur at any stage of a review, therefore explicit directions should be written in a protocol and discussed with the entire review team to ensure everyone involved understands the planned aims and methods of the research. A systematic review is therefore considered the gold standard approach to synthesising research, especially within health research where the conclusions from such reviews may be used to update clinical guidelines.

A systematic review has a clear structure which must be observed, irrelevant of the type of study design included. The Centre for Reviews and Dissemination (2009) has published a comprehensive guideline for completing a systematic review in healthcare research, which explains the necessary stages of a review and gives advice on the different techniques available. First, the reviewers must use the research question to develop the inclusion and exclusion criteria which potential studies must fulfil to be incorporated into the review. Furthermore, a strategy is created for searching the literature to identify all relevant research. This includes the databases to be searched and key words used and may include methods for searching for grey literature and abstract submissions for conferences. Studies identified by the search are then screened using the predefined criteria and all studies satisfying these criteria are included within the review. The quality of the included studies is then assessed using a standardised tool catered to the specific research design of the studies. For example, The Cochrane Collaboration have designed a 'Risk of Bias' tool for randomised controlled trials within a Cochrane Review (Higgins and Green, 2011). Preferably, the studies are independently screened, and quality assessed by at least two reviewers, as this ensures the studies are correctly evaluated and classified by reducing the chance of human error. Also, by independently assessing studies and then discussing differences in opinion, the risk of introducing reviewer bias is reduced. The outcomes of the quality assessment influence which papers are given greater weight when drawing conclusions from the collective results. The required data are extracted from the studies and combined either by narrative synthesis or quantitatively using meta-

analysis. The results of the review are then presented by the reviewers and used to answer the research question.

Ideally, the methodological theories behind the chosen method for each stage of the review should be explained in the published review (Boland, Cherry and Dickson, 2014). This demonstrates that the researchers have been systematic in the approach to the review and have conducted the research in such a way to produce high quality evidence with limited bias from the ambiguity of the review methods.

3.2 Methods of this systematic review

3.2.1 Objective

The objectives of this systematic review were to determine if parity is a risk factor for i) CHD and ii) stroke and to assess whether the risk of these diseases changes with each parity level.

3.2.2 Protocol

Once the final research question had been formed, as explained in chapter 2, the international register of prospective systematic reviews, PROSPERO (Booth *et al.*, 2012) was checked for any systematic reviews on the relationship of parity with future onset of CVD, and there were no ongoing or completed reviews registered. This meant that there were no systematic reviews being conducted on the same research question, so the development of the review protocol could begin.

A protocol was created using the template developed within the Research Institute for Primary Care and Health Sciences (iPCHS), which follows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Checklist (Moher *et al.*, 2009). As well as the PRISMA checklist, the Meta-analysis of Observational Studies in Epidemiology (MOOSE) Guidelines were followed throughout the course of this systematic review (Stroup *et al.*, 2000). As the specific question for the review was finalised during the early stages of the research, the protocol was adapted to ensure

the correct studies to answer the review question would be detected and included. A final version of the protocol is shown in Appendix A.

As well as using the protocol template created by the iPCHS systematic review team, the team were consulted during the protocol design for guidance on appropriate databases to search and eligibility criteria to set. The team also taught the author, Ashleigh Woodland (AW) how to produce a search strategy with appropriate wildcards, which will be discussed later in this chapter.

3.2.3 Inclusion criteria

In order for this systematic review to assemble publications which would answer the review question, potential studies had to satisfy several criteria which were outlined in the protocol. These inclusion criteria followed the PICOSS Framework (Schardt *et al.*, 2007) which facilitates the deconstruction of a research question into separate concepts. Table 3.1 demonstrates the PICOSS concepts and the correlating inclusion criteria used in this review.

Table 3.1 Inclusion criteria for this systematic review with the equivalent PICOSS concept.

Inclusion Criteria for Studies in this Systematic Review:	
PICOSS Concept:	**Inclusion Criteria:**
P is for the Population or Participants of interest	The participants in the studies must be adult women aged 16 years and over
I is for the Intervention or Exposure of interest	The exposure being analysed in the studies must be parity
C is for the Comparison group required	The studies must include a comparison group of nulliparous women
O is for the Outcomes of interest	The outcomes of studies must include morbidity or mortality from either CHD or stroke
S is for the Setting if a specific type is required	There was no restriction on the setting of research studies
S is for the Study design required	The studies must be observational, of either a cohort or case-control design

The biological theory behind this research is that the maternal adaptations during pregnancy have long term effects on the cardiovascular system, which increases the risk of developing CVD in the future. Thus, the population of included studies needed to be adult women as the exposure being assessed in this review was parity. It was therefore necessary for the included studies to use a

control group of nulliparous women in order to compare the difference in risk of CVD between women who had and had not undergone maternal adaptations during pregnancy.

The overall question of this research was to determine if parity was a risk factor for CHD and stroke, therefore the outcomes required from included studies were morbidity and/or mortality from any form of these diseases. This was building on the previous review (Lv *et al.*, 2015) which only used mortality from CVD as their outcome. As both of these diseases are managed in primary and secondary care, there was no restriction on the setting of studies for this review.

The inclusion criteria for the outcome of CHD in this review, included angina and myocardial infarction as well as unclassified CHD. The outcome of stroke included haemorrhagic and ischaemic stroke as well as transient ischaemic attack (TIA). This did not include subarachnoid haemorrhage as this has a distinct underlying pathophysiology (Nikolić, Banjanin and Stanojević, 2004). Studies which only examined the outcome of composite CVD were not included in this systematic review.

In order to determine if parity was a risk factor for CVD, the included studies needed to demonstrate temporality. Therefore, the only observational studies with an appropriate study design for this review were case-control and cohort studies.

As well as these specific points relating to the research question, the inclusion criteria also allowed for studies in any language, with no date restriction. This was to ensure all relevant studies were incorporated into the review.

3.2.4 Exclusion criteria

The inclusion criteria explained above were used to identify papers that would answer the research question, whereas the set of exclusion criteria were used to refine the list of included studies with concepts that were not part of the PICOSS Framework. The exclusion criteria also focussed on ensuring the studies had conducted appropriate analysis and reported the results in enough detail to be included in this review and meta-analysis.

The exclusion criteria were as follows:

Studies which included study participants with CVD before or during pregnancy and the puerperium e.g. pre-existing hypertension or pregnancy-induced hypertension, were excluded. This review was assessing if parity was a risk factor for CVD, therefore including women with pre-existing CVD or other significant risk factors e.g pre-eclampsia would not allow assessment of parity as a risk factor for CVD.

Studies that gave composite outcomes for men and women together were excluded. Some studies evaluate the association between child-rearing and CVD, and therefore can include men and women who have children. This review however, was focussed on the biological effect of being pregnant and therefore only results specific for child-bearing (parity) could be included.

Studies that did not analyse the exposure of parity separately from other exposures were excluded. Some studies looked at the effect of multiple reproductive factors on CVD, for example; age at menarche and menopause as well as parity. Also, some studies assessed gravidity, which is the total number of pregnancies a woman has had, including those ending before 24 weeks. As this review was dedicated to the exposure of parity, it was necessary to ensure all included studies had a separate analysis and estimates of risk for parity with sufficient data presented.

Studies were excluded if the outcome reported was not either in the form of adjusted risk estimates or raw numbers which could be used to calculate a risk estimate. This was necessary to ensure the results of included papers could be used in a meta-analysis.

The inclusion and exclusion criteria were used to construct the search strategy for this review and were used simultaneously during the screening process to identify all studies capable of answering the review question.

3.2.5 Search strategy

The search strategy for this review was to search three core medical databases: MEDLINE, EMBASE and CINAHL from the inception of the database to the most recent upload. MEDLINE is the US National Library of Medicine bibliographic database, which has a focus on biomedicine and health journals (US National Library of Medicine, 2017). MEDLINE was searched via the platform Ovid 'Ovid MEDLINE ® Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE ® Daily and Ovid MEDLINE ® 1946 to Present'. EMBASE is a bibliographic database published by Elsevier which contains journals for biomedicine and pharmacology (Elsevier, 2018). This database was also accessed through the platform Ovid. CINAHL is the Cumulative Index to Nursing and Allied Health Literature, which contains research studies relevant to nurses and allied health professionals. CINAHL Plus with Full Text was searched, which is an expanded form of the database, via the platform EBSCO Host (EBSCO, 2018)

Other databases such as AMED and Web of Science were also considered, however due to the time restraints of this research it was decided to restrict the search to three databases. The previous systematic reviews by Lv *et al.* (2015) and Rich-Edwards *et al.* (2014) both used MEDLINE, while Lv *et al.* (2015) also searched EMBASE and Web of Science. As MEDLINE and EMBASE were the largest medical databases available, they were incorporated into the search strategy. After researching the remaining databases, CINAHL was chosen for this systematic review as it incorporates research relevant to allied health professions, including midwifery. As the research was linked to cardiology, obstetrics and midwifery it was valuable to search this database as well as the medical databases. As this review was expanding on the previous publications, it was necessary to ensure the previous studies would also be detected using the search strategy. Therefore, once the search was complete, the previous reviews (Rich-Edwards *et al.*, 2014; Lv *et al.*, 2015) were checked to ensure all the studies included in these reviews had been found using the search strategy.

To construct the search strategy for each database, the key words from publications researching parity and CVD, identified in the scoping searches, were recorded, including the search terms used in the previous systematic reviews. Additional terms were sought by exploring alternative words or lay person terms for medical phrases. In addition, the American English spellings for several words were included as well as acronyms. It was necessary to include all these free text words to ensure the search would identify all relevant studies to the review question. As well as free text words, the subject headings for each database were examined to find headings which would be valuable to the search strategy.

Each search term was trialled in MEDLINE to assess how relevant the resulting articles were to the research question and was then adjusted or included accordingly. Wildcards, which are symbols programmed to modify a search term, were utilised to allow for the searching of part of a word with multiple suffixes (Boland, Cherry and Dickson, 2014). The use of these wildcards increased the effectiveness of the search strategy at finding all appropriate publications, without needing to add more search terms. To reduce the number of irrelevant search results the free text words were only searched in the title and abstract of articles. As this systematic review only included observational studies, several search terms were incorporated into the strategy to highlight articles which were of an observational study design or were aiming to assess risk factors for disease. This reduced the number of papers on randomised controlled trials in the search results, therefore improving the search strategy. Boolean operators 'OR' and 'AND' were used to refine the search to publications which contained at least one search term for parity, CVD and study design (Boland, Cherry and Dickson, 2014). Throughout the development of the search strategy the systematic review team at the iPCHS offered advice to ensure the wildcards and search terms would be effective enough to conduct a robust search. Once the search strategy had been finalised the systematic review was registered on the PROSPERO website (Booth *et al.*, 2012).

The original search strategy was created using MEDLINE, which had to be adapted to suit EMBASE and CINAHL. This is because each database uses different symbols for wildcards and has a unique set of subject headings. The final version of the search strategy for MEDLINE is shown in Appendix B and the free text search terms used are shown in table 3.2. The final searches for each database took place on 6th December 2017 for MEDLINE, 8th December 2017 for EMBASE and 2nd January 2018 for CINAHL. The search results from each database were exported into Legacy RefWorks, which is an online reference management software, and the duplicates were deleted.

Table 3.2 Free text terms used in the search strategy for this systematic review. Note: a forward slash (/) depicts where a wildcard would have been used to truncate a search term.

Exposure Terms:	**Outcome Terms:**	**Study Design Terms:**
Pregnant/Pregnancy/cies	Angina	Epidemiology
Multiparous/Multiparity	Myocardial infarct/Infarction	Etiology
Parity	MI	Risk/s
Parous	Myocardial ischaemia	Cohort/s
Gravidity	Ischaemic myocardium	Predict/s/Prediction
Live Birth/s	Ischaemic heart disease	
	CHD	
	Heart attack/s	
	Coronary artery disease	
	Coronary heart disease	
	Acute coronary syndrome/s	
	ACS	
	Stroke/s	
	Cerebral infarct/s/infarction	
	Cerebrovascular disease	
	Cerebrovascular accident/s	
	Cerebrovascular event/s	
	CVA	
	Cardiovascular disease/s	
	CVD	
	Cardiovascular outcome/s	

3.2.6 Screening Process

The selection process of studies for this systematic review was defined within the protocol in order to reduce bias and conflict between reviewers during screening, as studies can only be included if they fulfil the predefined criteria at each stage of screening. The titles of the imported studies were screened against the inclusion criteria, by a single reviewer, AW, within Legacy RefWorks. The studies which were deemed potentially eligible were imported into Covidence (Veritas Health Innovation Ltd, 2018), an online systematic review software which facilitates screening by multiple reviewers. Two reviewers; AW and Dr Pensée Wu (PW), independently screened all the abstracts of the studies against the inclusion and exclusion criteria. After this stage of screening, the full texts of the remaining studies were independently screened by the same two reviewers. Any conflicts which arose were dealt with by further scrutiny of the study in question as well as discussion between the two reviewers until a consensus was reached. The studies which satisfied the inclusion and exclusion criteria were included within the systematic review. The references of these included studies were searched for any eligible papers which had not been identified by the search strategy or the screening process.

3.2.7 Data extraction

To compare the characteristics and outcomes of the studies through a narrative synthesis and meta-analyses, a large amount of information was required from each paper. To ensure all the necessary information was extracted from the included studies a form was developed using Microsoft Excel 2013. It was important for the form to be clear and easily readable but also comprehensive. Therefore, the form was trialled before data extraction began to highlight any relevant data which may be missed or any elements of the form which were ambiguous in the potential information needed. The two reviewers AW and PW discussed the form to ensure both understood what was required in response to each item. A single reviewer, AW, extracted the data from all of the included studies whilst the second reviewer, PW, extracted data from 30% (six) of the included papers. The data were compared for the six papers which both reviewers had

extracted, to check for any mistakes in recording the information and any disagreements were resolved by discussion. The author of one paper (Peters *et al.*, 2016) was contacted for additional raw data which had not been published, and this was provided. The headings included in the data extraction form can be seen in Appendix C.

3.2.8 Quality Assessment

When conducting a systematic review, it is vital that the quality of included studies is assessed and taken into account when forming conclusions from the results. A standardised approach to assess quality must be used to reduce reviewer bias and to allow a clear comparison of studies within the review. The quality of a study is largely dependent on the internal validity, i.e. the risk of bias and confounding introduced through the research process, and the external validity, i.e. how generalisable the study results are to the whole population (Szklo and Nieto, 2014). The criteria used to judge the quality of a research paper differ depending on the study design. The quality assessment tool used in this review was the Newcastle-Ottawa Quality Assessment Scale (Wells *et al.*, 2014), and is presented in Appendix D. This tool was chosen because it was designed specifically to assess both case-control and cohort studies, which were included within this review. The Newcastle-Ottawa Scale (NOS) has been validated in terms of the content and inter-rater reliability (Wells *et al.,* 2014). Also, a Health Technology Assessment report (Deeks *et al.*, 2003) assessed several quality assessment tools and concluded that the NOS was one of the best tools and was suitable for use in a systematic review.

The NOS is comprised of eight questions within the three domains of selection, comparability and outcome for cohort studies and the domains of selection, comparability and exposure for case-control studies. All of the research methods that could have been used for each question are ranked and stars are awarded for the research method/s which are deemed of the highest quality. For example, the potential methods for the ascertainment of exposure in a study are: searching secure records such as medical notes, conducting a structured interview and participant self-report

through a questionnaire. As there is a greater potential for recall bias with an interview or questionnaire, in this question of the NOS, the star was awarded for the use of secure records, with the second best method being an interview and the self-report ranked lowest. This allocation of stars and ranking methods allows a reviewer to rate the overall quality of a study, by how many stars it has been awarded, but also assess in which areas the study has performed well or poorly.

The comparability domain within the NOS focussed on whether the exposed and unexposed participants within a cohort are sufficiently comparable, based on the management of risk factors in either the design or analysis of a study. Studies which adjusted for age, which was considered the most important confounding factor in these studies by the reviewers (AW and PW), were awarded a star. Studies which additionally adjusted for the physiological risk factors of CVD, smoking, hypercholesterolaemia, hypertension, obesity and diabetes mellitus, were awarded a second star. As well as this, adjustment for socioeconomic status indicators e.g. income and education level was credited with a second star.

One of the questions within the outcome domain of the NOS, asked whether the follow up of participants was long enough for the outcome to occur. For this review, the adequacy of follow up was dependent on the age of the participants at the start of follow up, as there is a considerable time difference between exposure and outcome. This lag effect (Yusuf *et al.*, 2001) is due to slow development of atherosclerosis over decades (Grech, 2003; Libby and Theroux, 2005) which in turn causes CHD and stroke (Strom and Libby, 2011). There is no defined age at which atherosclerosis will manifest in diagnosed CVD, meaning there is no clear definition of adequate follow up time for this review. However, according to data collated by the British Heart Foundation (BHF) (Townsend *et al.*, 2014) in 2013, the prevalence of angina and stroke did not surpass 1% of the female population until the age category of 55-64 years. Therefore, the definition of an adequate follow up in this systematic review was if the follow up was long enough for all of the participants to reach

at least 55 years of age. This represented the minimum length of follow up which would be adequate for a cohort study assessing the relationship between parity and CVD.

In this review, the questions within the NOS were incorporated into the data extraction sheet to simplify the extraction process. The data relevant to the quality assessment were then copied into a separate table which explicitly gave the results for each question of the NOS. The same method as for the data extraction was used for quality assessment, with AW assessing all of the studies and PW assessing six of the included papers. Both reviewers then compared results to discern any difference in opinions of the level of bias for each study and resolved conflicts in opinion through discussion. The blank quality assessment table can be seen in Appendix E.

3.2.9 Analysis (narrative / meta-analysis)

In order to easily compare the studies, a brief narrative synthesis was completed before the meta-analyses were performed. This involved tabulating the key characteristics and results of each study and summarising the similarities and differences between the studies. This element of the analysis focussed on the design, methods and key findings of each study as well as the quality assessment.

The main analysis in this systematic review was conducted quantitatively by performing meta-analyses, complemented by subgroup and sensitivity analyses. The studies included within the review were evaluated to decide whether the results were sufficiently homogenous to combine in a meta-analysis. Although the studies varied in follow up time and overall risk estimates, all the studies recruited adult women and examined the exposure of parity on either CHD or stroke incidence. It was therefore decided that the studies were sufficiently homogenous to conduct a meta-analysis. As there were small differences in the study designs, subgroup analyses were also completed by stratifying the studies by adequacy of follow up length and continent of origin.

In order to combine the results of several studies, the reference group for the risk estimates for each study had to be the same, which for this review was nulliparous women. As the included studies used different reference groups in the analysis, for example some studies used para 1

women, the risk estimates from each study were converted to all use nulliparous women as the reference group. This was achieved by the method derived by Hamling *et al.* (2008) using the conversion spreadsheet downloaded from www.pnlee.co.uk/software.htm. This method was also used to calculate ever parous vs nulliparous risk estimates from studies which only reported results per parity level. The risk estimates which were adjusted for the most confounders were extracted from the papers and used for these conversions. The risk estimates reported by the studies were either relative risk or hazard ratios, which were treated as equivalent in the analysis.

Once the data were in an appropriate, consistent form, Review Manager Version 5.3 (RevMan5), a software developed by The Cochrane Collaboration (2014) was used to conduct the meta-analyses. RevMan5 allows the input of adjusted risk estimates or raw numbers from which unadjusted risk estimates are calculated. The included studies were all slightly different from each other in terms of the exact population chosen or the recorded outcome. Therefore, random effects analyses were completed, as this method assumes each study has a different true effect size being examined (Boland, Cherry and Dickson, 2014). As the data available from the studies were dichotomous, either participants had a CVD event, or they did not, the raw data were analysed using the inverse variance method to create unadjusted risk ratios. The adjusted risk estimates, either hazard ratios or relative risk, were inputted using the generic inverse variance setting of the RevMan5 software. This utilised the DerSimonian and Laird method for conducting meta-analyses where the true effect size is different but related across studies (DerSimonian and Laird, 1986; Higgins and Green, 2011). To test the statistical heterogeneity between the studies and between the outcomes, an I^2 statistic was calculated. This statistic describes the percentage of difference between the study estimates which are due to heterogeneity rather than chance (Higgins and Green, 2011). A high I^2 statistic indicates large heterogeneity between the studies or stratified outcomes.

This analysis was completed for the cohort studies, stratified by the outcomes of CHD and stroke. The case-control studies were analysed separately to the cohort studies and all investigated the outcome of CHD, meaning there was no stratification by outcome for case-control studies. There

was not enough raw data from the case-control studies to conduct an unadjusted analysis, therefore only an adjusted random effects analysis was performed. Of the five case-control studies, three presented a relative risk estimate and two reported odds ratios. As there was a small number of outcomes in these studies, the odds ratios and relative risks were treated as equivalent. The final meta-analysis of the case-control studies was reported as an odds ratio.

These methods were used to assess ever parous versus nulliparous risk ratios and each parity level of 1 to 5+ versus nulliparous. One of the cohort studies (Magnus *et al.*, 2017) categorised women into para 4+ and so could not be compared with the 5+ parity levels used in other studies. For this study, only the parity levels 1, 2 and 3 were used in the analysis. Two of the cohort studies reported parity levels of 5 and 6+ and so could also not be directly compared to the remaining studies. The para 5 and 6+ levels from one of the studies (Steenland, Lally and Thun, 1996) was combined using the technique proposed by Hamling *et al.* (2008), to form a para 5+ risk estimate, which was used in the meta-analysis. However, the other study (Simons *et al.*, 2012) did not present enough raw data to transform the categories, meaning only the parity levels 1, 2, 3 and 4 were used in the analysis. Once the parity level analyses were performed, the pooled result for each parity level was transferred to a graph, to display the change in risk of CVD as parity increases.

3.2.10 Changes to the Protocol

As the systematic review progressed, it became necessary to update the protocol as certain issues arose which had not been considered at the time of protocol design. This was done so both reviewers were aware of the revised eligibility criteria during the screening process and to ensure both were clear on their role within the review.

Firstly, during the full text screening stage, it was recognised that some studies used gravidity as the exposure criteria rather than parity. Gravidity is the number of pregnancies a woman has had, including those ending before 24 weeks, which would therefore include parous women. However, as this review was based upon the biological mechanisms which occur throughout the three

trimesters of pregnancy, it was necessary to only include women who would have been exposed to the adaptations of carrying a pregnancy to term. The protocol was altered to reflect this by explicitly stating in the exclusion criteria that studies which only recorded exposure as gravidity or 'number of pregnancies' should be excluded.

Also, due to the time restraints of this review, it was decided that the second reviewer, PW, would not extract data from all the included studies, as was originally intended. Instead the first reviewer AW extracted data from all the studies and PW extracted from 30% of the studies to quality assess AW's extraction. The protocol was updated accordingly.

3.3 Conclusion

This systematic review screened the databases; CINAHL, MEDLINE and EMBASE for observational studies researching the association between parity and CVD, specifically CHD and stroke. The search results were screened against pre-specified criteria and all eligible papers were included within the review. These studies were then quality assessed using the NOS and the relevant data extracted. The individual risk estimates, for both ever parous and per parity level versus nulliparous, were then combined using random effects meta-analyses for CHD and stroke separately. The results are reported in the next chapter.

4 Systematic Review of association of parity with CVD: Results

The previous chapter discussed the development of systematic reviews as a distinct research category and the benefits systematic reviews provide for evidence based medicine. The main principles which underpin a systematic review were outlined as well as the generic process which is followed when conducting a review. The previous chapter also detailed the methods used to complete this systematic review of the association between parity and cardiovascular disease (CVD) and explained the reasons for selecting the methods employed.

This chapter will present the results of the systematic review, including a brief narrative synthesis of the included studies and a quantitative synthesis comprised of several meta-analyses. The chapter will detail the outputs from each section of the review process, starting with the selection and description of the included studies, the quality assessment and the individual results reported from each study. The meta-analyses will then be presented along with subgroup analyses.

4.1 Selection of Included Studies:

As explained in the previous methods chapter; three databases: MEDLINE, EMBASE and CINAHL were searched for any relevant publications. The published conference abstracts available from these databases were also searched but this did not reveal any studies related to parity and CVD. Figure 4.1 follows the PRISMA reporting guidelines (Moher *et al.*, 2009) and displays the number of studies reviewed at each stage of the selection process with the reasons for exclusion of studies. Even though the Web of Science database, used in the previous systematic review by Lv *et al.* (2015), was not included in the search strategy for this review, all the studies that were included in the Lv *et al.* review were also identified in the current search. Also, all of the studies included in the Rich-Edwards *et al.* (2014) review were identified by the search strategy for this review.

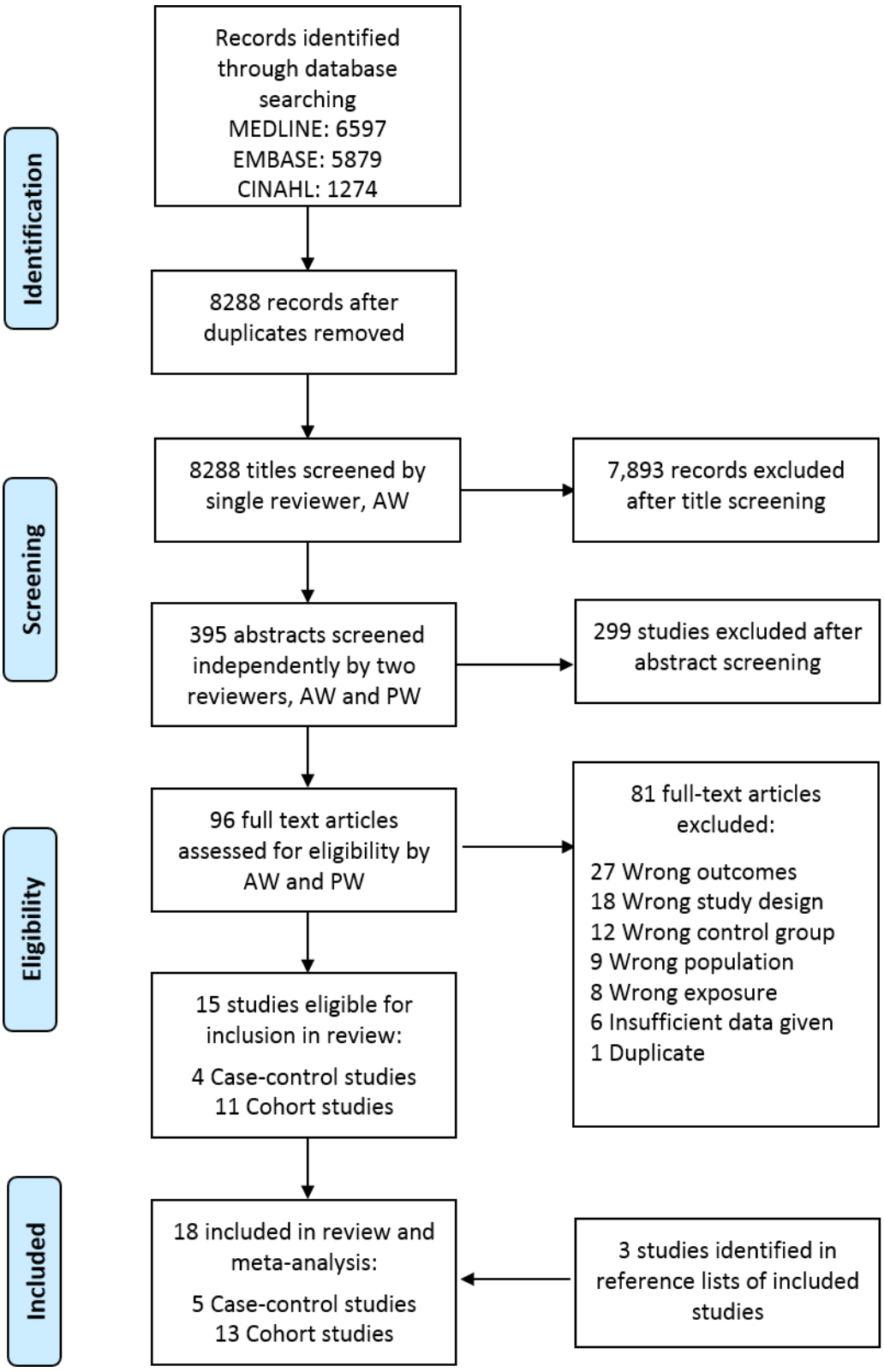

Figure 4.1 The screening process of studies identified by the search strategy for inclusion in this systematic review. Flow diagram adapted from the PRISMA guidelines (Moher et al., 2009).

4.2 Included Studies

The final number of studies included within the systematic review was eighteen, comprising thirteen cohort studies and five case-control studies. These studies provided information from 2,869,391 women, with the most participants originating from the cohort study by Parikh *et al.* (2010), which contributed 1,332,062 women. The smallest cohort study was Cooper *et al.* (1999) which included 867 women. Out of the participants from the cohort studies, 322,440 were nulliparous. This equates to approximately eight parous women: one nulliparous woman. In comparison to the cohort studies, the case-control studies included less women, with the smallest being (Beard, Fuster and Annegers, 1984) with 507 women and the largest being Rosenberg *et al.* (1999) with 2,112 women. Overall the case-control studies contributed 6,626 women to the total number of women included in this review.

The publication dates of the studies ranged from 1984 to 2017, although most of the papers (*n*=11) were published after 2000. Due to the varying length of follow up and time to publication, the studies may have started many years before publication. For example, (Jacobsen *et al.*, 2011) used data recorded from 1976-1988.

Of the eighteen studies in this review, nine focussed on the outcome of coronary heart disease (CHD), two studies assessed the risk of stroke, while seven of the studies assessed both CHD and stroke. All five of the case-control studies identified CHD cases only. Therefore, sufficient data were available to conduct meta-analyses for CHD and stroke separately. As explained in chapter 2, this systematic review included studies reporting on both morbidity and mortality from CVD with the outcome of CVD being specific to either CHD, stroke or both. Of the included studies, five used non-fatal CVD events as the outcome, four used fatal CVD events and nine reported outcomes of both fatal and non-fatal CVD events.

The included studies were carried out in several different countries; eight papers were carried out in the United States (US), seven papers were from European countries, two were from China and

one study was from Australia. Many of the papers from the US and Europe included predominately white participants, with Magnus *et al.* (2017), Cooper *et al.* (1999) and Colditz *et al.* (1987) reporting a study population with ≥95% white women. Vladutiu *et al.* (2017) oversampled black women in the US to create a cohort with approximately equal numbers of black and white women and reported separate results for each group. Also, Rosenberg *et al.* (1999) used data from the Black Women's Health Study in the US.

The study design and methods varied between studies, with some researchers using national databases and others using questionnaires or medical examinations to collect data. Some studies were carried out using data from previous trials which used cohorts whereas others recruited participants to form a new cohort. Of the cohort studies the median length of follow up ranged from six years (Magnus *et al.* 2017, Colditz *et al.*, 1987) to fifty-one years (Cooper *et al.*, 1999). As stated in chapter 3, an adequate length of follow up was defined as, a follow up long enough for all participants to reach at least 55 years of age. Which was achieved by seven of the cohort studies included in this review.

The main characteristics and findings of the cohort and the case-control studies are summarised in tables 4.1 and 4.2. The study ID is used to identify studies and is derived from the lead authors surname and the year of publication from each study.

Table 4.1 Main characteristics and results of cohort studies included in this systematic review.

Study ID	Country	Number of Participants	Age at start of follow up (years)	Outcome	Number of Outcome Events	Follow up Period	Length of Follow Up (years)	Adjusted HR/RR (95% CI) Parous vs Nulliparous	Adjusted Factors
Cohort Studies									
Colditz, 1987	United States	118,376	Range 30-55	Non-fatal MI and death due to CHD	299	1976-1982	Total 6	CHD: 0.83 (0.56-1.25)	Age
Cooper, 1999	United States	867	Range 63-81	Fatal and non-fatal CHD events	45	1935-1990	Range 51-56	CHD: 0.78 (0.40-1.51)	Age
Gallagher, 2011	China	256,023	Mean 52.4	Fatal CHD and stroke events	1054	1989-2000	Range 9-11	CHD: 0.87 (0.50-1.52) Stroke: 0.80 (0.51-1.26)	Age and smoking
Jacobsen, 2011	United States	9,863	Mean 64	Mortality from CHD and stroke	800	1976-1988	Mean 10.7	CHD: 0.93 (0.75-1.14) Stroke: 1.21 (0.85-1.71)	Marital status and level of education
Klingberg, 2017	Sweden	16,515	Mean 57.7	Fatal or non-fatal MI or stroke	1540	1991-2010	Median 15.8	CHD: 1.08 (0.86-1.36) Stroke: 1.05 (0.83-1.33)	Age, diet quality, smoking, exercise, education, HRT, country of birth, hx of miscarriage, BMI and weight change
Magnus, 2017	United Kingdom	180,626	Mean 55	Mortality and morbidity from CHD and stroke	1187	2006-2015	Median 6	CHD: 1.21 (0.99-1.48) Stroke: 0.85 (0.65-1.10)	Age, ethnicity, qualifications, income, Townsend deprivation index, fhx of CVD, smoking, alcohol, exercise, BMI, DM and high BP
Parikh, 2010	Sweden	1,322,032	All 50	Hospitalisation for CHD or stroke	59,143	1982-2005	Median 9.5	CHD: 1.06 (1.02-1.11) Stroke: 1.0 (0.97-1.03)	Age, birth year, income, education level and country of birth
Peters, 2016	10 European Countries	12,161	Mean 52.7	Fatal and non-fatal CHD events	4612	1991-2010	Median 11.1	CHD: 1.19 (1.01-1.41)	Age, education level, smoking, high BP, cholesterol, hx DM and BMI

Peters, 2017	China	289,573	Mean 50.5	Fatal and non-fatal CHD and stroke events	34,365	2004-	Median 7.1	CHD: 0.88 (0.77-1.0) Stroke: 0.97 (0.86-1.09)	Age, education level, income, exercise, smoking, alcohol, high BP, cholesterol, hx DM and BMI
Simons, 2012	Australia	1571	Mean 72.3	Mortality from CHD and ischaemic stroke	N/A	1988-2004	Total 16	N/A	Age, alcohol, smoking, peak flow, disability, health, AF, high BP, DM and BMI
Steenland, 1996	United States	585,445	Median 56	Mortality from CHD	4787	1981-1989	Total 7	CHD: 0.94 (0.85-1.04)	Age, race, smoking, baseline health, blue collar status, education, exercise, high BP, BMI, oestrogen use, and vegetable consumption
Vladutiu, 2017	United States	13,954	Mode 50-59	Fatal or non-fatal stroke	447	2003-2014	Mean 7.5	Stroke: White Women 0.9 (0.61-1.32) Black Women: 1.09 (0.71-1.69)	Age, race, education, marital status, income, location, smoking, alcohol, menopausal status, OC use and HRT
Yang, 2009	Sweden	45,729	Range 30-49	Fatal or non-fatal stroke	193	1991-2004	Mean 12.9	Stroke: 0.90 (0.5-1.4)	Age, BMI, education, alcohol, smoking, exercise, high BP and DM

Key: AF; Atrial Fibrillation, BMI; Body Mass Index, BP; Blood Pressure, CHD; Coronary Heart Disease, CI; Confidence Interval, CVD; Cardiovascular Disease, DM; Diabetes Mellitus, fhx; Family History, HR; Hazard Ratio, HRT; Hormone Replacement Therapy, hx; History, OC; Oral Contraceptive, RR; Rate Ratio.

Table 4.2 Main characteristics and results of case-control studies included in this systematic review.

Study ID	Country	Number of Participants	Age (years)	Outcome	Number of Outcome events	Follow up Period	Adjusted OR/RR (95% CI) Parous vs Nulliparous	Adjusted Factors
Case-control Studies								
Beard, 1984	United States	507	Less than 60	CHD mortality and morbidity	/	1960-1974	1.4 (0.9-2.1)	Unadjusted Ratio
Bertuccio, 2007	Italy	1715	Median 53 and 56	Non-fatal MI	1368	1983-2003	1.45 (1.07-1.97)	Age, education, BMI, smoking, coffee, alcohol, cholesterol, DM, obesity, high BP, HRT and fhx MI
La Vecchia, 1987	Italy	576	Median 45 and 47	Non-fatal MI	418	1983-1986	1.45 (0.92-2.27)	Age
Palmer, 1992	United Status	1716	Median 60	Non-fatal MI	1505	1986-1990	1.8 (1.0-3.30)	Age, smoking, high BP, cholesterol, DM, fhx MI, exercise, BMI, alcohol, education, oestrogen use, occupation, age at menarche, age at first birth, menopausal status, marital status, hx of hysterectomy and oopherectomy
Rosenberg, 1999	United States	2112	Median 38	CHD morbidity	1794	1995	1.67 (0.87-3.22)	Age, education, smoking, high BP,DM, cholesterol, fhx MI and height

Key: BMI; Body Mass Index, BP; Blood Pressure, CHD; Coronary Heart Disease, CI; Confidence Interval, DM; Diabetes Mellitus, fhx; Family History, HRT; Hormone Replacement Therapy, hx; History, OR; Odds Ratio, RR; Rate Ratio.

4.3 Quality Assessment

The Newcastle-Ottawa Scale (NOS) (Wells et al., 2014) was used to assess the quality of each study in the systematic review. The NOS assesses internal validity by evaluating the risk of selection bias, meaning the likelihood that the participants selected for the study will shape the results towards a certain outcome (Pannucci and Wilkins, 2010). For example, the study by Colditz *et al.* (1987) only recruited nurses to the study, who would have a greater knowledge of healthy lifestyle behaviours than the general population. The NOS also evaluates the risk of information bias, which occurs when the recording of outcomes is not consistent or reliable (Pannucci and Wilkins, 2010). A specific example of this can be seen in Cooper *et al.* (1999) as the participants were required to self-report the incidence of CHD after 51 years of follow up, which is reliant on the individual's ability to accurately recall this information, introducing recall bias or response bias (Sedgwick, 2014). However, as this condition causes chest pain and can require hospital admission, it is likely that a participant would remember this life event.

As explained in chapter 2, the NOS has distinct domains and questions to suit the differences in design of cohort and case-control studies. The results therefore are presented separately in table 4.3 for cohort and table 4.4 for case-control studies.

There was large variability in the results of the quality assessment, not only between studies but also between different domains of the same study. For example, Rosenberg *et al.* (1999) achieved 2/2 stars for the adjustment of confounding factors within the comparability domain but scored poorly, 1/3 stars, in the exposure domain. Similar trends are seen in many of the studies (Steenland, Lally and Thun, 1996; Bertuccio *et al.*, 2007; Simons *et al.*, 2012; Vladutiu *et al.*, 2017) which adjusted for multiple important confounders within the analysis of results, but potentially introduced bias through participant selection, or outcome assessment. A difference across domains can also be seen from (Peters *et al.*, 2017) which achieved all available stars in the selection and comparability domains but did not have a long enough follow up for the outcome of interest

(defined as all participants reaching at least 55 years of age) and therefore scored 2/3 in the outcome domain.

Due to the diversity of the study designs, there are differences in the quality assessment across studies. On the other hand, some consistencies exist between the studies. Firstly, for the 'selection of the non-exposed' element of the selection domain for cohort studies, all of the studies sampled nulliparous women from the same population as the parous women. All of the studies accomplished this by recruiting participants and then identifying the exposure status of the women. As explained previously in this chapter, this selection method is reflected in the low number of nulliparous women compared with parous women within the studies.

Another similarity between the studies is that the majority of studies (*n*=12), adjusted for multiple confounding factors and therefore attained the two available stars in the comparability domain. There was variation in the number of adjusted confounders, however, all studies, except Jacobsen *et al.* (2011), adjusted for age.

Within the case-control study quality assessment, all of the studies recruited controls which had no history of CVD and therefore gained a star in the 'definition of controls' section of the selection domain. Also, all the studies used the same method of ascertaining the exposure status for both cases and controls and therefore achieved a star in the 'same method of ascertainment for cases and controls' question of the exposure domain.

The study which, according to the NOS, had the highest quality was Parikh *et al.* (2010), as it met the criteria for all of the available stars in each domain. This is reassuring as this study was also the largest, contributing almost half of all the participants included in this review and therefore was given the greatest weight in the meta-analyses. Consequently, the meta-analyses were influenced most by a study with limited potential for bias in the design and analysis.

The studies which appeared to be of the lowest quality were Cooper *et al.* (1999), Gallagher *et al.* (2011) and Jacobsen *et al.* (2011), which all scored only one star in the selection domain. These

studies did not use women who were representative of the general female population or reliably ascertain exposure information or display temporality in the design methods. Jacobsen *et al.* (2011) was also not awarded any stars in the outcome domain, due to not reliably assessing the presence of the outcome and not having an adequate length of follow up for the outcome of interest to occur, therefore introducing information bias.

There was an additional feature of quality specific to the cohort studies included in this review, which was not assessed by the NOS. This was whether the assumption of proportional hazards had been met when conducting the Cox proportional hazards regression. All of the cohort studies used Cox proportional hazards regression, except Cooper *et al.* (1999), Colditz *et al.*, (1987) and Jacobsen *et al.*, (2011) which used logistic regression. These three studies reported risk estimates as risk ratios (RR), while the studies which used Cox regression reported hazard ratios (HR). Only six of the ten studies which used Cox regression reported that the assumption had been tested during statistical analysis and only two of these (Simons *et al.*, 2012; Peters *et al.*, 2017) declared that the assumption was met. The other four studies did not give an explanation as to whether the assumption had been tested.

Table 4.3 Results of the quality assessment of the cohort studies in this systematic review using the Newcastle-Ottawa Scale.

Study ID	Selection					Comparability		Outcome			
Surname, Year	Representativen-ess of the exposed cohort	Selection of non-exposed	Ascertainment of exposure	Tempora-lity in results	Total number of stars (max. 4)	Confounders adjusted for in analysis	Total number of stars (max. 2)	Assessment of outcome	Was follow up long enough?	Particip-ants lost to follow up	Total number of stars (max. 3)
Colditz, 1987	Selected group of nurses	Drawn from same community as exposed	Self-report	yes	2	Age	1	Record linkage	no	4.6% lost	2
Cooper, 1999	Somewhat representative. All college educated	Drawn from same community as exposed	Self-report	no	1	Age	1	Self-report	yes	8.06% lost	2
Gallagher, 2011	Selected group of factory workers	Drawn from same community as exposed	Self-report	no	1	Age	1	Record linkage	no	0% lost	2
Jacobsen, 2011	Selected group of Seventh Day Adventists	Drawn from same community as exposed	Self-report	no	1	Education	1	Self-report	no	Not explained	0
Klingberg, 2017	Truly representative	Drawn from same community as exposed	Self-report	yes	3	Age, smoking, education*	2	Record linkage	yes	Not explained	2
Magnus, 2017	Truly representative	Drawn from same community as exposed	Structured interview	yes	4	Age, smoking, education*	2	Record linkage	yes	Not explained	2
Parikh, 2010	Truly representative	Drawn from same	Swedish Multi	yes	4	Age, education*	2	Record linkage	yes	0% lost	3

		community as exposed	Generation Register								
Peters, 2016	Truly representative	Drawn from same community as exposed	Self-report	yes	3	Age, smoking education*	2	Record linkage and self-report	yes	Not explained	2
Peters, 2017	Truly representative	Drawn from same community as exposed	Structured interview	yes	4	Age, smoking education*	2	Record linkage	no	0% lost	2
Simons, 2012	Truly representative	Drawn from same community as exposed	Self-report	no	2	Age, smoking*	2	Self-report	yes	Virtually complete	2
Steenland, 1996	Somewhat representative. American Cancer Society volunteers and friends	Drawn from same community as exposed	Self-report	yes	2	Age, smoking, education*	2	Record linkage	no	0.2% lost	2
Vladutiu, 2017	Somewhat representative. Drawn from high risk stroke areas	Drawn from same community as exposed	Self-report	yes	2	Age, smoking, education*	2	Record linkage	yes	Not explained	2
Yang, 2009	Truly representative	Drawn from same community as exposed	Self-report	yes	3	Age, smoking, education*	2	Record linkage	no	Virtually complete	2

**Other confounders were adjusted for, however the maximum available stars for this question was 2, awarded for adjustment for age, or physiological factors, for example smoking or socioeconomic status, for example education. The confounders have therefore been summarised into age, smoking and education.*

Table 4.4 The results of the quality assessment of the case-control studies in this systematic review using the Newcastle-Ottawa Scale.

Study ID	Selection					Comparability		Exposure			
Surname, Year	Is the case definition adequate?	Representa-tiveness of the cases	Selection of controls	Definition of controls	Total number of Stars (max. 4)	Confounders adjusted for in the design or analysis*	Total number of stars (max. 2)	Ascertainment of exposure	Same method of ascertainment for cases and controls?	Non-response rate	Total number of stars (max. 3)
Beard, 1984	Not described	Representa-tive cases. Taken from community	Selected from community	No history of disease	2	Age	1	Medical record	Yes	Non-respond-ers described	1
Bertuccio, 2007	Not described	Representa-tive cases. Taken from hospitals	Selected from hospital	No history of disease	2	Age, smoking, education*	2	Interview	Yes	Same rate for cases and controls	2
La Vecchia, 1987	Validated medical records	Representa-tive cases. Taken from hospitals	Selected from hospital	No history of disease	2	Age	1	Interview	Yes	Same rate for cases and controls	2
Palmer, 1992	Validated medical records	Representa-tive cases. Taken from hospitals	Selected from community	No history of disease	4	Age, smoking, education*	2	Interview	Yes	Same rate for cases and controls	2
Rosenberg, 1999	Self report with 10% linked to medical records	Selected cases with a subscription to Essence magazine	Selected from community	No history of disease	2	Age, smoking, education*	2	Self report	Yes	Not described	1

**Other confounders were adjusted for, however the maximum available stars for this question was 2, awarded for adjustment for age, or physiological factors, for example smoking or socioeconomic status, for example education. The confounders have therefore been summarised into age, smoking and education.*

4.4 Meta-Analyses

There were sufficient studies in the review to allow separate meta-analyses for CHD and stroke. A generic inverse variance, random effects model was used to complete the meta-analyses for risk estimates of ever parity and per parity level versus nulliparity. Both unadjusted and adjusted results are presented in this section. Three of the included cohort studies could not be used in both the ever parous and per parity level analyses due to insufficient data. Therefore, Cooper *et al.* (1999) and Yang *et al.* (2009) were only incorporated in the ever parous meta-analyses for CHD and stroke, respectively. Simons *et al.* (2012) could only be included in the per parity level analyses for both outcomes. The study completed by Vladutiu *et al.* (2017) reported separate results for black and white women and treated these samples as separate cohorts and were therefore included as separate cohorts in the meta-analyses. The results, in the form of RR, from these analyses will be reported throughout as follows; RR estimate (95% Confidence Interval). RR denotes an unadjusted estimate and aRR represents an adjusted risk estimate.

4.4.1 Ever parous versus Nulliparous Results

Figure 4.2 displays the forest plot for the unadjusted meta-analysis of ever parous vs nulliparous risk of CHD and stroke from the cohort studies, which was calculated using the raw data from the studies.

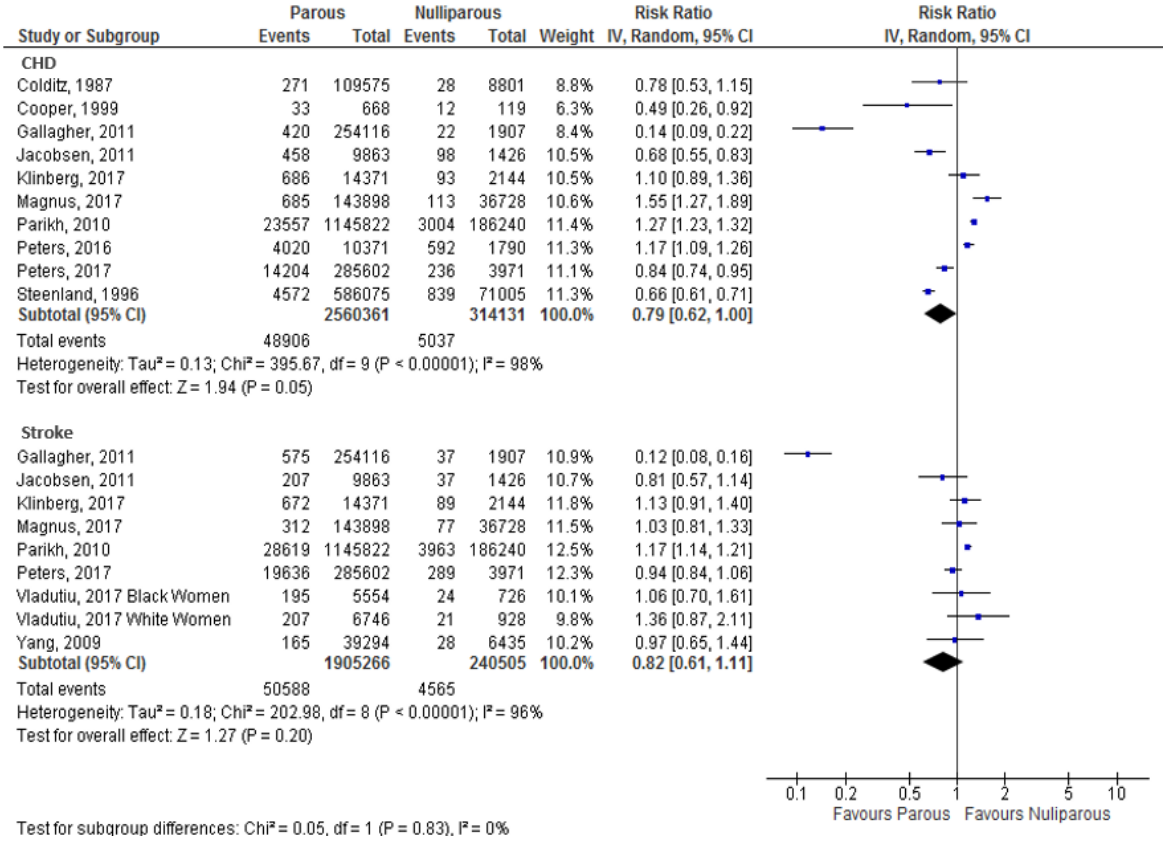

Figure 4.2 Forest plot demonstrating unadjusted ever parous versus nulliparous risk of CHD and stroke from cohort studies in this systematic review.

Twelve studies were included in this analysis, and as can be seen from the figure 4.2, six of these studies reported risk estimates for both CHD and stroke. This resulted in the use of ten studies in the CHD outcome and eight studies for stroke. For the outcome of CHD there were large variations in estimated risk, from Gallagher *et al.* (2011) reporting RR 0.14 (0.09-0.22) to Magnus *et al.* (2017) presenting RR 1.55 (1.27-1.89). Three studies reported statistically significant estimates implying an increased risk of CHD in ever parous women (Parikh *et al.*, 2010; Peters *et al.*, 2016; Magnus *et al.*, 2017) five reported statistically significant estimates indicating a reduced risk in ever parous women

(Steenland, Lally and Thun, 1996; Cooper *et al.*, 1999; Gallagher *et al.*, 2011; Jacobsen *et al.*, 2011; Peters *et al.*, 2017) and two studies had non-significant results. The pooled estimate for the CHD meta-analysis was therefore a borderline statistically significant reduced risk of CHD for ever parous women, RR 0.79 (0.62-1.00).

For the outcome of stroke the studies also varied in estimated risk, but with a smaller difference than seen for CHD. Again, Gallagher *et al.* (2011) reported an outlying low RR 0.12 (0.08-0.16). The Vladutiu *et al.* (2017) result for white women represented the largest risk increase for ever parous women compared to nulliparous with RR 1.36 (0.87-2.11). In contrast to the CHD outcome, all of the studies except Gallagher *et al.* (2011) were non-statistically significant. The pooled estimate for the stroke outcome was very similar to that of CHD, RR 0.82 (0.61-1.11), representing a reduced risk of stroke for ever parous women, however this was not statistically significant.

As can be seen from the I^2 statistics, there was large heterogeneity between the studies for the CHD and stroke outcomes (I^2 = 98% and 96% respectively). The heterogeneity between the CHD studies was only slightly altered (97%) when the outlying results from Gallagher *et al.* (2011) were excluded. However, when the results from Gallagher *et al.* were excluded from the stroke outcome, the pooled estimate increased to RR 1.05 (0.94-1.18) albeit non-statistically significant, with a reduced heterogeneity of I^2 = 64%.

A meta-analysis using adjusted data was performed and the resulting forest plot can be seen in figure 4.3. It must be noted that each study adjusted for different confounders, so even though the most adjusted data were taken from the primary papers, the results are not all adjusted for the same factors. The list of the confounders used in each study can be found in the previous section on quality assessment in table 4.3.

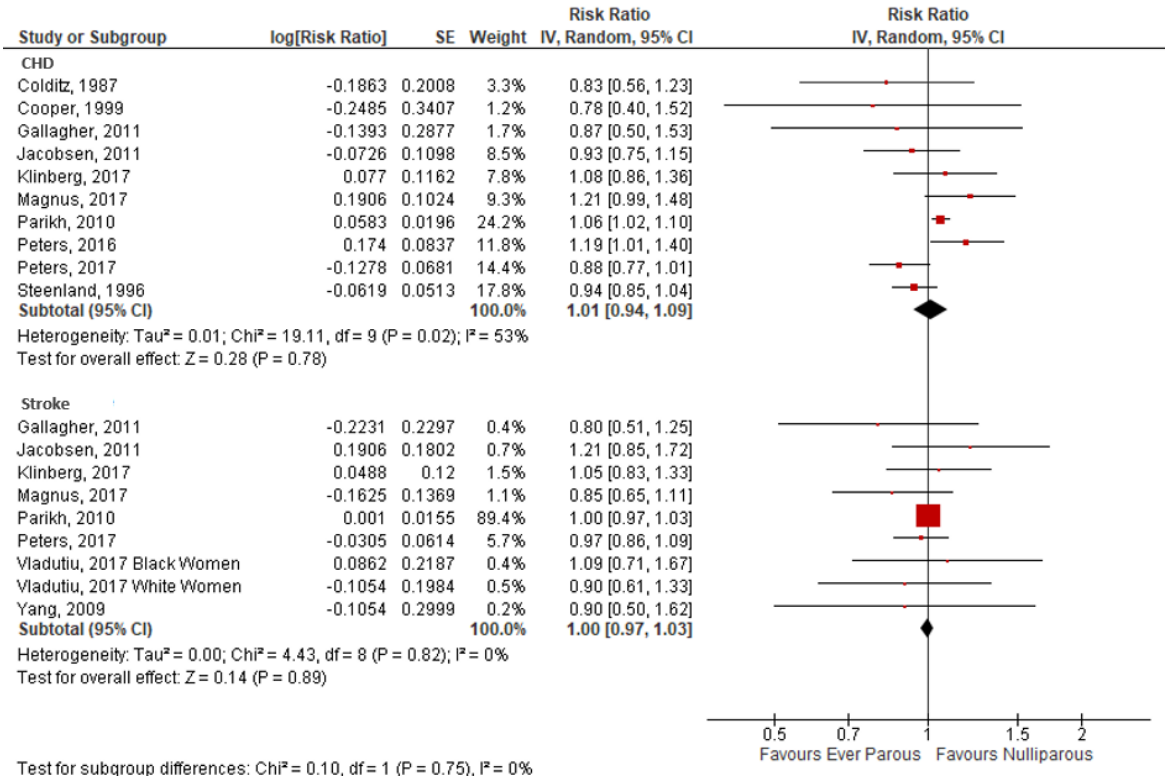

Figure 4.3 Forest plot demonstrating adjusted ever parous versus nulliparous risk of CHD and stroke from cohort studies in systematic review.

The range of risk estimates across the studies reduced after adjustment, with the lowest aRR estimate for CHD coming from Gallagher *et al.* (2011) with aRR 0.83 (0.56-1.23) and the highest at aRR 1.21 (0.99-1.48) from Magnus *et al.* (2017). Similar results were found in the stroke outcome with the risk estimates ranging from aRR 0.80 (0.51-1.25) in Gallagher *et al.* (2011) to aRR 1.21 (0.85-1.72) in Jacobsen *et al.* (2011). The adjustment lowered the heterogeneity in both outcomes, with $I^2 = 53\%$ for CHD and $I^2 = 0\%$ for stroke. The negligible heterogeneity between the stroke studies is likely due to the substantial weighting given to Parikh *et al.* (2010).

The pooled adjusted estimates for the ever parous versus nulliparous risk of both CHD and stroke were both close to the null, aRR 1.01 (0.94-1.09) and 1.00 (0.97-1.03) respectively. In the CHD outcome, most of the studies reported adjusted risk ratios of <1. However, the largest studies gave higher ratios and gained the majority of weighting in the analysis. Parikh *et al.* (2010) presented aRR 1.06 (1.02-1.10) as well as Magnus *et al.* (2017) with aRR 1.21 (0.99-1.48) and Peters *et al.* (2016) aRR 1.19 (1.01-1.40). Following adjustment only two of the risk estimates remained statistically significant, which were from Parikh *et al.* and Peters *et al.* due to the large sample sizes. This equated to a null pooled result when combined with the smaller studies reporting reduced risk ratios.

Within the stroke outcome, the majority of weight (89.4%) was given to Parikh *et al.* (2010) due to the very small standard error, resulting from the large sample size. The stroke events reported by Parikh *et al.* equated to 59% of all the stroke events (n=55,153), from the eight cohort studies recording this outcome. However, the reported aRR was not statistically significant, as were none of the other studies in the stroke outcome. The high RR favouring nulliparity for white women, from Vladutiu *et al.* (2017), in the unadjusted results became aRR 0.90 (0.61-1.33) after adjusment.

4.4.2 Per Parity Level versus Nulliparous Results

As well as analysing the risk of CHD and stroke in nulliparous compared to parous women, this systematic review aimed to determine whether the effect varied by number of pregnancies. Therefore, meta-analyses were conducted using data from each parity level (number of children), compared to nulliparous results.

4.4.2.1 CHD

The forest plot created from the meta-analysis for the unadjusted per parity level versus nulliparous risk of CHD is shown in figure 4.4. This analysis used the raw data from the studies to calculate the

pooled risk estimate. The parity levels used in this analysis were para 1, 2, 3, 4 and 5+ as the majority of the included studies reported the results in these categories. Eight papers were eligible for inclusion, however as Magnus *et al.* (2017) categorised participants into parity levels 1, 2, 3 and 4+, the risk estimates from this paper are only included in the first three levels; para 1, 2 and 3.

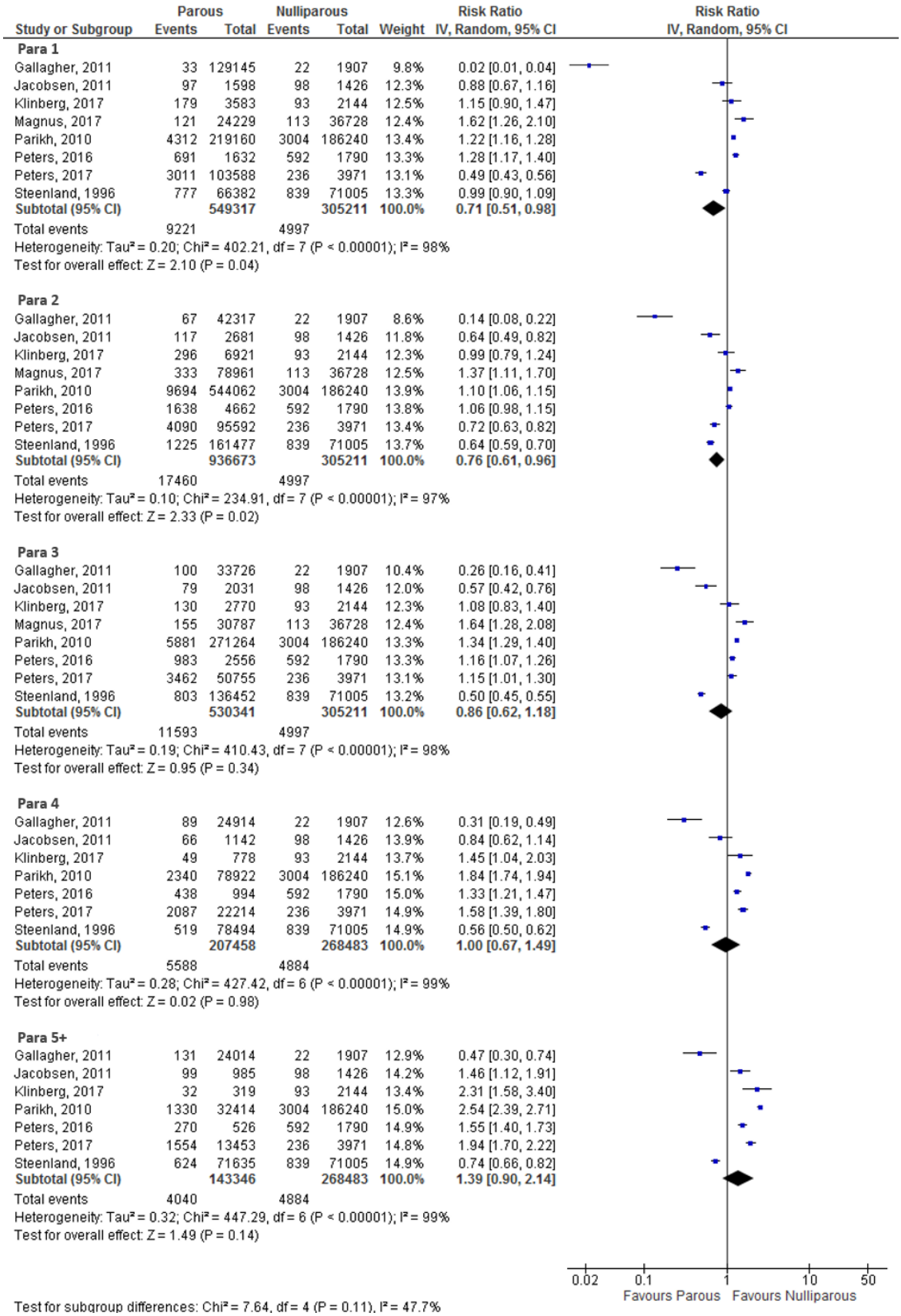

Figure 4.4 Forest plot demonstrating unadjusted per parity level versus nulliparous risk of CHD from cohort studies in this systematic review.

The unadjusted pooled estimates depict a clear trend of increasing risk ratios with ascending parity levels. Starting from para 1, the risk of CHD reduced, RR 0.71 (0.51-0.98), which increased to RR 0.76 (0.61-0.96) with two children, but still demonstrated a decreased risk compared to nulliparous women. The pooled result for para 3 indicated a higher risk of CHD compared to para 1 and 2, however this risk was still less than the nulliparous risk, RR 0.82 (0.62-1.18). At para 4 the risk of CHD was no different to that of nulliparity RR 1.00 (0.67-1.49), whereas the risk of CHD after having five of more children was much higher compared to being nulliparous, RR 1.39 (0.90-2.14). However, only the pooled risk estimates for the para 1 and 2 outcomes were statistically significant, and they were unadjusted for confounding factors such as age and other CVD risk factors.

Although there was a clear overall trend of increasing risk with each parity level from the pooled risk estimates, the pattern for individual studies was varied. The lowest estimated risk within parous women was found to be for para 1 in three of the studies (Gallagher *et al.*, 2011, Magnus *et al.*, 2017, Peters *et al.*, 2017), para 2 in three studies (Parikh *et al.*, 2010; Peters *et al.*, 2016; Klingberg *et al.*, 2017) and para 3 in two studies (Steenland, Lally and Thun, 1996; Jacobsen *et al.*, 2011). The highest risk was found to be in para 5+ for all of the studies except for Steenland, Lally and Thun (1996), which reported the highest risk of CHD in para 1. The heterogeneity for each parity level was therefore high, being at least $1^2 = 97\%$.

A meta-analysis was performed using adjusted data from the studies and figure 4.5 displays the resulting forest plot. Two additional papers (Simons *et al.*, 2012; Colditz *et al.*, 1987) were included in this analysis, as they did not present enough raw data to be used in the unadjusted pooling, but gave adjusted risk estimates to use directly in the adjusted analysis. This increased the number of studies to ten. Similar to the previous analysis, Magnus *et al.*, (2017) was used only in the first three parity levels and Simons *et al.* (2012) in the first four levels.

After adjustment for confounders, the pooled estimates for each parity level were largely altered from the unadjusted results. The estimates for para 1,2 and 3 were all close to the null, indicating no difference in risk between parous and nulliparous women. The risk of CHD increased in para 4 compared to nulliparity, aRR 1.10 (0.93-1.31) with the highest risk being at para 5+, aRR 1.21 (0.93-1.56). However, none of the pooled risk estimates were statistically significant.

The individual study estimates were more consistent after adjustment, with a noticeable difference in Gallagher *et al.* (2011), which was a low outlier in the unadjusted analysis, which then reported higher adjusted risk ratios, in keeping with the rest of the studies (para 1 RR 0.85 (0.48-1.51)). Also, there was a change in estimate from Steenland, Lally and Thun (1996) which reported para 1 as the highest risk in the unadjusted results, but para 5+ as the highest risk in the adjusted results. The introduction of Simons *et al.* (2012) into the analysis affected the pooled results, even though it was a small study with limited weighting, as the risk ratios from this paper were outliers, showing an increased risk of CHD in parous women, compared to nulliparous, at each level (para 2 RR 2.11 (1.11-4.01)). Colditz *et al.* (1987) reported conflicting results to Simons *et al.* as their risk estimates suggest a reduced risk of CHD for parous women at all parity levels. Despite the addition of more papers, the heterogeneity of each parity group was lower than in the unadjusted analysis, with the lowest being para 2 at I^2 = 61%.

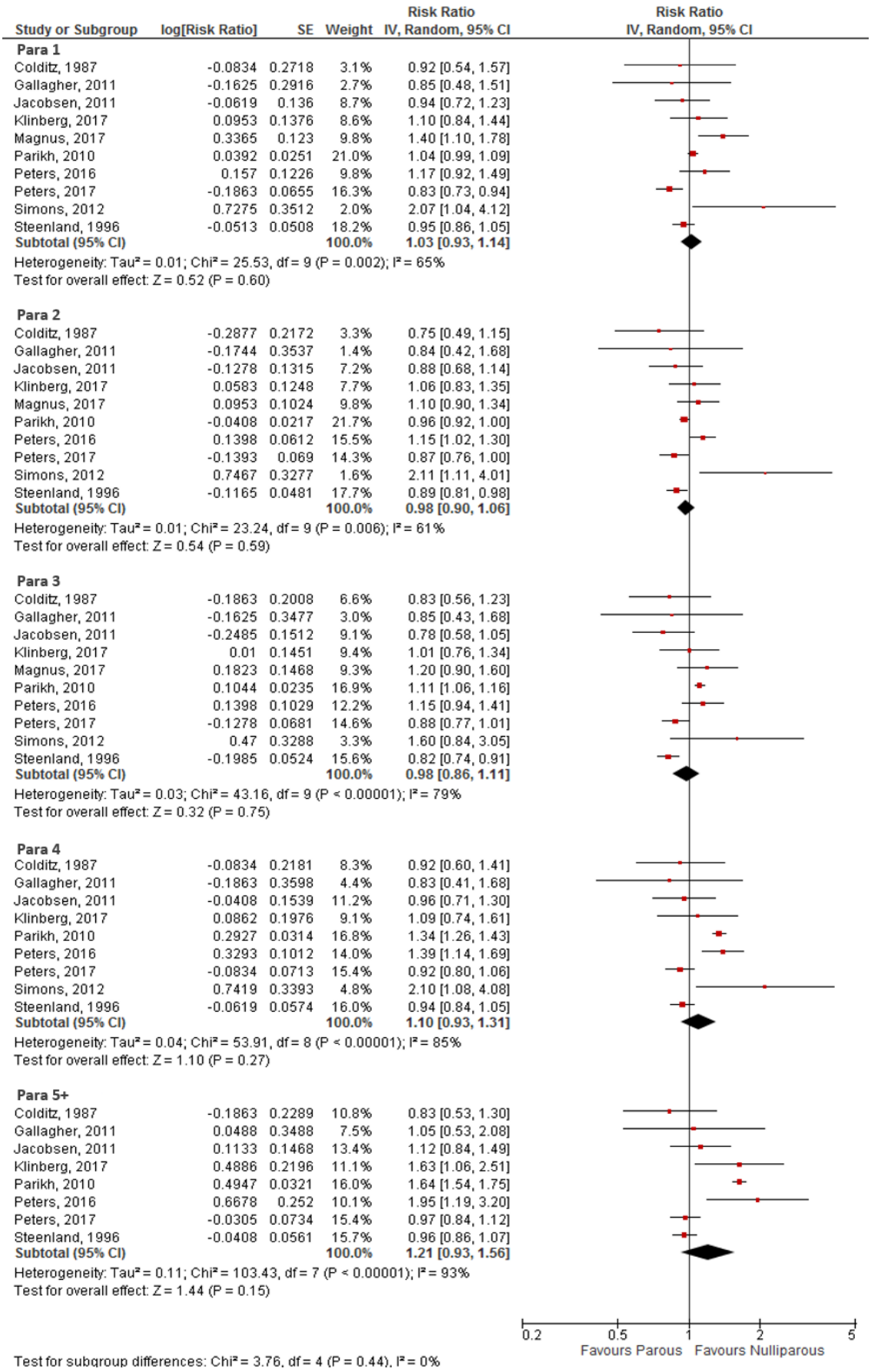

Figure 4.5 Forest plot demonstrating the adjusted per parity level versus nulliparous risk of CHD from cohort studies in this systematic review.

Figure 4.6 shows the change in risk of CHD with increasing parity using the pooled risk estimates from each parity level in the parous versus nulliparous adjusted meta-analysis. This demonstrates the equivalent risk of CHD for parous and nulliparous women in parity levels 1, 2 and 3 and the increased risk in parous women at para 4 and 5+. However as can be seen from the overlapping error bars on the graph, representing the confidence intervals, the results were not statistically significant.

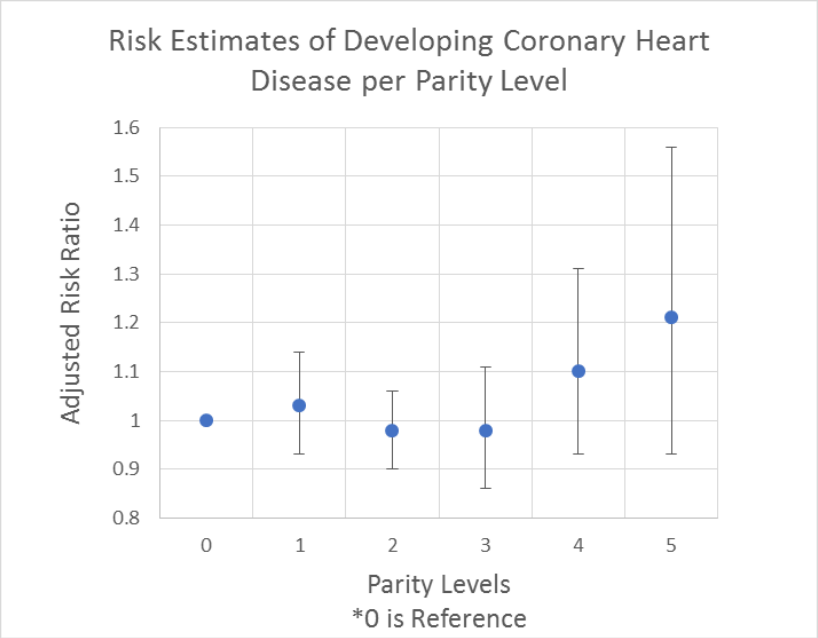

Figure 4.6 Per parity level versus nulliparous risk of CHD from cohort studies in this systematic review.

4.4.2.2 Stroke

The forest plot resulting from the meta-analysis of unadjusted stroke risk per parity level is shown in figure 4.7. Ten studies were included in this analysis, however Magnus *et al.* (2017) was only included in the estimates for para 1, 2 and 3. The trend is similar to that of CHD risk, with the risk of developing stroke increasing from para 1 onwards, however this was not statistically significant at any parity level.

There was once again a large variation between the studies within each parity group, with Gallagher *et al.* (2011) providing the lowest risk estimate for every parity level. However, the number of

statistically significant risk estimates from the individual studies increased per parity level, with only one study (Parikh *et al.*, 2010) for para 1, reaching five studies for para 5+ (Parikh *et al.*, 2010; Klingberg *et al.*, 2017; Peters *et al.*, 2017; Vladutiu *et al.*, 2017). The heterogeneity scores reflect these large differences between studies with all parity levels reaching at least I^2 = 98%.

When Gallagher *et al.* (2011) was excluded from the analysis the heterogeneity greatly reduced to as low as I^2 = 44% (para 5+) whilst still retaining the same pattern of increasing risk after para 1. The pooled risk estimates for Para 3, 4 and 5+, compared to nulliparity gained statistical significance (para 3 RR 1.20 (1.08-1.33), para 4 RR 1.52 (1.33-1.73), para 5 RR 1.96 (1.75-2.20)).

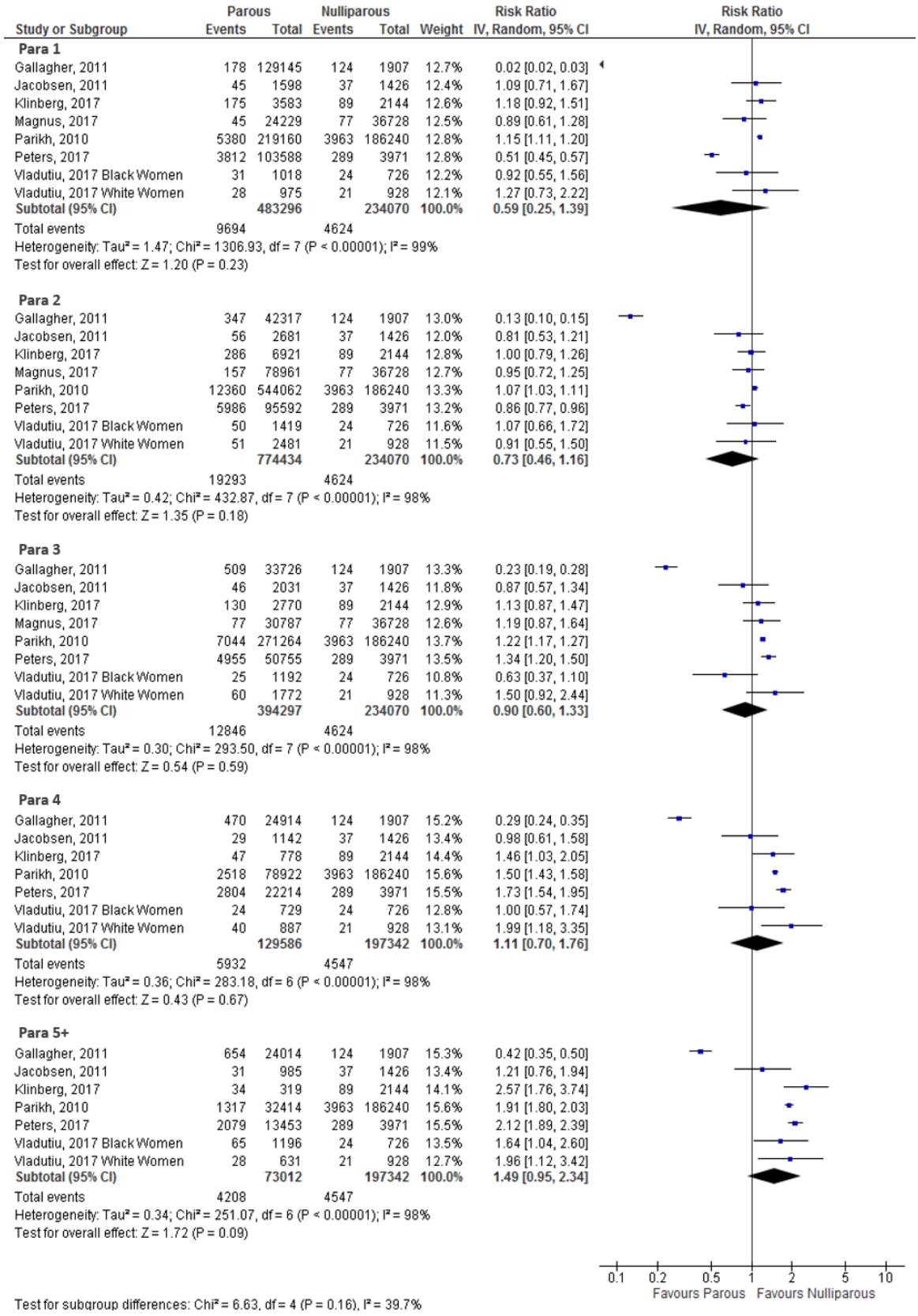

Figure 4.7 Forest plot demonstrating unadjusted per parity level versus nulliparous risk of stroke from cohort studies in this systematic review.

The adjusted study results were also pooled to estimate the risk of stroke per parity level, and the corresponding forest plot can be seen in figure 4.8. Simons *et al.* (2012) was included in the adjusted analysis, although it was given little weight due to the large standard errors associated with the estimates. After adjustment for age, the risk estimates from Gallagher *et al.* (2011) were more comparable with the other studies (Para 1 aRR 0.87 (0.55-1.38)). The changes in risk ratios from this study and others contributed to the large reduction in heterogeneity for the adjusted estimates compared to the unadjusted across all parity levels, with the lowest being at para 1 and 2 at $I^2 = 0\%$ and the highest in para 5+ with $I^2 = 50\%$.

Within each parity level, Parikh *et al.* (2010) was allocated the largest weight, with Peters *et al.* (2017) receiving the second largest percentage. Therefore, the overall trend of risk with parity levels closely follows the results from Parikh *et al.* Figure 4.9 shows the pooled risk estimates of stroke for each parity level which follow a 'J' shaped curve. The para 1 risk was equivalent to nulliparity, with the nadir of risk being at para 2 (aRR 0.94 (0.91-0.97)), the risk of stroke increased with each parity level thereafter, reaching a maximum at para 5+ (aRR 1.21 (1.06-1.39)). Only the risk estimates for para 2 and para 5+ were statistically significant.

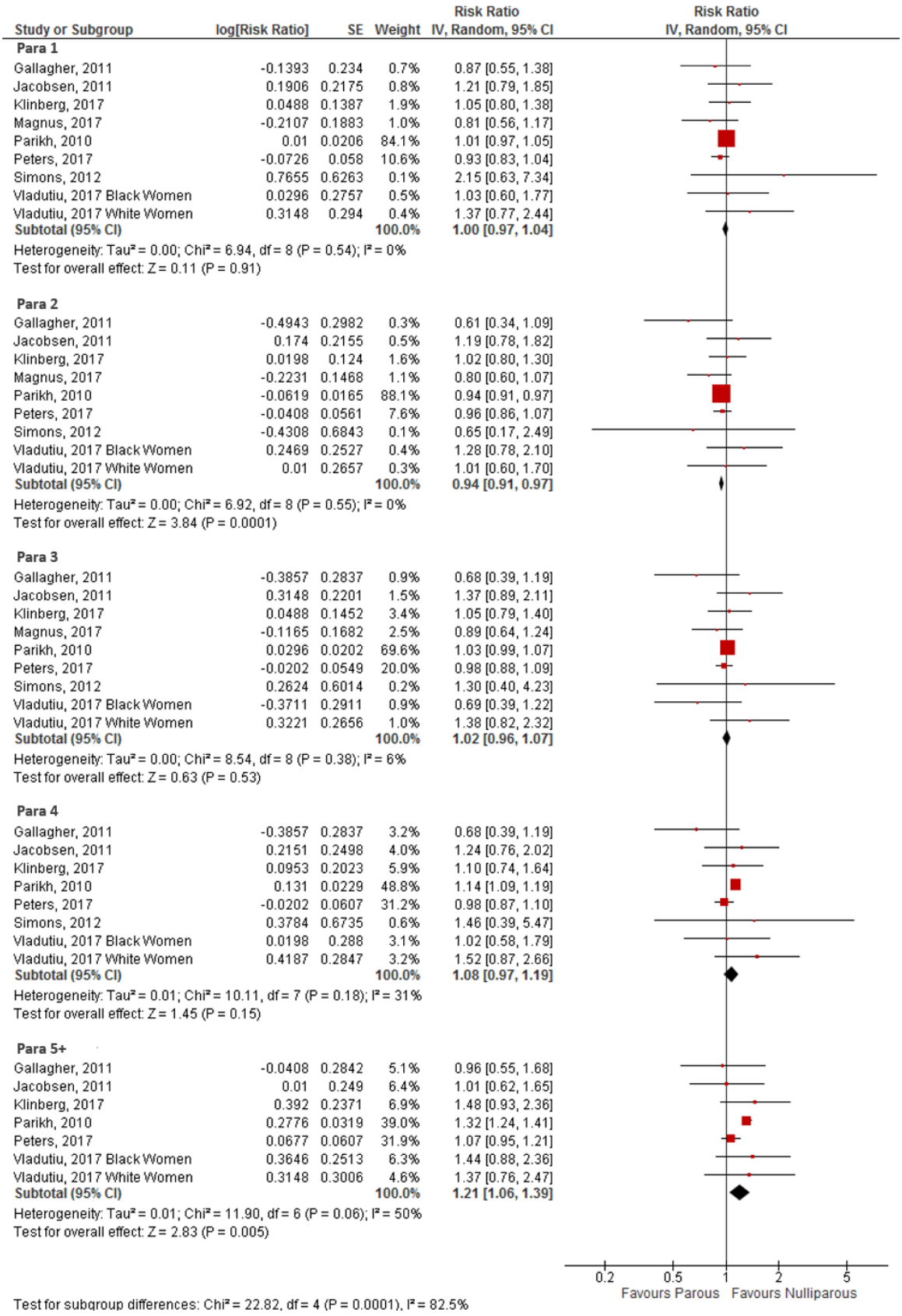

Figure 4.8 Forest plot demonstrating adjusted per parity level versus nulliparous risk of stroke from cohort studies in this systematic review.

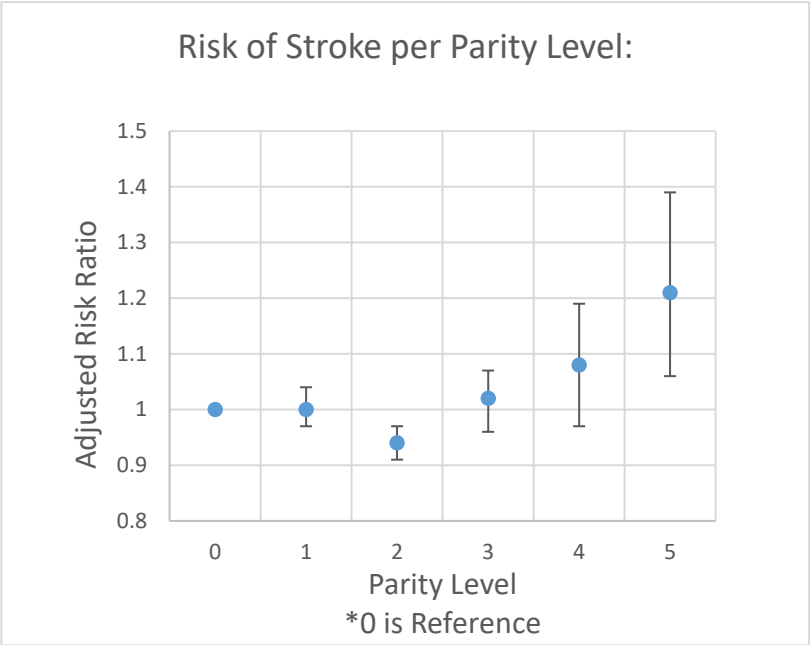

Figure 4.9 Per parity level versus nulliparous risk of stroke from cohort studies in this systematic review.

4.4.3 Subgroup Analysis

In order to determine whether differences in study characteristics affected the overall pooled results for the risk of CHD or stroke, subgroup analyses were performed. The studies were subgrouped based on study characteristics which were: the location in which the study took place, and whether the follow up time was adequate or not, based on the study sample and outcomes of interest. Subgroup analyses were performed for both CHD and stroke outcomes, using adjusted risk estimates. There were not enough studies to assess the risk of CHD and stroke per parity level within the subgroups. Therefore, only the ever parous versus nulliparous risk was assessed in the subgroup analyses. As a result, Simons *et al.* (2012) was excluded from the analyses as this study did not report enough data to produce an ever parous risk ratio.

Risk of CHD

The subgroup analyses for the risk of CHD in ever parous women comprised 10 studies. Figure 4.10 shows the results of the analysis based on location. The studies were categorised into three groups: US (four studies), Europe (four studies) and China (two studies). The pooled risk estimates were conflicting, with the studies from the US and China demonstrating a lower risk of CHD in ever parous women (aRR 0.93 (0.85-1.01) and aRR 0.88 (0.77-1.00) respectively) with the European studies indicating an increased risk (aRR 1.08 (1.03-1.14)). The largest study in the review, Parikh *et al.* (2010) was included in the European subgroup which may have contributed to this being the only subgroup with a statistically significant result. Having said this, the Chinese subgroup reached borderline statistical significance. The studies within each subgroup were very similar, indicated by a negligible I^2 statistic in the US and Chinese subgroups and only an I^2 of 9% in the European group. There was however, clear discrepancy between the subgroups highlighted by the I^2 = 85.9% result.

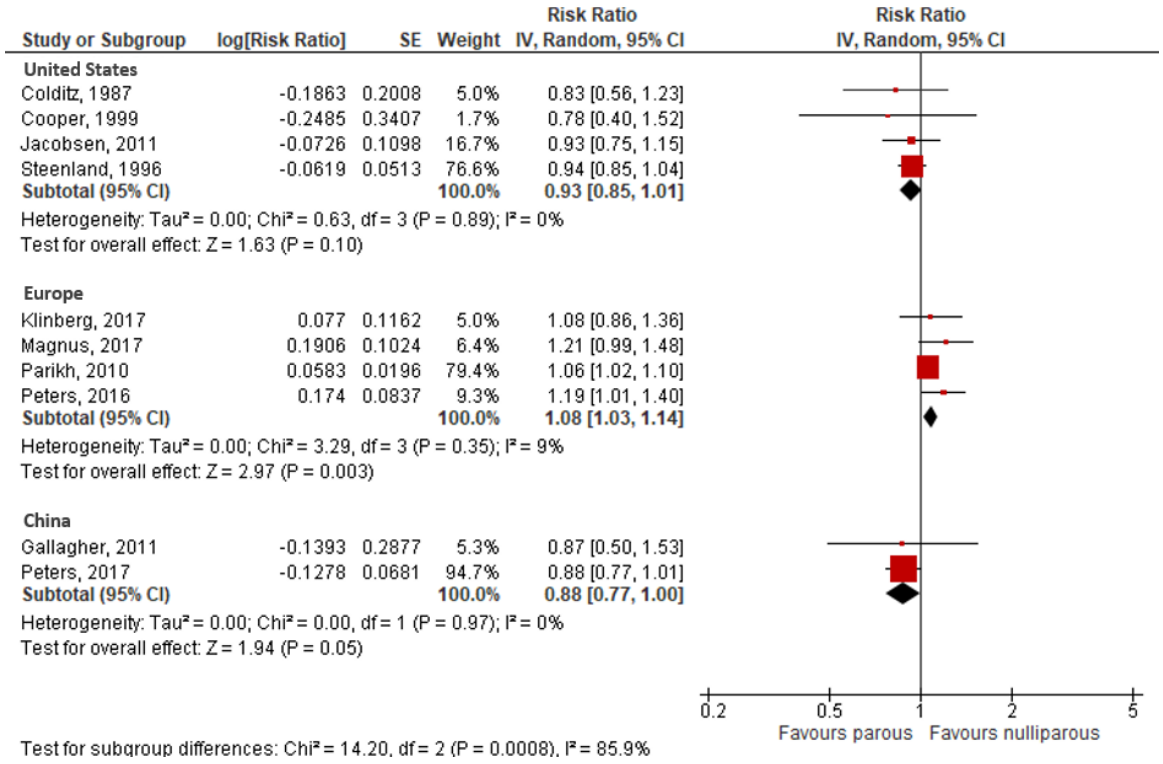

Figure 4.10 Forest plot demonstrating ever parous versus nulliparous risk of CHD in subgroup analysis based on location of study, of cohort studies included in this systematic review.

A subgroup analysis based on the adequacy of follow up was also conducted. These results can be seen in figure 4.11, where the 'Yes' group is those with an adequate follow up and the 'No' group is those studies which did not. The 'Yes' group showed a higher risk of CHD for parous women (aRR 1.08 (1.03-1.13)) and the 'No' group showed a reduced risk (aRR 0.92 (0.85-0.98)), with both estimates reaching statistical significance. This is reflected by the very slight heterogeneity within each subgroup and the high heterogeneity between the subgroups (I^2 = 92.6%).

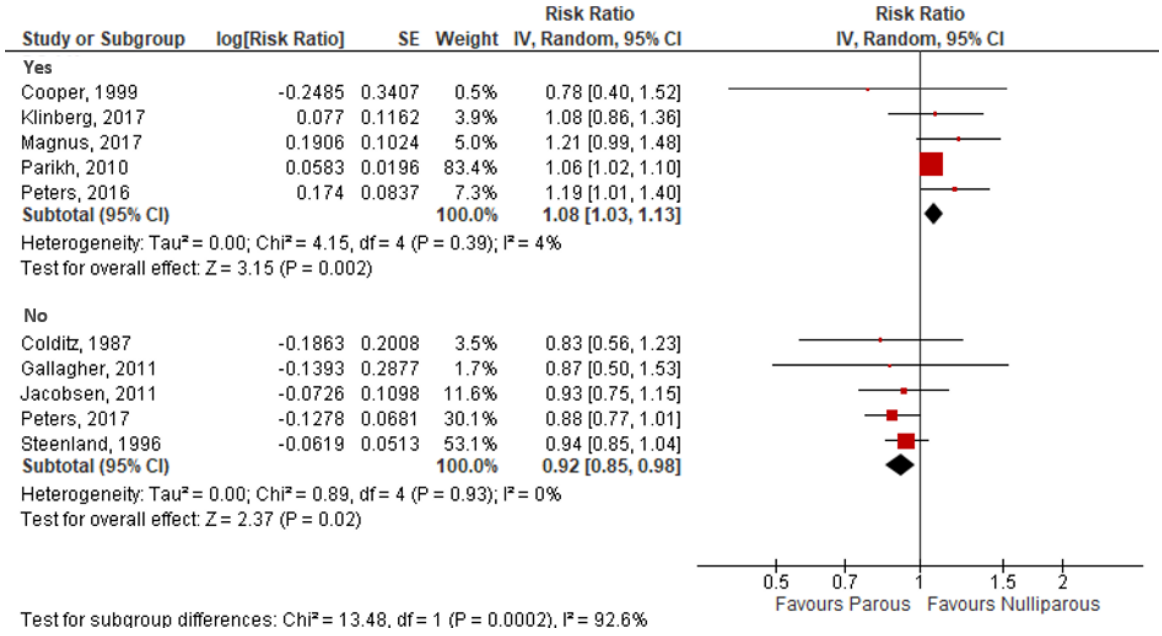

Figure 4.11 Forest plot demonstrating ever parous versus nulliparous risk of CHD in subgroup analysis based on the adequacy of follow up of cohort studies included in this systematic review.

4.4.3.1 Risk of stroke

The same subgroup analyses, as explained above, were also performed on eight studies assessing stroke risk. The first analysis based on location, produced varying results across the subgroups, as the US group of studies found a higher risk of stroke in parous women (aRR 1.13 (0.96-1.33)) while the Chinese group determined a slightly lower risk (aRR 0.96 (0.85-1.08)) and the European group found no difference (aRR 1.00 (0.97-1.03)). There was also an apparent null effect of parity on stroke risk when the studies were categorised into those with and without an adequate follow up time. For the subgroup analysis on adequacy of follow up, the risk estimate for the 'Yes' group was

aRR 1.00 (0.97-1.03) and for the 'No' group the risk was aRR 1.02 (0.87-1.19). None of the pooled results from either of the stroke subgroup analyses were statistically significant. Notably, due to the large number of participants in the Parikh *et al.* (2010) study and therefore small standard error, the study took the largest weight, at least 90%, in every subgroup to which it was assigned. Therefore, the pooled risk estimates of the subgroup analyses for Europe and adequate follow up period directly correspond to the risk estimate from the Parikh *et al.* study.

4.4.3.2 Summary of Subgroup Analyses

The results of the subgroup analyses for both CHD and stroke outcomes are summarised below in table 4.5.

Table 4.5 Results of the subgroup analysis for both CHD and stroke risk in ever parous versus nulliparous women, from the cohort studies included in this systematic review.

	Coronary Heart Disease Results:			**Stroke Results:**		
Subgroup:	Number of Studies:	Pooled Risk Ratio:	I^2value: (%)**	Number of Studies:	Pooled Risk Ratio:	I^2 value: (%)≠
Location:			85.9			24.7
United States	4	0.93 (0.85-1.01)		2*	1.13 (0.96-1.33)	
Europe	4	1.08 (1.03-1.14)		4	1.00 (0.97-1.03)	
China	2	0.88 (0.77-1.00)		2	0.96 (0.85-1.08)	
Follow up Adequate:			92.6			0
Yes	5	1.08 (1.03-1.13)		4*	1.0 (0.97-1.03)	
No	5	0.92 (0.85-0.98)		4	1.02 (0.87-1.19)	

**The number of studies appears to be 1 more than this value as separate results are reported from Vladutiu et al. (2017).*
≠The I^2 value corresponds to the heterogeneity between the subgroups

4.4.4 Meta-analysis of Case-control Studies

The adjusted odds ratios (aOR) from the five case-control studies were combined in a meta-analysis comparing risk of CHD in ever parous versus nulliparous women. There were insufficient data for an unadjusted analysis. Beard *et al.* (1984) categorised parous women into those with less than four live births or four and more live births. As the majority of women in England and Wales have less than four children (Office for National Statistics, 2017b), the results reported for the more than four

births category of parous women were used within the analysis. The forest plot corresponding to the adjusted meta-analysis is displayed in figure 4.12.

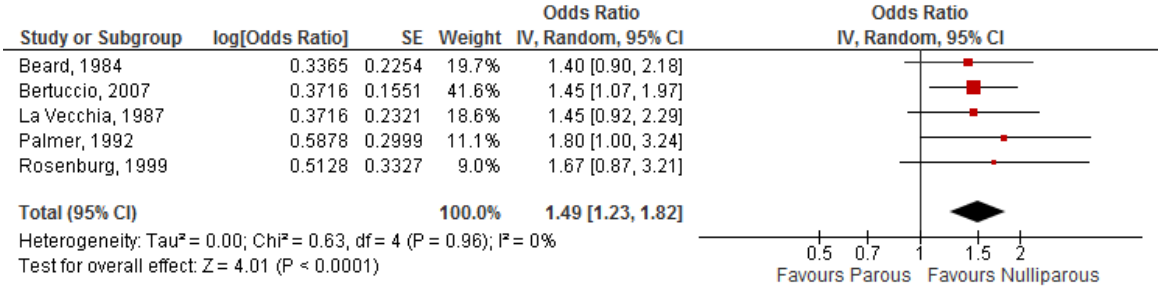

Figure 4.12 Forest plot demonstrating adjusted ever parous versus nulliparous risk of CHD from case-control studies in systematic review.

In the analysis Bertuccio *et al.* (2007) was allocated the largest weight, which also had a statistically significant individual aOR. The pooled aOR for CHD was significant and suggested almost a 50% increase in odds of CHD in parous women, aOR 1.49 (1.23-1.82), with low heterogeneity (I^2 = 0%).

4.4.5 Publication Bias

A funnel plot was created using the cohort studies from the adjusted ever parous versus nulliparous risk of CHD and stroke analysis seen in figure 4.3. The funnel plot in figure 4.13 displays the number of studies assessing stroke and CHD, therefore some studies are included twice. The graph highlights the lack of small studies included in this systematic review which present an increased risk of CVD in parous versus nulliparous women. Most of the studies favour parity rather than nulliparity.

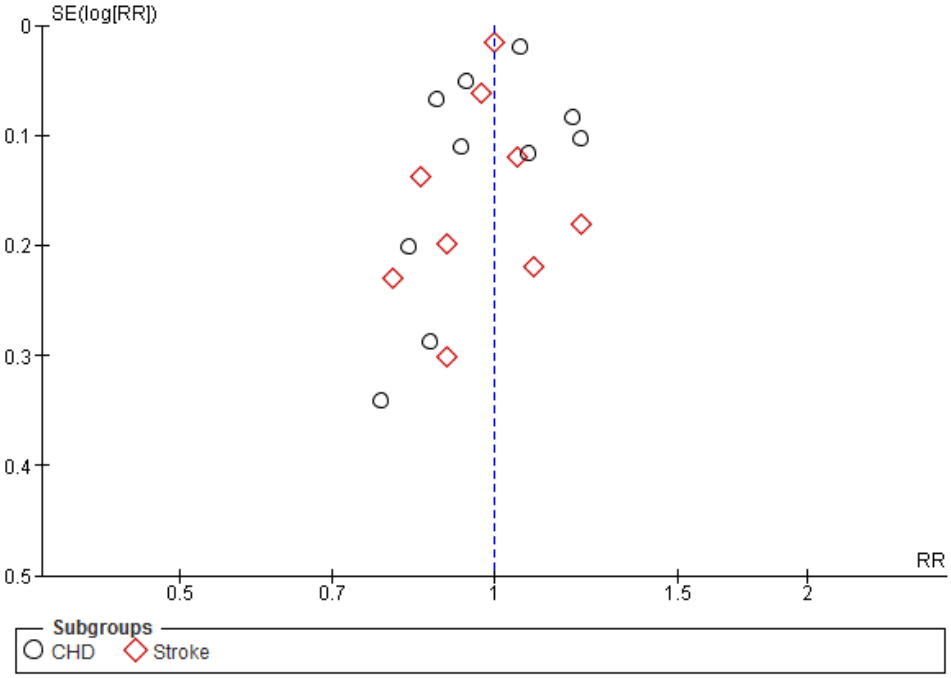

Figure 4.13 Funnel plot displaying the spread of risk estimates from the cohort studies included in this systematic review.

4.5 Discussion

The aim of this systematic review was to determine if parity was a risk factor for CVD, specifically for CHD and stroke. This discussion summarises the findings of the review, discusses the notable results in comparison to previous research and presents the strengths and limitations of the review.

4.5.1 Summary of findings

This systematic review included eighteen studies which assessed the association between parity and CVD, with the specific outcomes of either CHD and/or stroke. Of these publications, thirteen were cohort studies and five were case-control studies.

The quality of the studies was assessed using the NOS (Wells *et al.,* 2014). The results were varied across the studies with one (Parikh *et al.*, 2010) achieving all nine stars available for the research methods and some scoring poorly with only 2-4 stars (Beard et al., 1984; Cooper *et al.*, 1999; Gallagher *et al.*, 2011; Jacobsen *et al.*, 2011).

The unadjusted and adjusted results of the cohort studies were combined independently by random effects meta-analysis to evaluate the difference in risk of CVD in parous versus nulliparous women. The unadjusted analyses presented substantial diversity within the study risk estimates, which may be explained by the different ages and risk factor profiles of the individual sample populations. The adjusted ever parous versus nulliparous analysis for cohort studies reported no difference in risk for both CHD and stroke. Further analysis of risk per parity level suggested that the risk of these diseases changes with increasing parity. The CHD results suggest that the risk is equivalent for parous and nulliparous women until para 3, after which the risk increases, reaching a maximum at para 5+ (RR 1.21 (0.93-1.56)). For stroke risk the results follow a 'J' shaped curve, with a slightly reduced risk appearing at para 2 (RR 0.94 (0.91-0.97)) compared to para 0, and an increase in risk thereafter. Out of these analyses, only the pooled estimates for the parities with the lowest and highest risk of stroke were significant.

The subgroup analyses highlighted a difference in risk of CHD based on location, with the US and China reporting a reduced risk in ever parous women and the European studies stating an increased risk. These results were not consistent with the stroke analysis, which found an increased risk from the US subgroup and reduced risk from the Chinese studies, with no apparent effect of parity on stroke risk according to the European subgroup. Notably, the risk of CHD was significantly increased (RR 1.08 (1.03-1.13)) in the studies which did have an adequate follow up time compared to a reduced risk (RR 0.92 (0.85-0.98)) in those which did not.

The pooled result from the case-control studies represented a large increase in risk of CHD morbidity for ever parous women (aOR 1.49 (1.23-1.82)) compared to nulliparous women.

4.5.2 Notable Findings

The ratio of parous to nulliparous women within the 2,869,391 included in this review was 8:1. This high ratio is due to the majority of women worldwide having at least one child, for example in England and Wales in 2016, only 18% of menopausal women were nulliparous (Office for National

Statistics, 2017b). Therefore, when randomly sampling participants for a cohort study, the proportion of nulliparous women is comparatively low. It is necessary to note that many of these studies looked at different exposures or populations as well as those meeting the criteria for this review. Therefore, the total number of participants quoted in the original publications of these studies will be different to what is recorded in this chapter.

The death and disability adjusted life year (DALY) rates of CVD have been steadily declining since 1980, possibly due to improving disease prevention and healthcare provision (Bhatnagar *et al.*, 2016). Therefore, the CVD incidence from the most recent cohort studies is not directly comparable to the earliest studies. However, as the nulliparous and parous women in each study would have been treated by the same healthcare standards available for that time, the risk estimates produced for parous versus nulliparous women by each study are comparable.

The quality assessment of the studies included in this systematic review highlighted the poor research methods used in several of the studies, especially the six cohort studies which had an inadequate follow up time for the outcomes of interest to develop. This must be taken into account when drawing conclusions from the combined results of these studies, as an inadequate follow up time could bias the results towards the null. However, the studies which achieved the most stars were given the most weight in the meta-analyses due to their large sample sizes and consequent small standard error. This means the pooled risk estimates of this review are drawn mainly from results with limited potential for bias. Having said that, the study which achieved all available stars on the NOS (Parikh *et al.,* 2010), did not adjust for all traditional CVD risk factors e.g. smoking. Therefore, even the studies scoring highly in the quality assessment still present some potential for bias.

The pooled adjusted risk estimate of CHD in ever parous versus nulliparous women from the case-control analysis was much larger (aOR 1.49 (1.23-1.82) than that for the cohort studies (aRR (1.01 (0.94-1.09)). This may be because all of the case-control studies, except for a small proportion of

cases in the Beard *et al.* (1984) study, recorded outcomes only of CHD morbidity, whereas the cohort studies assessed both morbidity and mortality. Also, one of the studies (Beard *et al.*, 1984) only presented an unadjusted risk estimate which was included in the analysis, and one study (La Vecchia *et al.*, 1987) only adjusted for age. This means the 38.3% of the weighting in the case-control analysis given to these studies, represented data highly susceptible to confounding. Despite the potential confounding effect on the case-control pooled risk estimate, a study method used by the cohort studies may explain this discordance in results. All but three (Cooper *et al.*, 1999; Gallagher *et al.*, 2011; Jacobsen *et al.*, 2011) of the cohort studies excluded women with CHD at the start of follow up, to ensure temporality in the results. However, it was not possible to determine whether the CHD occurred before or after the exposure of pregnancy, as some of these cohorts began after women's reproductive years. Therefore, there may have been exclusion of women who were free from CVD before pregnancy and developed CVD afterwards. This exclusion may have introduced survival bias (Saracci, 2007) into the results of the cohort studies and diluted the association between parity and CHD.

From the cohort study meta-analyses, the adjusted risks of CHD and stroke were equal between ever parous and nulliparous women. This is potentially due to the change in risk per parity level, demonstrated in figures 4.5 and 4.8. As the variation in risk per parity level is relatively small, the parities with the lowest risk can attenuate the increased risk of the 4 or 5+ parities. Therefore, when these parities are combined to form an ever parous risk, there appears to be no effect. This contrasts with the previous systematic review by Lv *et al.* (2015) which suggested a protective effect of parity on CVD mortality (Hazard Ratio HR 0.79 (0.59-1.06)). However, that review only included studies of CVD mortality, therefore the outcome incidence from the studies would have been lower than this review, which also included morbidity cases. Also, the previous review included a study by Jaffe, Eisenbach and Manor (2011) which recorded mortality from all types of CVD, not only CHD and stroke. When this study was removed from the Lv *et al.*, meta-analysis the hazard ratio altered to only 0.91 (0.85-0.95), therefore still showing a protective effect but to a lesser extent.

Several of the studies which recorded outcomes of both CHD and stroke reported opposite risk estimates across the two diseases in the ever parous analysis. The risk ratio for CHD from Jacobsen *et al.* (2011) was aRR 0.93 (0.75-1.15) whereas the equivalent for stroke was aRR 1.21 (0.85-1.72), suggesting that the risk of stroke is greater with ever parity compared to a reduced risk of CHD. In contrast to this, Magnus *et al.* (2017) found parity to be protective against stroke, RR 0.85 (0.65-1.11), but increased the risk of CHD, RR 1.21 (0.99-1.48). These results suggest that there is an underlying difference in the effect of parity on the risk of these two diseases. However, neither of these papers' results were statistically significant and when the results of the individual studies are combined in both the ever parous and per parity level analyses the risk estimates for CHD and stroke are very similar.

The analysis of stroke risk per parity level demonstrated a 'J' shaped curve which was also reported by Parikh *et al.* (2010) and Lv *et al.* (2015). The results for both stroke, and to a certain extent CHD, compliment those from Parikh *et al.* which observed the lowest risk of these diseases to be at para 2 and the risk increasing with each parity level thereafter. This is due to the large weight which was given to this study in the meta-analyses. In contrast, the dose response analysis performed by Lv *et al.* found that women with four or five live births had the lowest risk of CVD mortality. This difference may again be due to the inclusion of both CVD morbidity and mortality in this review and the large weighting of the Parikh *et al.* study, whereas the Lv *et al.* review assessed the risk of CVD mortality alone.

The CHD subgroup analysis also demonstrated a statistically significant difference in results based on the location of the study. The studies from the US and China yielded a reduced risk of CHD in parous women, compared to a higher risk in Europe. However, this may be due to the inadequate follow up of the studies from China and the US compared to the studies from Europe which did have an adequate follow up length. Furthermore, all the studies from the US included women who had been college educated. Within the study by Colditz *et al.*, (1987) the study population consisted

only of nurses who have more knowledge of healthy lifestyle behaviours than the general population. As these characteristics would lower the prevalence of CVD risk factors in the study populations, the incident cases would have been proportionally lower than the studies from Europe which all recruited from the general population.

The two Chinese studies (Gallagher *et al.*, 2011, Peters *et al.*, 2017) show a clear trend of increased age of women at the higher parity levels within the cohorts. This is most likely due to the introduction of the one child policy in 1979 (Jiang, Feldman and Li, 2014) which greatly increased the number of women with only one child compared to the previous generations. Thus, the women of higher parity levels are much older than those in the para 1 group. This explains why the outlying unadjusted risk estimates from Gallagher *et al.* (2011) diminished after age adjustment. Both papers conclude that these intergenerational variations in parity have not affected the risk of CVD as the risk estimate favours parous women. However, the number of cases of stillbirth or spontaneous abortion were much greater in the nulliparous group in the Peters *et al.* (2017) study. Also, the nulliparous women were more likely to have never been married, which in Chinese culture is rare and shamed upon (Jiang, Feldman and Li, 2014). Both of these characteristics of nulliparous women suggest that underlying health problems or low socioeconomic status may account for their increased risk of CVD rather than the lack of physiological adaptations during pregnancy.

The stratified results reported by Vladutiu *et al.* (2017) reflect a small increase in stroke risk for ever parous black women compared to nulliparous black women, whereas the estimate for ever parous white women suggests a slightly reduced risk, RR 1.09 (0.71-1.67) and RR 0.90 (0.61-1.33) respectively. These results are congruous with those from the case-control study by Rosenberg *et al.* (1999) of black US women, which found a 67% increased risk of MI in parous women, RR 1.67 (0.87-3.22). The baseline characteristics of the Vladutiu *et al.* (2017) participants present skewed trends in parity, with the para 5+ women being more likely to be black, have limited education and low income and the para 1 women more likely to be white, college educated with a higher income.

Similar trends were found by Spence and Eberstein (2009) who compared all-cause mortality in black and white women, based on parity. These clear divides may infer that the difference in stroke risk between parous and nulliparous black and white women is not biological but due to a variety of biopsychosocial factors. Therefore, the conflicting risks of CVD reported by the Chinese and European studies in this review, may be due to contrasting cultures and psychosocial determinants and not solely due to differences in study design.

The CHD subgroup analysis revealed a significant difference between the studies which had an adequate follow up time, where there was a small increased risk with parity, compared to those which did not (a small decreased risk). An adequate follow up time in this review was defined as long enough for all of the participants to reach at least 55 years of age. This was a subjective cut-off for the dichotomisation of follow up length, as there is no defined time for the development of CVD. The results of this subgroup analysis may therefore change if a different cut-off was used. The difference in risk seen between the subgroups suggests that the proportional hazards assumption was not met in the studies which used Cox proportional hazards regression and did not declare the conclusion of testing the assumption. A similar result to this was found by Jacobs *et al.* (2012) which reported the association between number of pregnancies and CVD mortality were stronger after ten years of follow up. This highlights the effect poor quality studies have on the results of a review and the importance of designing studies with robust methodology which includes the consideration of the pathophysiological course of the outcome of interest. Additional studies with a long follow up are required to determine if this difference in risk of CHD is due to parity.

4.5.3 Strengths and Limitations

This systematic review is the largest to date, including 2,869,391 women from eighteen studies across four continents. The clear protocol which was determined before the research began ensured both reviewers were aware of their roles and of the strict criteria which were used to screen studies for inclusion. The search strategy covered two core medical databases, MEDLINE and

EMBASE and a further database, CINAHL Plus, which focussed on allied health professional's research, including midwifery. The search was therefore aimed at the relevant platforms to the research question and was successful in identifying all of the studies used in the previous two systematic reviews (Rich-Edwards *et al.*, 2014; Lv *et al.*, 2015) on parity and CVD. To prevent the introduction of publication bias, there was no restriction placed on the language of the studies included in this review and the abstracts from conference proceedings were searched. The search results were also screened independently by two reviewers and any conflicts in opinions discussed in relation to the pre-defined eligibility criteria. This prevented the introduction of reviewer bias during screening and the misclassification of studies (McDonagh *et al.*, 2013). Due to the revision of the exclusion criteria during the screening process, to discount studies which only assessed gravidity with CVD, we can be confident that the included studies address the research question by specifically investigating parity as the exposure. The quality assessment was completed using the NOS, which allowed an evaluation of the quality of a study overall, but the domain format also displayed which areas the included studies had design flaws or high standard research methods, in terms of reducing bias.

This systematic review was able to separately evaluate the risk of developing CHD and stroke, which had not been determined by the previous reviews (Rich-Edwards *et al.*, 2014; Lv *et al.*, 2015). These diseases have separate pathophysiological processes and implications on quality of life, therefore it was necessary to draw conclusions on the individual risk of these conditions rather than the umbrella term of CVD. This review included studies assessing both the morbidity and mortality from CHD and stroke, meaning the results from this review are closely reflective of the global burden of these diseases. The previous review (Lv *et al.*, 2015) only included mortality from CVD and therefore underestimated the incidence and risk of these conditions.

The inclusion of studies from a number of countries and races has allowed for a discussion on the effect of cultural or biological factors on parity and the risk of CHD and stroke. The results from this

study are also generalisable to a wider population as most of the studies, especially the largest by Parikh *et al.* (2010) recruited women from the general population.

Although this review presented novel data on the risk of two distinct CVDs, there are several limitations to the research. Firstly, focussing on the methods of this review, due to time restrictions, only one reviewer, Ashleigh Woodland (AW) conducted the data extraction and quality assessment of the studies. The second reviewer (Dr Pensée Wu, PW) completed this process for 30% of the studies in order to assess the quality of the first reviewer's technique. This may have introduced slight inaccuracies during quality assessment as this is a subjective process and is best assessed independently by at least two researchers. However, there were only small changes made after PW checked the data extraction and quality assessment completed by AW.

As explained in the methods chapter (see chapter 3), the data reported from the included studies was often not in the same format due to parities other than nulliparity being used as the reference group in reported analyses. Therefore, a technique proposed by Hamling *et al.* (2008) was utilised to convert some of the adjusted risk estimates from the studies into the ever parous and per parity level risk ratios used in the meta-analyses, with nulliparity made the reference category. The risk ratios used are therefore only estimates of the risk ratios which would have been derived based on the original data. Despite this, the risk ratios are still adjusted for the confounders included in each individual paper, and the method facilitated a more in-depth analysis of the studies than would have been possible using only the reported risk estimates.

As the studies did not present unadjusted risk ratios, the unadjusted meta-analyses in this review were calculated using only the raw data from the studies. This would not have taken into account the censoring of participants, which is a feature of the Cox regression analysis. However, the adjusted risk estimates were also presented, and these estimates were used to draw conclusions from the studies in the review.

Despite the conversion of data, there was still insufficient results to complete subgroup analyses for the risk of CVD per parity level. This would have been beneficial as the ever parous results do not represent a clear risk estimate for parity, as the risk changes with every pregnancy.

Due to the short time available for conducting this review, a formal dose response analysis was not performed, which would have further assessed the risk of CHD and stroke with increasing parity. However, the pooled adjusted risk estimates for each parity level were calculated and presented on graphs for easier interpretation.

Although attempts were made to find all relevant studies to the review, the grey literature was not searched extensively. Therefore, there may be studies which met the eligibility criteria for the review which were not identified. In addition, one study (Jacobs *et al.*, 2012) which was screened following the database search, reported a non-statistically significant weak inverse association of parity with CVD but did not present the results. Therefore, this study could not be included within the meta-analysis. This suggests there may be other studies which did not produce significant results and have therefore not been published, thus introducing reporting bias through selective outcome reporting (Sterne *et al.*, 2011). This may explain the apparent lack of small studies favouring nulliparity, especially for the outcome of CHD, in the funnel plot in figure 4.13. However, the graph is roughly symmetrical with a range of study sizes.

Throughout the review process limitations in the form of potential biases also arose from the studies themselves. The studies ranged in quality, with some studies scoring poorly for the possible introduction of selection bias. For example, the study by Colditz *et al.* (1987) only recruited nurses to the study. Also, due to the unreliable recording of outcomes, for example self-report, some studies may have introduced information bias or recall bias. Therefore, the results of the meta-analyses should be interpreted with this in mind. The largest studies within the analysis were however of good quality, meaning the largest weighting in the results comes from largely unbiased evidence.

Although the inclusion of participants with prior pregnancy complications, such as pre-eclampsia, was one of the exclusion criteria, seventeen of the included studies did not include this history in the baseline questionnaires. The only study which did ascertain a history of obstetric conditions from the participants was Parikh *et al.* (2010), which included exposed individuals but adjusted for this in the analysis. Therefore, the majority of studies will have involved patients with these risk factors but did not report it and therefore have been included in this systematic review. As discussed in chapter 2, pre-eclampsia carries a substantial increased risk of CVD for exposed women in the future (Wu *et al.,* 2017). The effect of this residual confounding may have altered the risk estimates of studies, potentially showing a false increased risk of CVD with parity. This will therefore have over-estimated the risk of CVD associated with parity.

However, a large Swedish study by Hernández-Díaz, Toh and Cnattingius (2009) found that the risk of pre-eclampsia is lower in parous women than nulliparous women. This suggests that the inclusion of women with previous pre-eclampsia is unlikely to have greatly affected the statistically significant result, for the increased risk of stroke at para 5+, found in this review. Also, several of the studies adjusted for diabetes and high blood pressure, although not specifically for obstetric conditions. The largest study by Parikh et al. (2010) included women with these obstetric conditions, but adjusted for them in the analysis and is therefore more accurate in assessing the independent risk associated with parity than the studies which did not ask about a history of these conditions.

4.5.4 Conclusion

The results of this systematic review suggest that ever parity is not a risk factor for CVD, however further research with longer follow up is needed to confirm this. The risk of CHD is equivalent for parous versus nulliparous women until para 3, after which the risk increases with each parity level. The risk of stroke in parous versus nulliparous women follows a 'J' shaped curve, as the lowest risk is at para 2, with the risk increasing thereafter. A statistically significant increase in stroke risk is

seen in women with five or more live births, compared to nulliparous women. As 10% of parous women have four or more children (Office for National Statistics, 2017b), this increased risk is potent within the parous population and should be acknowledged when assessing the likelihood of stroke or CHD, among other risk factors, in grand multiparous women.

The use of electronic health data recorded in primary care is increasingly being used to complete observational research and may be a potential resource for assessing the relationship between parity and CVD risk with a long follow up time. The next chapter describes the methods used to complete a feasibility cohort study, to assess this relationship, set within a local primary care database.

5 Cohort study set within primary care of association of parity with CVD: Methods

The previous two chapters explained the methods and results of the systematic review on the association between parity and cardiovascular disease (CVD). As this review collated data from participants across several continents, which all have a varied prevalence of disease and lifestyle behaviours, it is not possible to draw direct conclusions for specific populations. For example, the studies from China reported a higher risk of CVD in nulliparous women compared to parous women, which was the opposite of the European study results. This may have been due to the cultural differences, with nulliparous women in China being more likely to have an underlying health condition or be of low socioeconomic status.

Several of the studies included in the systematic review were of poor methodological quality as assessed by the Newcastle-Ottawa Scale (NOS) (Wells *et al.*, 2014). For example, one study only included nurses (Colditz *et al.*, 1987) while another only included college educated women (Cooper *et al.*, 1999). These studies would not have produced a cohort representative of the general female population. Four of the studies recorded outcome events based on self-reporting from participants (Cooper *et al.*, 1999; Jacobsen *et al.*, 2011; Simons *et al.*, 2012; Peters *et al.*, 2016)). This method of outcome ascertainment is susceptible to recall bias and would be improved by using medical records to identify CVD outcomes. The length of follow-up was also too short in many of the cohort studies included in the review. To overcome these limitations, it would be beneficial to conduct a cohort study which could assess the relationship of parity and CVD using longitudinal electronic health records (EHR) from general practice. EHR are becoming increasingly available for research and follow patients over a long period of time. Primary care is the gateway to the health service in the UK and over 95% of the population are registered with a general practice. Therefore, the cohort would be representative of the population registered to those practices, with no criteria based on educational level or occupation. Also, the ascertainment of exposure and outcomes would not be

subject to recall bias and should be more reliable as health data is recorded by healthcare professionals at the time of consultation.

A cohort study using the EHR of the local population of North Staffordshire was designed to investigate the strengths and limitations of using HER to assess the relationship between parity and CVD. The study therefore acted as a feasibility study, the methods of which could be developed further based on the findings of this feasibility study and applied to a larger, national general practice (GP) database, with longer follow-up time, to further explore the relationship in a wider population.

Stoke-on-Trent, the main city in North Staffordshire, is one of the most deprived local authorities in England, ranked 13th out of 326 districts (Department for Communities and Local Government, 2015), with 50% of its neighbourhoods falling within the most deprived national quintile (Public Health England, 2017b). The area fares worse than the national average for most health indicators (Public Health England, 2017b), including smoking and obesity prevalence, which are known risk factors for CVD. This study will therefore also give an indication of the potential relationship between parity and CVD in an area with high deprivation and CVD prevalence.

This chapter details the methods used to conduct the cohort study, including justification for the sample population, eligibility criteria and outcomes used, as well as details of the data analysis. The results of the cohort study are presented in chapter 6.

5.1 Aim of Study

The aim of the study was to explore the relationship between parity and CVD in the local population of North Staffordshire, in order to address the overall question of this research; Is parity a risk factor for CVD? This aim was achieved using routinely recorded electronic health data from the primary care setting.

5.2 Study Population

The participants for the cohort study were taken from the population of patients included within the Consultations in Primary Care Archive (CiPCA). CiPCA contains electronic primary care records of patients registered to currently nine GPs across North Staffordshire, with an annual registered population of just under 100,000. CiPCA is managed by the Research Institute for Primary Care and Health Sciences, Keele University. The practices which contribute to the database have undertaken training and quality assessments in recording health data electronically (Porcheret *et al.*, 2004). This patient data is recorded in the form of Read codes, which are a widely used set of hierarchical codes used for recording conditions, symptoms, procedures and test results in the United Kingdom (UK) primary care (Read, 1990; O'Neil, Payne and Read, 1995). Prescribed medications are also recorded. The data is pseudo-anonymised, meaning the names, addresses and free text of consultations of patients are not included within the data available to researchers, whilst year of birth is recorded rather than date of birth. Patients who have asked for their records not to be used in research have their records tagged at the practice, so they are not included in the extraction of data.

With sufficient completeness of recording, the Read codes can be used to estimate the prevalence or incidence of disease within this specific population and to track a patient's health over time. The completeness of coding from general practitioner consultations (i.e. percentage of consultations allocated a Read code) is over 90% in the CiPCA practices, which provide high quality patient records from 2000 to 2015 (Porcheret *et al.*, 2004). In a review comparing GP databases in 2001, the population contained within the CiPCA database was similar to the general population of England and Wales, in terms of the age and sex distribution (Jordan *et al.*, 2007).

5.3 Inclusion Criteria

As this cohort study aimed to assess the link between pregnancy and CVD in North Staffordshire, the study sample was women of childbearing age within the CiPCA database. This contrasts to some of the studies included in the systematic review which only included women who were

postmenopausal and therefore could not become pregnant during the follow up. However, as the CiPCA database only provided patient data from 2000 to 2015, the study required women to have a pregnancy code recorded during this time, and therefore would have been pre-menopausal at some point during the study period.

In order to evaluate the relationship between the exposure of parity and CVD, a comparison group of nulliparous women was required, which was also required of the cohort studies included in the systematic review. For this study, two unexposed (nulliparous) women were matched by age, within five years, to each exposed woman.

The accuracy of coding pregnancy outcomes, either livebirth, stillbirth or abortion, within the database was not deemed high quality enough to only include parous women, being women who delivered after 24 weeks gestation. To prevent the misclassification and subsequent incorrect inclusion or exclusion of participants, the exposure of interest in this study was broadened to encompass all pregnancies, including those of any gestational length. As the cardiovascular adaptations commence early in pregnancy, with blood pressure lowering within six weeks of conception (Sanghavi and Rutherford, 2014), a woman is still exposed to cardiovascular strain, even if the pregnancy terminates before 24 weeks. The results of this study can therefore still be used alongside previous studies which assessed the total number of pregnancies, for example (Ness *et al.*, 1993) in order to answer the research question of whether parity is a risk factor for CVD.

The inclusion criteria for participants in the study was therefore set as follows:

- Women between 15 and 45 years of age at any point between 1st January 2000 and 31st December 2015 and who were registered with a GP which contributed to the CiPCA.
- For the exposed group, women must have been recorded as pregnant at least once between 1st January 2000 and 31st December 2015.
- For the unexposed, comparison group, women must have no record of pregnancy between 2000 and 2015.

5.4 Exclusion Criteria

In order to gauge the relationship between pregnancy and CVD incidence, it was necessary for eligible participants to be free from cardio-metabolic disease at the beginning of the study follow up. This included the pregnancy complications of gestational diabetes and pre-eclampsia as these have been identified as independent risk factors for CVD (see chapter 2). Also, the participants must have not received a diagnosis or test result indicating the presence of the metabolic CVD risk factors, for example hypertension, which were being used as secondary outcomes in this study, prior to the record of pregnancy. Women with specific non-modifiable diseases (prior to pregnancy) which could increase the risk of CVD but had pathophysiological causes unrelated to the CVD outcomes, were also excluded. These included type 1 diabetes mellitus and secondary hypertension. To ensure these criteria were met, women were only included in the study if there were available data in CiPCA for one year preceding the first recorded pregnancy code, or for the comparison group, data for one year before follow up started. This was decided on the assumption that patients with a CVD or defined risk factor would present to the GP regarding these conditions at least once during a year and therefore would have this coded for on their record. These codes could then be identified, and the appropriate women excluded from the study.

The exclusion criteria for participants in this study were defined as:

- Women with a record of type 1 or type 2 diabetes mellitus before their first pregnancy
- Women with a record of CVD before their first pregnancy
- Women with a record of the metabolic CVD risk factors of hypertension and hyperlipidaemia prior to their first pregnancy
- Women with a record ever of gestational diabetes mellitus or pre-eclampsia
- Women without one year of records before the first recorded pregnancy code (index date)

For the never pregnant, unexposed group the same exclusions applied, however the index date was set as the latest of either the first recorded pregnancy date of the matched exposed woman or one

year after the start of the unexposed woman's records in CiPCA. This maximised the number of women able to be in the unexposed group.

5.5 Variables

5.5.1 Exposure Identification

As explained above, the exposure in this study was pregnancy of any outcome, including spontaneous or planned abortion, stillbirth or livebirth. To identify women with this exposure within the CiPCA database, a list of Read codes relating to pregnancy were compiled. This incorporated codes for a diagnosis of pregnancy and pregnancy related diseases, codes for the test or examination results within antenatal care as well as the codes for labour and outcomes of pregnancy. These codes were taken from previous published work (Tata *et al.*, 2005, 2007), except for the codes relating to abortions and miscarriages which were determined through the National Health Service (NHS) Clinical Terminology Browser, using the 5-byte Version 2 Read codes. The full list of exposure codes can be found in Appendix F.

5.5.2 Outcome Identification

The primary outcome in this cohort study was any outcome of CVD which was termed 'any CVD'. This outcome included codes for: CHD including myocardial infarction (MI), angina and heart operations used as treatment, heart failure, hypertension, peripheral vascular disease (PVD) and stroke including ischaemic and haemorrhagic stroke and transient ischaemic attack (TIA). CHD and stroke were chosen due to their combined burden of causing a third of all female deaths in Europe (Wilkins *et al.*, 2017). Heart failure was included as an outcome as it is the final outcome of cardiac disease of all forms, including CHD and hypertension, and therefore demonstrates severe disease (Chattterjee and Fifer, 2011). PVD has a similar pathogenesis to CHD including hypertension and atherosclerosis, whereby 40% of patients with atherosclerotic PVD also have significant CHD (Liang and Creager, 2011). Therefore, women could be acknowledged as at an increased risk of CHD due to the presence of PVD. Hypertension was included in this variable as although it is not a CVD, it is

a disease of the circulatory system and is commonly treated as a CVD in clinical practice (Lee, Williams and Lilly, 2011).

To identify women with these outcomes a list of Read codes was compiled, including codes for the diagnosis, monitoring and treatment of these diseases. This list incorporated codes which had been published as part of previous research (Kontopantelis *et al.*, 2014, 2015; Wright *et al.*, 2017; Zhong *et al.*, 2018). The list was reviewed by Dr Pensée Wu (PW), a consultant obstetrician, for the appropriateness of each code in identifying the outcome of interest. For the 'any CVD' outcome participants were censored after the first outcome event.

The outcomes of MI, CHD, stroke and type 2 diabetes mellitus (T2DM) were also investigated in isolation as secondary outcomes. Stroke and CHD were chosen as they are responsible for the majority of CVD deaths, with most of the CHD deaths attributable to MI (Townsend *et al.*, 2015). As the follow up time available through the CiPCA database was short, with a maximum of fifteen years, known metabolic risk factors for the development of CVD were included as secondary outcomes. These are type 2 diabetes mellitus and hypercholesterolaemia (GBD 2015 Risk Factors Collaborators, 2016). Therefore, given the likelihood of a limited number of cases of diagnosed CVD e.g. CHD and stroke, the incidence of risk factors could be used to determine how many participants were at an increased risk of CVD. Table 5.1 presents the primary and secondary outcomes for this cohort study.

Table 5.1 The primary and secondary outcomes for this cohort study.

Primary Outcome	Secondary Outcomes
Any CVD (including: CHD, stroke, PVD, hypertension and heart failure)	Coronary Heart Disease Myocardial Infarction Stroke Type 2 Diabetes Mellitus Hypercholesterolaemia

These outcomes were again identified through Read codes corresponding to the diagnosis and management of these conditions. The codes for diabetes and hypercholesterolaemia were found

through the NHS Clinical Terminology Browser, using the 5-byte Version 2 Read codes. The codes for hypertension were compiled using the previous research of Zhong *et al.* (2016) and Kontopantelis *et al.* (2015). The use of prescription codes of cardiovascular medications was considered for the identification of outcomes. However, as these medications are not specific to a certain condition and some have uses other than treating CVD, it was decided to only utilise the specific Read codes for each disease of interest. The full list of outcome codes can be found in Appendix G.

The follow up for participants started from their individual index date. For the exposed group this was the date of first pregnancy record and for the unexposed group it was the latest out of either the date of first pregnancy record of a matched exposed woman, or one year after the start of records for the unexposed participant. The participants were followed up from their index date until the earliest of either leaving the practice or the end of the study on 31st December 2015.

5.5.3 Covariates

There are several recognised risk factors for CVD which were explained in detail in the background chapter (see chapter 2). The metabolic risk factors which are independent diseases; T2DM, hypertension and hypercholesterolaemia were defined as outcomes in this study to indicate an increased risk of CVD due to the short follow up time for CVD development. Women with these risk factors at baseline were therefore excluded to ensure temporality in the results. The risk factors which were statistically adjusted for in this cohort study were: age, practice, obesity or being overweight, smoking and neighbourhood deprivation. Obesity and overweight were determined by the body mass index (BMI) which allows categorisation of patients into underweight, ideal, overweight and obese. This covariate was taken from the closest record of BMI before the participant's index date or the first record after if there was no record before the index date. Smoking status was dichotomised into 'ever smokers', meaning a record of being a current or ex-smoker at any point in the study period and 'non-smokers' being those recorded as a non-smoker at any point in the study period or those with no recorded smoking status. Those with no recorded

smoking status were defined as non-smokers as the Quality and Outcomes Framework (QOF), which is a voluntary reward programme for GP's, recommends that a non-smoker should be asked their smoking status until the age of 25, and if they have consistently been a non-smoker, at this point they do not need to be asked again (NHS Employers, 2014). Therefore, the absence of a smoking status code will most likely represent a non-smoker. The level of deprivation for each participant is reported as the index of multiple deprivation (IMD) score for the area which corresponds to the patient's postcode. The IMD is a score based on 37 indicators of deprivation within an area, which are categorised into 7 domains, for example 'employment deprivation' and 'barriers to housing and services' (Department for Communities and Local Government, 2015). These domains are then combined to give an overall IMD score. In CiPCA the deprivation scores reflect a weighted average of the IMD scores for the patient's wider area of residence, as a full postcode is not available. Deprivation was categorised into four groups from most to least deprived, based on the quartiles of IMD scores measured from the study participants.

These risk factors could act as confounders within the cohort study if the distribution of participants with these attributes was not equal between the exposed and non-exposed groups. Ensuring comparability between the cohort groups can be achieved by matching exposed to unexposed participants based on confounders, or by adjusting for these in the analysis (Szklo and Nieto, 2014). In this study each exposed participant was matched to up to two unexposed women whose age was within five years of the exposed woman's age. The participants were matched this way to increase the power compared to a 1:1 ratio of unexposed to exposed participants. Also, it was unlikely that there would have been enough unexposed to match more than two to each exposed participant. In order to maximise the number of unexposed participants included in the study, the participants were matched on age within five years. As the participants could not be matched on exact age, this was adjusted for along with the remaining risk factors in the analysis.

It is important to note that the CVD risk factors of family history, low physical activity and poor diet were not included in this analysis as these variables would not be extensively coded for in primary care records. However, the presence of obesity and hypercholesterolaemia, which were incorporated in the study, are indicators of a diet in high saturated fat and low physical activity (Strom and Libby, 2011).

The women in the database with these risk factors were detected by a list of Read codes corresponding to the diagnosis, treatment and monitoring of the conditions. The hypercholesterolaemia codes were derived from the NHS Clinical Terminology Browser, whereas the codes for smoking, and BMI were assembled using published code lists (Reeves *et al.*, 2014; Joseph *et al.*, 2017). The full list of Read codes for the confounders identified in this study can be found in Appendix H.

5.5.4 Codes for Excluded Conditions

To implement the exclusion criteria on the women within the database, a list of codes was compiled which matched the diagnoses or management of hereditary conditions or those which begin in childhood, for example, type 1 diabetes and pure hypercholesterolaemia. These codes were found using the NHS Clinical Terminology Browser. As well as this list of codes, all of the women with a cardiovascular outcome before their index date, were excluded.

5.6 Data Retrieval

The proposal for this research was reviewed by the CiPCA Academic Custodianship Committee to ensure the planned research met the ethical approval assigned to the CiPCA database for secondary analysis of the stored data. CiPCA has been approved as a research database by North West – Haydock REC ref: 17/NW/0232. Advice and guidance from the CiPCA Data Manager and the committee was incorporated into the research proposal throughout its development. Once the project proposal had been accepted, the data manager used the lists of Read codes to identify all

exposed women from the database for this cohort study and matched each woman to two unexposed women. The dataset was then cleaned by Professor Kelvin Jordan using IBM SPSS Statistics Data Editor Version 24. Ashleigh Woodland (AW) used this software to exclude participants who met each of the exclusion criteria and recoded variables to account for missing data, specifically the smoking, BMI and IMD variables. AW also used SPSS to complete all of the statistical analysis outlined below.

5.7 Analysis

The prevalence of the cardio-metabolic outcomes: 'any CVD', angina, MI, CHD, heart failure, stroke, hypertension, PVD, hypercholesterolaemia and T2DM was first calculated. This was also completed for the behavioural risk factors of ever smoking and obesity. This provided an indication of the burden of CVD in North Staffordshire. These prevalences were calculated using the frequency of outcome events, at any time between 2000 and 2015 in the original 15-45 year old cohort of women, before the exclusion criteria were applied. For the behavioural risk factor prevalences the frequency of participants with a relevant code was recorded.

A descriptive analysis of the participants within the final cohort (after exclusion criteria were applied) was completed to assess the baseline comparability between the exposed and unexposed groups. As all of the variables within the study except the matching variable of age were categorical, this was achieved using chi-squared tests. Statistical significance was set at a *p*-value of <0.05. For the continuous age variable, the mean and standard deviation with minimum and maximum ages for each group was calculated and an independent sample 2 tailed *t*-test was performed. To investigate the association between pregnancy and CVD risk, a Cox proportional hazards regression was performed. The time scale in the Cox model was follow-up time since cohort entry (index date), with censoring at time of first outcome event for the 'any CVD' variable, end of registration at

practice, or end of collected data (31st December 2015). Three models were created for the analysis, model 1 producing the unadjusted hazard ratio for ever pregnant versus never pregnant risk of cardio-metabolic disease ('any CVD') incidence. Model 2 adjusted this estimate for age at index date and model 3 included all the baseline covariates: age at index date, general practice, BMI, smoking status and index of multiple deprivation. Differences in the strength of association of the risk factors with cardio-metabolic disease were assessed between exposed and unexposed women using interaction terms. The analyses were repeated for the secondary outcomes of: MI, CHD, stroke and T2DM. There were only two outcome events for hypercholesterolaemia, meaning this outcome was not included in the Cox regression.

The assumption of proportional hazards was first tested for all outcomes in SPSS using the complementary log minus log plot, which showed that the data met the assumption. It was determined at a late stage of the analysis that the SPSS plots were incorrectly produced, as the statistical programme assumed that the data met the proportional hazards assumption when generating the complementary log minus log plot. The assumption was therefore tested again, this time by Professor Kelvin Jordan, using the Schoenfeld residuals in Stata and the assumption was met for the primary 'any CVD' outcome after adjustment for covariates. This method of testing the proportional hazards assumption is more accurate than using the log minus log plot as the plot is interpreted by visual inspection alone and can therefore be inaccurate in plots with a small hazard function.

5.8 Conclusion

This cohort study was completed using the CiPCA database which retrieves patient data from GPs within North Staffordshire. The exposure was pregnancy of any outcome and the comparison group was comprised of women with no recorded pregnancies. Women with pre-existing cardio-metabolic disease were excluded. The outcomes of interest were cardio-metabolic diseases which occurred during the follow up period which began on each individual's index date. Participants were

censored after the presence of an 'any CVD' outcome, leaving the GP practice or the end of follow up on 31st December 2015. A Cox proportional hazards regression model was used to investigate the association between pregnancy and cardio-metabolic disease. The next chapter presents the results of the cohort study.

6 Cohort Study set within primary care of association of parity with CVD: Results

The previous chapter explained the rationale behind conducting a cohort study using the Consultations in Primary Care Archive (CiPCA) to investigate the relationship between parity and cardiovascular disease (CVD). The methods used to complete the research were also detailed. This chapter presents the results of the study which include: the prevalences of cardio-metabolic diseases and risk factors within the population, the baseline characteristics of the study participants and the association of parity with CVD.

6.1 Formation of Cohort

In order to compile a cohort of women eligible for participation in the study, the CiPCA database was first searched for women who met the inclusion criteria. Using the exposure Read codes, the CiPCA data manager first extracted all women with a recorded pregnancy between 2000 and 2015 and able to be matched to two women (within five years of age) without a recorded pregnancy in this period. This yielded 12,024 exposed women matched to 24,048 unexposed women (36,072 in total). Women aged less than 15 or greater than 45 at their index date (as defined in chapter 5) were removed. This identified 31,715 women aged 15-45 years old between 2000 and 2015, with 11,721 of these being exposed women, matched to 19,994 unexposed women. Using the outcome and exclusion Read codes, the exclusion criteria were imposed on the participants resulting in a final eligible cohort of 20,513 women. To maximise numbers in the analysis, all initially identified unexposed and exposed women who fitted the inclusion and exclusion criteria were retained in the final analysis regardless of whether their matched unexposed or exposed woman/women was also retained. The, mean age of the two groups in the final analysis was very similar and age was included as covariate in final models. The flow chart in figure 6.1 depicts the exclusion process of potential participants for this cohort study.

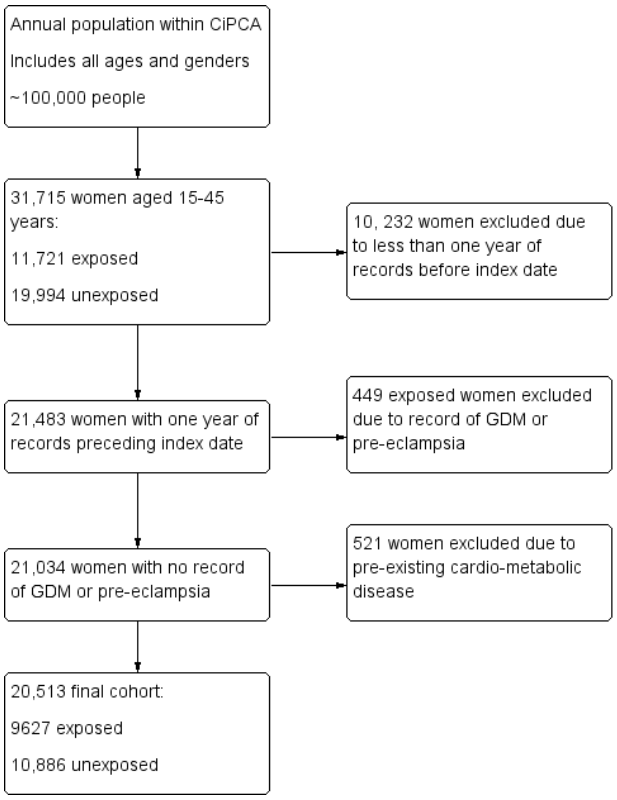

Figure 6.1 Flow diagram demonstrating the exclusion process of potential participants from the Consultations in Primary Care Archive, for this cohort study. GDM; Gestational Diabetes Mellitus.

6.2 Prevalence of Cardio-metabolic Disease

To determine the burden of disease within the local population of North Staffordshire the period prevalences of the cardio-metabolic outcomes were calculated for the study period 2000-2015. The original population of 31,715 women was used for this calculation as this would include all women who had an outcome at any time during the study period and gives an estimate for the prevalence of cardio-metabolic disease in women aged 15-45 years. Table 6.1 displays the period prevalences of the cardio-metabolic outcomes and behavioural risk factors in this study.

Table 6.1 Table depicting the period prevalence between 2000 and 2015 of cardio-metabolic disease for 31715 women in North Staffordshire aged 15-45 years≠.*

Cardio-metabolic Disease:	Total Period Prevalence: (%)	Unexposed Period Prevalence: (%)	Exposed Period Prevalence: (%)
Any CVD¥	3.5	2.9	4.5
Angina	0.1	0.1	0.1
Myocardial Infarction	0.1	0.1	0.1
Coronary Heart Disease∞	0.3	0.3	0.3
Heart Failure	0.1	0.1	0.1
Stroke	0.1	0.1	0.1
Hypertension	2.3	2.1	2.6
Peripheral Vascular Disease	1.1	0.7	1.9
Hypercholesterolaemia	0.02	0.02	0.03
Type 2 Diabetes Mellitus	1.8	1.6	2.2
Behavioural Risk Factor:			
Ever Smoker	41.4	29.8	61.2
Obesity (BMI>30)	22.0	20.9	23.1

** Not all women were followed up for 15 years. Maximum follow up was 15 years with a median follow up of 3.8 years*

≠ Women were aged 15-45 years at the start of follow up

¥ Any CVD = Cardiovascular Disease and includes all individual outcomes listed except hypercholesterolaemia and type 2 diabetes mellitus. For this variable, participants were censored after first CVD outcome.

∞ Coronary Heart Disease includes records of myocardial infarction, angina, unclassified coronary heart disease, and heart operations used as treatment for the disease.

6.3 Baseline Characteristics

The baseline characteristics of the 20,513 women included in the cohort study are presented in table 6.2. The *p*-values relating to the chi-squared tests for categorical variables and *t*-test for age, show that the differences in baseline characteristics for the exposed and unexposed groups were statistically significant. Although, the mean difference in age was small (0.24 years). These baseline characteristics were therefore included as covariates and adjusted for in the Cox regression models. The key disparities are in smoking status and deprivation. The majority of unexposed women were non-smokers (62.2%), whereas the majority of exposed women were ever smokers (62.0%). Most of the unexposed (27.8%) women were in the 2nd deprivation quartile while the largest percentage

of the exposed women (27.8%) were in the 3rd quartile for deprivation. It is important to note that there was missing data for deprivation and body mass index (BMI), with most of this originating from the unexposed group. Index of multiple deprivation (IMD) data was missing from 6.5% of the unexposed participants with full data for the exposed. A BMI code was not recorded for 29.5% of the unexposed and 9.5% of the exposed. 21% and 2% of the unexposed and exposed participants respectively did not have a smoking status code, however the participants without a smoking code were classified as non-smokers in the analysis.

Table 6.2 Baseline characteristics of participants within this cohort study, n=20513.

Characteristics:	Unexposed: n= 10886 % (n)	Exposed: n= 9627 % (n)	*p*-value: ≠ Significance at <0.05
Practice:			<0.001
1	14.6 (1588)	10.9 (1050)	
2	13.7 (1496)	10.6 (1020)	
3	12.1 (1315)	13.8 (1330)	
4	11.1 (1209)	11.3 (1090)	
5	4.1 (449)	2.9 (282)	
6	6.9 (752)	11.8 (1134)	
7	19.3 (2105)	12.7 (1227)	
8	8.2 (890)	9.9 (953)	
9	9.9 (1082)	16.0 (1541)	
Age: (SD)			0.028
Minimum	15	15	
Maximum	45	45	
Mean	27.80 (8.6)	28.04 (6.9)	
Index of Multiple Deprivation (IMD)*:			<0.001
Most Deprived	22.8 (2321)	25.3 (2438)	
2nd	27.8 (2834)	20.9 (2008)	
3rd	25.3 (2573)	27.8 (2680)	
Least Deprived	24.1 (2450)	26.0 (2501)	
Smoking Status:			<0.001
Non Smoker	62.2 (6774)	38.0 (3663)	
Ever Smoker	37.8 (4112)	62.0 (5964)	
Body Mass Index (BMI)*:			<0.001
Ideal (18.5-24)	48.4 (3717)	46.9 (4087)	
Underweight (<18.5)	6.6 (503)	4.6 (398)	
Overweight (25-29)	23.5 (1801)	27.0 (2356)	
Obese (≥30)	21.5 (1653)	21.5 (1872)	

** IMD and BMI contained missing data. 6.5% missing for IMD in unexposed. 29.5% missing for BMI in unexposed and 9.5% missing for exposed.*

≠ The *p*-value significance is for chi-squared test in all variables except age which is for an independent samples 2 tailed *t*-test

6.4 Incidence of Cardio-metabolic Disease

The participants were followed up for the outcomes explained in chapter 5 until they left the practice or the end of follow up on 31st December 2015. As all of the participants entered the study at different index dates, the length of follow up varies, from a minimum of one day to a maximum of fifteen years. The median length of follow up for the exposed women was 4.4 years compared to 3.4 years for the unexposed participants. During this follow up 761 participants had a recorded outcome, which corresponded to 3.7% of the cohort. There were 919 recorded outcomes in total, as participants could contribute more than one outcome, however only the first outcome was counted in the 'any CVD' variable. Of the recorded outcomes, 693 were CVD including hypertension, 224 were type 2 diabetes mellitus (T2DM) and 2 were hypercholesterolaemia. Table 6.3 depicts the number of events for each outcome in the exposed and unexposed groups.

Table 6.3 Incidence of primary and secondary outcomes during the follow up period 2000-2015, in this cohort study, n=20513.

Outcome:	Unexposed: n= 10886 % (n)	Exposed: n= 9627 % (n)
Any CVD*	2.9 (321)	2.9 (281)
Stroke	0.1 (15)	0.1 (10)
Coronary Heart Disease≠	0.2 (21)	0.1 (12)
Myocardial Infarction	0.1 (12)	0.1 (7)
Type 2 Diabetes Mellitus	1.1 (122)	1.1 (102)
Hypercholesterolaemia	0.02 (2)	0.0 (0)

** Any CVD = Cardiovascular Disease and includes all individual outcomes listed except Hypercholesterolaemia and Type 2 Diabetes Mellitus. For this variable, participants were censored after first CVD outcome.*

≠ Coronary Heart Disease includes records of myocardial infarction, angina, unclassified coronary heart disease, and heart operations used as treatment for the disease.

6.4.1 Cox Proportional Hazards Regression

In order to assess the association of pregnancy on the incidence of cardio-metabolic disease, a Cox proportional hazards regression was performed. As explained in chapter 5, the analysis was

undertaken for the primary outcome of 'any CVD', and the secondary outcomes: myocardial infarction (MI), coronary heart disease (CHD), stroke and T2DM. As previously stated, there were not enough outcome events to perform the regression for hypercholesterolaemia. Three models were fitted for each outcome; model 1 produced the unadjusted hazard ratio (HR), model 2 the age adjusted HR and model 3 HR adjusted for all the baseline covariates of: age at index date, general practice, smoking status, BMI and IMD. As there were only two records of hypercholesterolaemia in the follow up, this could not be used as a covariate in the analysis, for the other outcomes. None of the interaction terms for these covariates yielded statistically significant results, therefore these were not included in the final models. This was likely due to the lack of power from the small number of events. In order to maximise the number of participants within the analysis, those with missing codes for IMD and BMI were categorised into a 'missing' group for each covariate. This allowed the outcome data of these participants to be incorporated into the analysis. Table 6.4 presents the hazard ratio (HR) of incident cardio-metabolic disease in ever pregnant versus never pregnant women for each model of the Cox regression.

Table 6.4 Ever pregnant versus never pregnant risk of cardio-metabolic disease in women aged 15-45 years, during a 15 year follow up period between 2000-2015≠ n=20,513.*

	Hazard Ratio (95% Confidence Interval)		
Outcome†:	**Model 1₸**	**Model 2₸**	**Model 3₸**
Any CVD ¥	0.83 (0.70-0.97)	1.07 (0.91-1.27)	0.95 (0.78-1.13)
Myocardial Infarction	0.55 (0.22-1.39)	0.75 (0.28-1.96)	0.90 (0.32-2.54)
Coronary Heart Disease∞	0.54 (0.27-1.09)	0.91 (0.43-1.91)	1.12 (0.50-2.48)
Stroke	0.64 (0.29-1.43)	0.88 (0.38-2.01)	0.83 (0.35-1.99)
Type 2 Diabetes Mellitus	0.79 (0.60-1.02)	0.98 (0.75-1.28)	0.87 (0.66-1.16)

** Women were aged 15-45years at the start of follow up.*
≠ Maximum follow up was 15 years with a median follow up of 3.8 years.
¥ Any CVD = Cardiovascular Disease and includes all individual outcomes listed except Hypercholesterolaemia and Type 2 Diabetes Mellitus. For this variable participants were censored after first CVD outcome.
∞ Coronary Heart Disease includes records of myocardial infarction, angina, unclassified coronary heart disease, and heart operations used as treatment for the disease.
† Participants could contribute more than one outcome event except for the Any CVD outcome, where women were censored after the first outcome.
₸ Model 1 = Unadjusted HR. Model 2= Age adjusted. Model 3= Adjusted for: age, smoking status, body mass index, index of multiple deprivation and general practice.

As can be seen from table 6.4, the unadjusted HRs for each outcome suggest a reduced risk of cardio-metabolic disease for the exposed, ever pregnant women. These associations were attenuated by adjustment for age but still suggested a reduced risk for exposed women, except for the outcome of any CVD, which suggested a slightly increased risk. All the model 3 results, except for CHD, suggest that ever pregnancy is protective against cardio-metabolic disease incidence. However, these results were not statistically significant, with large confidence intervals (CI) due to the low number of outcomes. The results therefore suggest that there is no association between a history of pregnancy and the risk of future CVD.

Table 6.5 displays the risk estimates for the covariates from the model 3 regression for the primary outcome of 'any CVD'. As can be seen from the table, the risk of CVD differs between practises, and is higher in overweight or obese people. The risk of CVD is also higher in people living in deprived neighbourhoods (2nd quartile of IMD), older women and in those who have ever smoked.

Table 6.5 The effect of covariates within the model 3 Cox regression on the risk of the primary outcome, 'any cardiovascular disease', in this cohort study, with follow up between 2000 and 2015, n=20,513.

Model 3 Covariate:	Hazard Ratio (95% Confidence interval):	*p*-value: Significance at <0.05
General Practice:		
Practice 1	Reference	
Practice 2	0.70 (0.49-1.01)	0.057
Practice 3	0.58 (0.41-0.82)	0.002
Practice 4	0.67 (0.49-0.91)	0.011
Practice 5	0.73 (0.48-1.11)	0.140
Practice 6	0.84 (0.60-1.17)	0.295
Practice 7	0.67 (0.49-0.92)	0.012
Practice 8	0.68 (0.48-0.96)	0.028
Practice 9	0.85 (0.57-1.27)	0.426
IMD Quartile*:		
Most Deprived	Reference	
2nd Quartile	1.12 (0.82-1.5)	0.467
3rd Quartile	0.81 (0.58-1.15)	0.241
Least Deprived	0.82 (0.57-1.18)	0.281
Missing	0.23 (0.05-0.97)	0.045
Body Mass Index:		
Ideal	Reference	
Underweight	0.59 (0.28-1.27)	0.178
Overweight	1.52 (1.23-1.88)	<0.001
Obese	2.72 (2.24-3.31)	<0.001
Missing	0.37 (0.23-0.60)	<0.001
Age at index date≠:	1.10 (1.08-1.11)	<0.001
Ever smoker:	1.23 (1.03-1.47)	0.021

** IMD = Index of Multiple Deprivation*
≠ Women were aged between 15 and 45 years at the start of follow up

6.4.2 Sensitivity Analysis

Sensitivity analyses were performed to determine if the categorisation of missing data in the BMI covariate and allocation of participants without a smoking code into the non-smokers group, affected the results of the Cox regression. As both of these covariates had been statistically significant in the original 'any CVD' outcome analysis, this regression was repeated with the participants with missing data excluded. The results of the sensitivity analyses are presented in table 6.6. As can be seen from these results, the exclusion of women with missing data had no effect on the HR or statistical significance of the ever pregnant versus never pregnant risk of the primary outcome 'any CVD'. The sensitivity analysis results for the effect of each covariate on the risk of

each outcome was not greatly changed. The sensitivity analyses were only performed on the primary outcome due to the small number of events in the secondary outcomes.

Table 6.6 Results of model 3 Cox regression and sensitivity analysis after participants with missing data were excluded, for primary and secondary outcomes during a follow up period between 2000 and 2015.

		Ever Pregnant versus Never Pregnant Hazard Ratio (95% Confidence Interval)	
Outcome:	**Covariate:**	**Model 3:**	**Sensitivity Model with missing data excluded:**
Any CVD*	Body Mass Index	0.95 (0.80-1.13)	0.95 (0.79-1.12)
	Ever Smoker	0.95 (0.80-1.13)	0.94 (0.79-1.11)

**Any CVD = Cardiovascular Disease and includes all individual outcomes listed except Hypercholesterolaemia and Type 2 Diabetes Mellitus. For this variable, participants were censored after first CVD outcome.*

6.5 Discussion

The aim of this cohort study was to assess the relationship between parity and cardio-metabolic disease in the local population of North Staffordshire using general practice (GP) data from the CiPCA database. This discussion summarises the findings of the study as well as discussing the notable results. The strengths and limitations of the study are also presented with implications for future research.

6.5.1 Summary of findings

The cardio-metabolic diseases with the highest period prevalence in the 31,715 women, representing the female general population aged 15-45 within the CiPCA database, were hypertension at 2.3% and T2DM at 1.1%. Also, 41.4% of the total women were ever smokers and 22.0% were obese.

Once the exclusion criteria had been applied to this group a final cohort of 20,513 women was formed. There were dissimilarities between the unexposed and exposed groups, most notably the proportion of ever smokers was much higher in the exposed compared to the unexposed groups at

62% and 37.8% respectively. There was missing data for the BMI and IMD groups, with the majority of this corresponding to the unexposed participants, however, sensitivity analyses proved that this did not affect the results of the Cox regression analyses for the primary outcome, 'any CVD'.

The results of the Cox regression suggest that after adjustment for risk factors, the risk of developing cardio-metabolic disease is equivalent in ever pregnant versus never pregnant women ('any CVD' HR: 0.95 (0.78-1.13)). Due to the small number of outcomes the CIs were wide and none of the ever pregnant versus never pregnant risk estimates were statistically significant. All of the risk factors adjusted for in the model 3 'any CVD' regression were statistically significant.

6.5.2 Notable findings

The prevalences of cardio-metabolic diseases in this cohort were similar to the female United Kingdom (UK) figures for 16-44 year olds, according to GP data collected by the British Heart Foundation (BHF), (Townsend *et al.*, 2015). The prevalences of both MI and stroke, in the UK, were 0.1% for 2011. On the other hand, the prevalence of any CVD in this study, 3.5%, was greater than the UK average of 1.9% of 16-44 year old females. However, these figures are not directly comparable as the women in this cohort study were aged 15-45 years at their index date and the prevalence is over a median of 3.8 years rather than 1 year. The prevalence of obesity was comparable in this study population (22.0%) to the English figure for 2013 (24%), although this latter figure is for all women over 16 years (Townsend *et al.*, 2015) and so the results are not exactly comparable.

When assessing the baseline characteristics of the study participants there was missing data for smoking, BMI and IMD. The majority of the participants with missing codes for these covariates were unexposed women. This difference between the groups is likely to be because a woman's smoking status and BMI are required when completing an antenatal booking assessment. Therefore, the women who have been pregnant are more likely to have these risk factors recorded. As previously explained, the women without a smoking code were classified in the analysis reported

here as non-smokers due to the Quality and Outcomes Framework (QOF) recommendation to not ask for smoking status after the age of 25 years in non-smokers (NHS Employers, 2014). An alternative to this method would have been to use multiple imputation. However, sensitivity analysis showed there was no difference in results for the primary outcome, after excluding the women without a recorded smoking status. This was also true for the women with missing BMI data, and is probably due to the fact that healthcare practitioners are more likely to record the BMI of the women at the extreme ends of the BMI scale e.g underweight and obese. Therefore, the women with missing data likely represent those of an ideal weight.

As well as the missing data for the baseline characteristics, there was a clear distinction in the smoking status of exposed and unexposed women, with ever smokers comprising 62% and 37.8% respectively. When the women with missing data for smoking were excluded there was still a large distinction in the prevalence of ever smoking in exposed and unexposed women, with 59.2% and 40.8% respectively. This does not correspond to the expected prevalences of smoking in parous and nulliparous women, which in the pan-European study by Peters *et al.*, (2016) were similar at 21% and 25% respectively. Peters *et al.*, only recorded those who were current smokers which is why the figures are lower than those for this cohort study which reported ever smokers. However, using the Peters *et al.* study as an example it would be expected that the proportion of ever smokers would be similar across the exposed and unexposed groups. A potential reason for this deviation in the baseline characteristics could be that there is clear evidence that smoking can be harmful to a growing foetus (Mund *et al.*, 2013; Stone, Bailey and Khraisha, 2014). Smoking cessation advice and support is therefore given to pregnant women by healthcare professionals (Chamberlain *et al.*, 2017). Therefore, a combination of repeated questioning and lifestyle advice from healthcare professionals and a woman's desire for smoking cessation services during pregnancy will increase the likelihood that a woman will report a smoking habit. Unexposed women would not have experienced this period of intense smoking cessation advice and may therefore be less likely to

disclose their true smoking status. This could have led to the disproportionate number of ever smokers in the exposed group compared to the unexposed.

All of the risk factors were statistically significant as covariates in the Cox regression for the 'any CVD' outcome. This demonstrates the clear links between these risk factors and atherosclerosis and CVD progression, which have been proven by the Global Burden of Disease study (GBD 2015 Risk Factors Collaborators). Also, the trends seen in the covariate results were in keeping with the knowledge of these risk factors (Libby and Theroux, 2005; GBD 2015 Risk Factors Collaborators, 2016; Wilkins *et al.*, 2017), as the risk of an outcome was increased in the higher BMI groups and the ever smoker group. For example, compared to an ideal weight, the risk of T2DM incidence was HR: 2.9 (1.9-4.3) in the overweight category and HR: 7.6 (5.2-11.0) in the obese category.

6.5.3 Strengths and limitations

There are several strengths and limitations of this cohort study, some of which are due to the characteristics of GP databases and the assumptions which come with using this recorded data, whilst others reflect the specific design of this cohort study.

A GP is the usual gateway to healthcare in the UK, with all medical conditions, other than emergencies, first being reported at this level. GP visits are available free of charge for all under the National Health Service (NHS) in the UK, where 98% of residents are registered with a GP (Herrett *et al.*, 2015). Information regarding the management of diseases within secondary care, is also fed back to GP's to enable continuity of care. Primary care databases therefore provide invaluable health data for research, as the records from these consultations depict broad health profiles for the specific population served by that GP. Furthermore, when the records from individual practices are compiled into a database, the resulting health data is highly representative of the general UK population (Jordan *et al.*, 2007). GP databases are therefore increasingly being used for research, for example the CiPCA database has been used for over 30 research papers

(www.keele.ac.uk/mrr/publications/), while the Clinical Practice Research Datalink (CPRD) a national database, has been used for over 1,800 publications since 1988 (CPRD, 2018).

The records stored within these databases allow for the follow up of patients over time, without the cost of repeated correspondence with participants. Also, the potential for reporting bias, being the incorrect recall by a participant of an exposure or outcome event (Sedgwick, 2014), is abolished as the conditions and symptoms are coded at the time of presentation. The size of the registered population allows for research into rare diseases or studies where the outcome incidence may be low, as in this cohort study.

Since the introduction of the QOF, which is a voluntary reward scheme for general practices based on the coding of specific indicators (NHS Employers, 2014), the completeness of coding has improved (Herrett *et al.*, 2015). Specific to this study, the percentage of consultations coded from the GPs contributing to the CiPCA database was 93% in 2004 (Jordan, Porcheret and Croft, 2004).

As well as the associated strengths of primary care databases, certain aspects of this specific cohort study are noteworthy. Firstly, the CiPCA database is comprised of North Staffordshire practices and is therefore an excellent resource for health data from the specific population of Stoke on Trent, Newcastle under Lyme and the Staffordshire Moorlands. As this area is in the 10% most deprived localities within the UK (Public Health England, 2017b), it was useful to conduct research in this location on the incidence of CVD, for which low socioeconomic status is a risk factor (Mackenbach *et al.*, 2000; Cubbin *et al.*, 2006).

The Read codes used to identify the exposure, outcomes and confounders in the study participants, were compiled from several previous code lists, and the NHS Clinical Browser, with the duplicates removed. These lists were also discussed with Dr Pensée Wu (PW) for clinical expertise in assessing the suitability of each code for identifying the required conditions. Therefore, the final lists are likely to contain all of the relevant read codes for each variable, thereby reducing bias from the incorrect recording of exposure or outcomes in this study.

The GPs which contribute data to the CiPCA database have ongoing training and assessment in accurate and complete coding of morbidities, meaning the data provided is of high quality (Porcheret *et al.*, 2004). Furthermore, the variables of CHD, smoking status, heart failure, hypertension and diabetes mellitus recorded in this study are included in the QOF, therefore GPs have an incentive to code these conditions to a high standard (NHS Employers, 2014). This means the incidence and prevalence of these variables should closely reflect the true values within the population.

Within the model 3 Cox regression, four risk factors of cardio-metabolic disease were adjusted for: age, socioeconomic status (defined by IMD score), BMI and smoking status. This meant the HRs produced by the analysis more closely reflected the true, independent relationship between parity and cardio-metabolic disease. The age covariate was statistically significant in every outcome regression with all of the covariates being statistically significant in the 'any CVD' analysis. This confirmed the requirement to adjust for these risk factors. This strength in the study improves upon five of the cohort studies included in the systematic review, presented in chapter 4, which either did not adjust for age (Jacobsen *et al.*, 2011), or adjusted for age only (Colditz *et al.*, 1987; Cooper *et al.*, 1999) or adjusted for age and only one other risk factor (Parikh *et al.*, 2010; Gallagher *et al.*, 2011).

This study does however present some limitations. Despite the strengths of primary care databases, the data is not recorded specifically for research purposes, leading to limitations in its use. Firstly, the coding of morbidities is subjective, with the potential for certain codes to be used more than others based on the preference of the healthcare professional (Jordan, Porcheret and Croft, 2004). Also, during multi-complaint consultations only the most important issues may be coded, whilst long standing conditions may not be recorded at every presentation and may only be coded for if the treatment changes (Jordan *et al.*, 2007). These subjective aspects of the recording process produce disparities in coding both between and within practices, meaning the quality of coding and

use of specific codes across practices may not be directly comparable. This was demonstrated by Porcheret *et al.* (2004) who found a variation in coding completeness of 5% to 97% in seven practices, however coding has improved since this paper was published (Herrett *et al.,* 2015).

Inaccurate or incomplete data coding may also arise due to the inadequate transcription of secondary care records onto the primary care coding system, and morbidities being written as free text rather than coded in the correct format (Herrett *et al.*, 2015). Also, the GPs can only code for conditions which are consulted for, meaning complaints which do not require medical treatment or are not regarded as significant by a patient will not be recorded. For example, a general limitation of using GP records is that the availability of over the counter medicines for common ailments will reduce the number of patients consulting for these problems. All of these inconsistencies either due to the subjective nature of coding or inaccurate and missing codes, may result in the underestimation of prevalence and incidence of morbidities in research, both with cross-sectional and longitudinal study designs (Jordan *et al.*, 2007). This is built upon the necessary assumption that the lack of a Read code is due to the absence of that disease in any particular patient (Herrett *et al.*, 2015).

The most significant weakness in this research study is the short follow up of participants due to the lack of sufficient data within the CiPCA database before 2000. Therefore, the longest potential follow up was fifteen years which is not in accordance with the disease processes of the study outcomes. This is due to a lag effect between the exposure to a risk factor and the development of CVD (Yusuf *et al.*, 2001). As explained in the chapter 2, the main pathophysiological process causing CVD is atherosclerosis, which is a development of fatty plaques within the arteries over decades (Strom and Libby, 2011). This is demonstrated by data collected by the BHF (Townsend *et al.*, 2014) which shows the prevalence of both angina and stroke respectively do not surpass 1% of the UK population until the age of 55-64 years. As the mean age of the exposed and unexposed women in this study at the index date were 28.14 years and 27.69 years respectively, the maximum potential

follow up of fifteen years would not have been sufficient. Therefore, the incidence rates of CVD reported in the results will be an underestimate of the true value if a longer follow-up had been identified. This study can however be used to determine the risk of early onset cardio-metabolic disease in exposed and unexposed women.

Another limitation is that there may have been participants in this study which met the exclusion criteria, for example type 1 diabetes mellitus, but due to inconsistent coding of chronic conditions at each presentation or due to non-consultation, were not coded for this in the one year preceding the study start. These participants would therefore have been incorrectly included within the cohort and may have biased results by overestimating the incidence of cardio-metabolic disease.

Furthermore, the time frame of the study restricted the assessment of exposure status, as women may have had pregnancies before 2000 which were not recorded in the CiPCA database. This poses several limitations to the research as this may have caused incorrect classification of unexposed and exposed women. Firstly, women who had a pregnancy before 2000 and no pregnancies after this point would have been assigned into the unexposed group. Women with pre-eclampsia or GDM during a pregnancy before 2000 would also have been included in the unexposed group. The incidence of an outcome for these women would have been recorded in the unexposed group and therefore underestimated the association between pregnancy and cardio-metabolic disease. Secondly, women with CVD following a pregnancy which occurred before 2000 would have been excluded from the study, as it would have appeared that this CVD preceded their first pregnancy after 2000. This exclusion of exposed women with a CVD outcome would have led to an underestimate of the relationship between parity and cardio-metabolic disease. Also, due to the use of the first pregnancy code as the index date, CVD beginning in pregnancy but after the first record would have been classified as an outcome of CVD after pregnancy. Thirdly, only ever pregnant versus never pregnant analysis could be completed as there was insufficient information in the coding of pregnancies to determine the parity level of women.

As explained in chapter 2, the maternal cardiovascular adaptations are greater during a multiple pregnancy compared to a singleton, and the long term effects on the cardiovascular system may be increased (Tan and Tan, 2013; Sanghavi and Rutherford, 2014). However, due to incomplete coding it was not feasible to determine whether the pregnancies of the exposed group were singleton or multiple pregnancies. As multiple pregnancies could not be adjusted for in the Cox regression, this study could not determine if the relationship between pregnancy and cardio-metabolic disease is different in multiple pregnancies compared to singletons. However multiple pregnancies account for only 1% of pregnancies (Sagili and Divers, 2007) meaning any potential effect will have been small.

The original proposal for this study was to determine a participant's ethnicity and incorporate this into the analysis, as the systematic review presented in chapter 4, proposed varied CVD risks across different ethnicities. However, the completeness of coding of ethnicity within the CiPCA database was poor and therefore could not be included as a variable. This is consistent with data from the Clinical Practice Research Datalink (CPRD), a national GP database, which only reports an ethnicity code for half of patients (Herrett *et al.*, 2015). As a result, this cohort study was unable to examine the effect of ethnicity on CVD risk.

As the majority, 82%, of women in the UK, have at least one child (Office of National Statistics), there was not a sufficient number of nulliparous, unexposed women to match two to every one parous women by exact age. However, to overcome this, age was adjusted for in the analysis. It was also not possible to match all exposed women to 2 unexposed women.

Due to all of these limitations it is likely that the results of this study, which suggest there is no association between a history of pregnancy and cardio-metabolic disease, are an underestimate of the true effect of parity on future cardio-metabolic disease. Using data from a national database with a longer period of records would overcome many of these limitations. There would have been more data available to determine exposure status and longer follow up of patients for outcomes.

As this study was a feasibility study, the recommendations for future research in this area are explained in chapter 7.

6.5.4 Conclusion

The results of this study could suggest there is no association between parity and short term CVD outcomes. The study has highlighted the importance of adjusting for risk factors in analyses of this kind and presented the strengths and limitations of using GP records in observational research. Similar to the conclusions of the systematic review in chapter 4, this study has demonstrated the requirement of a follow up duration which suits the population and outcomes of interest. This feasibility study can be used to improve the methods of future studies carried out using CPRD or an equivalent database with a longer period of available data for follow up.

In the next chapter, the results of this study and the systematic review presented in chapter 4, will be compared to the current published literature on the association of parity with CVD, which was not included in the systematic review. Recommendations for future research and clinical practice will also be presented.

7 Discussion of Research

The aim of this research was to investigate the relationship between parity and future cardiovascular disease (CVD) and to determine if parity is a risk factor for CVD. This was achieved by completing a systematic review of the published literature, which was presented in chapters 3 and 4, and conducting a cohort study using the Consultations in Primary Care Archive (CiPCA) database, which was presented in chapters 5 and 6. This discussion will summarise the main findings of this research as well as the strengths and limitations. The results will be compared to previous publications on the topic. The potential mechanisms through which parity increases the risk of CVD will be explored and a conclusion will be reached as to whether parity is a risk factor for CVD. The implications this research has on future clinical practice and research will also be discussed.

7.1 Summary of Findings

The systematic review included eighteen studies in total, with thirteen cohort and five case-control studies. Separate meta-analyses of the cohort study results were conducted for the outcomes of morbidity or mortality from coronary heart disease (CHD) and from stroke, while the case-control studies only assessed the outcome of CHD. Both the CHD and stroke results suggested that the risk of CVD is increased from para 4 onwards.

The cohort study, included the records of 20,513 women from the CiPCA database, aged 15-45 years at baseline, between 2000 and 2015. There was no association between a history of pregnancy and future CVD. However, the covariates of: age, smoking status and body mass index (BMI) were all statistically significantly associated with CVD. The study acts as a feasibility study for future research into the association of parity with CVD. It provides a template of methods to be applied to another database, with a lengthier duration of patient records, to allow a longer follow up of participants.

There are three main findings of this research. Firstly, more research is needed to assess the risk of CVD in ever parous versus nulliparous women, as both the systematic review and CiPCA cohort study found no association. However, the subgroup analysis of the systematic review found that studies with an adequate follow up were more likely to find an association. For the purpose of this research an adequate follow up was defined as all of the study participants being 55 years and over at the end of follow up. Therefore, the results from the ever parous/ever pregnant analyses presented here may be an underestimate of the true relationship, due to the short follow up time in the CiPCA cohort study and in some of the studies included in the review. This theory is in accordance with the increased risk of CHD for ever parous women found in the case-control meta-analysis.

The second main finding of this research is that the risk of CVD is not the same for each parity level. Therefore, research into the relationship between parity and CVD should not only focus on ever parity but also each individual parity level as an exposure. Finally, the results of the meta-analysis for stroke demonstrated a 'J' shaped trend in risk per parity level. The lowest risk compared to nulliparity was at para 2, after which the risk increased with each subsequent pregnancy, with para 5+ women having a statistically significant increased risk of stroke.

7.2 Previous Research

Both the systematic review (see chapter 4) and CiPCA cohort study (see chapter 6) results suggested there was no difference in CVD risk between ever parous and nulliparous women. These results are similar to the ever parous versus nulliparous risk of all-cause CVD mortality found in the previous systematic review (Lv *et al.*, 2015), which suggested a protective effect of parity but with a non-statistically significant risk estimate. These findings are in contrast with a cross-sectional study by Catov *et al.* (2008), which found a statistically significant increase in stroke risk for ever parous women compared to nulliparous, in women whose mean age was 80 years. This study was not included in this systematic review (see chapters 3 and 4) as it was a cross-sectional study. The meta-

analysis of the case-control studies in this systematic review also found a statistically significant increased risk of CHD in ever parous women. Although these studies were not of a cohort design, it adds weight to the conclusion that a longer follow up of participants in a cohort study may yield statistically significant results for this risk estimate.

Within the published literature assessing the association between parity and CVD, there is a growing body of evidence demonstrating that grand multiparity increases the risk of CVD, as was seen in this systematic review. However, this systematic review did not include all published studies focussing on the association of parity with CVD, due to the specific exclusion criteria set within the protocol. For example, studies which examined composite cardiovascular disease risk, gravidity rather than parity, did not have a nulliparous comparison group, or utilised a cross-sectional design, were excluded. The following paragraph will compare the results of those excluded studies with this systematic review.

In concordance with this systematic review (see chapter 4), some studies report a 'J' shaped association of CVD risk with increasing parity (Green, Beral and Moser, 1988; Lawlor *et al.*, 2003; Dior *et al.*, 2013; Lv *et al.*, 2015), while others indicate a 'U' shaped curve (Koski-Rahikkala *et al.*, 2006; Catov *et al.*, 2008; Jaffe, Eisenbach and Manor, 2011) or a positive linear association (Ness *et al.*, 1993; Kvale, Heuch and Nilssen, 1994; Qureshi *et al.*, 1997; Chang *et al.*, 2011; Kim *et al.*, 2016). In contrast to these studies and the systematic review, a minority of studies report a negative linear association of risk with increasing parity (Sakauchi, 2007; Jacobs *et al.*, 2012).

These heterogeneous results are due to large disparities in the study design, population, exposure and outcomes of interest in the studies. The exposure of interest in these studies varies between gravidity (Ness *et al.*, 1993; Qureshi *et al.*, 1997; Kim *et al.*, 2016), meaning number of pregnancies, which was the exposure in the CiPCA cohort study (see chapter 5) and parity, being number of live or potentially live births. This means the results are not directly comparable at the lower

parity/gravidity levels, as a woman who has suffered three miscarriages would be a para 0 but would also be a gravida 3.

The inclusion of women with miscarriages in para 0 may partly explain the 'U' shaped and 'J' shaped associations reported by studies, in the form of negative health selection (Grundy and Tomassini, 2005; Jacobs *et al.*, 2012). Women with poor health, for example polycystic ovary syndrome, may be unable to conceive or may experience miscarriages, due to the associated subfertility (Lawlor *et al.*, 2003; Goodman *et al.*, 2015). Also, due to the underlying medical problem they are at an increased risk of CVD (Goodman *et al.*, 2015) compared to women who are healthy enough to support several pregnancies (positive health selection). Therefore, the 'J' or 'U' shaped trends of risk per parity level may reflect the increased risk of women who are unable to conceive due to health problems, rather than parity bearing a protective effect for women of para 1 or 2. Similar to this, the increased risk of CVD attributed to para 1 in some studies may be due to women who had a pregnancy complication, such as pre-eclampsia, which are more likely to occur in first pregnancies and due to this did not reconceive (Hernández-Díaz, Toh and Cnattingius, 2009). As these complications are independent risk factors for CVD, and were not adjusted for in all studies, the para 1 women appear to be more at risk of CVD compared to women of para 2. Para 2 women most commonly represent the parity at lowest risk of CVD, as was seen in the stroke results in this systematic review (see chapter 4).

Although multiple studies have investigated the association of parity with CVD, the specific outcomes used are different between the studies: mortality only, morbidity only, mortality and morbidity, CHD only, stroke only, CHD and stroke and composite CVD. The systematic review (see chapter 4), showed no substantial difference between the estimated risk of CHD and stroke with parity. The apparent irrelevance of specific CVD outcomes in determining results, may be due to the common pathophysiological process of atherosclerosis behind these different diseases.

Few of the results reported by the excluded studies are statistically significant, which was similar to the studies included in the systematic review. This may be due to the wide parity categories used by some, for example Chang *et al.* (2011) grouped para 0-4 women together, which does not account for the varying risks for each of these parities. This was evident in the null results of the ever parous analysis in this systematic review (see chapter 4) and CiPCA cohort study (see chapter 6). Other potential reasons for the non-significant results are inadequate follow up lengths or small cohorts leading to a lack of outcome events, which were both limitations of the cohort study presented in chapter 6. Also, the results will be subject to confounding as not all of the studies completed rigorous adjustment for metabolic and behavioural risk factors.

Despite the lack of significance for the majority of results on parity level 1-4, the unifying element of all of these studies is the increased risk of CVD morbidity in grand multiparous women (para 5+). In many studies, including several incorporated in this systematic review (see chapter 4), the risk estimate for grand multiparity was statistically significant. This research therefore adds to the current evidence supporting the relationship between grand multiparity and an increased risk of CVD.

7.3 Strengths and Limitations

There are specific strengths and limitations of the individual analyses within this thesis which were discussed in chapters 4 and 6. The strengths and limitations related to this research as a whole are as follows. Firstly, a strength is that the objectives set out at the beginning of this research were met, as both a systematic review with meta-analysis and cohort study using the CiPCA database were conducted. Another strength of the research is the large number of participants included in the systematic review and cohort study. This systematic review was the first to assess the relationship between parity and the outcomes of CHD and stroke separately and include both morbidity and mortality events from these diseases. The outcomes of CHD and stroke were

investigated individually, as these diseases have different pathological causes, which may have led to a difference in risk with parity. Also, these diseases account for the majority of CVD disability adjusted life years (DALYs) in England (Newton *et al.*, 2015). Therefore, the results accurately represent the large burden of CVD within the study populations.

There are limitations to this research in answering the research question of whether parity is a risk factor for CVD. Firstly, due to the lack of accurate pregnancy coding in the CiPCA database, the exposure recorded in the cohort study was gravidity rather than parity. Although this will have included parous women, the results are not directly comparable to that of the systematic review or previous research assessing parity. However, Jacobs *et al.*, (2012) investigated both exposures with CVD and found that the trends in risk are similar. The CiPCA cohort study was unable to assess the effect of ethnicity on CVD risk with parity. Therefore, future research should aim to address this.

Due to the single analysis of ever pregnant versus never pregnant women in the cohort study and wide parity categories used in previous research (Koski-Rahikkala *et al.*, 2006; Chang *et al.*, 2011; Dior *et al.*, 2013), there were not sufficient results to fully investigate the risk of CVD for individual parity levels, specifically para 3 and 4. Also, there is a potential for incorrect recording of exposure or outcomes in the studies in the systematic review, due to poor confirmatory methods, for example only using self-reported medical diagnoses.

Another major limitation of the research is the poor quality of some of the studies included in the systematic review and the limitations of the cohort study conducted using the CiPCA database. The poor quality arose mainly from the short length of follow up in the studies which pose limitations for both younger and older cohorts. A short follow up in younger women will not give adequate time for the CVD to manifest, yielding a small number of outcomes, as was the case in the CiPCA cohort study (see chapter 6). On the other hand, the initiation of follow up starting later in life allows the potential for survival related bias to influence results (Saracci, 2007). This occurs when exposed women die earlier in life due to the increased risk of mortality. This means any analysis

with a baseline at older age, will only include women who survived and may have been less exposed, causing the risk of death in exposed women to be biased towards the null (Saracci, 2007).

Finally, the results of both the systematic review and cohort study were not fully adjusted for confounders. This is because the individual studies in the review all adjusted for different CVD risk factors, and due to the insufficient recording of ethnicity and socioeconomic status, among other risk factors, in the CiPCA database.

7.4 Mechanisms for the Effect of Parity on future CVD

The relationship between parity and CVD is thought to be due to three potential mechanisms. These are: the cardio-metabolic effects of pregnancy, the lifestyle factors influenced by raising children and the social inequalities which undermine health. The interaction of these three mechanisms are displayed in figure 7.1 which was adapted from Magnus *et al.*, (2017).

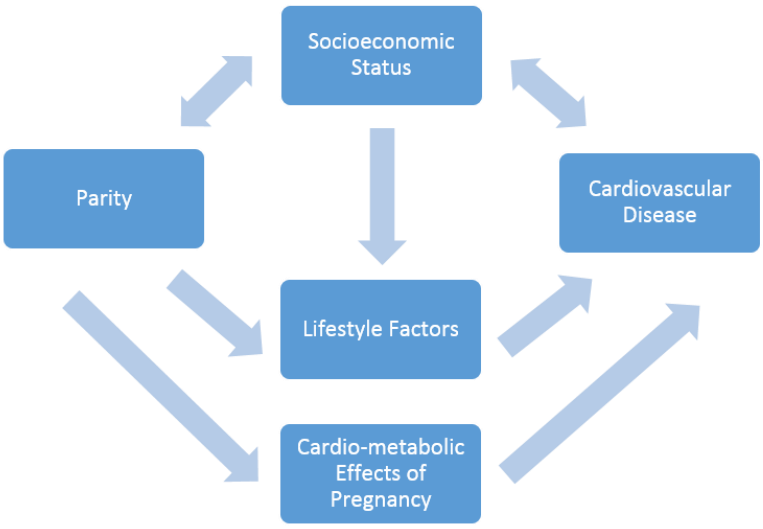

Figure 7.1 Potential mechanisms for the relationship between parity and cardiovascular disease.

There are several biological theories which have been presented for the association between parity and future disease. These include the disposable soma theory which considers a trade-off between fertility and life expectancy (Kirkwood, 1977; Lorenzini, Stamato and Sell, 2011). This is due to the division of resources, being energy and macronutrients between either maintenance of healthy

cells or reproduction. Therefore, multiparous women have reduced their resources available for maintenance, allowing development of disease. The theories of positive and negative health selection have already been discussed earlier in this chapter. Following on from these theories, the concept of reverse causality may affect the relationship between parity and CVD (Szklo and Nieto, 2014). However, these theories do not focus specifically on CVD development, therefore the biological factors discussed in this section will focus on the maternal adaptations to pregnancy which have been linked to future CVD.

7.4.1 Cardio-metabolic Effects of Pregnancy

The physiological mechanisms through which parity increases the risk of CVD were explained in detail in chapter 2. These adaptations have long term effects on the cardiovascular system, for example, left ventricular diastolic dysfunction is more common in grand multiparous women than all other parity levels (Aggarwal *et al.*, 2017; Keskin *et al.*, 2017). The fluctuations in metabolic markers and oxidative stress during pregnancy accelerate atherosclerosis development (Eren *et al.*, 2013, Skilton *et al.*, 2010). The effects of the maternal adaptations have been shown to increase with each subsequent pregnancy (Clapp and Capeless, 1997). This is reflected in the results of the systematic review (see chapter 4), as the risk of CHD and stroke increased with parity, however the results were not statistically significant after adjustment until para 5+. A recent systematic review (Li *et al.*, 2016) determined that grand multiparity is an independent risk factor for T2DM. A longitudinal study using record linkage in England and Wales by Grundy and Tomassini (2005) found that although the risk of all-cause mortality was greatest in para 5+ women, there was no statistically significant difference between para 1-4, which is in concordance with the results from this systematic review (see chapter 4). The authors therefore suggest that the effects of subsequent pregnancies are not cumulative, but that a mother's physiology can tolerate the adaptations until para 5. At this point a threshold is reached where the consequences of the cardio-metabolic adaptations manifest. Although, the results of this systematic review support this hypothesis, this threshold may be lower in a woman with underlying health problems.

7.4.2 Lifestyle Factors

Another potential mechanism through which parity mediates the increase in risk of CVD is through lifestyle choices which are behavioural risk factors for CVD. The characteristics of women in several studies assessing parity and mortality have shown that the prevalence of CVD risk factors such as smoking, obesity and low physical activity increase with increasing parity (Lawlor *et al.*, 2003; Koski-Rahikkala *et al.*, 2006; Catov *et al.*, 2008). This was also apparent in the results of the cohort study completed as part of this research, as the preponderance of exposed women were ever smokers, accounting for 62%, compared to only 37.8% in the unexposed women. This trend may be due to the busy lifestyle associated with raising children and the psychological and financial stress on parents (Ross and Mirowsky, 2002; Lee and Ryff, 2016).

A study by Lawlor *et al.* (2003) involving 8,538 participants from the British Regional Heart Study and the British Women's Heart and Health Study found that there was a statistically significant increase in smoking, obesity and inactivity with increasing parity for women. For example, the likelihood of smoking increased by odds ratio (OR) of 1.20 (1.11-1.30) for every increase of parity level. Furthermore, only the association with obesity was present in the male participants, where the number of children rather than parity was investigated. This raises questions in regard to the roles of men and women as parents, as having more children was linked to higher smoking rates and lower physical activity in women but not in men. Despite these statistically significant trends of CVD risk factors only occurring in women, Lawlor *et al.* (2003) found that there was not a statistically significant difference in risk of CVD between men and women for each number of children. As men have not undergone the cardiometabolic adaptations which occur in pregnancy, this result suggests that the increase in risk of CVD with parity in women is due to these behavioural risk factors rather than the biological effects of pregnancy. Other studies which propose that lifestyle choices are responsible for the link between parity and CVD are Jaffe *et al.* (2010) and Magnus *et al.* (2017). The latter of which was included in the systematic review presented in chapter 4.

7.4.3 Social Inequalities

The interaction between parity and socioeconomic status forms the third pathway through which the increase in risk of CVD is seen in grand multiparous women. As explained in chapter 2, parity increases inversely to socioeconomic status. Women of para 5+ are more likely to have completed fewer years of education, have lower income and be unemployed or in routine manual occupations than any other parity level (Lawlor *et al.*, 2003; Dior *et al.*, 2013; Office for National Statistics, 2016). Socioeconomic status impacts greatly on health as the prevalence of risk factors, such as poor diet and smoking, is higher in lower socioeconomic positions (Public Health England, 2017a).

Data from the 2010 report 'Fair Society, Healthy Lives' (The Marmot Review, 2010) show that the most deprived people will live shorter lives with more disability from ill health, compared to the least deprived. In the United Kingdom (UK), the difference in disability free life expectancy at birth, from the richest neighbourhoods compared to the poorest is seventeen years. Furthermore, a person with poor health or poor access to childcare may be unable to work and therefore cannot progress to higher social positions. This displays the complex nature of the social determinants of health and highlights the need to tackle social inequalities to improve the health of society as a whole.

These social inequalities are apparent in the literature addressing parity and CVD (Kington, Lillard and Rogowski, 1997; Lawlor *et al.*, 2003; Koski-Rahikkala *et al.*, 2006; Catov *et al.*, 2008; Dior *et al.*, 2013). In the Lawlor *et al.* (2003) study the independent adjustment for socioeconomic status attenuated the ORs, of CHD risk with increasing parity, more than any other metabolic or behavioural risk factor (Age adjusted OR: 1.31 (95% CI 1.18-1.44), Socioeconomic status adjusted OR: 1.22 (95% CI 1.10-1.35)). Thus, demonstrating the large weight socioeconomic status bears on parity and health outcomes.

In the Lawlor *et al.* (2003) study, the percentage of women of low socioeconomic status, as both an adult and a child, increased with parity level (p <0.001 and p 0.002 respectively). The social class a

woman is born into therefore influences her reproductive patterns and risk of CVD. For example, the rate of teenage pregnancies is higher in women of lower socioeconomic status (Lee and Ryff, 2016; Office for National Statistics, 2016). A systematic review (Rosendaal and Pirkle, 2017) has shown that the younger a woman is at her first birth, the higher her risk of CVD. This is due to a dynamic interplay of social and physiological factors as discussed above. Younger women gain and retain more weight during pregnancy and through the risk of obesity are more likely to develop CVD (Rosendaal and Pirkle 2017). Also, a young woman with a child will likely not complete high school or further education and is unable to work due to the need for childcare, meaning she will likely remain in the low socioeconomic position she was born into throughout adulthood (Lee and Ryff, 2016; Rosendaal and Pirkle 2017). This perpetuating cycle of adversity affects every generation of a family. Children born to mothers of low socioeconomic status will bear the same shorter disability free life expectancy as their mothers. Low socioeconomic status in early childhood has been shown to increase the risk of CHD in the future after adjustment for adult socioeconomic status and lifestyle factors (Hamil-Luker and O'Rand, 2007).

The studies in the systematic review (see chapter 4) were adjusted for several behavioural and metabolic risk factors as well as socioeconomic status and the para 5+ stroke risk estimate retained statistical significance. This suggests that the increased risk of CHD and stroke in para 5+ women is due to biological factors. However, not all of the cohort studies adjusted for these CVD risk factors, suggesting that a definite conclusion cannot be made from this research. Furthermore, Rosendaal and Pirkle (2017) propose that adjusting for metabolic factors such as T2DM and hypercholesterolaemia, which form part of the biological pathway between parity and CVD, masks the true relationship. Therefore, the adjusted risk ratios (aRR) presented in chapter 4 may be an underestimate of the effect parity has on woman's physiology and CVD risk in later life. Despite the statistically significant results after adjustment for socioeconomic status in both the systematic review and the Lawlor *et al.* (2003) study, high parity is clearly associated with social inequalities.

Therefore, further research into the association, with the aim of identifying the underlying mechanism, is required.

7.5 Parity as a risk factor for CVD

The aim of this research was to determine if parity is a risk factor for CVD. Several elements of the research compiled in this thesis can be used to answer this question. Firstly, the non-linear associations between parity and CVD identified in this systematic review (see chapter 4) and the previous published literature have demonstrated that parity should not just be classified as a dichotomous exposure. Future research should focus not only on ever parity as an exposure but also individual parity levels.

Although there is not clear evidence for an association between ever parity and CVD, one has been identified for para 5+ (grand multiparity). The systematic review in chapter 4, and previous studies (Ness *et al.*, 1993; Kvale, Heuch and Nilssen, 1994; Dior *et al.*, 2013) presented a statistically significant association between grand multiparity and CVD. The magnitude of this association was also noteworthy, with the systematic review in chapter 4 suggesting a 21% increase in stroke risk for para 5+ women (aRR 1.21 (95% CI 1.06-1.39)). In addition, as discussed above, due to the adjustment for risk factors which are influenced by parity, such as obesity, cholesterol and diabetes, the strength and magnitude of study results may be an underestimate of the true relationship. However, by adjusting for CVD risk factors, the studies included in this review have illustrated that the biological effects of pregnancy reach a threshold effect in grand multiparous women which increases their risk of CVD. This biological plausibility adds weight to the association. This research therefore suggests that grand multiparity is a risk factor for CVD, however more research is needed with a longer follow up of participants to form a definite conclusion.

7.6 Future Implications for Research

This thesis has highlighted several implications for future research into the association between parity and CVD. These are derived from the strengths and limitations of this research and those identified in the current published literature.

The main strength of this research is the inclusion of morbidity and mortality from CVD as outcomes, as this reflects the true burden of CVD. This should therefore be continued in future studies. However, this systematic review was unable to conduct meta-analyses for morbidity and mortality separately, due to a limited number of studies once stratified by CHD and stroke outcome. This has also not been achieved by other published studies on this topic. Therefore, it would be beneficial for future studies to complete analysis for the risk of composite CVD morbidity and mortality separately.

The main limitation of this research is the short duration of follow up in cohort studies, which does not reflect the lag effect between the exposure of parity and development of CVD. However, studies which only include an older population of women are susceptible to survival related bias (Saracci, 2007). Studies which utilised an adequate follow up duration were more likely to find an association between parity and CVD. Therefore, to build upon the current knowledge, there is a requirement for future studies to begin follow up during women's childbearing years and continue recording outcomes until the death of each participant. As this may not be feasible due to the cost and time required to complete such as study, follow up could continue until a defined study end date which would allow all participants to have reached 75 years. The age of 75 years would be a reasonable end point as death before this is considered to be premature mortality (Townsend *et al.*, 2015). Taking into account this long length of follow up required, case-control studies assessing the relationship between parity and CVD may be more appropriate in the short term.

As this research was unable to determine the effect of certain parity levels, specifically para 1, 3 and 4, it is imperative that each parity level is recorded and analysed in isolation in future research,

as the risk of CVD may differ between parity levels. Following this, it is important that future studies exclude women with pregnancy complications which are risk factors for CVD, such as pre-eclampsia. Also, women with CVD or T2DM before pregnancy should be excluded. These exclusions would facilitate a clearer evaluation of the effect parity exerts on the risk of CVD especially in nulliparous and primiparous women. By starting follow up during a woman's reproductive years, the risk of recall bias when identifying these exposures will be reduced. However, the risk of recall bias is particularly low in research on parity, due to the life-changing nature of the exposure.

In a future cohort study, the measurement of metabolic risk factors, such as cholesterol level and hypertension, as well as behavioural risk factors, for example, BMI and smoking status, should be recorded at baseline and at set intervals, as time-varying covariates, during follow up. This would allow the investigation of the changes in these risk factors with increasing parity. As Rosendaal and Pirkle (2017) suggest that adjusting for these risk factors underestimates the association between parity and CVD, presenting serial measurements could add weight to the theory that pregnancy causes these deviations in metabolic risk factors. Moreover, adjusting for these risk factors as continuous variables rather than categorical, where possible, would reflect the trend in risk, for example with increasing cholesterol levels, even within the normal range of results. Potential factors to adjust for in future research would include those related to pregnancy: age at first birth, history of the pregnancy complications pre-eclampsia, gestational diabetes mellitus (GDM), small birth weight and preterm birth as well as history of miscarriage and subfertility. Metabolic risk factors should also be adjusted for, including: hypertension, total cholesterol and lipoprotein levels and fasting plasma glucose level. The behavioural risk factors which should be adjusted for are: smoking, BMI, low physical activity and poor diet. Socioeconomic status should also be adjusted for, through measuring the IMD or other measure of socioeconomic status e.g. income or level of education. The non-modifiable risk factors of: age, family history of CVD and personal history of CVD or diabetes mellitus should also be adjusted for in future research.

As these recommendations present time consuming and costly study methods, it would be beneficial to conduct a cohort study fulfilling these criteria using electronic health records (EHR) from a general practice (GP) database such as the Clinical Practice Research Datalink (CPRD). This would facilitate the follow up of participants until death, without large resource implications, as well as abolishing recall bias when determining exposure or outcomes events. The methods employed in the cohort study presented in chapter 5 could be used to conduct such a study. However, as there are limited unexposed women compared to parous women, it would not be feasible to match two unexposed women to one woman of each parity level. Therefore, age would need to be adjusted for in the statistical analysis. The participants could be identified by selecting women from the database and then excluding all women with an exclusion code, as outlined in chapter 5.

In a study that utilises EHR, the index date for all participants could be the date of their 15th birthday. As the participants would not be matched, therefore the classification of women into parous and nulliparous could occur at the end of follow up. Starting follow up from fifteen years of age, allows for an accurate measurement of parity for both ever parity and per parity level. The frequency of birth/labour codes on a participant's record could be used to identify the specific parity level. Alternatively, the frequency of pregnancy codes could be used to identify parity level, however this would be more complicated, as a woman may have several pregnancy codes recorded during one pregnancy. Therefore, a gap of time between clusters of pregnancy codes could be set to determine that a new pregnancy has begun. For example, a gap of twleve months between pregnancy codes could be used to indicate two separate pregnancies. The participants could be followed up for CVD outcomes until death, or at least until their 75th birthday. This is currently not feasible in British national databases such as CPRD as the duration of records is not long enough. However, as the databases continue to record patient data this will be achievable in the future.

As socioeconomic status was strongly associated with both high parity and CVD, it would be beneficial to conduct an intergenerational study assessing parity and CVD. However, this could not be completed within a GP database as it would require information on years of education attained and occupation, which are not recorded in GP consultations. The IMD could not be used as an indicator of socioeconomic status in this situation as it is dependent on postcode and is therefore not specific to different individuals of one family, living in the same neighbourhood. Promisingly, this study could be done using the Multigeneration Register for Sweden (Ekbom, 2011) which has information on all people born in Sweden from 1932 onwards and has linkage to parental information. This would allow the investigation of the effects of childhood socioeconomic status and mother's parity on the relationship with adult socioeconomic status and reproductive patterns.

7.7 Future Implications for Clinical Practice

This research has identified two implications for future clinical practice. Firstly, grand multiparity is a likely risk factor for CVD and should therefore be considered by healthcare professionals when evaluating a woman's CVD risk. Explaining this risk to women may encourage them to engage in healthier lifestyle behaviours, for example smoking cessation. If healthcare professionals recognise this increased risk, it may also lead to the earlier instigation of medications for primary prevention of CVD. This would assist in the reduction of CVD incidence. The inclusion of grand multiparity in a risk assessment tool, such as the QRISK3 (Hippisley-Cox, Coupland and Brindle, 2017), which is part of the National Institute for Health and Care Excellence (NICE) Guidelines for CVD prevention (NICE, 2010), would enable healthcare professionals to recognise this increased risk. However, more studies are needed to confirm the increased risk, as the European Society of Cardiology (Piepoli *et al.*, 2016) and American Heart Association (Mosca *et al.*, 2011) do not yet recognise grand multiparity as risk factor for CVD, and as such do not recommend the consideration of this exposure in CVD risk assessment.

Secondly, this thesis has highlighted relevant literature showing that grand multiparity is associated with low socioeconomic status, which influences the health and lifestyle of children born to these women. Therefore, healthcare professionals should utilise the increased encounters with grand multiparous women during pregnancy, to encourage healthier lifestyle behaviours and signpost to social support if required. This is in line with the European Society of Cardiology (Piepoli *et al.*, 2016) and American Heart Association (Mosca *et al.*, 2011) guidelines on opportunistic screening for CVD risk assessment. This advice could improve the CVD risk of these women as well as their children.

7.8 Final Conclusion

This thesis has highlighted the importance of research into parity as a risk factor for poor health and CVD prevention. The research has explored the association of parity with CVD morbidity and mortality through a systematic review of the published literature and a cohort study using an EHR database. This facilitated an investigation of CVD risk in ever parous women and per parity level, compared to nulliparous women. There are complex interactions between parity and biological, social and lifestyle factors, which can determine both reproductive patterns and CVD development. This research has identified an increased risk of CVD in grand multiparous women, which should be considered by healthcare professionals when assessing a woman's CVD risk and explained to patients to encourage healthy lifestyle behaviours. As the current literature presents conflicting results for the trend in risk per parity level, more high quality research is needed in this area, to establish the relationship between parity and future CVD.

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Appendix

Appendix A

Arthritis Research UK Primary Care Centre
Systematic Review Protocol & Support Template
Version 5, last updated 0ctober 2016

Title of the review	Is Parity a Risk Factor for Cardiovascular Disease: a Systematic Review and Meta-Analysis
First reviewer	Ashleigh Woodland (AW)
Other reviewers (with role/contribution in the review)	Dr Pensée Wu (PW) Prof Kelvin Jordan
Clinical Portfolio Group	
Funding source	Part of an MPhil project.
PROSPERO registration number	

Amendments to the protocol	Addition of an exclusion criteria: Exclude studies if the only exposure is number of pregnancies (gravidity). Due to the time restraints of the review PW was unable to extract all of the data from the studies as part of the dual extraction. Change to the process of data extraction: AW will extract data from all the studies and PW will extract from 30% of the studies to quality assess AW's extraction.

1. Background to review

Brief introduction to the subject of the review, including rationale for undertaking the review and overall aim

It is known that a women's cardiovascular system undergoes significant changes during pregnancy, to support the developing fetus[1]. These maternal adaptations increase cardiac load and have been shown to affect the cardiac function long term[2]. Based on this knowledge, there have been many studies comparing parity and future cardiovascular disease (CVD). A study by Keskin et al[2] found that grand multiparous women (>5 pregnancies) had significantly reduced left ventricular diastolic function when compared to nulliparous women[2]. A systematic review by Lv et al[3] found that parity was inversely proportional to CVD risk until a woman's fourth live birth and with every pregnancy after this the risk of CVD increases. However, this study only looked at CVD mortality as a whole, rather than the individual diseases which are included within the umbrella term of CVD. The study also had significant heterogeneity of results and differences based on the country of origin[3]. By carrying out a systematic review and meta-analysis, we aim to assess the literature to identify if multiparity is an independent risk factor for ischaemic heart disease and cerebrovascular disease. This is because the two diseases cause the most mortality and morbidity, compared to all other types of CVD[4]. The results of this study can be used to inform women of their cardiovascular risk when discussing family planning and long term health. As well as allowing health professionals to consider parity as a risk factor for cardiovascular disease when assessing a woman's future health.

1. May L. Cardiac Physiology of Pregnancy. Compr Physiol. 2015 Jul 1;5(3):1325-44. DOI: 10.1002/cphy.c140043. Review. PubMed PMID: 26140720.
2. Keskin M, Avşar Ş, Hayıroğlu Mİ, Keskin T, Börklü EB, Kaya A, Uzun AO, Akyol B, Güvenç TS, Kozan Ö. Relation of the Number of Parity to Left Ventricular Diastolic Function in Pregnancy. Am J Cardiol. 2017 Jul 1;120(1):154-159. DOI: 10.1016/j.amjcard.2017.03.244. Epub 2017 Apr 12. PubMed PMID: 28479168.
3. Lv, H. *et al. Parity and Cardiovascular Disease Mortality: a Dose-Response Meta-Analysis of Cohort Studies*. *Sci. Rep.* **5**, 13411; doi: 10.1038/srep13411 (2015)
4. Newton JN, Briggs ADM, Murray CJL, Dicker D, Foreman KJ, Wang H, *et al.* Changes in health in England, with analysis by English regions and areas of deprivation, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. The Lancet 2015;386(10010):2257-2274.

2. Specific objectives/questions the review will address

Is parity an independent risk factor for cardiovascular disease, specifically for coronary heart disease and stroke?

At what number of pregnancies is the risk of developing cardiovascular disease the highest/lowest?

3. a) Eligibility Criteria for including studies in the review If the PICOS format does not fit the research question of interest, please split up the question into separate concepts and put one under each heading	
i. Population, or participants and conditions of interest	Adult women, aged 15 years and over
ii. Interventions/Exposure/item of interest	Parity
iii. Comparisons or control groups, if any	Adult nulliparous women, aged 15 years and over
iv. Outcomes of interest	Morbidity and mortality from coronary heart disease (CHD) and stroke CHD includes angina, myocardial infarction and unclassified CHD Stroke includes hemorrhagic and ischaemic stroke and transient ischaemic attack
v. Setting	No restriction
vi. Study designs	Cohort studies and case-control studies

3. b) Criteria for excluding studies not covered in inclusion criteria Any specific populations excluded, date range, language, whether abstracts or full text available, etc
Exclude studies if they are not case-control or cohort studies. Exclude studies where the study participants had cardiovascular disease before or during pregnancy and puerperium e.g. pre-existing hypertension or pregnancy-induced hypertension. Exclude studies if they do not compare multiparous women with nulliparous women. Some studies have different groups of patients e.g. 1-2 pregnancies, 3-5 pregnancies. These studies can be included as long as there is a nulliparous group. Exclude studies that give composite outcomes for men and women together. Exclude a study if the exposure of parity is not analysed separately from other exposures. Exclude a study if the outcome reporting is not either crude numbers (sufficient to calculate risk) or most adjusted results in form of risk ratio, relative risk, hazard ratio, odds ratio, etc. Exclude a study if the exposure is number of pregnancies (gravidity).

4. Search methods	
Electronic databases & websites Please list all databases that are to be searched and include the interface (eg NHS HDAS, EBSCO, OVID etc) and date ranges searched for each.	The databases to be searched are: EMBASE and MEDLINE through Ovid and CINAHL through EBSCO. Articles will be searched from inception of database to present.
Other methods used for identifying relevant research ie contacting experts and reference checking, citation tracking	The reference lists of included studies will be searched for any relevant articles to include.
Journals hand searched If any are to be hand searched, please list which journals and date searched from, including a rationale.	Due to time restraints of this systematic review, journals will not be hand searched.

5. Methods of review	
How will search results be managed & documented? ie which reference management software, how duplicates dealt with	The search results will be exported into Refworks to remove the duplicates and be screened by study title. The studies which are included after the title screen will be exported into Covidence for the abstract and full text screening.
Selection process Number of reviewers, how agreements to be reached and disagreements dealt with, etc.	There will be one reviewer AW during the initial selection using titles only. Then there will be two reviewers AW & PW for the selection using abstracts and full text. If there is a disagreement between reviewers then a decision will be made based on discussion. However, PW will make the final decision.
Quality assessment Tools or checklists used with references or URLs, was this piloted? Is it to be carried out at same time as data extraction?	The Newcastle- Ottawa Quality Assessment Scale (Wells *et al.*, 2014) will be used for quality assessment as follows: 1. Is the exposed population representative of the general population. 2. Is the exposed cohort similar to the non-exposed cohort? 3. How is the exposure ascertained? 4. Is the outcome of interest present at the beginning of the study? 5. Comparability of cohort? 6. How reliable is the ascertainment of the outcome? 7. How long is the follow up? 8. What is the attrition rate? The scale has been used in previous publications by PW. The scale has been piloted on several possible studies to be included in this review.

How is data to be extracted? What information is to be collected on each included study? If databases or forms on Word or Excel are used, were these piloted and how is this recorded and by how many reviewers?	The data to be extracted from each paper will be: Main author, year of publication, country of study, study design, number of exposure and control participants, age range of participants, categories of parity, the outcome risk, length of follow up, % participants lost to follow up and what confounders the results have been adjusted for. The outcomes listed below will be extracted from the data. The outcome reporting must be either crude numbers (sufficient to calculate risk) or most adjusted results in form of risk ratio, relative risk, hazard ratio, odds ratio, etc. The forms which will be used to record the data in Excel have been trialled on several studies which may be included in this review. AW will extract data from all the studies and PW will extract from 30% of the studies to quality assess AW's extraction.
Outcomes to be extracted & hierarchy/priority of measures	In order of preference: Morbidity from CHD and stroke. Meaning receiving a diagnosis of either of these diseases. Mortality from CHD and stroke. Meaning the cause of death being identified as one of these diseases.
Narrative synthesis Details of what methods, how synthesis will be done and by whom. Is the Narrative Synthesis Framework to be used?	We plan to conduct a thorough meta-analysis which will be complemented with a brief narrative synthesis. We will tabulate the main characteristics of each study to begin the synthesis as well as writing an overall description of the included studies. As we are analysing the results separately for CHD and stroke the synthesis will focus on the results of a few studies at a time which are all recording the same disease outcome. If there are not enough studies looking at the same disease outcome then the narrative synthesis will describe the cardiovascular disease risk as a whole involving all of the studies.

Meta-analysis Details of what and how analysis and testing will be done. If no meta-analysis is to be conducted, please give reason.	We aim to complete a meta-analysis for each disease outcome (CHD and stroke). However, if there are not enough studies to allow this, we will instead complete a meta-analysis for the composite cardiovascular risk using all of the outcomes together. We will use RevMan version 5.3.5 (Nordic Cochrane Center) to conduct random-effects meta-analysis using the inverse variance method for pooling log risk ratios. We will use random effects because the preliminary searches showed studies which were conducted in a wide range of settings and in different populations. Where possible, we will pool adjusted risk estimates from primary studies and if the data is not available, we will use raw data to calculate unadjusted risk estimates. We will complete a meta-analyses for the ever parous versus nulliparous results and the per parity level versus nulliparous risk of each disease outcome.
Will the overall strength of evidence be assessed? If so, how? ie GRADE?	As most of the studies will be cohort or case control studies, they will all be observational evidence and of a similar strength of evidence using the GRADE score. We therefore do not plan to assess the strength of the evidence of the included studies.

6. Presentation of results	
Outputs from review Papers and target journals, conference presentations, reports, etc	This systematic review will be forming part of an MPhil dissertation. The target journal for publication is *Circulation: Cardiovascular Quality and Outcomes*.

7. Timeline for review – when do you aim to complete each stage of the review	
Protocol	
Literature searching	2 weeks
Quality appraisal	2 weeks
Data extraction	2 weeks

Synthesis	2 months
Writing up	2 months

Support – please state if advice/training or required at each stage	
SR overview	
Protocol development	
Literature searching	Will require assistance with literature search
Quality appraisal	
Data Extraction	
Synthesis	
Writing up	

Appendix B

Final Search Strategy: MEDLINE

1 pregnan*
2 multipar*.ti,ab.
3 parity.ti,ab.
4 parous.ti,ab.
5 Parity/
6 gravidity.ti,ab.
7 Gravidity/
8 live birth*.ti,ab.
9 or/1-8
10 angina.ti,ab.
11 myocardial infarct*.ti,ab.
12 MI.ti,ab.
13 (myocard* adj5 isch?emi*).ti,ab.
14 isch?emic heart disease.ti,ab.
15 IHD.ti,ab.
16 heart attack*.ti,ab.
17 (coronary adj4 disease).ti,ab.
18 acute coronary syndrom*.ti,ab.
19 ACS.ti,ab.
20 exp Myocardial Ischemia/
21 stroke*.ti,ab.
22 cerebral infarct*.ti,ab.
23 cerebrovascular disease.ti,ab.
24 cerebrovascular accident*.ti,ab.
25 cerebrovascular event*.ti,ab.
26 CVA.ti,ab.
27 exp Stroke/
28 cardiovascular diseas*.ti,ab.
29 CVD.ti,ab.
30 cardiovascular outcome*.ti,ab.
31 Cardiovascular Diseases/
32 or/10-31
33 exp Epidemiology/
34 exp Epidemiologic Methods/

35 epidemiology.fs.
36 etiology.fs.
37 risk*.ti,ab.
38 Risk Factors/
39 cohort*.ti,ab.
40 predict*.ti,ab.
41 or/33-40
42 9 and 32 and 41

Appendix C

Headings within data extraction table for the cohort studies in the systematic review.

Study Characteristics

First Author - Surname, Initial e.g Jones, A
Year - Year of publication
Country of Study - Countries where participants were followed up
Study Design - cohort or case control
Name of Trial or Cohort - If a cohort e.g. Framingham Heart Study was used to collect data. If individual study with no name put N/A

Participant Characteristics

Total number of participants - If a study included men and women, only give total number of women in study
Number of Exposed Women - Total number of parous women included
Number of Unexposed Women - Total number of nulliparous women included
Categories of Parity - What levels of parity are used? E.g. 1-2, 3-5, >5 etc.
Number of Participants per Parity Level - Give number of participants per parity level. If not stated in paper put ?
Age of Unexposed - Mean age ± SD nulliparous women
Age of Exposed - Mean age ± SD parous women

Study Methods & Quality Assessment using Newcastle Ottawa Scale

Selection of Exposed – How were parous participants selected?
Representativeness – How representative were the exposed group to the general population? Please see extra guidance for NOS
Exposure Confirmation – How was the level of parity for each participant ascertained? See extra guidance for NOS
Selection of Unexposed – How were nulliparous participants selected? See extra guidance for NOS
Matching – How were the participants matched? If not matched put N/A if not stated put ?
Temporality – Was there a demonstration that the outcome of interest was not present at start of study? Yes/No
Length of Follow Up – How long were the participants followed up for? Cohort studies only
Adequate Follow Up? – Was follow up long enough for outcomes ot occur? Yes/No
Attrition Rate – How many participants were lost to follow up? See extra guidance for NOS
Outcomes – Give all outcomes recorded in order of priority
Outcome Definition – What were the definitions of outcomes if stated e.g. mortality or morbidity
Outcome Confirmation - How was the presence of outcomes confirmed? See guidance for NOS

Results

Crude Numbers Exposed – Give crude number of outcomes in parous women. If stated e.g 1/10 that developed CVD. Give for each outcome in same order as previously. If not stated put ?
Crude Numbers Unexposed – Give crude number of outcomes in nulliparous women if stated. Give for each outcome in same order as previously. If not stated put ?

Unadjusted Ratio – Can be risk, hazard or odds ratio. Give for each outcome with 95% confidence interval.
Adjusted Ratio – Can be risk, hazard or odds ratio for each outcome. Give with 95% confidence interval. Choose most adjusted result.
Confounders – Which confounders were adjusted for in the analysis?

Any additional comments about the study which do not fit into these boxes

Headings within data extraction table for the case-control studies in the systematic review.

Study Characteristics

First Author - Surname, Initial e.g Jones, A
Year - Year of publication
Country of Study - Countries where participants were followed up
Study Design - cohort or case control
Name of Trial or Cohort - If a cohort e.g. Framingham Heart Study was used to collect data. If individual study with no name put N/A

Participant Characteristics

Total number of participants - If a study included men and women, only give total number of women in study
Number of cases - Total number of cases included
Number of controls - Total number of controls women included
Categories of Parity - What levels of parity are used? E.g. 1-2, 3-5, >5 etc.
Number of Participants per Parity Level - Give number of participants per parity level. If not stated in paper put ?
Age of Controls - Mean age ± SD
Age of Cases - Mean age ± SD

Study Methods & Quality Assessment using Newcastle Ottawa Scale

Outcomes – Give all outcomes recorded in order of priority
Outcome Definition – What were the definitions of outcomes if stated e.g. mortality or morbidity
Selection of cases – How were participants selected?
Outcome Confirmation - How was the presence of outcomes confirmed? See guidance for NOS
Representativeness – How representative were the cases to the general population? Please see extra guidance for NOS
Selection of Unexposed – How were controls selected? See extra guidance for NOS
Matching – How were the controls matched to cases? If not matched put N/A if not stated put ?
Definition of controls – Were the controls free from the outcomes of interest? See NOS guidance
Exposure definition – What was the exposure e.g. number of children, number of live births etc.
Exposure Confirmation – How was the level of parity for each participant ascertained? See extra guidance for NOS
Ascertainment of Exposure – Was the method of exposure ascertainment the same for cases and controls. See extra guidance for NOS
Non-response Rate– How many participants did not respond for exposure ascertainment? See extra guidance for NOS

Results

Crude Numbers Cases – Give crude number of exposed cases out of all cases. If stated e.g 1/10 that developed CVD. Give for each outcome in same order as previously. If not stated put ?

Crude Numbers Controls– Give crude number of exposed controls out of all controls. If stated. Give for each outcome in same order as previously. If not stated put ?
Unadjusted Ratio – Can be risk, hazard or odds ratio for each outcome. Give with 95% confidence interval.
Adjusted Ratio – Can be risk, hazard or odds ratio for each outcome. Give with 95% confidence interval. Choose most adjusted result.
Confounders – Which confounders were adjusted for in the analysis?
Any additional comments about the study which do not fit into these boxes

Appendix D

Wells, G. *et al.* (2014) *The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta- analyses*, *The Ottawa Hospital Research Institute*. Available at: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp (Accessed: 28 June 2018).

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE
CASE CONTROL STUDIES

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Exposure categories. A maximum of two stars can be given for Comparability.

Selection

1) Is the case definition adequate?
   a) yes, with independent validation ✸
   b) yes, eg record linkage or based on self reports
   c) no description
2) Representativeness of the cases
   a) consecutive or obviously representative series of cases ✸
   b) potential for selection biases or not stated
3) Selection of Controls
   a) community controls ✸
   b) hospital controls
   c) no description
4) Definition of Controls
   a) no history of disease (endpoint) ✸
   b) no description of source

Comparability

1) Comparability of cases and controls on the basis of the design or analysis
   a) study controls for _____ (Select the most important factor.) ✸
   b) study controls for any additional factor ✸ (This criteria could be modified to indicate specific control for a second important factor.)

Exposure

1) Ascertainment of exposure
   a) secure record (eg surgical records) ✸
   b) structured interview where blind to case/control status ✸
   c) interview not blinded to case/control status
   d) written self report or medical record only
   e) no description
2) Same method of ascertainment for cases and controls
   a) yes ✸
   b) no
3) Non-Response rate
   a) same rate for both groups ✸
   b) non respondents described
   c) rate different and no designation

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE
COHORT STUDIES

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability

Selection

1) Representativeness of the exposed cohort
   a) truly representative of the average _____ (describe) in the community ✸
   b) somewhat representative of the average _____ in the community ✸
   c) selected group of users eg nurses, volunteers
   d) no description of the derivation of the cohort
2) Selection of the non exposed cohort
   a) drawn from the same community as the exposed cohort ✸
   b) drawn from a different source
   c) no description of the derivation of the non exposed cohort
3) Ascertainment of exposure
   a) secure record (eg surgical records) ✸
   b) structured interview ✸
   c) written self report
   d) no description
4) Demonstration that outcome of interest was not present at start of study
   a) yes ✸
   b) no

Comparability

1) Comparability of cohorts on the basis of the design or analysis
   a) study controls for _____ (select the most important factor) ✸
   b) study controls for any additional factor ✸ (This criteria could be modified to indicate specific control for a second important factor.)

Outcome

1) Assessment of outcome
   a) independent blind assessment ✸
   b) record linkage ✸
   c) self report
   d) no description
2) Was follow-up long enough for outcomes to occur
   a) yes (select an adequate follow up period for outcome of interest) ✸
   b) no
   3) Adequacy of follow up of cohorts
a) complete follow up - all subjects accounted for ✸
   b) subjects lost to follow up unlikely to introduce bias - small number lost - > ____ % (select an adequate %) follow up, or description provided of those lost) ✸
   c) follow up rate < ____% (select an adequate %) and no description of those lost
   d) no statement

Appendix E

Blank table for the quality assessment of cohort studies included in the systematic review, using the Newcastle-Ottawa Scale.

Study ID:	Selection					Comparability		Exposure			
Surname, Year	Is the case definition adequate?	Representa-tiveness of the cases	Selection of controls	Definition of controls	Total number of Stars (max. 4)	Confounders adjusted for in the design or analysis*	Total number of stars (max. 2)	Ascertainment of exposure	Same method of ascertainment for cases and controls?	Non-response rate	Total number of stars (max. 3)

Blank table for the quality assessment of case-control studies included in the systematic review, using the Newcastle-Ottawa Scale.

Study ID	Selection					Comparability		Outcome			
Surname, Year	Representativen-ess of the exposed cohort	Selection of non-exposed	Ascertainment of exposure	Tempora-lity in results	Total number of stars (max. 4)	Confounders adjusted for in analysis	Total number of stars (max. 2)	Assessment of outcome	Was follow up long enough?	Particip-ants lost to follow up	Total number of stars (max. 3)

Appendix F

Highest level exposure Read codes used in cohort study. All daughter codes were included.

Pregnancy	
13H8.00	Illegitimate pregnancy
13H7	Unwanted pregnancy
13Hd.00	Teenage pregnancy
13S..00	Pregnancy benefits
1541	H/O: stillbirth
1544	H/O: ectopic pregnancy
1547	H/O: medical termination of pregnancy
13SZ.00	PREGNANCY BENEFIT NOS
2684	O/E - VE - gravid uterus
14Y0.00	Born by caesarean section
14Y1.00	Born by forceps delivery
14Y2.00	Born by elective caesarean section
14Y3.00	Born by normal vaginal delivery
14Y4.00	Born by breech delivery
14Y5.00	Born by ventouse delivery
14Y6.00	Born by emergency caesarean section
271..	O/E - gravid uterus size
2723	O/E - oblique lie
2724	O/E - transverse lie
271B.00	O/E - fundal size = dates
271Z.00	O/E - GRAVID UTERUS SIZE NOS
2722	O/E - breech presentation
44Cy	Serum pregnancy associated plasma protein-A MoM measurement
4453	Serum pregnancy test positive
4654	URINE PREGNANCY TEST POSITIVE
584..	Fetal U-S scan
615C.00	IUD failure - pregnant
615C.11	PREGNANT, IUD FAILURE
615H.00	Breast feeding problem
6166	Pregnant, diaphragm failure
6174	Pregnant, sheath failure
62…	PATIENT PREGNANT
63…	Birth details
64	Bottle fed at birth
640	Breast fed at birth
648..11	Baby length centiles
6556	Pertussis vaccination in pregnancy
65560	Pertussis vacc in pregnancy given by other healthcre providr
66AX.00	Diabetes: shared care in pregnancy - diabetol and obstet
67A2	Diet in pregnancy advice
67A3	Pregnancy smoking advice

67A4	Pregnancy exercise advice
67A5	Pregnancy alcohol advice
67A6	Drugs in pregnancy advice
67A7	Pregnancy dental advice
67AE	Folic acid advice in first trimester of pregnancy
67B..00	Ante-natal relaxation classes
7F…	Obstetric operations
7N61100	[SO]Gravid uterus
7N61111	[SO]Delivered uterus
7N61200	[SO]Fetus
7N61300	[SO]Placenta
7N61400	[SO]Amniotic membrane
851..00	Haemorrhage control by packing
851Z.00	Haemorr. control by pack NOS
8B68.00	Pregnancy prophylactic therapy
8B7..11	Pregnancy vitamin/iron prophyl
8B74.00	IRON SUPPLEMENT IN PREGNANCY
8HHf	Refer to early pregnancy unit
8IEc	Pertussis vaccination in pregnancy declined
8B75.00	Vitamin supplement - pregnancy
8M6..00	REQUESTS PREGNANCY TERMINATION
942	Medical cert. of still-birth
9Ea0.00	Risk life pregnant woman greater than if pregnancy terminatd
9G7	Notification of birth
9kv	Pertussis vaccinatn progrme pregnant women enhan srvce admin
9mK	Pertussis vaccination in pregnancy invitation
9mK0	Pertussis vaccination in pregnancy invitation first letter
9mK1	Pertussis vaccination in pregnancy invitation second letter
9mK2	Pertussis vaccination in pregnancy invitation third letter
9Nif	Did not attend pertussis vaccination in pregnancy
9NkN	Seen in early pregnancy unit
H472.00	Asp pneumonitis due to anaesthesia during labour and deliv
L....	COMPLICATIONS OF PREGNANCY, CHILDBIRTH AND THE PUERPERIUM
L030000	Delivery of viable fetus in abdominal pregnancy
L08..	Failed attempted abortion
L0A..	Failed attempted abortion
L1…	PREGNANCY COMPLICATIONS
L2…	RISK FACTORS IN PREGNANCY
L3…	Complications occurring during labour and delivery
L4…	Complications of the puerperium
L5…	Maternal care for fetus
Ly…	COMPLICATIONS OF PREGNANCY,CHILDBIRTH OR THE PUERPERIUM OS
Lz…	Complications of pregnancy,childbirth and the puerperium NOS

M240500	ALOPECIA OF PREGNANCY
M290300	CHLOASMA GRAVIDARUM
Q01..00	Fetus/neonate affected by maternal complication of pregnancy
Q021.00	Fetus/neonate affect other placental separation/haemorrhage
Q022200	Fetus or neonate affected by placental insufficiency
Q030.00	Fetus or neonate affected by breech delivery and extraction
Q030.11	Fetus affected by breech delivery
Q031.00	Fetus/neonate affected by malposition/disproportion-delivery
Q031.11	Fetus affected by malpresentation during delivery
Q031400	Fetus or neonate affected by transverse lie in labour/deliv
Q031y00	Fetus/neonate affected malpos/malpres/disprop - lab/deliv OS
Q032.00	Fetus or neonate affected by forceps delivery
Q033.00	Fetus or neonate affected by vacuum extraction delivery
Q034.00	Fetus or neonate affected by caesarean section
Q100.00	Fetus small-for-dates, without mention of malnutrition
Q100.11	Fetus small-for-dates (SFD), without mention of malnutrition
Q101.00	Fetus small-for-dates with signs of malnutrition
Q101.11	Fetus small-for-dates (SFD) with signs of malnutrition
Q10z.00	Fetal growth retardation NOS
Q10z.11	Intrauterine growth retardation
Q11..	Short gestation and unspecified low birthweight problems
Q12..	Disorders relating to long gestation and high birthweight
Q13..	Light for gestational age
Q212.00	Liveborn with prelabour fetal distress
Q213.00	Liveborn with labour fetal distress
Q214.00	Liveborn with fetal distress, unspecified
Q21z.00	Liveborn with birth asphyxia NOS
Q432.00	Preterm delivery associated jaundice
Q44..00	Perinatal endocrine and metabolic problems
Q44z.00	Perinatal endocrine or metabolic problem NOS
Q48D	[X] Stillbirth
Q4z-5	Stillbirth NEC
Qyu1000	[X]Other low birth weight
Qyu1100	[X]Other preterm infants
Qyu1200	heavy for gestational age infants
Z1H4.00	Pain relief in labour
Z2…	Pregnancy, childbirth and puerperium observations
ZC2CB00	Dietary advice for gestational diabetes
ZV13900	[V]PH comp of pregnancy, childbirth and the puerperium
ZV22.00	[V]Normal pregnancy
ZV23.00	[V]High-risk pregnancy supervision
ZV24000	[V]Examination immediately after delivery
ZV27.00	[V]Outcome of delivery
ZV3..00	[V]Healthy liveborn infants according to type of birth
ZV4J000	[V]PROBLEMS RELATED TO UNWANTED PREGNANCY

ZV61800	[V]ILLEGITIMATE PREGNANCY
ZV61900	[V]Other unwanted pregnancy
ZVu2300	[X]Supervision of other normal pregnancy
ZVu2400	[X]Supervision of preg with oth poor reprod obstet history
ZVu2500	[X]Supervision of other high-risk pregnancies
DEGRADE_EVENT_1730_48	Patient pregnant
EMISREQ|4654	Urine pregnancy test positive
EMISREQ|62	Patient pregnant
L04..	Spontaneous abortion
L04..11	Miscarriage
1542	H/O: miscarriage
L02..00	Missed Abortion
L02..11	Missed Miscarriage
L02..12	Silent Miscarriage
6755	Post miscarriage counseling
L044..00	Inevitable abortion incomplete
L044..11	Inevitable miscarriage incomplete
L045..00	Inevitable abortion complete
L045..11	Inevitable miscarriage complete
L043..00	Inevitable abortion unspecified
L043..11	Inevitable miscarriage unspecified
1543	H/O: abortion
1543..11	H/O: termination
L0312	Tubal abortion
L07..00	Unspecified abortion
6776	Preg. termination counselling
6776..11	Abortion counselling
6776..13	Termination counselling
6776..12	TOP counselling
L05..00	Legally induced abortion
L05..11	Elective abortion
L05..13	Therapeutic abortion
L05..12	Termination of pregnancy
L06..00	Illegally induced abortion
L06..11	Criminal abortion
L06..12	Self-induced abortion
956..00	HSA1-therap. abort. green form
L097.00	Readmission for abortive pregnancy (NHS Codes)
L097.11	Readmission for retained products of conception
7E08800	Dilation andf curettage removal of missed abortion
L09..00	Complications following abortion and ectopic and molar pregnancies
L09..11	Complications following abortion and ectopic and molar pregnancies

8Cg..00	Pregnancy termination care
7E086.00	Termination of pregnancy NEC
8M6..00	Requests pregnancy termination
7E08400	Suction termination of pregnancy
7E08411	Vacuum termination of pregnancy
9Ea.00	Reason for termiantion of pregnancy
L051700	Incomplete medical abortion
L051711	Incomplete termination of pregnancy
8HHV.00	Referral for termination of pregnancy
389B.00	Assessment for termination of pregnancy
7E06600	Hysterotomy and termination of pregnancy
8Hh3.00	Self referral to termination of pregnancy service
7E07100	Curettage of products of conception from uterus
7E07111	Curettage of term pregnancy NEC
7E07112	Curettage of retained products of conception
7E07113	Curettage of uterus for termination of pregnancy
7E07114	Curettage of uterus for termination of pregnancy NEC
Q48G.00	Complication of termination of pregnancy, affecting fetus newborn
7E08500	Dilation of cervix and extraction termination of pregnancy
7E07000	Dilation of cervix uteri and curettage for termination of pregnancy
7E07011	Dilation of cervix uteri and curettage for termination of pregnancy
7E07012	Dilation cerv & curettage RPC

Appendix G

Highest level outcome Read codes used in cohort study. All daughter codes were included.

Myocardial Infarction	
G30..	Acute myocardial infarction
G35..	Subsequent myocardial infarction
G36..	Certain current complications following acute myocardial infarction
G38..	Postoperative myocardial infarction
G501.	Post infarction pericarditis
323..	ECG: myocardial infarction (excl. 3231. ECG: no myocardial infarction & 3232. ECG: old myocardial infarction)
889A.	Diabetes mellitus insulin-glucose infusion in acute myocardial infarction
G3115	Acute coronary syndrome
G31y1	Microinfarction of heart
G344.00	Silent myocardial infarction
Gyu34	[X]Acute transmural myocardial infarction of unspecified site
Gyu35	[X]Subsequent myocardial infarction of other sites
Gyu36	[X]Subsequent myocardial infarction of unspecified site
G32..	Old myocardial infarction
322..	ECG: myocardial ischaemia
Stroke (or just use G6… Cerebrovascular disease plus non G6 codes below)	
G61..	Intracerebral haemorrhage
G610.	Cortical haemorrhage
G611.	Internal capsule haemorrhage
G612.	Basal nucleus haemorrhage
G613.	Cerebellar haemorrhage
G614.	Pontine haemorrhage
G615.	Bulbar haemorrhage
G616.	External capsule haemorrhage
G617.	Intracerebral haemorrhage, intraventricular
G618.	Intracerebral haemorrhage, multiple localized
G61X.	Intracerebral haemorrhage in hemisphere, unspecified
G61X0	Left sided intracerebral haemorrhage, unspecified
G61X1	Right sided intracerebral haemorrhage, unspecified
G61z.	Intracerebral haemorrhage NOS
G63y1	Cerebral infarction due to embolism of precerebral arteries
G64z.	Cerebral infarction NOS
G64z0	Brainstem infarction
G64z1	Wallenberg syndrome
G64z2	Left sided cerebral infarction
G64z3	Right sided cerebral infarction
G64z4	Infarction of basal ganglia
G66..	Stroke and cerebrovascular accident unspecified
G660.	Middle cerebral artery syndrome

G661.	Anterior cerebral artery syndrome
G662.	Posterior cerebral artery syndrome
G663.	Brain stem stroke syndrome
G664.	Cerebellar stroke syndrome
G665.	Pure motor lacunar syndrome
G666.	Pure sensory lacunar syndrome
G667.	Left sided CVA
G668.	Right sided CVA
G677.	Occlusion and stenosis of cerebral arteries, not resulting in cerebral infarction
G6770	Occlusion and stenosis of middle cerebral artery
G6771	Occlusion and stenosis of anterior cerebral artery
G6772	Occlusion and stenosis of posterior cerebral artery
G6773	Occlusion and stenosis of cerebellar arteries
G6774	Occlusion and stenosis of multiple and bilateral cerebral arteries
G683.	Sequelae of cerebral infarction
G6W..	Cerebral infarction due to unspecified occlusion or stenosis of precerebral arteries
G6X..	Cerebral infarction due to unspecified occlusion or stenosis of cerebral arteries
G6z..	Cerebrovascular disease NOS
G63y0	Cerebral infarct due to thrombosis of precerebral arteries
Gyu6.	[X]Cerebrovascular diseases
G60..	Subarachnoid haemorrhage
G68..	Late effects of cerebrovascular disease
1M4..	Central post-stroke pain
E0324	Acute confusional state; of cerebrovascular origin
E0334	Subacute confusional state; of cerebrovascular origin
Hypertensive disease	
G2...	Hypertensive disease
662P0	Hypertension 9 month review
9OI..	Hypertension monitoring admin.
Angina	
662K.	Angina control
G31..	Other acute and subacute IHD
G3111	Unstable angina
G3112	Angina at rest
G3113	Refractory angina
G3114	Worsening angina
G33..	Angina pectoris
Heart failure	
G58..	Heart failure
1O1..	Heart failure confirmed
8B29.	Cardiac failure therapy
8CeC.	Preferred place of care for next exacerbation heart failure

8CL3.	Heart failure care plan discussed with patient
8CMK.	Has heart failure management plan
8CMW8	Heart failure clinical pathway
8H2S.	Admit heart failure emergency
G5yy9	Left ventricular systolic dysfunction
G5yyA	Left ventricular diastolic dysfunction
585f.	Echocardiogram shows left ventricular systolic dysfunction
585g.	Echocardiogram shows left ventricular diastolic dysfunction
Ischaemic Heart Disease	
G3…	Ischaemic heart disease
G34..	Other chronic IHD
Gyu3	[X]Ischaemic heart diseases
Other Heart disease (not included elsewhere) OR broad G…. Circulatory disease	
G5…	Other forms of heart disease
G7…	Arterial, arteriole and capillary disease
G8…	Vein, lymphatic and circulatory diseases NOS
Gyu7.	[X]Diseases of arteries, arterioles and capillaries
Procedures	
7A4..	Iliac and femoral artery operations
7A1..	Aorta operations
792	Coronary artery operations
79...	Heart operations
7A...	Artery and vein operations
Hypercholesterolaemia:	
Xa9As	Hypercholesterolaemia
X40Wz	Primary Hypercholesterolaemia
XaYQB	Dietary surveillance in hypercholesterolaemia
X40X6	Secondary hypercholesterolaemia
X40X0	Polygenic hypercholesterolaemia
XaX3u	Possible familial hypercholesterolaemia
C320y	Other specified pure hypercholesterolaemia
Type 2 Diabetes Mellitus	
C10	Diabetes
66A..	Diabetes monitoring
9OL..	Diabetes monitoring admin.
1434	H/O Diabetes Mellitus
6872	Diabetes mellitus screen
9NM0.	Attending diabetes clinic
XaZq8	Pre-diabetes
R102.	Glucose tolerance test abnormal

Appendix H

All Read codes for confounders in the cohort study.

Smoking	
137..00	Tobacco consumption
137..11	Smoker - amount smoked
1372	Trivial smoker - < 1 cig/day
1372.11	Occasional smoker
1373	Light smoker - 1-9 cigs/day
1374	Moderate smoker - 10-19 cigs/d
1375	Heavy smoker - 20-39 cigs/day
1376	Very heavy smoker - 40+cigs/d
1377	Ex-trivial smoker (<1/day)
1378	Ex-light smoker (1-9/day)
1379	Ex-moderate smoker (10-19/day)
137a.00	Pipe tobacco consumption
137b.00	Ready to stop smoking
137c.00	Thinking about stopping smoking
137d.00	Not interested in stopping smoking
137e.00	Smoking restarted
137F.00	Ex-smoker - amount unknown
137G.00	Trying to give up smoking
137H.00	Pipe smoker
137I.00	Passive smoker
137j.00	Ex-cigarette smoker
137K.00	Stopped smoking
137K000	Recently stopped smoking
137l.00	Ex roll-up cigarette smoker
137m.00	Failed attempt to stop smoking
137N.00	Ex pipe smoker
137O.00	Ex cigar smoker
137P.00	Cigarette smoker
137P.11	Smoker
137Q.00	Smoking started
137Q.11	Smoking restarted
137R.00	Current smoker
137S.00	Ex smoker
137T.00	Date ceased smoking
137V.00	Smoking reduced
137W.00	Chews tobacco
137X.00	Cigarette consumption
137Y.00	Cigar consumption
137Z.00	Tobacco consumption NOS
8CAL.00	Smoking cessation advice
8H7i.00	Referral to smoking cessation advisor

8HkQ.00	Referral to NHS stop smoking service
8HTK.00	Referral to stop-smoking clinic
8IAj.00	Smoking cessation advice declined
9km..00	Ex-smoker annual review - enhanced services administration
9km..11	Ex-smoker annual review
9ko..00	Current smoker annual review - enhanced services admin
9ko..11	Current smoker annual review
9NS0200	Referral for smoking cessation service offered
13p..00	Smoking cessation milestones
13p0.00	Negotiated date for cessation of smoking
13p1.00	Smoking status at 4 weeks
13p2.00	Smoking status between 4 and 52 weeks
13p3.00	Smoking status at 52 weeks
13p4.00	Smoking free weeks
13p5.00	Smoking cessation programme start date
13p5000	Practice based smoking cessation programme start date
13p6.00	Carbon monoxide reading at 4 weeks
13p7.00	Smoking status at 12 weeks
13p8.00	Lost to smoking cessation follow-up
38DH.00	Fagerstrom test for nicotine dependence
6791	Health ed. - smoking
67H6.00	Brief intervention for smoking cessation
745H.00	Smoking cessation therapy
745H000	Nicotine replacement therapy using nicotine patches
745H100	Nicotine replacement therapy using nicotine gum
745H200	Nicotine replacement therapy using nicotine inhalator
745H300	Nicotine replacement therapy using nicotine lozenges
745H400	Smoking cessation drug therapy
745Hy00	Other specified smoking cessation therapy
745Hz00	Smoking cessation therapy NOS
8B2B.00	Nicotine replacement therapy
8B3f.00	Nicotine replacement therapy provided free
8B3Y.00	Over the counter nicotine replacement therapy
8BP3.00	Nicotine replacement therapy provided by community pharmacis
8CAg.00	Smoking cessation advice provided by community pharmacist
8CdB.00	Stop smoking service opportunity signposted
8HBM.00	Stop smoking face to face follow-up
8HBP.00	Smoking cessation 12 week follow-up
8I2I.00	Nicotine replacement therapy contraindicated
8I2J.00	Bupropion contraindicated
9N2k.00	Seen by smoking cessation advisor
E023.00	Nicotine withdrawal
E251.00	Tobacco dependence
E251000	Tobacco dependence; unspecified
E251100	Tobacco dependence; continuous

E251300	Tobacco dependence in remission
E251z00	Tobacco dependence NOS
ZG23300	Advice on smoking
ZRaM.00	Motives for smoking scale
ZRao.00	Occasions for smoking scale
ZRBm200	Fagerstrom test for nicotine dependence
ZRBm211	FTND - Fagerstrom test for nicotine dependence
ZRh4.00	Reasons for smoking scale
ZRh4.11	RFS - Reasons for smoking scale
ZV11600	[V]Personal history of tobacco abuse
ZV4K000	[V]Tobacco use
ZV6D800	[V]Tobacco abuse counselling
Body Mass Index	
22A..00	O/E - weight
22A1.00	O/E - weight > 20% below ideal
22A2.00	O/E -weight 10-20% below ideal
22A3.00	O/E - weight within 10% ideal
22A4.00	O/E - weight 10-20% over ideal
22A4.11	O/E - overweight
22A5.00	O/E - weight > 20% over ideal
22A5.11	O/E - obese
22A6.00	O/E - Underweight
22K..00	Body Mass Index
22K1.00	Body Mass Index normal K/M2
22K2.00	Body Mass Index high K/M2
22K3.00	Body Mass Index low K/M2
22K4.00	Body mass index index 25-29 - overweight
22K5.00	Body mass index 30+ - obesity
22K6.00	Body mass index less than 20
22K7.00	Body mass index 40+ - severely obese
22K8.00	Body mass index 20-24 - normal
22Z..00	Height and Weight
229..	Height