Investigating relationships between maternal micronutrient intakes, dietary patterns and alcohol consumption during pregnancy

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By

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Abstract

Background: Evidence from animal models of fetal alcohol spectrum disorders (FASD) suggests that the risk of ethanol-induced harm to the developing fetus is greater in the presence of inadequate maternal nutrition. Findings from observational studies in the general population have also indicated that heavy drinking is associated with poorer quality diets.

Aims: The aims of this thesis were to explore relationships between patterns of alcohol consumption and dietary intake during pregnancy, and to investigate whether they influence the risk of adverse infant and childhood outcomes.

Methods: Five interrelated studies were undertaken. The initial study involved the design and validation of a food frequency questionnaire (FFQ). This was followed by two cross-sectional studies to explore relationships between dietary intake and alcohol consumption in two different samples of pregnant women: 1) a small sample recruited from across the UK (n=350); 2) a secondary analysis of the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort (n=11,457). This was then followed by two further studies, which comprised of secondary analyses of the ALSPAC cohort to investigate whether relationships between diet and alcohol influence the risk of adverse offspring outcomes: 1) small for gestational age (SGA) (n=9,935); and 2) IQ scores at 8 years (n=5,557).

Results: Results suggest that heavy drinking during pregnancy is associated with diets characterised by low intakes of fruit and vegetables, and high intakes of processed, fried foods, which showed weak correlation with important micronutrients. Women in quartile one for vitamin E intake, who reported binge drinking, were significantly more likely to have a SGA baby compared to women in other quartiles who also reported binge drinking during pregnancy. Micronutrient intakes were not associated with childhood IQ scores at 8 years in women who reported alcohol consumption during pregnancy.

Conclusion: The investigations undertaken as part of this thesis have highlighted a number of new and important findings. The results highlighted the clustering of potentially harmful patterns of dietary intake and alcohol consumption during pregnancy, and suggest that the potential harm from antenatal alcohol consumption may be exacerbated in the presence of inadequate micronutrient intakes.

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Abbreviations and acronyms

24-HR	24-hour recall interview
ADH	Alcohol Dehydrogenase
ALDH	Aldehyde Dehydrogenase
ALSPAC	Avon Longitudinal Study of Parents and Children
ANOVA	Analysis of Variance
ARBD	Alcohol Related Birth Defects
ARND	Alcohol Related Neurodevelopmental Disorder
AUDIT-C	The Alcohol Use Disorders Identification Test
BAC	Blood Alcohol Concentration
BMI	Body Mass Index
CARE	Caffeine and Pregnancy Outcomes
CC	Correlation Coefficient
CI	Confidence Interval
CNS	Central Nervous System
DNA	Deoxyribonucleic Acid
FA	Factor Analysis
FAS	Fetal Alcohol Syndrome
FASD	Fetal Alcohol Spectrum Disorder
FFQ	Food Frequency Questionnaire
IFS	Infant Feeding Survey
IMD	Index of Multiple Deprivation
IQ	Intelligence Quotient
IQR	Inter-quartile Range
LBW	Low Birth Weight
MCS	Millennium Cohort Study
NICE	National Institute for Clinical Excellence
OCM	One Carbon Metabolism
OR	Odds Ratio
PCA	Principle Components Analysis
PRAMS	Pregnancy Risk Assessment Monitoring System
Q1	Quartile 1
Q4	Quartile 4
Ref	Reference category
RRR	Reduced Rank Regression
s.d.	Standard Deviation
SCOPE	Screening for Pregnancy Endpoints
SES	Socio-economic Status

SGA	Small-for-gestational-age
TLFB	Time-line Follow Back
VC	Validity Coefficient

Common definitions

Low to Moderate alcohol consumption: Refers to drinking less than one alcoholic drink per day, unless otherwise stated

Heavy alcohol consumption: Refers to drinking one or more alcoholic drinks per day, unless otherwise stated

Binge drinking:

Study 1A and 1B use the definition of six or more units in one drinking occasion.

Study 2A, 2B and 2C use the definition of four or more units in one day

Chapter 1: Introduction

1.1 General introduction

This introductory chapter provides a summary of the background information and rationale for conducting this programme of work. It begins with a description of the current guidelines and prevalence of antenatal alcohol consumption in the United Kingdom (UK) followed by a description of the burden of harm attributable to antenatal alcohol consumption in the UK and the potentially mediating role of maternal dietary intake. The primary research aims investigated in this programme of work are then stated and a brief overview of the structure of this thesis is provided.

1.1.1 Antenatal alcohol consumption: Current UK guidelines

Current UK guidelines, published by the National Institute for Clinical Excellence (NICE), advise pregnant women and women planning to become pregnant to avoid alcohol, particularly during the first trimester, but if they choose to drink alcohol, to drink no more than one to two units of alcohol, once or twice a week, and always to avoid binge drinking[1]. The definition of binge drinking varies in the literature. Typically, binge drinking is defined as six or more units per drinking occasion (Smith & Foxcroft 2009), however, other definitions have been reported ranging from four or five units per occasion (Kesmodel et al. 2012; Cooper et al. 2013; Coles et al. 2015) to 7.5 units per occasion (NICE 2014a).

1.1.2 Prevalence of antenatal alcohol consumption in the UK

A recent study compared the prevalence of antenatal alcohol consumption across three cohort studies in Ireland, Australia and New Zealand, and found that the proportion of women who reported alcohol consumption during pregnancy varied widely from 20% to 80% (O'Keeffe et al. 2015).

There are many biological markers of alcohol consumption available, however, these all have their strengths and limitations, and mean that there is currently no reliable, cost-effective biomarker to estimate light to moderate alcohol consumption during pregnancy, which has resulted in a number of challenges when trying to accurately estimate alcohol consumption in epidemiological studies.

Biochemical markers of alcohol consumption

There are two broad categories of biomarkers, ranging broadly in their sensitivity and specificity; biomarkers of alcohol pathology and biomarkers of alcohol metabolites. The three most commonly used biomarkers of alcohol pathology are Gamma-Glutamyltransferase (GGT), a liver enzyme;

[1] Since this thesis was originally examined, the guidelines have now changed and women are advised to avoid alcohol completely during their pregnancy (NICE 2016)

Carbohydrate-Deficient Transferrin (CDT), a modified form of the iron-transporting protein transferrin; and Mean Corpuscular Volume (MCV), a measure of average red blood cell volume. While they are reliable biomarkers, they are only appropriate for detecting chronic alcohol consumption (Tavakoli et al. 2011).

The second category of biomarkers include those of alcohol metabolites. The three most common metabolites are Fatty Acid Ethyl Esters (FAEE), Ethyl Glucuronide (EtG) and Ethyl Sulfate (EtS), and Phosphatidylethanol (PEth), which can be found in breath, urine, blood and hair. While these are the most sensitive and specific markers of alcohol consumption, alcohol and its main metabolite, acetaldehyde, break down quickly and the window for detection is very small (Fig 1.1). This means their clinical application is relatively limited in this context, as pregnant women typically consume alcohol in an intermittent pattern (Peterson 2005).

There have been promising results from the exploration of these biomarkers in clinical samples from neonates. A number of studies have shown that FEE, EtG and EtS being reliable markers of in utero ethanol exposure (Peterson et al. 2008; Morini et al. 2010). While these results are encouraging, the samples can be challenging to collect and are complex and costly to analyse (Bakhireva & Savage 2011).

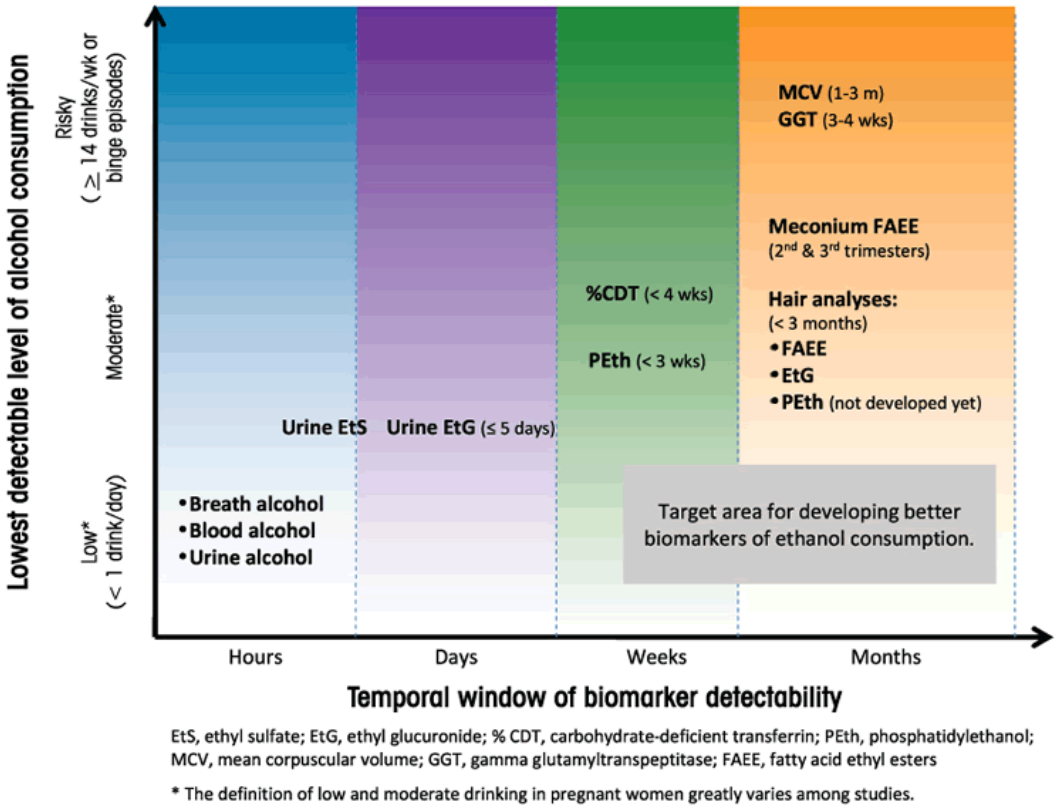

Figure 1.1 The period of time during which alcohol consumption can be detected and the lowest levels of alcohol consumption detectable by current alcohol biomarkers (Bakhireva & Savage 2011)

Because there is currently no reliable and cost-effective biomarker to measure light to moderate alcohol consumption during pregnancy, healthcare professionals and researchers must rely upon self-reported estimates of intake. Whilst cultural and lifestyle differences between countries will contribute to varied prevalence, methodological differences are also accountable for the large reported variability.

Differences in prevalence of any antenatal alcohol consumption are also reported in studies conducted across the UK. Estimates of 30% to 40% were reported by two large, cohort studies, The Millennium Cohort Study (MCS) (Kelly et al. 2009) and the Infant Feeding Survey (IFS) (Mc Andrew et al. 2012), respectively. However, estimates of prevalence were higher in smaller UK studies ranging from 53% in Caffeine and Reproductive Health (CARE) to 75% in Screening for Pregnancy Endpoints (SCOPE) (O'Keeffe et al. 2015). Quantities of reported alcohol consumption during pregnancy in the UK also vary greatly in the literature. Results from the IFS indicated that only 3% of women reported drinking two or more units per week whilst pregnant (Mc Andrew et al. 2012), compared with 33% of women in SCOPE reporting eight or more units per week, and reported binge drinking ranged from 9% (Cooper et al. 2013) to 33% (O'Keeffe et al. 2015) in two cohort studies.

The wide range in UK estimates of prevalence may be due to a number of methodological differences. Factors influencing reported rates include when data were collected, how they were collected and who collected them (Jacobson et al. 2002; Del Boca & Darkes 2003; Alvik et al. 2006). Data were collected during pregnancy in SCOPE and CARE, whereas the IFS and MCS both collected data during the postnatal period. In general, data collected during pregnancy are more reliable due to a lower risk of response bias because of the shorter recall period (Dawson 2003). However, there is evidence to suggest that women provide more accurate reports of antenatal alcohol consumption during the postnatal period. This is believed to be because if women give birth to a healthy baby, with no evidence to alcohol induced harm, they don't feel under the same social pressures and will report their consumption more accurately (Alvik et al. 2006). Nonetheless, it is not clear whether their levels of postnatal alcohol consumption influence recall.

Alcohol consumption is considered typically higher during the first and second trimesters (O'Keeffe et al. 2015). Alcohol data were collected at a number of different time points throughout the gestational period in SCOPE and CARE. In the IFS and MCS, alcohol consumption was measured as an average over the whole pregnancy. Shorter periods of recall are more likely to yield more reliable estimates of intake (Dawson 2003). The higher prevalence reported in the SOCPE study may be due to the methods of data collection. Research midwives collected data and may have been able to build rapport and trust with women. Data collection techniques were also not standardised, enabling midwives to prompt women when asking alcohol questions (O'Keeffe et al. 2015).

The methodological challenges have resulted in some scepticism about current estimates of prevalence. Based on the upper and lower estimates of prevalence (reported between 2004 and 2012), it is possible that in 2014 the number of alcohol exposed births may have ranged from approximately 208,579 to 521,425 and the number of births exposed to binge drinking may have ranged from approximately 19,777 to 217, 547. Because many pregnancies in the UK are unplanned (Wellings et al. 2013) and women might not be aware they are pregnant until weeks 4 to 8 of gestation, these estimates may still underestimate the true prevalence of alcohol exposed births.

1.1.3 Predictors of antenatal alcohol consumption

A number of studies have identified factors that are associated with alcohol consumption during pregnancy, of which many are also associated with poorer birth and childhood outcomes. It is imperative to establish what these factors are in order to understand the effect they have and how they may interact with each other. A systematic review and a cross-cohort comparison reported that smoking during pregnancy, drinking before pregnancy, being Caucasian and being exposed to abuse or violence (Skagerström et al. 2011; O'Keeffe et al. 2015) were the only consistent predictors of antenatal alcohol consumption.

Negative health behaviours typically cluster and studies have consistently shown that women who smoke during pregnancy are also more likely to drink alcohol (Martin et al. 2008; Skagerström et al. 2011; Hutchinson et al. 2013; Powers et al. 2013). This phenomenon is also observed in non-pregnant populations (Cohn et al. 2015); however, the mechanisms for this co-occurrence are still not fully understood (Spring et al. 2012). All studies measuring pre-pregnancy alcohol consumption reported it to be a significant predictor of antenatal consumption (Skagerström et al. 2011). As pre-pregnancy consumption increased, so did the probability of drinking during pregnancy. This has also been observed in more recent, UK studies (Mc Andrew et al. 2012; Nykjaer et al. 2014) and may be a result of habitual behaviour and social norms around drinking (O'Tousa & Grahame 2014).

Many other studies have also reported ethnicity as a predictor of maternal alcohol consumption and evidence suggests this may be attributable to cultural norms, namely traditional values, familial expectations and religious beliefs (Hurcombe et al. 2010). Women of white, European descent are more likely to drink alcohol prior to, and during, pregnancy compared to women of other ethnic origins (Maloney et al. 2011; Mullally et al. 2011; Hutchinson et al. 2013). Findings from the IFS also indicated that women of non-white ethnic origins, who did drink alcohol prior to pregnancy, were more likely to stop drinking alcohol when they became pregnant (Mc Andrew et al. 2012).

While previous research has suggested that other socio-demographic characteristics, including maternal age, income, education, parity and having a partner, are predictors of alcohol consumption, the evidence is not as consistent. Light to moderate alcohol consumption during pregnancy has been

associated with higher maternal age, income, education and parity (Kelly et al. 2010; Skagerström et al. 2011; Mc Andrew et al. 2012; Cooper et al. 2013; Nykjaer et al. 2014; Hutchinson et al. 2013; O'Keeffe et al. 2015). In contrast, other studies have reported heavy patterns of alcohol consumption (including binge drinking) during pregnancy are associated with younger maternal age, lower educational attainment, unemployment and being single (Kelly et al. 2010; Cooper et al. 2013; Powers et al. 2013; Chambers et al. 2014). The two opposing socio-demographic profiles for both drinking patterns are also observed in non-pregnant populations (Smith & Foxcroft 2009) and may be a result of personality traits and social factors associated with SES (Kuntsche et al. 2004).

Many of these predictors are also associated with particular outcomes in childhood and later life, meaning they are confounders. A confounding variable is an unobserved variable that is associated with both the exposure and outcome, potentially distorting the relationship under investigation. Observational studies in this area are particularly vulnerable to confounders, as alcohol consumption is associated with many other lifestyle and socio-demographic factors that are also associated with birth and childhood outcomes, making it difficult to tease apart these relationships (Gronbaek et al. 1994).

While it is possible to adjust for confounders in the design of a study, residual confounding may remain; this refers to confounding that persists, either because it has not been measured or it has been measured inappropriately. This can produce spurious associations (false association) or hide a true association (Niclasen 2013). Therefore, it is crucial that predictors are well understood to ensure robust analysis of observational data.

1.1.4 Harms of antenatal alcohol consumption

The harms from alcohol exposure are discussed in more detail in Chapter 2. Briefly, antenatal alcohol consumption can result in a wide range of physical, behavioural and cognitive deficits in children, which are permanent, pervasive and often devastating. The wide range of symptoms can be present in many combinations and vary greatly in severity. This has led to the use of 'Fetal Alcohol Spectrum Disorders' (FASD); a clinical definition encompassing the range of disorders resulting from antenatal alcohol consumption. The prevalence of FASD in the UK is presently unknown, but a worldwide estimate of 1 in 100 children would mean that approximately 6,000 to 7,000 babies each year may be born in the UK with some form of alcohol related damage (Gray et al. 2009).

There is currently no reliable and cost-effective biomarker for alcohol consumption in pregnancy which means that clinicians and researchers rely on self-reported methods in all but exceptional cases (see section 1.1.2). The sensitive nature of antenatal alcohol consumption, coupled with retrospective recall methods, means that self-reports are vulnerable to misclassification. This can make diagnosing alcohol related harm very difficult, particularly in less severe cases, where no

physical abnormalities are present and cognitive outcomes may be confounded by socio-demographic characteristics. Because of these challenges, it is likely that many cases of alcohol related harm during pregnancy have not been identified (Gray et al. 2009).

1.1.5 The role of maternal dietary intake

There is a growing body of evidence to suggest that dietary intake may play a role in mediating the relationship between antenatal alcohol consumption and related fetal harms. A number of studies have presented findings from cell cultures and animal models that indicate that inadequate intakes of dietary antioxidants and micronutrients involved in One-Carbon Metabolism (OCM) might increase the risk of fetal harm in alcohol-exposed pregnancies (Cohen-kerem & Koren 2003; Ballard et al. 2012). To date, there has been limited research conducted in humans to evaluate this hypothesis.

1.2 Overall aims and objectives

The overall aims of this thesis were to explore the relationships between maternal dietary intake and alcohol consumption patterns during pregnancy, and examine possible ways they may affect the risk of adverse infant and child outcomes.

Specific objectives were:

- To measure the alcohol consumption patterns in a population of pregnant women in the UK
- To describe typical dietary patterns in a population of pregnant women in the UK, based on the frequency of food and drink items consumed
- To estimate the mean daily intakes of dietary antioxidants and OCM micronutrients in a population of pregnant women in the UK
- To determine relationships between maternal dietary patterns, micronutrient intakes and patterns of alcohol consumption during pregnancy in women in the UK
- To evaluate whether maternal dietary intake modifies the risk of having a small for gestational age infant from antenatal alcohol exposure
- To evaluate whether maternal dietary intake modifies the risk of offspring having low IQ scores and behavioural problems in childhood from antenatal alcohol exposure.

Key aims and objectives of each study are described at the beginning of each chapter.

Five studies were undertaken to provide insight into these aims. The first three studies focused on whether or not particular patterns of alcohol consumption are associated with particular patterns of dietary behaviour before and during pregnancy, to establish whether some women may be putting their baby at increased risk of alcohol related harm (Studies 1A, 1B & 2A). The final two studies focused on exploring whether particular dietary patterns modify the relationship between maternal alcohol consumption and the risk of adverse infant and childhood outcomes (Studies 2B & 2C).

1.3 Thesis overview

Chapter 1: *General introduction and thesis overview*

This chapter has presented a brief summary of the rationale for this thesis, the overall aims of this programme of research and an overview of the thesis structure.

Chapter 2: *The potential harms of antenatal alcohol consumption and the role of maternal dietary intake*

Chapter two presents a more detailed review of the current evidence base relevant to this thesis. The discussion will also highlight gaps in the current knowledge.

Chapter 3: *Methodology*

The general methodological considerations associated with the five studies in this thesis will be discussed, including the measures put in place to minimise the risk of bias and how these might affect the interpretation of results. The specific methods used in each study will be discussed in the relevant results chapter.

Chapter 4: *Validation of a Food Frequency Questionnaire (FFQ) (Study 1A)*

At the beginning of this programme of research, no dietary assessment tool was available to measure the habitual intake of folate, choline, betaine, vitamin C and carotenoids in women of reproductive age. Study 1A presents details of the development and validation of a FFQ specifically designed for use in study 1B (Chapter 5).

Chapter 5: *Cross-sectional survey of micronutrient intakes, dietary patterns and alcohol consumption in pregnant women in the UK (Study 1B)*

Chapter 5 presents the findings from Study 1B, which explores relationships between alcohol consumption patterns, dietary patterns and micronutrient intakes in a sample of pregnant women in the UK. To do this a cross-sectional survey consisting of socio-demographic, alcohol and dietary questions was conducted with a sample of pregnant women recruited through antenatal clinics and social media platforms in the UK. Dietary data were collected using the FFQ previously discussed in study 1A (Chapter 4).

Chapter 6: *Exploration of micronutrient intakes, dietary patterns and alcohol consumption in pregnant women in the Avon Longitudinal Study of Parents and Children (ALSPAC) (Study 2A)*

The sample recruited in Study 1B was small, homogenous and reported very low levels of alcohol consumption, meaning it was not possible to address the original aims of this thesis. Therefore, a secondary analysis of data from the ALSPAC cohort was undertaken to explore the relationships between maternal alcohol consumption patterns and dietary intake during pregnancy.

Chapter 7: *Exploration of maternal diet, alcohol consumption and fetal growth (Study 2B)*

Study 2B explored whether dietary intakes modified the risk of fetal growth restriction in alcohol-exposed pregnancies using data from the ALSPAC cohort.

Chapter 8*: Exploration of maternal diet, alcohol consumption and childhood cognitive performance (Study 2C)*

Study 2C explored whether dietary intakes modified childhood IQ scores at 8 years of age in alcohol-exposed pregnancies using data from the ALSPAC cohort.

Chapter 9: *General discussion and conclusions*

The final chapter provides a discussion of the main findings as a whole, focusing on the strengths and limitations of this thesis. This is followed by clinical and policy implications, recommendations for future research and the overall conclusions of the thesis.

Chapter 2: The potential harms of antenatal alcohol consumption and the role of maternal dietary intake

2.1 Introduction

The potential harms from antenatal alcohol consumption have been documented in the scientific literature for decades. Despite this, social norms engrained in British culture (Measham & Ostergaard 2009), unclear guidance from government bodies and inconsistent advice from healthcare professionals (Raymond et al. 2009; Kesmodel & Kesmodel 2011) have all contributed to continued antenatal alcohol consumption. This chapter will begin with a brief overview of ethanol metabolism, followed by the potential harms associated with different patterns of antenatal alcohol consumption. The next part of this chapter will then provide a summary of research published about the interactions between particular micronutrients and ethanol, and the potential implications for fetal development. The paucity of data collected from studies in humans means this section will focus on research from studies using cell culture and animal models. This will then be followed by an overview of the relationships between patterns of alcohol consumption and dietary intake in human populations, identifying patterns of behaviour that may leave offspring at increased risk of harm.

2.2 Ethanol metabolism

The subsequent harm from alcohol consumption varies based on Blood Alcohol Concentration (BAC), which differs from person-to-person and is determined by a number of factors influencing the speed at which ethanol is emptied from the stomach and undergoes metabolism in the stomach and liver. These factors include body size and composition, the ingestion of food, the type of alcoholic beverage and genetic factors (Zakhari 2006). Alcohol is freely distributed in total body water which means that alcohol concentrations in the fetus are comparable with those of the mother (Burd et al. 2012).

Alcohol is metabolized primarily in the liver by a number of different pathways: the alcohol dehydrogenase (ADH), cytochrome P450 2E1 (CYP2E1) and catalase pathways. The most common pathway is the ADH pathway, which is responsible for the majority of alcohol metabolised in the liver and the pathway that the remainder of this review will focus on. The ADH pathway involves two enzymes, ADH and aldehyde dehydrogenase (ALDH); firstly, ethanol is metabolized into acetaldehyde, which is a highly toxic by-product and has the potential to cause significant damage. Secondly, acetaldehyde is metabolized into acetate (Edenberg 2007), a less toxic substance that is finally broken down into carbon dioxide and water (NIH 2007).

There are slightly different forms of ADH and ALDH, which are encoded by different genes. In addition, there are several variants (alleles) of these genes that encode these enzymes, resulting in slightly different outcomes. Some people may have slightly different forms of ADH or ALDH that convert ethanol to acetaldehyde or acetaldehyde to acetate more or less quickly than others. For example,

fast metabolism of ethanol to acetaldehyde, but slow conversion of acetaldehyde to acetate means there will be a build-up of acetaldehyde, potentially resulting in a greater level of harm from alcohol consumption. Nevertheless, people with these forms of ADH and ALDH will also feel the negative effects of acetaldehyde more quickly, such as hot flushes, light-headedness, rapid heartbeats and nausea, which has been linked with lower levels of alcohol consumption (Oota et al. 2004; NIH 2007; Edenberg 2007).

Genetic variants related to ethanol metabolism are also associated with levels of harm from antenatal alcohol consumption. A study conducted in the Western Cape in South Africa investigated genetic variations of the ADH2 gene in a population of mothers and their children. The authors reported lower rates of FASD in children born to mothers with the ADH2*2 allele, which is associated with higher rates of ethanol metabolism, resulting in a build of acetaldehyde (Viljoen et al. 2001). Another study conducted in the UK explored the genetic variant ADH1B, which is also associated with fast ethanol metabolism and a build-up of acetaldehyde, in a population of pregnant women and found it was associated with abstaining from alcohol prior to pregnancy and throughout the first trimester (Zuccolo et al. 2009).

2.3 Potential harms of alcohol consumption during pregnancy

2.3.1 Search strategy (Search 1)

A literature search was conducted in three electronic databases: MEDLINE, EMBASE and PsychInfo. Grey literature was not searched. Reference lists of relevant studies were hand searched for additional articles. A mixture of MeSH headings and key word searches were conducted using the search terms in Table 2.1. Terms were grouped using Boolean operators and searches were limited to human participants and English language articles. Fetal Alcohol Syndrome (FAS) was first described in the scientific literature in 1973; therefore, each search was limited to a publication date between 1973 and present day. Inclusion and exclusion criteria are presented in Table 2.2. The full search string for each database can be found in Appendix A.

Table 2.1 Data base search terms (search 1)

Search terms
Pregnan*/ Antenatal/ Prenatal
Alcohol*/ Ethanol/ Alcohol drinking/ Alcohol-related disorder/ Alcohol exposure/ Binge drinking/ Drinking/ Heavy episodic drinking
Risk/ harm/ pregnancy outcome/ growth/ weight/ size/ F?etal alcohol*/ FAS*/ Spontaneous abortion/ Still birth/ Preterm*/ Premature/ birth weight/ Head circumference/ Child development/ F?etal development/ F?etal growth/ IUGR/ Intrauterine growth re/ SGA/ small for gestational age/ ADHD/ Attention deficit*/ IQ/ Intelligen*/ Cognit*/ Neuro?behav*/ Motor skills/ Executive function/ Behaviour/ Congenital A*/ Birth defect/ Learning

Table 2.2 Eligibility criteria for database search (search 1)

Inclusion criteria:	Exclusion criteria:
Pregnant, human populations	Animal model
English language article	No quantitative measure of alcohol
Peer reviewed journal	Exposure to alcohol before or after antenatal period
Observational study design	
Quantifiable measure of alcohol consumption	
Alcohol exposure occurs during pregnancy	

2.3.2 Heavy alcohol consumption

The evidence from chronic alcohol consumption during pregnancy is well established in the scientific literature. Despite recognition of the potential harms from antenatal alcohol consumption since London's gin epidemic in the 1700s (Calhoun & Warren 2007), the first study to describe the pathology of chronic alcohol consumption during pregnancy as FAS was by Jones and Smith in 1973 (Jones & Smith 1973). Their article described the devastating effects chronic alcohol exposure in utero can have on fetal development, including the damage to the Central Nervous System (CNS), stunted growth and a range of distinct facial characteristics observed in children (Table 2.3). There is currently no objective diagnostic test for FAS; instead a diagnosis relies on the recognition of these three symptoms by clinicians. Confirmation of maternal alcohol consumption is not always deemed essential for a diagnosis of FAS: as long as other conditions have been ruled out, the symptoms can be clear enough to provide a diagnosis (O'Leary 2004).

In addition to dose, the timing and duration of alcohol consumption during pregnancy are also important factors that will influence the severity of symptoms in infants. Major morphological abnormalities typically result from alcohol consumption during the early stages of pregnancy. In

contrast, growth deficits tend to result from exposure during the late stages, and damage to the CNS can happen at any stage during pregnancy (Larkby et al. 1997). Subsequently, providing a diagnosis can be challenging in some cases, particularly those exposed to lower doses of alcohol or during a specific period in pregnancy, and not all of the symptoms for a diagnosis of FAS may be present. In such cases, infants may be diagnosed with Fetal Alcohol Spectrum Disorders (FASD), which is a clinical description referring to a group of conditions that result from antenatal alcohol consumption, but vary in their presentation (Table 2.3).

Damage to the CNS can be devastating and may occur without additional physical characteristics. Subsequently, symptoms of FASD may not manifest themselves until later in life (Hellemans et al. 2010). Heavy doses of alcohol during pregnancy have consistently been associated with a reduction in intellectual functioning (Coles et al. 1991; Spohr et al. 1993; Mattson & Riley 2000) verbal communication skills (Church & Abel 1998; Korkman et al. 2010), balance and other motor development (Roebuck et al. 1998; Connor et al. 2006), executive functioning and memory (Coles et al. 1991; Burden et al. 2005), and behavioural problems (Kelly et al. 2000; Sood et al. 2001). Without confirmation of maternal alcohol consumption, an incorrect diagnosis can be made, exacerbating the challenges associated with estimating prevalence and harm. While there is strong evidence to support a link between heavy alcohol consumption during pregnancy and harm to the developing fetus, studies assessing the harm from lower intakes of antenatal alcohol exposure have produced inconsistent, and often conflicting results.

Table 2.3 Fetal Alcohol Spectrum Disorders (FASD)

Condition	Clinical presentation
Fetal Alcohol Syndrome (FAS)	Growth deficiency (height/weight at or below 10th percentile) All three of the following facial characteristics: Thin vermilion (Upper lip) Smooth philtrum Small palpebral fissures Neurocognitive impairment Confirmed or unknown antenatal alcohol consumption
Partial Fetal Alcohol Syndrome (PFAS)	Confirmed antenatal alcohol consumption May or may not be growth deficient May have some, but not all, characteristic facial features Neurocognitive impairment
Alcohol Related Birth Defects (ARBD)	Confirmed antenatal alcohol consumption Congenital abnormalities that are related to alcohol exposure, but the child does not present with classic FASD
Alcohol Related Neurodevelopmental Disorder (ARND)	Confirmed antenatal alcohol consumption Normal or minimally deficient growth No or minimal distinctive facial characteristics Cognitive impairment

2.3.3 Light to moderate alcohol consumption

Research studies investigating low to moderate levels of alcohol exposure during pregnancy have produced contrasting and sometimes paradoxical findings, highlighting the methodological challenges of conducting robust observational studies within this field.

A systematic review analysed the findings of 66 studies (1970-2005) assessing the intake of low to moderate alcohol consumption, defined as less than one drink (12g ethanol) per day during pregnancy (Henderson et al. 2007). A number of the included studies reported better neonatal outcomes in light to moderate drinkers compared to women who did not report any antenatal alcohol consumption; however, the authors acknowledged this may be a result of residual confounding. On this basis, the authors concluded that there was no consistent evidence of increased risk of spontaneous abortion, poor fetal growth, preterm birth, stillbirth, antepartum haemorrhage or neurodevelopment outcomes from light to moderate alcohol consumption during pregnancy. However, the authors also acknowledged many methodological weaknesses in included studies, which need to be addressed in further research before the harm from light to moderate antenatal alcohol exposure can be robustly estimated (Henderson et al. 2007).

Subsequent research has not provided convincing evidence to support or refute the hypothesis that light to moderate alcohol increases the risk of adverse infant and childhood outcomes. A number of studies have indicated that low to moderate doses of alcohol are associated with lower IQ (Streissguth et al. 1990; Zuccolo et al. 2013), poorer memory and learning (Burden et al. 2005), behaviour (Underbjerg et al. 2012), speech and communication (Faden & Graubard 2000), and balance ability (Humphriss et al. 2013). In contrast, findings from other studies have suggested there are no associations between low to moderate antenatal alcohol consumption with IQ (Alati et al. 2008; Underbjerg et al. 2012), behaviour (Kelly et al. 2010; Skogerbø et al. 2013), and speech and communication (O'Leary et al. 2009). Some researchers have even reported a positive association between low to moderate alcohol exposure and child IQ (Kelly et al. 2010; Kesmodel et al. 2012).

There are a number of methodological factors that may be attributable to these contrasting findings. Firstly, alcohol assessment methods employed by large, epidemiological studies are self-reported and prone to measurement error; many low to moderate drinkers may be misclassified as non-drinkers, making it difficult to detect differences in outcomes (Dawson 2003). Secondly, alcohol consumption is associated with many other social and environmental factors that are also related to infant and childhood outcomes, and therefore, results are vulnerable to residual confounding which can lead to erroneous results (Gronbaek et al. 1994).

To minimize the limitations of observational studies, many studies have focused on Mendelian randomization; a technique that enables researchers to assess causal associations using genetic information and observational data in the presence of confounding factors (Burgess et al. 2012). The technique works on the basis that particular genetic variants are associated with certain exposure patterns (e.g. alcohol consumption). Because the allocation of genetic variants from parents to offspring is random, they should be unrelated to confounding factors, making it possible to explore causal associations (Smith & Ebrahim 2003).

As discussed previously, the genetic variant rs1229984 (ADH1B), is associated with accelerated ethanol metabolism and a build-up of acetaldehyde. An investigation into its relationship with antenatal alcohol consumption indicated the variant was associated with abstaining from alcohol consumption during the first trimester, not drinking prior to pregnancy and being less likely to binge drink before or during pregnancy (Zuccolo et al. 2009). The same group of researchers then categorised women on the basis of their reported alcohol consumption during the first trimester and whether they had this genetic variant or not, and then compared their children's IQ scores at 8 years. The findings were compared with a conventional analysis using regression models, adjusted for confounders. The conventional analysis suggested that low to moderate intakes of alcohol during the first trimester were associated with higher IQ scores at 8 years; in contrast, results from the Mendelian randomization indicated a small detrimental effect of low to moderate alcohol consumption during pregnancy (Zuccolo et al. 2013). Overall, the findings suggest that low to moderate levels of alcohol consumption

may be harmful to offspring, but traditional methods of investigation are unable to detect these relationships due to measurement error and residual confounding.

2.3.4 Binge drinking

Evidence from animal models indicates that Blood Alcohol Concentration (BAC) is responsible for the level of harm caused to the CNS in offspring (Burd et al. 2012) and there has been a recent shift to focus on patterns of binge drinking (high quantities of alcohol consumed over a short period of time). While experimental research using animal models of FASD has produced convincing evidence about the harm from binge drinking (Bonthius et al. 1988; Maier et al. 1999; West et al. 1989; Maier & West 2001), observational studies in humans have produced less consistent findings.

A systematic review synthesized the results from 14 studies published before 2006 investigated the effects of binge drinking (typically defined as 4 to 7 units of alcohol consumed in one drinking occasion) during pregnancy on birth weight, gestational age at birth, birth defects and neurological development (Henderson et al. 2007). Despite a lack of strong evidence to suggest a causal relationship between binge drinking and adverse birth outcomes, three out of four included studies reported binge drinkers had children at increased risk of 'disinhibited behaviour' (Nulman et al. 2004), delinquent behaviour, lower verbal IQ (Bailey et al. 2004) and learning difficulties (Streissguth et al. 1983; Streissguth et al. 1989; Streissguth et al. 1990). However, the differences were small and the authors acknowledged methodological weaknesses, which included inappropriately adjusted confounders and varying definitions of binge drinking. One study defined women as binge drinkers if they reported binge drinking throughout pregnancy, in contrast to other studies, which tend to use the definition of at least one reported episode of binge drinking during pregnancy (Bailey et al. 2004). Overall, the authors concluded that the evidence was not strong enough to support a causal link between antenatal binge drinking and adverse birth outcomes, but did indicate that a relationship may exist between binge drinking and a delay in neurological development (Henderson et al. 2007).

Since 2005, further research has been conducted to further explore these relationships. A study conducted in Canada concluded that even occasional binge drinking episodes during pregnancy were associated with lower birth weights, head circumference and poorer visual acuity at 6 months (Fraser et al. 2012). Whereas, a study conducted in Switzerland reported no relationships between binge drinking, gestation length, birth weight, SGA or preterm birth (Meyer-Leu et al. 2011). A number of studies have also reported associations between binge drinking and increased risk of psychiatric problems in adult life (Barr et al. 2006), hyperactivity and inattention (Sayal et al. 2009; Alvik et al. 2013), behavioural issues (Alvik et al. 2013; Niclasen et al. 2014), lower IQ (O'Callaghan et al. 2007) and poorer sleep quality in childhood (Alvik et al. 2011). Conversely, there is evidence to suggest that there is no relationship between binge drinking and child IQ (Alati et al. 2008; Sayal et al. 2009;

Kesmodel et al. 2012; Underbjerg et al. 2012; Skogerbø et al. 2012), behaviour (Sayal et al. 2009; Day et al. 2013; Skogerbø et al. 2013) and attention (Underbjerg et al. 2012).

It is likely that these contrasting findings are attributable to methodological differences between studies. While, a number of studies report using detailed exposure measures, , such as the Time Line Follow Back (TLFB) method (Fraser et al. 2012) or face-to-face interviews with probing questions (Skogerbø et al. 2013), some report using brief exposure measurement tools to estimate alcohol consumption. Brief tools, such as the AUDIT-C obtain crude estimates of quantity and frequency consumed throughout pregnancy (O'Callaghan et al. 2007; Sayal et al. 2009; Meyer-Leu et al. 2011).

The classification of 'binge drinkers' also varied between studies. O'Callaghan et al. (2007) defined binge drinkers as women who reported consuming six or more units per drinking occasion, and then categorised binge drinkers into two groups: those binge drinking less than half the time in early pregnancy and those binge drinking more than half of the time during early pregnancy. Whereas, it is more common in studies for women to be classified as binge drinkers if they report at least one episode during their pregnancy (Alati et al. 2008; Sayal et al. 2009). Research published from the Danish Birth cohort reported no difference in IQ when binge drinkers were categorised by ever having binged, by the number of binge episodes or by the timing of binge episodes (Skogerbø et al. 2012; Skogerbø et al. 2013). Kesmodel et al. (2012) even reported a protective effect of binge drinking; women who reported two or more binge sessions in early pregnancy were consistently (but not significantly) more likely to have children with higher IQs. Interestingly, women who were binge drinkers had significantly higher IQs compared to those who were not and non-drinkers were more likely to be younger, less educated, smokers and from suboptimal living conditions, indicating the conclusion may be a result of residual confounding (Kesmodel et al. 2012).

While there is convincing evidence to support a relationship between heavy or chronic antenatal alcohol consumption and adverse infant and childhood outcomes, the evidence for other patterns of alcohol consumption, such as light to moderate or binge drinking, is less conclusive. However, results from studies using Mendelian randomization are promising and suggest that even at low to moderate levels of alcohol can have detrimental effects on childhood cognitive performance, indicating a lack of association in studies using traditional analysis methods may be attributable to residual confounding.

2.4 Potential mechanisms of harm

Animal studies play a central role in FASD research due to the practical and ethical barriers associated with using human participants, enabling researchers to understand more about the potential mechanisms of harm from antenatal alcohol consumption. While the exact mechanisms are still unclear, many different theories have been developed in order to explain the teratogenic effects of

alcohol on the developing fetus. For the purpose of this thesis, two mechanisms of harm will be explored in more detail: oxidative stress and effects on DNA methylation.

2.4.1 Search strategy (Search 2)

A literature search was conducted in two electronic databases: MEDLINE and EMBASE. Grey literature was not searched. Reference lists of relevant studies were hand searched for additional articles. A mixture of MeSH headings and key word searches were conducted using the search terms in Table 2.4. Terms were grouped using Boolean operators and searches were limited to English language articles. FAS was first described in the scientific literature in 1973; therefore, each search was limited to a publication date between 1973 and present day. Inclusion and exclusion criteria are presented in Table 2.5. The full search string for each database can be found in Appendix A.

Table 2.4 Database search terms (search 2)

Search terms
Pregnan*/ Antenatal/ Prenatal
Alcohol*/ Ethanol/ Alcohol drinking/ Alcohol-related disorder/ Alcohol exposure/ Binge drinking/ Drinking/ Heavy episodic drinking
Diet*/ nutrient/ vitamin*/ mineral/antioxidant/ DNA methylation/ methyl group/ methyl donor/ one carbon metabolism/ oxidative stress/ reactive oxygen species/ROS/ free radical/ Folate/ Folic acid/ Choline/ Betaine/ Methionine/ Homocysteine/ B vitamin*/ Ascorbic acid/ Tocopherol/ Caroten/ Diet?supplement*

Table 2.5 Eligibility criteria (search 2)

Inclusion criteria:	**Exclusion criteria:**
Pregnant populations (animal or human)	No quantitative measure of alcohol
English language article	No dietary element explored
Peer reviewed journal	No offspring outcomes reported
Experimental study design	
Quantifiable measure of alcohol consumption	
Alcohol exposure occurs during pregnancy	

2.4.2 Oxidative stress

Free radicals and Reactive Oxygen Species (ROS) are by-products of normal cellular metabolism and are required to regulate several signalling pathways. They have one or more unpaired electrons, which make them highly chemically reactive (Figure 2.1). A complex system of antioxidants acts as a defence to counteract the production of free radicals and ROS, maintaining equilibrium; however, in certain

conditions, ROS production can exceed antioxidant defences and overwhelm the system. This imbalance is called Oxidative Stress (OS) and in this state, damage can occur to surrounding lipids, proteins and DNA. Sustained OS can result in cell death (apoptosis and necrosis) (Wang & Bieberich 2010) and epigenetic modification (changes in gene expression, without changes to the DNA sequence) (Wang et al. 2009; Brocardo et al. 2011).

Ethanol can induce OS in a number of ways. Firstly, ROS are produced as by-products during the breakdown of ethanol (Figure 2.1), and are mostly observed in the liver (Lieber 1991) and brain (Montoliu et al. 1995). Secondly, the formation of free radicals which react with cellular components (Cohen-kerem & Koren 2003). Thirdly, ethanol can also induce OS indirectly by reducing antioxidant defences (Fernandez-Checa et al. 1991; Bailey et al. 2001).

Ethanol is largely metabolised in the liver and brain, where ethanol metabolising enzymes are most abundant. The brain is particularly vulnerable to the generation of free radicals and ROS for two reasons. Firstly, the brain metabolises oxygen at the highest rate compared to any other organ in the body. Secondly, brain tissues are rich in unsaturated fatty acids, iron and neurotransmitters that can spontaneously react with oxygen (Halliwell 1992; Gerlach et al. 1994). Despite this, the brains antioxidant system is surprisingly restricted (Floyd & Carney 1992); it is even more compromised in the fetal brain, consisting of less than half the concentration of antioxidants compared to adult levels (Henderson et al. 1999; Bergamini et al. 2004).

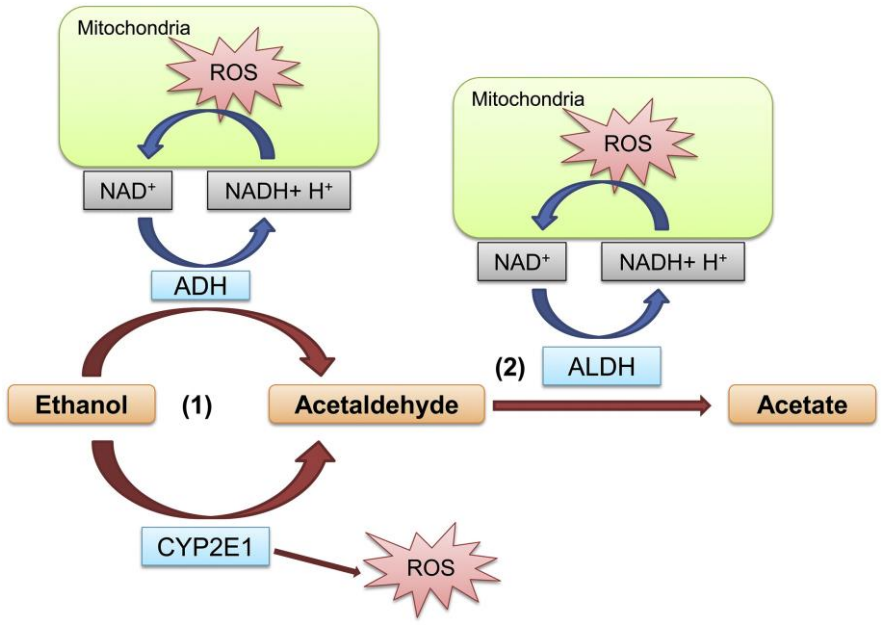

Figure 2.1 Production of reactive oxygen species during ethanol metabolism (Brocardo et al. 2011)

A number of studies have provided evidence that OS plays an important role in ethanol-induced harm in pregnancy. Findings from experimental research using animal models have suggested there is a reduction in antioxidants (Reyes et al. 1993; Henderson et al. 1999; Dembele et al. 2006) and increased markers of oxidation (Petkov et al. 1992; Chu et al. 2007; Henderson et al. 1999; Dong et al. 2010) after chronic exposure to ethanol. Similar results were reported after acute administration of ethanol, simulating binge drinking (Ramachandran et al. 2001).

There is also evidence to suggest that OS plays a role in spontaneous abortion and fetal growth restriction in ethanol exposed pregnancies (Gundogan et al. 2010). Ethanol exposure induces OS in the human placental villi, which allow maximum contact between the mother and fetus for the transfer of gas and nutrients, and can affect the placental blood flow regulation (Kay et al. 2006). Pregnancy loss may be attributable to placental apoptosis (programmed cell death) and necrosis (death of cells due to injury), which has been observed in rats that were chronically exposed to alcohol during pregnancy. Ischemia (inadequate blood supply), infarction (obstructed blood flow) and reduced thickness due to increased cellular necrosis have also been observed in ethanol-exposed placentas, which may account for impaired nutrient delivery and intrauterine growth restriction (Gundogan et al. 2008). A number of studies have reported a relationship between low birth weight and OS, with a focus on three particular types of dietary antioxidants; vitamin C, vitamin E and carotenoids (Matsubasa et al. 2002; Lee et al. 2004; Kim et al. 2005; Scholl et al. 2006; Osorio et al. 2011; Weber et al. 2014).

2.4.3 The role of dietary antioxidants in oxidative stress

Vitamin C

Vitamin C is a water-soluble micronutrient that cannot be stored in the body, making it an essential micronutrient. It is referred to as a scavenger, as it donates electrons to ROS and free radicals, preventing further damage to surrounding cells. When it donates an electron, vitamin C is itself oxidized, but produces a much more stable free radical in its place (Padayatty et al. 2003). A prospective study conducted in South Korea reported a positive correlation with plasma vitamin C concentration during pregnancy and birth weight (Lee et al. 2004).

Vitamin E

Vitamin E is a fat-soluble micronutrient, meaning it is possible to store it in the liver and fat throughout the body. It is composed of a group of tocopherols and tocotrienols, of which α-tocopherol is the most biologically active. Vitamin E also acts as a scavenger in the same way as vitamin C (Niki 2014), and low maternal status during pregnancy has been associated with poor fetal growth (Scholl et al. 2006).

Carotenoids

Carotenoids are a class of pigments that are found in plants and are responsible for the vibrant colours found in particular fruits and vegetables, and the five most commonly consumed are α-carotene, β-

carotene, lutein, lycopene and β-cryptoxanthin (Burrows et al. 2015). Many carotenoids also have pro-vitamin A activity, including α-carotene, β-carotene and β-cryptoxanthin, meaning they can be converted into retinol (vitamin A). Carotenoids, in particular, pro-vitamin A carotenoids, have been suggested as an important factor in healthy fetal development (Strobel et al. 2007).

2.4.4 The role of antioxidants in models of FASD

A number of studies have investigated the modifying effects of antioxidants in models of FASD. There are a number of different types of antioxidants that have been explored, including vitamin C (Peng et al. 2005; Nash et al. 2007), vitamin E (Mitchell et al. 1999a; Heaton et al. 2004; Marino et al. 2004; Wentzel et al. 2006; Nash et al. 2007), carotenoids (Mitchell et al. 1999a) and flavonoids (Grange et al. 1999; Edwards et al. 2000; Chen et al. 2004).

Cell cultures

Fetal hippocampal cells taken from rats were exposed to ethanol and were starved of glucose and exposed to an anoxic environment (without oxygen) in order to replicate ischemia. Neuronal viability was significantly higher in cultures treated with vitamin E or beta-carotene compared to those exposed to ethanol only. Despite these promising findings, the concentrations of ethanol exposure were very high and no measure of oxidative stress was recorded in order to provide a plausible mechanism of protection (Mitchell et al. 1999b; Mitchell et al. 1999c).

A similar study was conducted which exposed hepatocyte cells from fetal rat livers to much lower concentrations compared to those by Peng et al. (2005); the authors concluded that pre-supplementation with vitamin E prevented lipid peroxidation but did not prevent the reduction of glutathione (markers of oxidative stress) (Devi et al. 1993). A more recent set of studies by Heaton et al. also explored the use of vitamin E as an effective antioxidant in fetal rat cell cultures; neonatal cerebellar granule cell cultures were divided into one of four exposure groups: (1) control; (2) ethanol only (in varying concentrations from 200 to 1600mg/dl); (3) control plus vitamin E (50μM); (4) ethanol (in varying concentrations from 200 to 1600mg/dl) plus vitamin E (50μM). While, apoptosis was significantly increased in those in group 2 compared to group 1, vitamin E improved the survival of cells in all ethanol treated cell culture. Interestingly, cell cultures in group 3 had a significantly higher survival rate compared to controls in group 1 (Heaton et al. 2004).

Animal models

Studies using animal models have also reported similar findings. Peng et al (2005) developed a model of FAS using frog (Xenopus Laevis) embryos. Ethanol exposure resulted in reduced brain size (microcephaly) and retarded growth of tadpoles. A number of embryos were either pre-treated with 100μM of ascorbic acid (vitamin C) two hours prior to ethanol exposure or were treated concurrently. Vitamin C treatment inhibited the production of ROS and protected against microcephaly (abnormal

brain development) and growth retardation (Peng et al. 2005). Nash et al (2007) also explored the use of vitamin C in an animal model and reported similar findings. Pregnant guinea pigs were either fed a sucrose drink or ethanol (4g/kg), with or without a mixture of vitamin C (250mg) and vitamin E (100mg). Ethanol exposed offspring had significantly lower brain weights compared to controls, but those given vitamin C and E supplements had offspring with higher hippocampal weights compared to those exposed to ethanol alone (Nash et al. 2007).

Evidence also suggests there is a protective effect of vitamin E treatment alone against ethanol induced harm. Findings have suggested that vitamin E protected fetal liver cells from ethanol-induced oxidative stress and reduced the number of birth malformations (Wentzel et al. 2006). Studies exploring similar effects in neonatal rats, which represent late gestation brain development in humans, reported vitamin E ameliorated the increase in oxidative stress, loss of hippocampal cells (Marino et al. 2004) and purkinje cells in ethanol exposed neonatal rats (Heaton et al. 2000). However, a study by Tran et al (2005) reported conflicting results when pregnant rats were exposed to ethanol, with or without vitamin E supplements. Neonatal rat pups exposed to ethanol and vitamin E showed no signs of improved functional and structural damage to cerebellum (Tran et al. 2005). The different findings may be a result of the dose of ethanol; neonatal rats were given lower doses of ethanol compared to those in other studies (Marino et al. 2004)

The antioxidant properties of another group of phytochemicals, flavonoids, have also been explored as a potential method of protecting against ethanol-induced damage in fetal models of FAS. Findings have suggested that they reduced oxidative stress (Grange et al. 1999; Edwards et al. 2000) and the incidence of forelimb malformations in mice (Chen et al. 2004) in ethanol exposed rat and mice fetuses, respectively.

While there is convincing evidence to support a protective effect of antioxidants against physiological changes to fetal brain and liver cells due to alcohol exposure, the findings supporting a protective effect against behavioural deficits are not as consistent. Studies investigating flavonoids, reported offspring of rats exposed to a combination of ethanol and flavonoids performed better at the radial arm maze task (Busby et al. 2002; Neese et al. 2004) and social recognition tests (Reid et al. 1999) compared to those exposed to ethanol only. Vitamins C and E mitigated the ethanol-induced deficit in offspring performance of the water maze test. However, in control group 2 (sucrose drink + vitamins), exposure to high doses of vitamins C and E resulted in poorer test performance compared to rats in control group 1 (sucrose drink only) (Nash et al. 2007). However, two studies reported that vitamin E did not mitigate the cognitive deficits of ethanol-induced oxidative stress (Marino et al. 2004; Tran et al. 2005). The great heterogeneity between research methods means the differences are likely to be a result of variations in timing and concentration of exposures and the methods employed to measure outcomes (Tran et al. 2005).

2.4.5 One carbon metabolism

The early stage of pregnancy (embryogenesis) is a period of rapid cell proliferation, which is particularly vulnerable to environmental factors, which may cause disruptions to optimal physiological development (Anderson et al. 2012). DNA methylation plays a crucial role in healthy cell development and refers to a process where methyl groups are added to DNA. This process alters the expression of genes in cells as they are dividing and changing from stem cells into specific tissues. One carbon metabolism (OCM) is a complex cycle of chemical reactions that generates methyl donors required for healthy DNA methylation. Carbon units are partitioned from specific amino acids through three pathways: the folate cycle, the methionine cycle and the transsulfuration pathway (Figure 2.2). One important aspect of OCM is the conversion of homocysteine (Hcy) to methionine; elevated maternal Hcy levels are indicative of poor maternal one carbon metabolism. A number of studies have reported findings to suggest an association between elevated plasma homocysteine concentrations and increased risk of adverse birth outcomes (Hogeveen et al. 2012; Kalhan & Marczewski 2012), although the exact mechanisms are not fully understood. A number of micronutrients play key roles; including folate, betaine, methionine, vitamin B12 and B6.

There is evidence to suggest that DNA methylation is inhibited in animal models of fetal ethanol exposure. Pregnant mice exposed to ethanol between gestational days 9 and 11 showed signs of hypomethylation (decrease in methylation) in fetal DNA (Garro et al. 1991). In other animal models, ethanol exposure resulted in alterations in DNA methylation in the hippocampus and pre-frontal cortex (Otero et al. 2012; Resendiz et al. 2013).

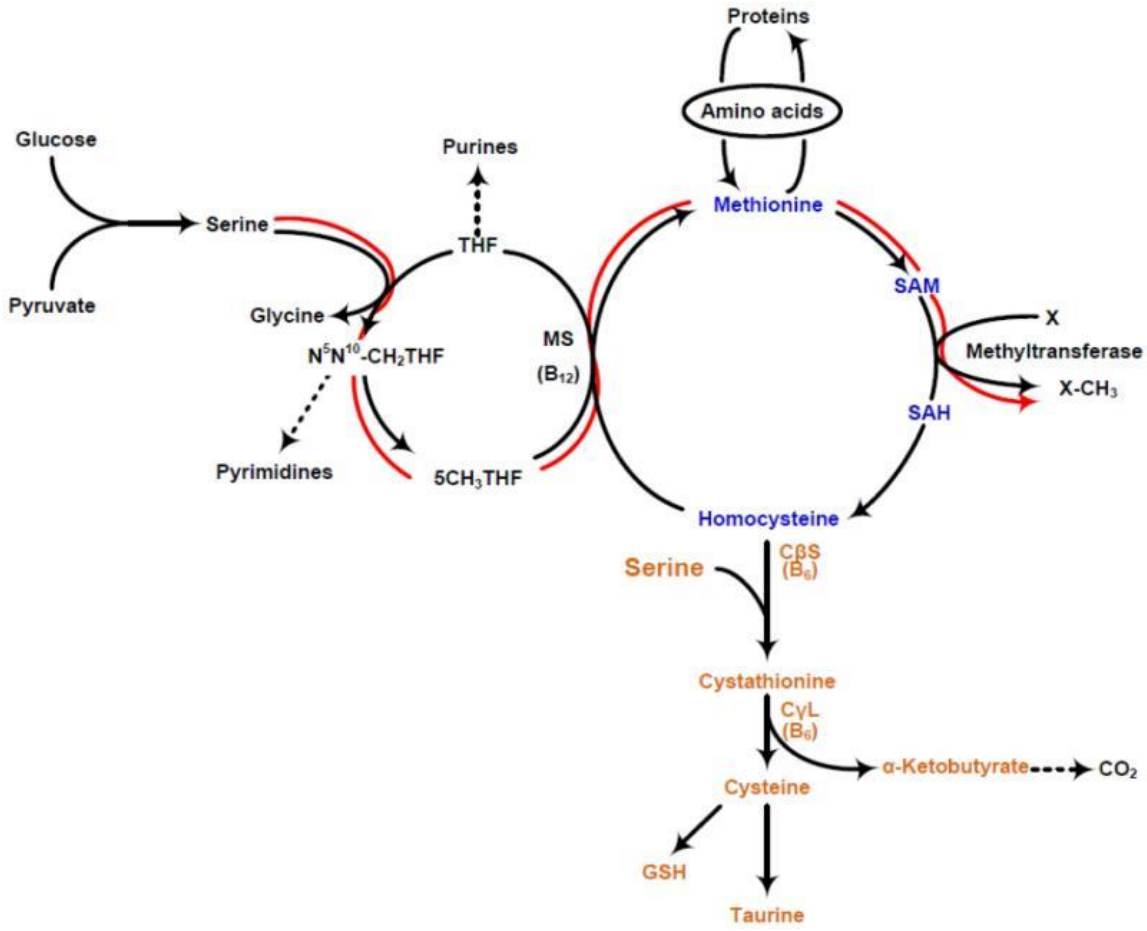

Figure 2.2 One-carbon metabolism (Kalhan & Marczewski 2012)

Black = folate cycle, blue = methionine cycle, orange = transsulfuration pathway

'Folate begins the cycle by entering the cells where it is reduced to THF, which is then converted to me-THF by SHMT. Me-THF is then reduced to mTHF, where it is demethylated to complete the cycle. The carbon unit is then donated to the methionine cycle through the methylation of Hcy to methionine. SAM is demethylated to SAH and converts back to Hcy where it enters the transsulfuration pathway; it is here that cysteine is generated in order to produce glutathione and taurine. Perturbations in methyl transfer can have profound effects on cell function, growth and proliferation' (Kalhan & Marczewski 2012).

Although ethanol is known to perturb OCM, the underlying mechanism is still not fully understood (Kruman & Fowler 2014). It is possible that acetaldehyde, the toxic by-product of ethanol metabolism, may interact with components of OCM, interrupting crucial chemical reactions involved in DNA methylation (Barak et al. 1993; Villanueva & Halsted 2004); ethanol-induced damage to methionine synthase prevents the conversion of 5-methylTHF to THF (Villanueva & Halsted, 2004). Another suggested mechanism of harm is the result of inhibited folate absorption and increased excretion (Halsted et al. 2002). A study reported elevated plasma concentrations of Hcy and methionine in ethanol exposed pregnant dams (Ngai et al. 2015). The complex and tightly interrelated pathways involved in DNA methylation mean OCM is sensitive to alterations in micronutrient status and ethanol-

induced teratogenic insult. However, there is a growing body of evidence to suggest that micronutrient intakes may play a mediating role in the relationship between ethanol and fetal harm.

2.4.6 The role of micronutrients in one-carbon metabolism

Folate

Folate (Vitamin B9) is an essential micronutrient and acts as a co-enzyme in the transfer of one-carbon units; it also has a crucial role to play in DNA and RNA synthesis, methylation and gene expression (SACN 2006). The demand for folate is highest during pregnancy due to the rapid rate of cell proliferation resulting from the sharp rise in red cell mass required for maternal and fetal growth (Jadavji et al. 2015). The current Recommended Nutrient Intake (RNI) for women planning to become pregnant in the UK is 400µg/day prior to pregnancy and during the first 12 weeks of gestation. Folate is of particular importance during the first few weeks of gestation when the neural tube closes; it is well established that maternal folate deficiency during this period increases the risk of Neural Tube Defects (NTD) (Pitkin 2007).

Betaine and choline

Betaine is an amino acid that high concentrations in wheat-based foods and also plays a key role in OCM, and choline is a precursor to betaine and found in animal products. Betaine dependent methylation compensates for folate deficiency by providing one-carbon units for the methylation of Hcy to methionine in OCM (Niculescu & Zeisel 2002). Like folate, low levels of choline and betaine are also associated with elevated levels of plasma homocysteine and increased risk of adverse birth outcomes (Shaw et al. 2004). There are currently no UK RNI for choline and betaine.

Methionine

Methionine is an amino acid required for the transfer of methyl groups from folate to S-adenosylmethioine (SAM) and deficiency can lead to growth retardation of the developing fetus (Rees et al. 2006). However, high levels of methionine can also lead to adverse fetal development by disrupting DNA methylation; excess methionine requires glycine which may divert it away from the fetus (Jackson et al. 2002). Therefore, a balance of methionine with other important dietary factors is important for healthy fetal growth and development.

Vitamins B12 and B6

Vitamins B12 and B6 are important cofactors in OCM, with vitamin B12 (cobalamin) acting as a coenzyme for methionine synthase. While deficiency in the UK is relatively uncommon (Ruston et al. 2004), it can result in adverse birth outcomes, such as birth defects
(Ray et al. 2007; Molloy et al. 2009; Mobasheri et al. 2010), SGA births (Lindblad et al. 2005; Yajnik et al. 2005) and preterm deliveries (Ronnenberg et al. 2002). Vitamin B6 acts as a coenzyme in the transsulfuration of homocysteine to cysteine, and evidence suggests that women with low vitamin B6

status are more likely to experience early pregnancy loss and a lower probability of conception (Ronnenberg et al. 2007). Vitamin B12 and B6 plasma concentrations decrease during the third trimester, even without any sign of clinical symptoms of deficiency, which indicates that it may be a normal aspect of physiological development during gestation; this makes it difficult to quantify and define deficiency during this period (Morris et al. 2008; Dror & Allen 2012).

2.4.7 The role of OCM micronutrients in models of FASD

Similar results have also been reported in studies investigating the role of micronutrients involved in OCM in animal models of FASD. In a study exploring the role of folic acid in ethanol teratogenicity, pregnant rats were allocated to receive either a low, medium or high dose of ethanol, with or without the addition of Folic Acid (FA). The findings indicated FA was able to suppress ethanol-induced harm; embryo growth and development was significantly increased in the offspring of rats in the FA groups, compared to those exposed to ethanol alone (Wang et al. 2009). A similar study was conducted exploring the effects of low and high doses of ethanol in pregnant rats either consuming a commercially prepared feed or a folate deficient diet. The authors concluded that in the presence of inadequate folate intake, low doses of ethanol have similar teratogenic effects as higher doses with an adequate folate intake (Gutierrez et al. 2007). Other animal models of FASD have also reported FA supplementation reduced the risk of ethanol-induced cardiac birth defects in ethanol exposed offspring (Serrano et al. 2010; Sarmah & Marrs 2013).

A number of promising findings surrounding the role of choline and betaine in FASD have been published in recent years. Initial results from research by Thomas and colleagues in the United States of America (USA) have indicated that intakes of choline during pregnancy may mitigate the harmful effects of ethanol. Offspring born to rats exposed to ethanol and given choline supplements had significantly higher birth and brain weights compared to offspring born rats who were exposed to ethanol alone (Thomas et al. 2009). The research team also explored the effect of choline supplements on behavioural outcomes and reported that choline mitigated the cognitive deficits attributable to ethanol exposure (Thomas et al. 2010), behavioural (Thomas et al. 2009) and eyeblink conditioning deficits (Thomas & Tran 2012).

While animal models are vital for progressing knowledge of medical fields where it isn't feasible to randomize humans to particular treatment strands, the information they provide also has limitations. The vast physiological and environmental differences means findings don't immediately translate to human populations; however, two recent studies in human populations have provided evidence to support the role of micronutrients in FASD. A study conducted in Canada showed a significant reduction in maternal to fetal transport of folate in pregnancies with heavy ethanol exposure (Hutson et al. 2012), however, women in this study were consuming alcohol in heavy quantities throughout pregnancy, so the results are not generalisable.

Coles et al (2015) have also published promising results from an RCT conducted in Western Ukraine, which involved randomising a sample of pregnant women who did and did not report drinking alcohol, into one of three intervention arms: 1) daily multivitamin and mineral supplements; 2) daily multivitamin and 3) mineral supplements plus additional choline supplement (750mg); and control group. The findings suggested that male infants born to mothers in the control group who continued to drink alcohol had the poorest developmental scores at 6 months of age, compared to infants born to mothers from any other exposure groups (Coles et al. 2015). While the findings are encouraging, the study did have limitations. Firstly, details of the multivitamin and mineral composition were not provided; therefore, it is not clear what component may be having this effect. Secondly, there was no group who were given daily choline supplement only, without the multivitamin and mineral, so it was not possible to explore the potential of choline supplement exclusively. Thirdly, cognitive outcomes were only measured until 6 months; many subtle measures of cognitive development are not easily detected until later in childhood (Coles et al. 2015).

Additional research is required to investigate this further and explore the interrelationships between micronutrients and ethanol teratogenicity. Many challenges faced in this area include the social patterning of both diet and alcohol consumption, making it difficult to tease apart the underlying causes of particular outcomes.

2.5 Relationships between dietary intake and alcohol consumption during pregnancy

2.5.1 Search strategy (search 3)

A literature search was conducted in two electronic databases: MEDLINE and PsychInfo. Grey literature was not searched. Reference lists of relevant studies were hand searched for additional articles. A mixture of MeSH headings and key word searches were conducted using the search terms in Table 2.6. Terms were grouped using Boolean operators and searches were limited to human participants and English language articles. No date restrictions were placed on this search. Inclusion and exclusion criteria are presented in Table 2.7. The full search string for each database can be found in Appendix A.

Table 2.6 Database search terms (search 3)

Search terms
Alcohol*/ Ethanol/ Alcohol drinking/ Alcohol-related disorder/ Alcohol exposure/ Binge drinking/ Drinking/ Heavy episodic drinking
Diet*/ Nutrient/ Vitamin*/ Mineral/antioxidant/ Dietary pattern/ Food habits/ Nutrition assessment/ Nutrition survey/ Nutrition/ Food/ Food preference/ Diet?supplement*

Table 2.7 Eligibility criteria (search 3)

Inclusion criteria:	Exclusion criteria:
Adult human populations	No quantitative measure of alcohol
English language article	No dietary element explored
Peer reviewed journal	No women in sample
Observational study design	Sample population <18 years old
Quantifiable measure of alcohol consumption	Disease group specific
Dietary measure	Exploring in relation to outcome
Healthy adult population	No details of how dietary intake was derived

2.5.2 Dietary patterns and alcohol consumption

Diet and alcohol consumption are both socially patterned health behaviours that are related to Socio-Economic Status (SES), and findings from observational studies in the general population suggest that frequency and quantity of alcohol consumption is associated with certain dietary habits. Two contrasting patterns were consistently described within the literature.

The first relationship that was observed was between light to moderate alcohol consumption and dietary intakes. A number of studies compared the dietary habits of individuals who reported any alcohol consumption with those who reported never drinking and found that never drinkers tended to have poorer quality diets, characterised by lower intakes of fresh fruit and vegetables compared to ever drinkers (Barefoot et al. 2002; Breslow et al. 2010). It is likely that this relationship is a result of SES; low to moderate alcohol consumption is typically associated with higher age, income and education (Tjønneland et al. 1999; Casswell et al. 2003; Touvier et al. 2014), and existing evidence suggests that dietary patterns are also associated with markers of SES (Northstone et al. 2008).

However, when varying quantities of alcohol consumption were explored, another relationship became apparent throughout the literature. As the quantity of alcohol consumed increased, the quality of diet typically decreased. A number of cross-sectional studies have previously reported that as mean daily alcohol consumption increased, intakes of fruit, vegetables and dairy decreased, while red, processed meat consumption and egg intakes increased in adult populations (La Vecchia et al. 1992; Ruf et al. 2005; Valencia-Martin et al. 2011; Herbeth et al. 2012; Touvier et al. 2014).

Ruf et al (2005) estimated the dietary intakes of approximately 13,000 adult women in Germany and found that when comparing dietary intakes between average alcohol consumption per day, differences were minimal until daily alcohol consumption exceeded 20g. This was also observed in two other studies conducted in Denmark (Tjønneland et al. 1999) and the US (Breslow et al. 2006). Breslow et al (2006) investigated both frequency and quantity of alcohol consumption in adult women and

compared this with the Health Eating Index (HEI) score; a composite score based on the intake of 12 food groups. A higher HEI score indicates a better quality diet. As frequency of alcohol consumption increased, HEI also increased, however, as quantity per occasion increased, HEI score decreased. Individuals in the highest quartile for quantity and lowest quartile for frequency had the lowest HEI scores compared to all other categories (Breslow et al. 2006).

A cross-sectional survey exploring binge patterns of drinking in Spain measured intakes of food groups and dietary habits. The authors concluded that binge drinking was associated with lower intakes of fruit and vegetables; higher intakes of red and processed meats and increased likelihood of skipping meals (Valencia-Martin et al. 2011).

Many studies have also estimated micronutrient intakes and the relationships with alcohol consumption, however, the findings vary greatly between studies and no consistent findings have been reported. Some studies have indicated drinkers have higher intakes of micronutrients compared to never drinkers (Breslow et al. 2010), however, many others have reported no differences (Colditz et al. 1991; La Vecchia et al. 1992; D'Avanzo et al. 1997). The great heterogeneity in dietary assessment methods, sample populations, study settings and types of food and drinks typically consumed means it is difficult to draw comparisons between individual study findings of micronutrient intakes.

Overall, low-to-moderate amounts of alcohol consumption appear to be associated with more frequent intakes of fruit, vegetables and dairy products. In contrast, heavy or binge patterns of alcohol consumption appear to be associated with higher intakes of red and processed meats, and low intakes of dairy, fresh fruit and vegetables. These patterns suggest that more harmful patterns of alcohol consumption may be associated with lower intakes of important food groups and key micronutrients in populations of adult women.

These findings are of particular concern as evidence suggests that dietary patterns alter very little during pregnancy (Crozier et al. 2011), meaning it is likely that these potentially harmful relationships may persist into pregnancy; a time of rapid growth and greater maternal demand for micronutrients. There is currently no published research that explores whether these relationships do persist into pregnancy.

2.6 Summary and conclusions

This chapter has reviewed a wide range of literature, summarizing the current understanding of the possible effects of women's antenatal alcohol consumption, dietary patterns and micronutrient intakes on their developing baby and identifying important areas for further research.

A number of different micronutrients and dietary antioxidants were highlighted as playing an important role during pregnancy, particularly in the presence of alcohol consumption. Micronutrients involved in OCM play critical roles in DNA methylation, and include folate, choline, betaine, methionine, vitamin B12 and B6. Deficiency can lead to errors in methylation during pregnancy, which has been linked with congenital abnormalities and poor childhood cognitive performance. Evidence from animal models of FASD also indicated that antioxidants may play an important role in fetal development. Oxidative stress refers to a state of imbalance between levels of ROS and antioxidant defences, which can result in damage to fetal tissues. Ethanol interacts with both OCM and redox balance, by interfering with DNA methylation pathways and by increasing the production of ROS, respectively. This means that alcohol consumption in the presence of inadequate maternal nutrition may increase the risk of adverse fetal outcomes.

Animal studies have provided evidence to suggest that folate, choline and various non-enzymatic antioxidants may mitigate the toxic effects of alcohol, thereby reducing the risk of adverse birth outcomes and long-term cognitive deficits.

In addition to this, results from observational studies in the general population suggest that particular alcohol consumption patterns, such as binge drinking and heavy consumption, are associated with certain dietary habits. Binge patterns of drinking and heavy alcohol consumption are typically associated with lower intakes of fruit and vegetables, higher consumption of processed and fried meats compared with intake in people consuming lower levels of alcohol or abstaining.

There is a paucity of research exploring how maternal alcohol consumption related to dietary intake during pregnancy and whether these relationships modify the risk of adverse infant and childhood outcomes. A better understanding of these associations is important in determining potential targets for prevention strategies and nutritional interventions for women who consume alcohol during pregnancy.

Chapter 3: Methodology

3.1 Chapter overview

This chapter outlines the overall research strategy adopted to address the aims and objectives set out in Chapter 1. The initial strategy used a mixed-methods design comprising a cross sectional survey of a convenience sample of pregnant women, and in-depth interviews with a purposive sample of pregnant women. A mixed methods design was originally employed as using both quantitative and qualitative data would lead to a more detailed understanding of the complex, socially driven patterns of dietary intake and alcohol consumption (Dures et al. 2011). However, due to difficulties with recruitment the strategy was revised to include a secondary analysis of a pregnancy cohort study with three distinct studies to address the overall aims and objectives.

The first section of this chapter outlines the design of the initial strategy involving a mixed-methods study (Strategy 1), including the development and validation of a new Food Frequency Questionnaire (FFQ) (Study 1A), and the methodological considerations related to doing so. The second section outlines the design of a cross sectional survey of a convenience sample of pregnant women (Study 1B) in which the new FFQ and other instruments were used to measure maternal alcohol consumption patterns and describe dietary intake and the methodological considerations related to this.

The third section sets out the rationale for revising the initial research strategy and developing an alternative (Study 2A) to address the main aims and objectives of Study 1B. It outlines the design of Study 2A, a secondary analysis of a well-established cohort study, the Avon Longitudinal Study of Parents and Children (ALSPAC), and the methodological considerations in using this dataset.

The ALSPAC cohort also provided a wealth of neonatal and childhood outcome measures. Therefore, additional aims and objectives were developed to make use of this important data. Research Strategy 2 also aimed to explore how the associations between maternal alcohol consumption and aspects of dietary intake influence fetal growth (Study 2B) and childhood cognitive performance (Study 2C).

In this chapter, I will describe the methodological issues associated with assessing alcohol consumption, dietary patterns and micronutrient intakes and the rationale for adopting the specific methods used in the studies, which make up the programme of work described in this thesis. I will discuss the methodological considerations in Strategy 1 and then those relevant to Strategy 2. Specific methods employed for each individual study will be discussed in relevant chapters.

3.2 Overview of Strategy 1

Chapter 2 highlighted the lack of research assessing relationships between dietary intake and alcohol consumption during pregnancy and the potential harms to the developing fetus. Therefore, the original aims of research Strategy 1 were to explore relationships between maternal micronutrient intakes, dietary patterns, and alcohol consumption in a population of pregnant women in the UK.

To address these aims, a mixed-methods study (Strategy 1) was developed and comprised of a cross-sectional survey of a convenience sample of women attending antenatal clinics in England. To provide contextual information and explore how between diet and alcohol develop, in-depth interviews were conducted with a purposive sub-sample of women. To identify women who were drinking alcohol during pregnancy, a short screening questionnaire was conducted in antenatal clinics. Women who reported drinking any alcohol during their current pregnancy were then invited to take part in the next stages of the research (Study 1B). Because of the limitations with existing instruments, a new dietary assessment tool was designed and validated, for use alongside an alcohol questionnaire (Study 1A).

3.3 Strategy 1

3.3.1 Objectives

While a number of studies have explored alcohol consumption during pregnancy or dietary intake during pregnancy, no previous research studies have explored the maternal dietary intake in relation to alcohol consumption during pregnancy. The objective of Strategy 1 was to measure maternal alcohol consumption patterns, describe maternal dietary patterns, estimate relative micronutrient intakes during pregnancy, and to explore relationships between these variables.

3.3.2 Biochemical vs. self-reported measures

A number of dietary and alcohol assessment methods are available and can be categorised into two broad groups; biochemical markers and self-reported measures. Both have strengths and limitations, which will be discussed in relation to the aims of this study.

Collecting alcohol and dietary intake data are both subject to similar challenges and biases, and the biggest limitation of self-reported methods is the subjective nature of estimates, which are vulnerable to recall and social desirability biases (Schatzkin & Kipnis 2004). Biochemical markers of exposure can overcome this and provide an objective measure that is not susceptible to these biases (see section 1.1.3). There are a number available to estimate alcohol exposure and dietary intake, however, they also have limitations. Firstly, the collection and analysis of biological samples can be costly, time consuming and invasive. Secondly, they provide a proxy of actual intake, and in the case of alcohol, reflect either chronic or very acute exposures (Peterson 2005). Finally, additional factors including,

environmental exposures (e.g. smoking), underlying diseases and genetic traits can all influence the estimation of dietary intakes and alcohol consumption using biochemical markers (Peterson 2005; Hedrick et al. 2012).

The collection, preparation and analysis of biological samples were not feasible within the context of this study. Therefore, it was deemed more advantageous to sacrifice precision for a cruder estimate and use self-reported measures of both dietary intake and alcohol consumption. The remainder of this chapter will discuss the methodological considerations pertaining to self-reported measures.

3.3.3 Prospective vs. retrospective methods

One of the aims of this study was to estimate usual intake of food and drink items over the course of pregnancy. Prospective methods include food and drink diaries, where the participants describe all food and drink items, as and when they consume them, often also weighing items and leftovers. They are often recommended for use as they can provide researchers with a more accurate measure of intake (W. C. Willett 2013). However, they place a large burden on participants, requiring them to be highly motivated and literate; collecting more than three days' worth of data can often cause participant fatigue, resulting in incomplete datasets and potentially bias the sample population (Coulston & Boushey 2008). In addition to this, recording consumption can often lead to altered behaviour, due to a heightened awareness of actions. Because the aim of this study was to measure usual intakes, rather than absolute exposures, the potential threat of altered behaviour was seen as a methodological weakness.

Retrospective data collection methods include those where participants are asked to recall their consumption over a defined period. Response bias refers to cognitive biases that influence how a participant will answer questions. Participants may provide inaccurate responses due to poor memory (recall bias) or purposefully provide an inaccurate response that is believed to be more favourable (social desirability bias) (Paulhus 1991; Crutzen & Göritz 2010). Despite these limitations, retrospective methods were considered the most feasible within the context of this study.

3.3.4 Mode of delivery

Diet and alcohol consumption in the context of pregnancy can be a sensitive issue for many, as women may feel under additional scrutiny to adhere to particular guidelines or expectations (Croghan 2005; Szwajcer et al. 2007). While interviewer administered techniques allow researchers to prompt participants and obtain a more detailed understanding of exposures, they require considerable time and funding to implement, and evidence suggests that socially desirable responses are more common when socially sensitive questions are being asked (King & Bruner 2000). Studies exploring the delivery of sensitive questionnaires topics (e.g. alcohol, smoking and illicit drug use), reported that there were higher levels of social desirability bias using interviewer administered techniques compared to self-

completion methods (Kreuter et al. 2008). Therefore, self-completed data collection methods were considered the most appropriate for this study.

3.3.5 Assessment of maternal alcohol consumption

Self-reported measures of alcohol consumption in pregnancy are plagued with measurement error. Evidence suggests that up to 40% of pregnant women underreport alcohol use (Ernhart & Morrow-tlucak 1988; Morrow-Tlucak et al. 1989; Czeizel et al. 2004). Because the aims of this study also included the collection of dietary data and socio-demographic details, it was deemed inappropriate to use a time intensive method to collect alcohol data, such as the self-completed, web-based Time-Line Follow-Back method (TLFB) (Rueger et al. 2012); lengthy and repetitive assessments can lead to erroneous or incomplete responses (Del Boca & Darkes 2003).

Two methods of alcohol assessment that are commonly used in surveys include, Graduated Frequency (GF) and Quantity-Frequency (QF) methods. The GF approach initially asks participants about the quantity and type of alcohol consumed on the heaviest drinking days, and then gradually moves onto light drinking days, providing detail about the quantity, frequency and type of alcohol consumed. Despite collecting relatively detailed information about previous alcohol consumption, studies suggest that GF methods overestimate alcohol consumption (Midanik 1994; Heeb & Gmel 2001). QF methods were developed to classify participants into broad categories of 'usual' alcohol consumption by asking the number of day's alcohol is consumed on in a typical week, and the number of drinks consumed on typical drinking days. The main criticism of QF methods is that they are unable to capture less frequent patterns of drinking, such as binge drinking (Greenfield & Kerr 2008) and the National Institute on Alcohol Abuse and Alcoholism (NIAAA) in the US recommended that if QF questions are used, additional questions that capture information on less frequent patterns of consumption should also be included (NIAAA 2003).

The AUDIT-C is a shortened version of the Alcohol Use Disorders Identification Test (AUDIT) and includes three QF questions about frequency of alcohol consumption in a typical week over a specified period, quantity of alcohol consumed on a typical drinking day and the frequency of consuming six or more units (binge drinking) on one occasion. It was considered the most appropriate short, QF alcohol assessment tool for this study, as it is simple, short and is a valid and reliable measure, providing relevant information in order to assess patterns of alcohol consumption. The AUDIT-C has been used extensively in alcohol research to identify individuals who are drinking in potentially hazardous or harmful patterns during pregnancy (Dawson et al. 2005; Burns et al. 2010). Results from a method comparison study suggested that the AUDIT-C was more sensitive to identifying binge drinkers compared to standard QF questionnaires (Shakeshaft et al. 1998). Other brief, validated screening tools for alcohol use are available, including the CAGE, TWEAK, TACE, SMAST and BMAST, but do not

ask QF questions. Rather focus on dependency symptoms, which are not appropriate measures to address the objectives of this thesis.

Despite the strengths discussed previously, there are limitations to using the AUDIT-C questionnaire that must be considered when interpreting the result in this thesis. The tool asks participants to estimate their average intake of units over the course of their pregnancy, which can be challenging for respondents who do not consume alcohol in regular patterns. In addition to this, units are an relatively abstract measure that many individuals in the UK have difficulty interpreting, and a recent UK study suggests that the adult population, particularly those who are heavy or irregular drinkers, cannot accurately estimate their unit intake and typically underestimate their consumption (Boniface & Shelton 2013).

3.3.6 Assessment of maternal dietary intake

The aims of Study 1B were to estimate patterns of dietary intake and micronutrient intakes during pregnancy. While 24-hour dietary recall interviews (24-HR) can provide a detailed measure of intake that can be used to calculate absolute estimates of dietary intake, they are also labour intensive and costly, even self-completed versions (Subar et al. 2012). In addition to this, depending on the micronutrients under investigation, the number of 24-HR interviews required can vary between three and 38 for energy and carotenoid intakes, respectively (Nelson 1989; Smith-Warner et al. 1997; unsheng et al. 2009). A high number of recalls can place a large burden on the participant and researcher, which may increase attrition and research costs (W. C. Willett 2013).

In the past decade, Food Frequency Questionnaires (FFQ) have become more commonly used in epidemiological research and an FFQ was chosen as the most suitable dietary assessment method for Study 1B, due to a number of reasons. Firstly, they are less burdensome for the research and participant. Secondly, they are easy and low cost to administer. Thirdly, they are easily adapted for use in various formats, including paper based and electronic. Their use in epidemiological studies has greatly increased over the past decade. Nevertheless, they are susceptible to measurement error, which means they can only provide a crude estimate of intake. This is useful when comparing relative values, but means absolute estimates of micronutrient intakes cannot be derived (W. Willett 2013).

One of the objectives of this study was to measure the intake of selected antioxidants and micronutrients involved in OCM. Therefore, a comprehensive assessment of all dietary intake was deemed unnecessary and time-consuming, leading to a risk of participant fatigue. At the time of designing the study, there were no validated FFQs to measure the intake of micronutrient methyl donors. Therefore, an FFQ was designed for the purpose of this study (Study 1A).

Having a pre-specified list of food and drink items mean FFQs are vulnerable to systematic bias, which cannot be overcome by increasing sample size. Therefore, when tailoring FFQs for study requirements, items should be commonly consumed by the target population, and high in concentration, or low in concentration but consumed in high quantities (W. C. Willett 2013). Therefore, it is recommended that the validity and reliability of any FFQ be tested prior to use in the target sample population. A description of the methods used to design and assess the construct validity of the FFQ are discussed in Chapter 4.

3.3.6.1 Portion sizes

The use of portion sizes varies between FFQ, some include no portion size and only measure frequency, others allow participants to describe typical portions and some provide 'average' portion sizes to food items. While a study conducted in Germany concluded that the majority of intra-individual variation in nutrient intakes was due to the frequency of consumption (Noethlings et al. 2003), a review of FFQ design characteristics reported that FFQs including portion sizes that were described by participants, had slightly higher correlations with the reference method compared to FFQs with 'average' portion sizes or no portion size information at all (Cade 2004). Therefore, in a bid to obtain the most accurate estimates of micronutrient intakes, portion size estimates were included in the design of the FFQ.

The use of portion sizes is also dependent upon what nutrients are being studied and the length of the FFQ. A systematic review reported that the energy adjusted correlation coefficient for nutrients typically increased with the inclusion of portion size estimates, with the exception of vitamin C; average correlation decreased from 0.80 to 0.60 (Molag et al. 2007). Therefore, for the purpose of this study, some food items also had portion size questions using a UK reference guide (Food Standards Agency 1988), whereas others had allocated standard portion sizes, particularly those that contributed to vitamin C intake.

3.3.7 Assessment of micronutrient intakes

As discussed previously, FFQs are not appropriate for obtaining measures of absolute micronutrient intake (Willett & Lenart 2013). However, relative estimates of micronutrient intakes still remain a useful measure when exploring relationships between exposures and outcomes. When calculating micronutrient intakes, a number of factors were considered to increase the validity of the estimates.

3.3.7.1 Choice of dietary composition database

A dietary composition database is necessary when converting raw FFQ data into micronutrient intakes. Variability between growing environments, product composition and the types of food available between different countries, means that the choice of database can have considerable implications

on the outcomes of the study. Concentrations of nutrients found in these databases only provide an average measure, particularly for items such as fruit, vegetables and meat, as the concentrations can vary depending on season, country of origin and growing conditions.

The aims of this study included estimating the relative intakes of folate, choline, betaine, vitamin C and carotenoids during pregnancy in a sample of women in England. Therefore, the most suitable database for this study was McCance and Widdowson's (R A McCance & Widdowson 2002), which contains the composition of almost 3,000 different foods available in the UK. Unfortunately, this database does not contain the concentrations of choline or betaine. Therefore, the USDA composition table was used instead (Patterson et al. 2008). The main limitation of using values from this database is that concentrations of choline and betaine may not accurately reflect those found in UK foods and this must be taken into consideration when interpreting the results in this thesis.

3.3.8 Dietary pattern analysis

Nutrient intakes provide valuable insight into the diet quality of populations. However, there are limitations to single nutrient analysis, which have been discussed in a number of review papers (Hu 2002; Newby & Tucker 2004). Briefly, the effect of a single nutrient may be too small to detect on its own, and nutrients are not consumed in isolation; therefore, the measurement of a single nutrient may actually be a proxy for the effect of a group of nutrients being consumed together. Dietary patterns provide a better reflection of 'true' dietary intake by focusing on how food and drinks are consumed together, which may be a more powerful indicator of diet quality (Hu 2002). It does not replace single nutrient methods, but compliments it, by providing a more holistic view of dietary intake.

However, there are number of limitations to dietary pattern analysis. Firstly, due to variability in FFQ items and analytic decisions, it is difficult to make direct comparisons between studies using different methods. Secondly, because of this variation, the findings do not easily translate into dietary recommendations. Thirdly, because many aspects of a diet are under investigation, the biological mechanisms underlying any relationships with health outcomes are not immediately obvious (Kant 2004). By measuring micronutrient intakes in addition to describing dietary patterns, some of these limitations may be minimised.

There are a number of methods that have been used previously to derive dietary patterns. The first is using dietary indices, an 'a priori' method, which fits the dietary data to current indices of diet quality (usually based on dietary guidelines). This method can be easier to understand, as data are compared to current standards of an optimal diet. However, guidelines may not be based on current scientific evidence or they may not exist in order to answer particular aims (Smith et al. 2011). Cluster analysis, Factor Analysis (FA) and Principle Component Analysis (PCA) are 'a posteriori' methods, deriving

patterns from the actual data, by searching for maximum variation between linear combinations of variables. While cluster analysis simplifies the data by essentially grouping individuals with similar dietary patterns into 'clusters', there is no differentiation between individuals who are in the same clusters, but have relatively diverse dietary habits. FA and PCA develop dietary patterns (or factors) that are typically consumed in conjunction or avoidance with each other, producing correlation coefficients with food and drink variables, allowing users to identify distinct characteristics of each dietary pattern. FA and PCA are similar methods and often considered the same method (Reedy et al. 2010). However, the distinction between FA and PCA lies in the reduction of dietary data into dietary patterns. FA reduces a set of dietary data to a smaller set by creating new dietary patterns, whereas, PCA reduces sets of attributes and the user selects those that account for the majority of variation in the data. PCA is the most commonly used method in nutritional epidemiology and in order to draw comparisons with the findings of this thesis and wider literature about dietary patterns during pregnancy, it was chosen as the most appropriate method of dietary patterns analysis for this study (Crozier et al. 2006).

3.4 Strategy 2

3.4.1 Overview

The sample of women who were recruited into Study 1A reported very low intakes of alcohol consumption during pregnancy (three women reported at least one episode of binge drinking and the remainder reported drinking less than 1-2 units, no more than once per week). A number of attempts were made to recruit women who were consuming heavier amounts of alcohol. Women attending specialist substance misuse antenatal clinics in Newcastle, Northumbria, Brighton and Gloucester were invited to participate by midwives. Only one participant was recruited using this strategy. Based on the data gathered in Study 1B, it was not possible to address the original aims of the study. Therefore, an additional data set was sought for this purpose.

Two other large birth cohort studies were considered: the Southampton Women's Study (SWS) and Screening for Pregnancy Endpoints study (SCOPE). The Avon Longitudinal Study of Parents and Children (ALSPAC) was selected as it had collected data on maternal dietary intake and alcohol consumption during pregnancy from a large number of women in the West of England. The ALSPAC cohort also included a wide range of birth and childhood outcomes, which made it possible to explore additional aims around birth and cognitive outcomes (Studies 2B & 2C).

3.4.2 Avon Longitudinal study of Parents and Children (ALSPAC)

ALSPAC is an on-going population-based study that has followed pregnant women and their children from eight weeks gestation to present day. The overall aim of ALSPAC was to determine how genotypes and their environment influence the health and development of parents and offspring

(Fraser et al. 2013). Women were eligible for inclusion if they resided in a pre-defined area within the county of Avon and their estimated delivery date was between 1st April 1991 and 31st December 1992 (Golding, Pembrey & Jones 2001). ALSPAC initially enrolled 14,541 pregnant women into the study. A total of 647 women were excluded, leaving 13,761 unique women enrolled, and a total of 14,062 live births (Fraser et al. 2013). The ALSPAC team have collected a variety of data from questionnaires, clinical measurements and biological samples, from mothers, partners and children, at different time points since 1991. At 7 years post-delivery, an additional 706 children were recruited; these were eligible cases that were unable to participate in the original study, making a total of 14,775 live births. The ALSPAC team invited a random 10% sample of participants to take part in the Children in Focus (CiF) groups, which included clinical assessments between 4 and 61 months of age. A variety of measurements were recorded, including, anthropometric, biological and cognitive. Specific strengths and limitations pertaining to the use of the ALSPAC dataset are discussed in relevant chapters.

3.4.3 General methodological considerations of strategy 2

A secondary analysis is a well-established methodology that involves analysing existing data to answer new research questions (Dunn et al. 2015). There are a number of general strengths and limitations to this methodology that will be discussed, along with specific methodological considerations relating to using the ALSPAC dataset.

A major advantage to conducting a secondary analysis is the avoidance of data collection and the many challenges that it presents. Using data from existing research studies is significantly less resource demanding; the time and financial resources required to recruit a research team, gain necessary approvals and complete data collection can be considerable. Another major advantage is the typically large sample size, which generally provide more precise estimates, reduce the likelihood of spurious results (Button et al. 2013) and produce findings that are more likely to be generalizable (Schlomer & Copp 2014). Fraser and colleagues (2013) compared the total ALSPAC sample population with those residing in Avon and across Great Britain, using data from the 1991 census. Overall, ALSPAC participants were broadly representative of Great Britain and Avon. However, they tended to be of higher socio-economic status, which is a common phenomenon reported in many large, epidemiological studies (Marmot 2010; Withall et al. 2011).

Secondary data analysis methods also allow many researchers to access data on hard to reach participants that may not have been possible otherwise. There has been a reduction in the proportion of women who report drinking large quantities of alcohol during pregnancy (Mc Andrew et al. 2012), which can make them a difficult population reach without considerable amounts of time or financial resources. ALSPAC recruited a wide variety of women, with 22% reporting heavy (1+ drink/day) or binge patterns (4+ units in one day) of drinking during pregnancy. Using pre-existing, de-identified

data also avoids duplication of data collection procedures, minimizing the burden to participants (Dunn et al. 2015).

While the advantages of conducting a secondary analysis using ALSPAC data are evident, there are also many challenges that are faced when using this methodological approach. The most notable disadvantage is that the data was originally collected with a different purpose in mind. Researchers have no control over important design aspect of the data collection, such as the choice of variables measured; the tools used to measure them; the sample population chosen; and the time period for data collection. Depending on the research questions under investigation, these factors can contribute to unreliable estimates and inappropriate adjustment for confounders, increasing the risk of spurious results. In the case of ALSPAC, these factors provide a number of challenges. Firstly, the validity or reproducibility of the alcohol assessment tool and FFQ were not tested before use. As discussed in section 3.3.6, even subtle changes in design can alter performance (Willett & Lenart 2013). Secondly, the alcohol and dietary variables were not contemporaneously measured, which can pose challenges when investigating potential interactions between two exposures. While current evidence suggests that alcohol consumption levels are inconsistent over the course of pregnancy (O'Keeffe et al. 2015), dietary intakes appear to be more constant, with research indicating that maternal dietary patterns differ very little from before pregnancy (Crozier et al. 2011). Thirdly, the most commonly used definition of binge drinking in the UK for women is six or more units of alcohol during one occasion, and this definition is used to describe 'binge drinkers' in Study 1. However, in ALSPAC, women were asked the number of days they have consumed four or more units across one day. There may be great variation between the drinking patterns of women defined as 'binge drinkers' in Study 2.

Another consideration of conducting a secondary analysis can be the lag time between data collection and analysis. This can often render data out-dated and irrelevant (Schlomer & Copp 2014; Dunn et al. 2015). The alcohol and dietary exposure data in ALSPAC were collected more than two decade ago, which means the reported exposure patterns may not reflect present consumption. However, the longitudinal study design has provided a sufficient follow up period, facilitating the exploration of childhood outcomes. This was deemed another major advantage to using the ALSPAC dataset and additional aims and objectives were developed as a result.

3.5 Summary and conclusions

This chapter has described the methodological considerations of both primary and secondary analysis methodologies to answer the research questions of this thesis. Strategy 1 used a purposefully designed FFQ, validated alcohol assessment tools and measured exposures contemporaneously. However, significant recruitment difficulties were faced which resulted in a small, homogenous sample population. In contrast, Strategy 2 involved a large, generalizable sample, with a wide variety of data, including outcome measures into childhood. However, this gain was met with a lack of control in

design, which resulted in a number of weaknesses relating to measurement tools and time periods of data collection. Overall, this chapter has emphasized the strengths and limitations of both research strategies and ultimately, the trade-offs that exist when conducting primary research or a secondary analysis of an existing dataset.

Chapter 4. Development and Validation of a Food Frequency Questionnaire for use in a sample of women of reproductive age in the UK (Study 1A)

Parts of this chapter have been published as a research article in a peer reviewed journal: Coathup, V., Wheeler, S. & Smith, L., 2015. A method comparison of a food frequency questionnaire to measure folate, choline, betaine, vitamin C and carotenoids with 24-h dietary recalls in women of reproductive age. Eur J Clin Nutr. (Appendix B)

4.1 Introduction

Chapter 2 discussed the prevalence of antenatal alcohol consumption in the UK, highlighting the fact that many women still continue to drink alcohol during pregnancy. Because of the lag time between conception and pregnancy recognition, it is possible that the true prevalence of alcohol-exposed pregnancies is much higher than reported in these studies; drinking habits may not change until women are aware of their pregnancy.

Evidence from research conducted in non-pregnant populations indicates that potentially harmful patterns of alcohol consumption and dietary intake are related, with individuals reporting binge drinking to have diets characterised by low intakes of fresh fruit and vegetables and high intakes of red and processed meats. This raises important questions about whether these relationships exist in pregnant populations, as research using animal models of Fetal Alcohol Spectrum Disorders (FASD) suggest that maternal alcohol consumption in the presence of inadequate intakes of micronutrients involved in One Carbon Metabolism (OCM) and antioxidants, may increase the risk of fetal growth restriction, and cognitive and behavioural deficits associated with antenatal alcohol exposure (Mitchell et al. 1999; Heaton et al. 2000; Cohen-kerem & Koren 2003; Wang et al. 2009; Thomas et al. 2010). Therefore, measuring alcohol and dietary exposures during pregnancy and understanding how they are related may highlight populations of women at risk and also provide targets for nutritional interventions

Chapter 3 highlighted the methodological challenges associated with measuring alcohol and dietary intakes using self-reported estimates during pregnancy. In particular, it highlighted the persistent conflict between accuracy and feasibility in nutritional epidemiology. Prospective dietary assessment methods usually provide more accurate estimates of exposure, however, they can be costly and burdensome to both participants and researchers. Food Frequency Questionnaires (FFQ) contain a pre-defined list of food and drink items, and the participant reports how frequently they have consumed each of these items over a specified period. They have become frequently used in epidemiological research in the past decade, due to their low cost and ease of distribution; they can be self-completed relatively quickly and in the privacy of the participant's home. However, there is a

trade-off and the convenience of FFQs is also coupled with crude estimates of intake that are prone to recall and social-desirability bias.

The choice and presentation of food and drink items can have significant effects on the estimates of dietary intake; even subtle changes can result in systematic error that sample size cannot overcome. To minimise the risk of measurement error, the pre-defined list of food and drink items means FFQs should be tailored and validated before use (W. C. Willett 2013).

At the time of Study 1B, there was no FFQ available to estimate mean intakes of folate, choline, betaine, vitamin C and carotenoids in populations of pregnant women, using an FFQ developed for a different purpose may have resulted in spurious findings. Therefore, Study 1A was designed to develop an FFQ specifically for the purpose of Study 1B. This chapter will outline the design, development and validation of the dietary assessment tool for use in Study 1B.

4.2 Aims and objectives

The overall aim of this study was to determine the concurrent validity of a purposefully designed FFQ to estimate usual dietary intake of micronutrient methyl donors (folate, choline, and betaine) and antioxidants (vitamin C and carotenoids: α-carotene, β-carotene, lutein, lycopene and β-cryptoxanthin) in women of reproductive age in the UK, by comparing data with that from multiple-pass 24-hour dietary recall interviews and plasma folate concentrations.

Specific objectives of this study were:

- To design an FFQ comprising of commonly consumed food and drink items contributing to intakes of folate, choline, betaine, vitamin C and carotenoids (α-carotene, β-carotene, lutein, lycopene and β-cryptoxanthin).
- To measure concentrations of plasma folate in a sample of women of reproductive age.
- To measure mean daily intakes of folate, choline, betaine, vitamin C and carotenoids (α-carotene, β-carotene, lutein, lycopene and β-cryptoxanthin) using the FFQ in women of reproductive age.
- To measure mean daily intakes of folate, choline, betaine, vitamin C and carotenoids (α-carotene, β-carotene, lutein, lycopene and β-cryptoxanthin) using 24-hour dietary recalls in women of reproductive age.
- To compare estimates from all three measures to determine the concurrent validity of the FFQ in women of reproductive age.

4.3 Methods

4.3.1 Sample population

The choice of sample population to assess the validity of an FFQ can also influence the results of a study due to behavioural differences between populations. To minimise this bias, a random sample of the target population would ideally be chosen i.e. a random sample of pregnant women across the UK. However, in the context of this study, it was not deemed feasible. Women were required to fast overnight before providing a sample of plasma and it was considered unethical to ask pregnant women to do this. A convenience sample comprising of 67 British women of reproductive age (18-40) was recruited online, using social media adverts and posters in local university departments, hospital waiting rooms and shop windows. Adverts included the contact details of the researcher. Women were invited to email the researcher if they were interested in participating. The researcher then responded and sent the participant information sheet and consent form. After 24 hours, the researcher contacted the potential participant to discuss the project and answer any questions. If the women still agreed to take part, they would arrange a day and time that the woman could come into the lab to participate in the project. The study aimed to recruit at least 62 participants, which was calculated to provide 80% power to detect an expected correlation of r = 0.35 between the mean nutrient intakes estimated by the two dietary assessment methods with significance at the 5% level (Altman 1991). Whilst 0.35 is a moderate correlation coefficient, it is typical for FFQs (Kabagambe et al. 2001; Pufulete et al. 2002; McNaughton et al. 2005).

Women were eligible for inclusion into the study if they met the following criteria:

- Aged between 18 and 40 years of age
- At the time of data collection, were not:
    - Pregnant
    - Breastfeeding
    - Suffering with gastrointestinal or metabolic conditions
    - Taking medication that interfered with folate metabolism.

Women visited the study site once for anthropometric measurements; they completed a background questionnaire, which included questions about supplement use, then provided a fasting capillary blood sample, before completing the FFQ and returning it to the research team. This was followed by the first 24-hour dietary recall interview.

4.3.2 Food Frequency Questionnaire

A previously validated FFQ for the assessment of folate intake (Pufulete et al. 2002) was updated using the most recent UK dietary survey data (Bates et al. 2010) on folate intake and modified to also measure usual intake of betaine, choline, vitamin C and common dietary carotenoids (α-carotene, β-

carotene, lutein, lycopene and β-cryptoxanthin). Food and drink items were eligible for inclusion if they were either:

1) Commonly consumed foods that greatly contributed to the dietary intake of selected micronutrients and antioxidants (Nelson et al. 2007; Bidulescu et al. 2009; Xu et al. 2009; Bates et al. 2011).
2) Rich food sources of selected micronutrients and antioxidants (Robert Alexanda McCance & Widdowson 2002; Patterson et al. 2008).

The final FFQ included 113 food and drink items grouped into 32 categories. The FFQ was designed to measure recent intake over approximately a three-month period, so it could be used to measure dietary intake in pregnant women. An abbreviated portion size booklet was also modified from a published food atlas (Nelson et al. 1997). Typical portion size estimates for items without food photographs were taken from an existing UK reference (Food Standards Agency 1988).

4.3.2.1 Frequency responses

The number of possible responses for frequency of intake varies between FFQs and can greatly affect the accuracy of data collected. Studies comparing broad response categories and detailed assessment methods found the two methods had a low correlation; very broad response categories resulted in a loss of discrimination between participants (W. C. Willett 2013). However, open-ended responses do not yield more precise intake estimates. A qualitative study evaluating methods to improve FFQs suggested that multiple choice responses with a higher number of categories as frequency increases, allowing more detail to be captured, are the most effective way of collecting frequency data (Subar et al. 1995). Therefore, response categories included 'once per month', 'once per fortnight' and 'number of days per week' (days ranged from 1 to 7).

4.3.3 Reference method

There is currently no gold standard of dietary assessment, so estimates must be compared to that of a superior, but still imperfect, method. As previously discussed in Chapter 3, weighed food diaries are considered the most accurate method to estimate nutrient intake. However, participant motivation, literacy and available funding can make prospective dietary assessment methods difficult to conduct; women need to be trained to use food diaries, which imposes a substantial burden onto the participant for data collection. The primary alternative to food diaries are 24-hour dietary recall interviews (Willett et al. 1985).

To measure the intake of certain micronutrients, many recalls are required, as one 24-HR interview is not sufficient to capture usual intake due to day-to-day variation in dietary habits. The number of recalls chosen as the reference method in validation studies of FFQs varies from study-to-study, with the majority choosing between 2 and 10 (Resnicow et al. 2000; Johansson et al. 2007; Mouratidou et

al. 2007; Segovia-Siapco et al. 2007; Haftenberger et al. 2010; Iqbal et al. 2014; Barbieri et al. 2015). The average of three multiple-pass 24-hour dietary recall (24-HR) interviews was the reference method (Thompson & Subar 2008).

Interviews were conducted on three, non-consecutive days over a two-week period (two weekdays and one weekend day) in order to capture intra-individual variability in dietary patterns (Bingham et al. 1994). A trained nutritionist conducted all three interviews; the first was conducted face-to-face, after the completion of the FFQ. The following two interviews were conducted by phone over the following 14 day period (Yanek et al. 2000). Multiple-pass recall interviews were conducted. Initially, women were asked to provide a quick overview of their day, giving the interviewer details of the previous day, including foods eaten, the location and time they were consumed and a brief overview of activities. The next phase involved the interviewer prompting for more details on foods eaten, such as brands and portion sizes consumed, and using the activities of the day to prompt for any other items that may have been forgotten. The final stage involved the interviewer reading back the dietary recall details to the participant, in a bid to capture any final mistakes or missing items. This format provides numerous cues and prompts to minimise recall bias (Moshfegh et al. 2008). Women with one or more days' worth of missing 24-hour dietary recall data were excluded from the present analysis.

Women reported the mean frequency of food and drink items consumed and then estimated portion sizes using a photographic booklet (Nelson et al. 1997). If a suitable photo was not included, the weight was estimated from participant's descriptions or using average portion sizes (Food Standards Agency 1988). Mean daily intakes of folate, vitamin C and carotenoids were calculated using UK dietary composition tables (Robert Alexanda McCance & Widdowson 2002). Mean daily intakes of choline and betaine were calculated using US dietary composition tables (Patterson et al. 2008), as there is currently no UK or European data on the concentration of choline or betaine in foods. Mean daily intakes were calculated using the following formula:

Concentration per g x (portion size (g) x weekly frequency) / 7

4.3.4 Anthropometric measurements

Height was measured using a mobile stadiometer (Seca 217, Seca Inc. Birmingham, UK), and Body Mass Index (BMI) and body composition (fat mass) were measured using a multi-frequency segmental body composition analyser (Tanita BC-418MA, Tanita Inc. Amsterdam, The Netherlands).

4.3.5 Plasma folate

One limitation of using 24-HRs as the reference method is that the errors cannot be considered independent; both methods (FFQ and 24-HR) rely on retrospective recall of dietary information, and it is likely that the errors may be correlated in some way, over-estimating the validity between the two

tools (Ocke & Kaaks 1997). Plasma folate concentrations were analysed, providing an additional estimate of folate intake with uncorrelated errors (Yokota et al. 2010). The method of triads can then be used to estimate the correlation between each measure of folate intake and the true intake (Ocke & Kaaks 1997).

Overnight fasting capillary blood samples were collected from women upon arrival. Samples were collected in microtainers pre-treated with EDTA and immediately centrifuged at 4000rpm for 10 minutes. 250µl of plasma was collected and stored at -70°C until analysed. Plasma folate concentration was analysed in a single batch using an immunoassay kit (Folate III, Roche Diagnostics GmbH, Mannheim, Germany) on a COBAS e411 analyser (Roche Diagnostics GmbH, Mannheim, Germany). The minimum detectable amount was 1.45nmol/l and intra-assay coefficient of variation was 5.8%. Haemolysed samples were excluded from analysis due to high content of folate in red blood cells. Due to the high cost of analysing additional samples, it was beyond the remit of the study to analyse for plasma vitamin C, carotenoids, choline and betaine.

4.3.6 Statistical analysis

Non-normally distributed variables were log transformed for analysis, but medians and inter-quartile ranges (IQRs) are presented in tables for ease of interpretation.

The validity was assessed in a number of different ways. Validity refers to the accuracy of an estimate; the extent to which the FFQ measures what it has been designed to measure. In this case, it was the mean daily intake of folate, choline, betaine, vitamin C and carotenoids in women of reproductive age. There are a number of different types of validity that can be assessed, including face, content, construct, criterion, concurrent and predictive (Koh & Owen 2000). The tests used in this study were assessing the concurrent validity of the FFQ. The performance of the FFQ was being assessed in relation to intakes of folate, choline, betaine, vitamin C and carotenoids measured using 24-hour dietary recall interviews from the same time period.

Reliability refers to the precision of a tool; the degree to which the FFQ produces consistent measures when used by the same participant (Elia & Stratton 2011). However, the reliability of the FFQ was not estimated in this study. The lack of this data means it was not possible to assess how well the FFQ repeatedly measures nutrient intake. However, the reliability of the original FFQ was 0.72, and it may be reasonable to expect a similar finding (Pufulete et al. 2002).

Pearson's correlation coefficients were used to assess the strength of the relationship between mean daily micronutrient intakes (µg or mg/day) between the FFQ and 24-HR. To explore the role of dietary supplements, correlation coefficients were calculated with and without dietary supplement intakes.

The following definitions were used to describe the strength of the correlation (Dancey & Reidy 2004):

- Very strong if >0.80
- Strong if 0.60 – 0.79
- Moderate if 0.40 – 0.59
- Weak if 0.20 – 0.39
- Negligible if >0 - <0.20

As discussed in Chapter 3, individual's dietary intake can vary greatly from day-to-day. This means there is usually significant within-subject variation, which can attenuate correlations. To adjust for this, Pearson's correlation coefficients were deattenuated using the following formula (Kabagambe et al. 2001):

$Cr = r \surd 1 + (\lambda x / nx))$

(Cr = the corrected correlation coefficient, r = crude correlation coefficient, λx = ratio of intra to inter subject variation, and nx = number of dietary records for each subject)

Bland-Altman (BA) plots provide a visual representation of agreement between two methods and have a number of advantages over correlation coefficients. Firstly, the plots are not vulnerable to extreme values. Secondly, they can reveal systematic or random bias acting on the measurement tool (Van Stralen et al. 2008). Agreement is assessed by plotting the mean values of the FFQ and 24-HR against the differences between the two measures. The Limits Of Agreement (LOA) are calculated using the mean difference ± 1.96 SD of the differences and 95% of the estimates should lie between these parameters. Agreement is assessed based on the mean difference, the width of the LOA and how many observations lie beyond them, and whether there is any evidence of systematic bias (Bland & Altman 1986). When the difference between the two measurements was not normally distributed, data were log transformed and plotted (Sedgwick 2013).

To evaluate the ranking ability of the FFQ compared with the 24-HR, women were classified into quartiles based on their mean daily nutrient intakes. Quartile classifications were compared by calculating the percentage of women classified to within one quartile and the weighted Kappa coefficient. The following values were used to evaluate agreement between the dietary methods (Viera & Garrett 2005):

- Very good if >0.80
- Good if 0.61-0.80
- Moderate if 0.41 - 0.60
- Fair if 0.21- 0.40
- Poor if <0.20

Both the FFQ and 24-HR rely on retrospective recall of dietary information, and it is likely that the errors may be correlated in some way, over-estimating the validity between the two tools (Ocke & Kaaks 1997). Therefore, the method of triads was used to help overcome measurement errors for the

assessment of folate. The method of triads is based on assumptions that any random errors in the dietary assessment methods are uncorrelated and that there is positive linear correlation between the estimates of dietary assessment methods and the true intake values (Ocke & Kaaks 1997; Andersen et al. 2002). Pearson's correlation coefficients between each of the three dietary assessment methods for folate intake, including supplements, were used to calculate a Validity Coefficient (VC), and by taking 1,000 bootstrap samples using random sampling with replacement (n=64), 95% Confidence Intervals (CI) for the VC were estimated (Ocke & Kaaks 1997).

Analyses were performed using SPSS statistical software package (Version 19; SPSS Inc. Chicago, IL, USA).

4.3.7 Ethical approval

Full ethical approval was provided by Oxford Brookes University Ethics Committee (UREC) in May 2012

4.4 Results

4.4.1 Socio-demographic characteristics

Characteristic of the sample population are presented in Table 4.1. A total of 67 women were recruited. Three were excluded from the present analysis due to missing dietary data. The mean age of women in the sample was 26 years and the majority were white British students or graduates. Women had relatively low BMIs, regularly took dietary supplements and very few reported smoking.

Table 4.1 Socio-demographic characteristics of sample population (n=64)

Age (years) (mean ± sd)	26±4
BMI (mean ± sd)	22±3
IMD score (mean ± sd)	16±9
Employed (%)	
Full time	41
Part time	5
Student	54
Ethnicity (%)	
White	84
Mixed: White/Black African	3
Mixed: White/Black Caribbean	5
Mixed other	2
Indian	2
Chinese	3
Smoking status (%)	
Current	3
Former	8
Occasional	22
Never	68
Supplements (%)	
Folic acid	21
Vitamin C	21
Education (%)	
GCSE/A-Levels	3
Bachelor's degree	34
Postgraduate degree	23

SD = Standard deviation
BMI =Body Mass Index
IMD = Index of Multiple Deprivation

4.4.2 Micronutrient intakes

The median intakes estimated by the FFQ were all higher compared with the 24-HR (Table 4.2), with the largest discrepancies observed for α-carotene, β-carotene and lycopene. The mean crude and deattenuated correlation coefficients between the FFQ and 24-HR for all nutrients are presented in Table 4.2. Strong correlations were observed for folate and choline, while moderate correlations were observed for vitamin C and lycopene. Weaker correlations were observed for betaine, α-carotene, β-carotene, lutein and β-cryptoxanthin. When values for dietary supplements were removed from the nutrient intake calculations, the crude and deattenuated correlation coefficients for folate and vitamin C decreased.

Table 4.2 Median daily intake of micronutrients and crude and deattenuated Pearson's correlation coefficients (FFQ and 24-HR) (n=64)

Dietary Factor	FFQ		24-hour recalls		Pearson correlation	Deattenuated correlation coefficients
	Median	IQR	Median	IQR	coefficients (r)	
Folate (µg)						
Diet + supplements	369	292 – 479	260	216 – 348	0.74**	0.80**
Diet only	347	278 – 419	241	195 – 307	0.39**	0.47**
Choline (mg)	285	221 – 355	255	201 – 331	0.60**	0.68**
Betaine (mg)	130	91 – 164	130	81 – 202	0.38**	0.39**
Vitamin C (mg)^						
Diet + supplements	171	114 – 229	120	72 – 169	0.49**	0.50**
Diet only	167	144 – 219	106	69 – 166	0.35**	0.37**
α-carotene (µg)^	1620	824 – 3329	128	43 – 1050	0.21	0.26
β-carotene (µg)^	6115	3478 – 25235	2152	1126– 4195	0.25*	0.29*
Lycopene (µg)^	6144	2526 – 10540	851	201 – 4034	0.39**	0.43**
Lutein (µg)^	885	509 – 1847	293	150 – 512	0.30*	0.38*
β-cryptoxanthin (µg)^	152	82 – 355	94	0 – 195	0.25*	0.31*
Total carotenoids					0.28	0.33

^Log transformed values

* p=<0.05

**p=<0.001

FFQ = Food Frequency Questionnaire; 24-HR = 24-hour dietary recalls

Mean differences and LOA are presented in Table 4.3. The BA plots for folate (Figure 4.1), betaine (Figure 4.2), choline (Figure 4.3), vitamin C (Figure 4.4) and carotenoids (Figures 4.5-4.8) indicated that the FFQ estimated higher nutrient intakes compared to the 24-HR. In contrast, the plot for betaine (Figure 4.2) indicated the FFQ estimated slightly lower intakes compared to the 24-HRs. Approximately 95% of observations were plotted within the LOA for all nutrients. There was evidence of systematic bias in the plot for α-carotene; the FFQ over-estimated lower intakes and under-estimated higher intakes compared to the 24-HR. When BA plots for folate and vitamin C were plotted with and without supplements, the shape didn't differ and the mean differences were similar (Coathup et al. 2015).

Table 4.3 Mean difference and limits of agreement between estimates using FFQ and 24-HR

Dietary factor	Mean difference (95% CI)	LOA		% >2SD LOA
		-1.96 SD	+1.96 SD	
Folate (µg)	97 (72, 121)	-95	289	4
Choline (mg)	15 (5, 26)	-151	182	3
Betaine (mg)	-18 (-20, -8)	-176	141	6
Vitamin C (mg)	52 (29, 75)	-131	235	4
α-carotene (µg) ◊	9 (5, 15)	0.1	528.5	3
β-carotene (µg) ◊	5 (4, 7)	0.2	97.7	2
Lycopene (µg) ◊	3 (2, 6)	0.0	212.7	3
Lutein (µg) ◊	3 (2, 5)	0.2	45.6	3
β-cryptoxanthin (µg) ◊	2 (1, 3)	0.1	78.3	4

◊Antilog values

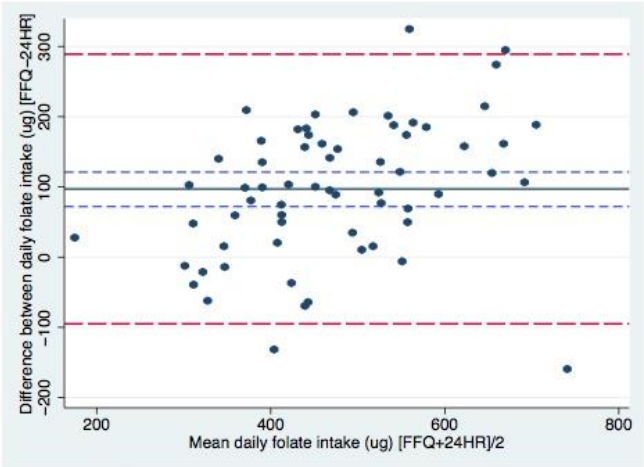

Figure 4.1 Bland-Altman plot of the difference between folate intake estimated by the FFQ and 24-hour dietary recalls (red lines = LOA; blue lines = 95% CI; grey line = Mean)

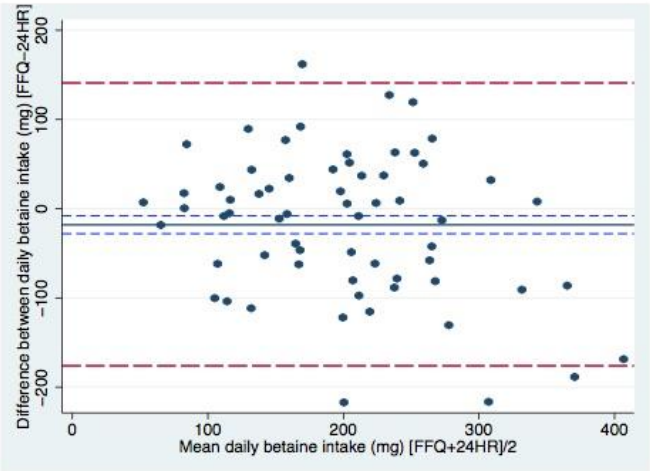

Figure 4.2 Bland-Altman plot of the difference between betaine intake estimated by the FFQ and 24-hour dietary recalls (red lines = LOA; blue lines = 95% CI; grey line = Mean)

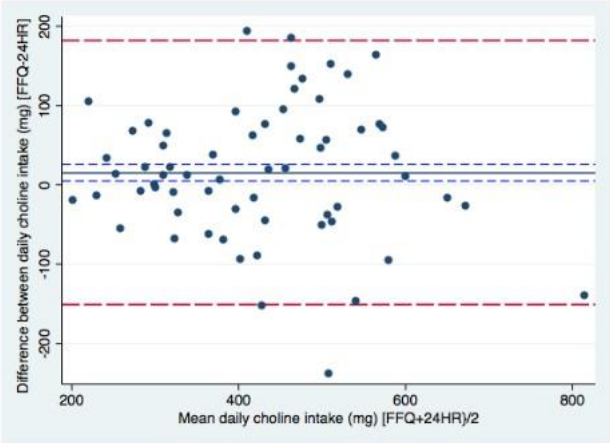

Figure 4.3 Bland-Altman plot of the difference between choline intake estimated by the FFQ and 24-hour dietary recalls (red lines = LOA; blue lines = 95% CI; grey line = Mean)

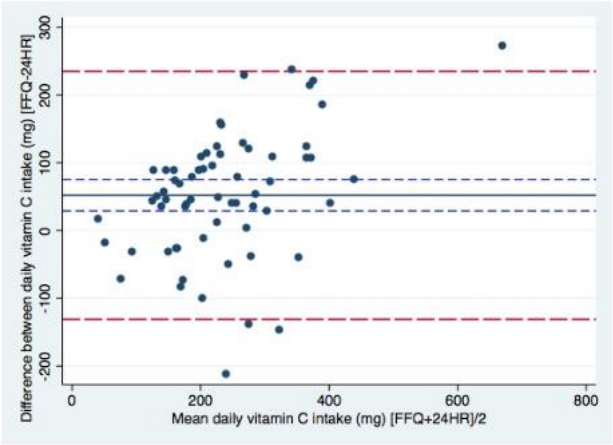

Figure 4.4 Bland-Altman plot of the difference between vitamin C intake estimated by the FFQ and 24-hour dietary recalls (red lines = LOA; blue lines = 95% CI; grey line = Mean)

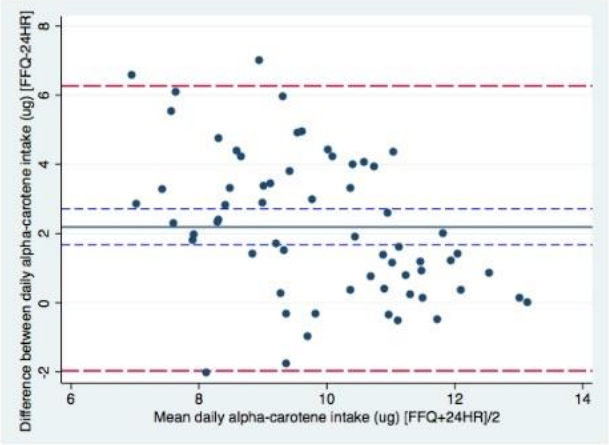

Figure 4.5 Bland-Altman plot of the difference between alpha-carotene intake estimated by the FFQ and 24-hour dietary recalls (red lines = LOA; blue lines = 95% CI; grey line = Mean)

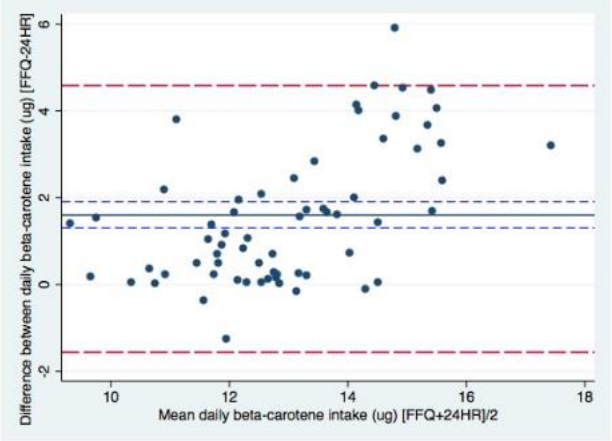

Figure 4.6 Bland-Altman plot of the difference between beta-carotene intake estimated by the FFQ and 24-hour dietary recalls (red lines = LOA; blue lines = 95% CI; grey line = Mean)

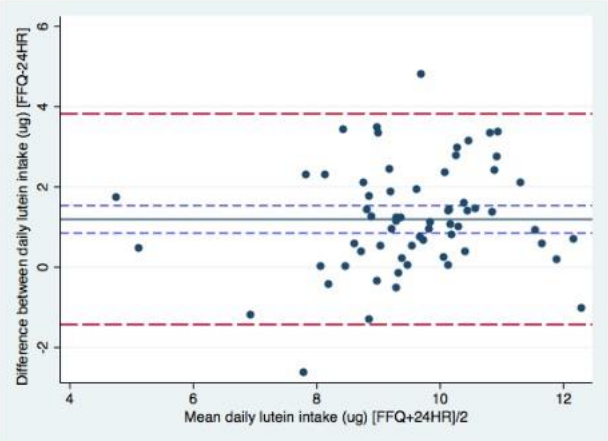

Figure 4.7 Bland-Altman plot of the difference between lutein intake estimated by the FFQ and 24-hour dietary recalls (red lines = LOA; blue lines = 95% CI; grey line = Mean)

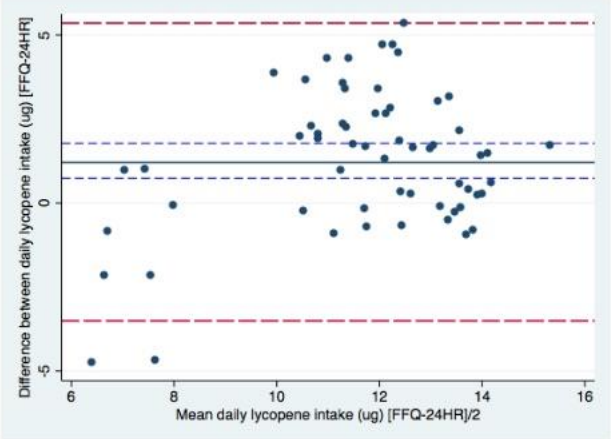

Figure 4.8 Bland-Altman plot of the difference between lycopene intake estimated by the FFQ and 24-hour dietary recalls (red lines = LOA; blue lines = 95% CI; grey line = Mean)

Kappa agreement between the FFQ and 24-HR was strong for folate, choline and betaine, however, agreement for vitamin C and carotenoids was poor (Table 4.4). The mean percentage agreement between the two methods was relatively high when looking at those classified to within one quartile, with a mean agreement of 73%. Gross misclassification was highest for beta-carotene and lutein.

Table 4.4 Weighted Kappa agreement between estimates of nutrient intake measured by the FFQ and 24-HR, for participant classification into quartiles of each nutrient or dietary factor (n=64)

Dietary factor	Within one quartile (%)	Gross misclassification (%)	Weighted Kappa statistic
Folate	86	0	0.75
Choline	84	2	0.72
Betaine	70	5	0.64
Vitamin C	73	8	0.14
α-carotene	72	8	0.16
β-carotene	63	11	0.06
Lycopene	70	8	0.11
Lutein	69	11	0.11
β-cryptoxanthin	78	9	0.16

Gross misclassification = extreme opposite quartile

Three samples of blood were haemolysed during collection (breakage of red blood cells) and excluded from the analysis. Pearson's correlation coefficients and validity coefficients between dietary assessment methods and plasma biomarkers for folate intake are presented in Table 4.5. The mean plasma folate concentration was 21.48nmol/l (SD=9.45nmol/l). The Pearson's correlation coefficients between the FFQ and plasma folate biomarker were weak (r=0.32), and the highest correlation observed was between the FFQ and 24-HRs (r=0.80). The VCs for the FFQ, 24-HR and plasma biomarkers for folate were 0.77, 1.0 and 0.43, respectively.

The VC for the intake of folate between the 24-HR and biomarker was over 1. This is termed a 'Heywood case' and is indicative of either one correlation being much lower than the others or it suggests a violation of the method of triads.

Table 4.5 Pearson's correlation coefficients and corresponding validity coefficients, calculated using the method of triads

Dietary Factor	Correlation coefficients			Validity Coefficients		
	QR	RB	QB	FFQ QT (95% CI)	24-hour recalls RT (95% CI)	Plasma biomarker MT (95% CI)
Folate						
Diet + supplements	0.80	0.41	0.32	0.78(0.36 - 0.89)	>1*(1.80 - 0.96)	0.41(0.1 - 0.63)

QR is Pearson's correlation coefficient between FFQ and 24-HR; RB is Pearson's correlation coefficient between 24-HR and plasma biomarker; QB is Pearson's correlation coefficient between FFQ and plasma biomarker

QT is the FFQ validity coefficient; RT is the 24-HR validity coefficient; MT is the Plasma biomarker validity coefficient

*A VC of >1 is a Heywood case. Due to one correlation coefficient being much lower than the other two, or a violation of the method of triads

4.5 Discussion

This chapter presents the results arising from the development and validation of the first FFQ to measure usual intake of folate, choline, betaine, vitamin C and carotenoids against 24-hour dietary recall interviews and plasma folate biomarker data.

The crude and deattenuated correlation between the FFQ and 24-HR for folate was stronger than reported for another UK study in a similar setting with a similar sample population (r=0.31) (Pufulete et al. 2002). However, this may be due to the use of dietary supplements by the women, as when FA supplements were removed from nutrient calculations in a sensitivity analysis, the correlation decreased to a similar value (0.39). This trend has been reported in other studies (Munger et al. 1992; Messerer et al. 2004) and may be a result of greater distinction between very high and low intakes of nutrients subsequently increasing correlation. While this is encouraging, the Bland-Altman plots indicated that the agreement between the FFQ and 24-HRs was not strong, with the FFQ overestimating folate intake by a mean of 100μg/day. This was much higher compared to other studies, with similar sample populations (Mouratidou et al. 2007).

The FFQ VC was relatively high (0.78), and comparable to studies also conducted in women of reproductive age. Studies conducted in the UK (Pufulete et al. 2002) and the Netherlands (Verkleij-Hagoort et al. 2007) reported VCs of 0.72 and 0.94, respectively. However, due to the small sample size, findings must be interpreted with caution and 0.78 should be considered as the upper limit of the 'true' VC (Ocke & Kaaks 1997). The 24-HR VC was >1, which is known as a 'Heywood case'. This can occur due to one correlation coefficient being much lower than the other two or it can indicate a violation of the method of triads, such as the independence of errors (Ocke & Kaaks 1997). The correlation coefficient for the plasma biomarker was much lower than the others and because the FFQ and 24-HR are both retrospective recall methods, it is possible that the errors are not independent. Therefore, the cause of the Heywood case is not clear. Biomarkers are not only influenced by dietary intake, but also genetic and metabolic factors and lower correlations between plasma folate concentrations and other dietary assessment methods has been observed in studies with similar sample populations (Verkleij-Hagoort et al. 2007).

Another possible reason for this may be because the FFQ measured intake over a three-month period, whereas plasma folate concentration provides a better reflection of very recent folate intake. Erythrocyte folate provides a better long-term indicator of folate intake (Piyathilake et al. 2007); however, it was not feasible to collect and store whole blood to analyse erythrocyte folate.

The crude and deattenuated correlation coefficients observed for choline and betaine were moderate, and comparable with another study with a similar sample population in Belgium which reported crude correlation coefficients of 0.34 and 0.39 for choline and betaine, respectively (Pauwels et al. 2014). The Bland-Altman plot and percentage agreement for choline and betaine indicate that there is good agreement between the two methods, with mean differences of only 15mg/day and 18mg/day, respectively. Daily recommendations for the intake of choline and betaine are currently unavailable, making it difficult to fully interpret the clinical significance of these results.

The crude and deattenuated correlation coefficients observed for vitamin C were stronger compared to results published in other studies assessing the validity of FFQs. A study conducted in Brazil (n=93) reported crude and deattenuated correlation coefficients of 0.23 and 0.39, respectively (Cardoso et al. 2010). However, when supplements were removed from nutrient calculations, the same trend was observed as for folate; the crude and deattenuated correlation coefficients decreased (Cardoso et al. 2010). The BA plots also revealed that the FFQ reported higher mean estimates of vitamin C intake compared to the 24-HR, with a difference of approximately 52mg/day.

The agreement between the FFQ and 24-HR was fairly weak, with BA plots suggesting systematic bias was present when measuring α-carotene, β-carotene and lycopene. However, the correlation coefficients appeared to be similar to other published studies. Two studies conducted in Australia and Costa Rica reported slightly stronger correlations between their FFQ and 24-HRs for β-carotene, which may be due to including men in their sample. A study by Nelson and colleagues indicated that there is more day-to-day variation in women's diets compared to men's (Nelson 1989). The weaker agreement between the FFQ and 24-HR for carotenoids may be a result of the high intra-individual intake that is often observed for carotenoids; they exist in few foods, but in large concentrations. Conducting 38 24-hour dietary recall interviews is recommended to capture usual daily intake of dietary carotenoids (Nelson 1989). This would require large amounts of time and money, and was not feasible in the context of this study.

4.5.1 Strengths and limitations

One strength included unscheduled appointments for the follow up recall interviews to minimise the risk that women may alter their diet on purpose. The FFQ has also been developed to measure the intake of specific groups of nutrients and antioxidants, reducing completion time, potentially reducing participant burden. Finally, validity of the FFQ was assessed using Bland-Altman plots, weighted Kappa and the method of triads, providing a more comprehensive analysis of this purposely designed FFQ.

This study was conducted with restricted resources, which has resulted in a number of limitations. Firstly, the small and homogenous sample population, comprised of mainly young, well-educated,

white British women, despite efforts to recruit from the wider community in Oxford, UK. This is a common phenomenon within health research; many sample populations often consist of white, middle-class women, even when they are specifically targeted at low-income populations (Marmot 2010; Withall et al. 2011). The lack of reliability data means it was not possible to assess how well the FFQ repeatedly measures nutrient intake. However, the reliability of the original FFQ was 0.72 and it may be reasonable to expect a similar finding (Pufulete et al. 2002). Furthermore, although three 24-HR interviews have been used in other validation studies (Resnicow et al. 2000; Fayet et al. 2011), increasing the number of interviews is likely to improve assessment of agreement between the dietary assessment methods. Finally, the lack of biomarker data for other nutrients meant VCs could only be calculated for folate intake.

4.6 Summary and conclusions

The findings from Study 1A have highlighted a number of issues related to the design and performance of the FFQ. Firstly, the results indicated that the FFQ was able to adequately rank women according to categories of their nutrient intake. Secondly, the correlations between the FFQ and 24-HRs were strong for folate and choline, moderate for vitamin C, and weak for betaine and total carotenoids, and were comparable with wider literature. Thirdly, the VC from the method of triads also produced a strong validity coefficient for folate intake measured by the FFQ. However, the Bland-Altman plots did highlight considerable variation between the estimates and indicated that the FFQ overestimated the intakes of folate, choline, vitamin C and carotenoids in comparison to the reference method.

The results from all analyses emphasize the challenges when measuring carotenoids; they are present in high quantities, in few foods. Therefore, estimates in study 1B should be interpreted with some caution. While the findings from Study 1A indicate that this FFQ is suitable for use in Study 1B, the limitations of the small, homogenous sample mean further research should be conducted before this FFQ is used in studies specifically designed to target minority populations. Overall, this FFQ can be considered a useful instrument for measuring the relative intake of folate, choline, betaine, vitamin C and carotenoids in women of reproductive age. The next chapter will present the results from Study 1B, where the FFQ was conducted in a population of pregnant UK women.

Chapter 5. Exploration of dietary patterns and alcohol consumption in pregnant women in the UK (Study 1B)

5.1 Introduction

Study 1A (Chapter 4), described the design and development of an FFQ specifically for the purpose of this study. Various aspects of the FFQs validity were also tested. Overall, the FFQs ability to rank women in relation to their relative intake was adequate and correlation coefficients between the intakes estimated using the FFQ and reference method (24-hour dietary recalls) were comparable to the wider literature. However, the results from the Bland-Altman plots did indicate there to be differences in mean estimates between the FFQ and reference method. This chapter will present the findings from study 1B, which aimed to use the purposefully designed FFQ within a population of pregnant women in the UK to measure the intakes of folate, choline, betaine, vitamin C and carotenoids. The findings from Study 1A are important when interpreting the results of Study 1B.

There is a wealth of evidence indicating a clustering of health-compromising behaviours, such as alcohol consumption, smoking, drug-use, poor dietary intakes and physical inactivity (Padrão et al. 2007; Lanting et al. 2009). These interrelationships are multi-dimensional and can significantly increase the risk of chronic health issues in individuals. Results from observational studies in the general population suggest that harmful patterns of alcohol consumption, such as binge drinking (6 or more units per drinking occasion), are associated with diets characterised by processed foods, high in fat and sugar that are low in important nutrients. A number of studies have indicated that as the volume of alcohol consumed per day increased, the intakes of fresh fruit, vegetables and dairy decreased, and the consumption of red and processed meats increased (Tjønneland et al. 1991; Barefoot et al. 2002; Valencia-Martin et al. 2011; Touvier et al. 2014).

In contrast, a number of studies have also reported that alcohol consumption is associated with better quality diets. A study conducted in the US reported that individuals who reported any alcohol consumption scored significantly higher on the Healthy Eating Index (HEI) (Breslow et al. 2006). The HEI is a composite score based on a number of dietary factors; the higher the score, the 'better' quality the diet. When this association was explored in more detail, two key relationships were observed. Increasing frequency of alcohol consumption was associated with higher HEI scores, when the quantity of alcohol consumed per occasion was low. In contrast, the lowest HEI scores were observed for participants who reported high quantities of alcohol consumed coupled with low drinking frequency; indicating binge patterns of drinking (Breslow et al. 2006).

While there are a number of studies exploring relationships between various aspects of alcohol consumption and dietary intake in the general population

(La Vecchia et al. 1992; Tjønneland et al. 1999; Chatenoud et al. 2000; Sieri et al. 2002; Ruf et al. 2005; Padrão et al. 2007; Touvier et al. 2014), there is currently no research looking at whether these relationships persist into pregnancy.

It is imperative that these relationships are explored during pregnancy, as there is a growing body of evidence to suggest alcohol consumption in the presence of poor maternal nutrition can increase the risk of harm to the developing fetus. In particular, animal models of FASD have indicated that antioxidants and micronutrient involved in One Carbon Metabolism (OCM) may mediate the relationship between alcohol and harm (Gutierrez et al. 2007; Naseer et al. 2010; Thomas et al. 2010; Hewitt et al. 2011). A number of studies have reported a protective effect of folate, choline, vitamin E and carotenoids, when pregnant rats were exposed to ethanol. Their offspring had higher birth and brain weights (Thomas et al. 2009), less damage to their brain structure (Mitchell et al. 1999c; Heaton et al. 2000a; Marino et al. 2004), and showed fewer signs of cognitive and behavioural deficits associated with antenatal ethanol exposure (Thomas et al. 2009; Thomas et al. 2010).

Women who continue to drink alcohol while they are pregnant may be putting their unborn baby at increased risk of harm including, congenital birth defects, restricted growth, and cognitive or behavioural deficits in childhood and later life (Muralidharan et al. 2013). Understanding relationships between patterns of alcohol consumption and dietary intake, and how these choices are made, may facilitate identification of at risk populations of women and highlight targets for nutritional interventions.

5.2 Aims and objectives (1B)

The overall aims of this study were to investigate associations between maternal alcohol consumption and dietary intake and to explore what influences women's choices around eating and drinking in a sample of pregnant women across the UK.

The specific objectives of the study were:

- To describe the following aspects of alcohol consumption, before and during pregnancy:
    - Frequency and quantity of alcohol consumption
    - Frequency of binge drinking (six or more units of alcohol on one occasion)
- To estimate the relative mean, daily intakes of OCM micronutrients (folate, choline and betaine) and antioxidants (vitamin C and carotenoids)
- To describe relationships between patterns of alcohol consumption (before and during pregnancy) and relative intakes of folate, choline, betaine, vitamin C and carotenoids
- To derive dietary patterns during pregnancy

- To explore the relationship between maternal dietary patterns and folic acid supplement use during pregnancy
- To describe relationships between patterns of alcohol consumption (before and during pregnancy) and maternal dietary pattern
- To explore what influences the choices women make about what to eat and drink during pregnancy

5.3 Methods

5.3.1 Design

A mixed method approach was selected, combining quantitative and qualitative data in order to provide a richer understanding of the relationships between diet and alcohol during pregnancy than could be achieved by using one method alone (Johnson & Onwuegbuzie 2004).

A two-phase, explanatory design was implemented whereby quantitative and qualitative data were collected sequentially (Fig. 5.1). This design was chosen for a number of reasons. Firstly, it enables the researcher to use the qualitative phase to explore and provide context to findings from the quantitative phase. In this study, phase 1 described levels of diet and alcohol consumption during pregnancy and the relationships between them, while phase 2 provided insights into the variety of beliefs, concerns and situations that gave rise to these patterns of consumption. Secondly, the sequential design, with two clear phases of research, facilitates the collection and analysis of data in a more focused way (Creswell & Plano Clark 2007). Phase one consisted of a cross-sectional survey, which included a purposefully design FFQ that provided detailed data on both levels of alcohol and micronutrient consumption and on patterns of maternal dietary intake. This was followed by phase two, which comprised of semi-structured, in-depth interviews to explore how these patterns were produced. Greater weight was given to the quantitative phase due to the fundamental importance of providing accurate descriptions of levels and patterns of alcohol consumption and nutritional intake.

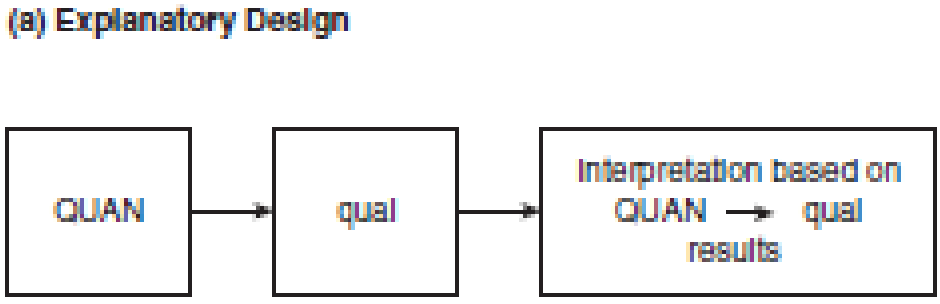

Figure 5.1 Explanatory Design: Follow-up Explanations Model (Creswell & Plano Clark 2007)

5.3.2 Sample population

Initially, women were eligible for inclusion if they were pregnant, aged 16 years or older, were attending their 12 week antenatal scan at an antenatal clinic in Gloucestershire, and reported drinking any alcohol consumption during their current pregnancy. Women were excluded if they did not speak English and reported suffering with hyperemesis gravidarum (severe morning sickness). Due to recruitment challenges, eligibility criteria were widened to include pregnant women, aged 16 years or older, living anywhere in the UK, and who did and did not report alcohol consumption during their current pregnancy. A sub-sample of women were recruited from the quantitative phase to participate in the qualitative phase. All were pregnant at the time of interview.

5.3.3 Recruitment

5.3.3.1 Phase 1: Quantitative study

Potential participants were contacted through three different routes (routes A, B and C). Initially, recruitment route A was employed, but due to low reported drinking levels, route B was also introduced. Finally, due to continuing poor recruitment rates, route C was adopted.

A. Antenatal clinics (Gloucestershire)

All women attending their 12-week scan at five antenatal clinics across Gloucestershire were given a short screening questionnaire upon arrival, by their midwife, a midwifery assistant or the receptionist, and were invited to complete it in the waiting room. The questionnaire included the AUDIT-C (Bush 1998), which was used to measure quantity and frequency of alcohol consumption since they had been pregnant. Women were invited to provide their contact details if they wished to participate in the second phase of the study, which included a further, more detailed questionnaire on diet and alcohol. Women completed the short screening questionnaire and placed it in a blank envelope (provided by the receptionist), sealed it and placed the envelope in a collection box in the waiting room. Submitting a completed screening questionnaire implied consent.

Women who reported drinking any alcohol since they had been pregnant were contacted and invited to participate in phase 2 of the study. Women were invited to participate in one of two ways:
The questionnaire was posted to women, and they were invited to complete it and return it in the self-addressed, pre-paid envelope provided. Submitting a completed questionnaire implied consent.
A link to the questionnaire was emailed to women, and they were invited to complete it online. Completing and submitting an online questionnaire implied consent.

B. Substance misuse antenatal clinics (Northumbria and Brighton)

All women attending either of two specialist substance misuse antenatal clinics, who reported drinking alcohol during their pregnancy, were informed about the study by their clinician (Obstetrician, paediatrician or specialist substance misuse midwife). Women who were interested in participating were invited to discuss the study with the researcher in private. When the researcher was not able to be present, women were given a participant information sheet, with a reply slip attached, by the clinician and, if they were interested in participating, asked to provide their details on the reply slip or send a text message or email to the researcher. Women who wished to participate were sent a copy of the questionnaire by post or a link to the online questionnaire was sent via email. Submission of a completed questionnaire implied consent.

C. Online recruitment

In June 2013, recruitment criteria were widened and an online recruitment strategy was employed. The study was advertised on social media platforms (Twitter and Facebook) and information provided to direct those interested to a website where they could complete a questionnaire on-line. By submitting a completed questionnaire, consent was implied.

Incentives

Whatever their route of recruitment, women were invited to enter into a prize draw to win one of five £50 Amazon vouchers, as remuneration for taking part. To enter into the prize draw, women ticked a box on the questionnaire and provided contact details. The contact details were stored separately from the questionnaire responses to maintain anonymity. Women who participated in an interview were given a £20 voucher for one of two UK supermarkets (Marks & Spencer or Sainsbury's) as remuneration for taking part.

5.3.3.2. Phase 2: Qualitative study

Whatever the route of recruitment, the final page of the questionnaire that participants completed included an invitation to women to take part in an interview. Those who were interested were asked to provide their contact details (email or telephone number) and were subsequently sent a participant information sheet and consent form. A minimum of 24 hours later, the researcher contacted the potential participant and discussed with her what was entailed in the interview. If they were still interested in taking part, a day, time and location for the interview was arranged. Each participant was contacted again the day before the scheduled interview to confirm that the day, time and location were still suitable.

5.3.4 Measures

Using a cross-sectional design, women completed a questionnaire, which was comprised of three sections; they enquired about alcohol consumption, dietary intake (frequency and quantity of selected food and drink items) and socio-demographic characteristics during their current pregnancy.

5.3.4.1 Alcohol consumption

Alcohol consumption was measured using the AUDIT-C (Bush 1998). Women were asked questions about their alcohol consumption in relation to two time periods; the 12 months before their current pregnancy and the time during their current pregnancy. Women were asked the following questions:

- 'Before you were pregnant, how often did you have a drink containing alcohol?' and responses included 'Never', 'Monthly or less', '2-4 times per month', '2-3 times per week', '4+ times per week'. These were recoded into 'Monthly or less', '2-4 times per month', 2-3 times per week or more' for analysis.
- 'Before you were pregnant, how many units of alcohol did you drink in a typical day when you were drinking?' and responses included '1-2', '3-4', '5-6', '7-9', '10+'. These were re-coded for the analysis into '1-2', '3-4' '5+'. Women who reported 'Never' in the previous question were coded as '0'.
- 'Before you were pregnant how often did you have six or more units on a single occasion?' and responses included 'Never', 'Less than monthly', 'Monthly', 'Weekly', 'Daily or almost daily'. These were recoded into 'Never', 'Less than monthly' and 'Monthly or more' for the analysis.
- 'Since you have been pregnant, how often have you had a drink containing alcohol?' and responses included 'Never', 'Monthly or less', '2-4 times per month', '2-3 times per week', '4+ times per week'. These were recoded into 'Never', 'Monthly or less' and '2-4 times per month' for analysis.
- 'Since you have been pregnant, how many units of alcohol do you drink in a typical day when you are drinking?' and responses included '1-2', '3-4', '5-6', '7-9', '10+'. Reported quantities were very low and it was not possible to explore this variable in the analysis.
- 'Since you have been pregnant, how often have you had six or more units on a single occasion?' and responses included 'Never', 'Less than monthly', 'Monthly', 'Weekly', 'Daily or almost daily'. Reported frequencies were very low and it was not possible to explore this variable in the analysis.
- Women were also asked at what stage (how many weeks gestation) when they found out they were pregnant. If women reported alcohol consumption before pregnancy, they were asked at what time point they reduced their intake or stopped drinking. Response categories included 'before trying to conceive', 'while trying to conceive', 'when I found out I was pregnant', 'during

the first trimester', 'during the second trimester' and 'during the third trimester'. This provided information on the level of alcohol exposure during the early stages of pregnancy.

5.3.4.2 Dietary intake

Dietary intake was measured using the modified FFQ, which was designed, developed and tested in Study 1A. A full description of the FFQ is provided in Chapter 4. Briefly, the FFQ was modified from an existing FFQ (Pufulete et al. 2002) to derive dietary patterns and measure the habitual intake of folate, choline, betaine, vitamin C and carotenoids (α-carotene, β-carotene, lutein, lycopene and β-cryptoxanthin).

Micronutrient intakes

Estimates of daily nutrient intakes were calculated by multiplying weekly portion sizes by nutrient concentrations per gram, and then dividing weekly nutrient intakes by seven:

Daily intake = Concentration per g x (portion size (g) x weekly frequency) / 7

For each included food and drink item on the FFQ, the average concentrations of folate, vitamin C and carotenoids were referenced from a UK dietary composition table (R A McCance & Widdowson 2002). Concentrations of choline and betaine were referenced from US dietary composition tables (USDA) (Patterson et al. 2008), as no UK equivalent data was available at the time.

Dietary pattern scores

The reported frequency of certain food and drink items differed i.e. tea and coffee was reported as number of cups per day, but meat and vegetables were reported as portions per week. Therefore, before dietary pattern analysis, all frequencies were converted to weekly intakes.

Principle Components Analysis (PCA) with varimax rotation was performed on the standardised food and drink items (Northstone & Emmett 2008; Smith et al. 2011). Eigenvalues were produced in a scree plot, to provide a visual assessment of which component factors explain the most variability in the data. The screen plot indicated that two components should be investigated further. Food and drink items with a factor loading of ≤-0.3 or ≥0.3, which suggested a strong negative or positive association (Northstone et al. 2008), respectively, and were considered to be significant descriptive characteristics of that dietary pattern. A dietary pattern label was provided to each component investigated based on these characteristics.

A total dietary pattern score for each participant was calculated by multiplying the factor loadings by the corresponding z-score and summing all values. A summary score is created for each participant

and dietary pattern. The mean score is zero, with a score above or below indicating a higher or lower adherence to that dietary pattern, respectively (Hu 2002; K Northstone et al. 2008; Smith et al. 2011).

Folic acid supplements

The questionnaire also comprised of questions relating to dietary supplement use. Women were asked if they had taken any dietary supplements during their current pregnancy and were also asked to provide the frequency of consumption throughout the pregnancy. Responses included: 'Daily', 'Almost daily', 'Once or twice a week', 'Less than once or twice per week' or 'Never'. Because of variations in compositions between commercial brands of supplements women were also asked to provide the brand and type of supplements they had been taking.

For women who reported taking folic acid supplements on a daily or almost daily basis during pregnancy, an additional variable was derived. Where possible, the type and brand of dietary supplement was searched, and the concentration of folic acid per tablet was added to the average, daily dietary folate intakes. In cases where women did not provide the brand name or the supplement could not be found, 400μg was added to the average daily intake values calculated from dietary sources.

5.3.4.3 Socio-demographic characteristics

The following socio-demographic characteristics were self-reported:

- Age – women provided their age in years when completing the questionnaire. This variable was categorised as '<25', '25-29', '30-34' and '35+' for analysis.
- Parity – women reported number of children they had (excluding adopted or step children). This variable was dichotomised into 'Primiparous' (first time pregnancy) and 'Multiparous' (Has been pregnant two or more times) for analysis.
- Education – women reported highest level of educational attainment. Categories included '< secondary school', 'GCSE', 'A-levels', 'Vocational', 'Bachelor's degree', and 'Postgraduate'. Categories were dichotomised into '<bachelor's degree' and '≥bachelor's degree' for analysis.
- Occupation – women reported their current occupation. Categories included 'Higher managerial and professional', 'Lower managerial and professional', Skilled non-manual', 'Skilled manual', 'Unskilled manual', 'Self-employed', 'Student' and 'Unemployed'. Categories were recoded as 'Unemployed', 'Non-managerial/professional', and 'Managerial/professional' for analysis.
- Ethnicity – women were asked to report their ethnicity, by ticking one of the following response categories; 'White', 'Mixed', 'Asian or Asian British', 'Black or Black British', 'Chinese', and 'Other', which were dichotomised to 'White' and 'Other' for analysis.

- Index of Multiple Deprivation (IMD) score – women were invited to provide their postcode in order to calculate IMD score. The score is an indicator of social deprivation and is based on a number of factors, including, education, income, crime and access to healthcare in the local area (Department for Communities and Local Government 2015). This variable was treated as a continuous measure.
- Marital status – women were asked to report their marital status. Responses included 'Married', 'Single', 'Partner, cohabiting', and 'Partner, living separately'. These categories were dichotomised into 'Married' and 'Not married' for analysis.
- Smoking – women reported frequency of smoking and quantity of cigarettes smoked per day of smoking during this pregnancy. These variables were used to produce a dichotomous variable 'Smoker' and 'Non-smoker' for analysis.

5.3.4.4 Interviews

Those women who had agreed to do so took part in an in-depth, semi-structured interview that was conducted face-to-face and audio recorded. Most were conducted in the home of the participant and some in a local café. If conducted in a café, the interviewer chose a place away from other people, to ensure the participant's privacy. Women were asked to consent to the audio recording of the interview and informed that if they did not want to answer particular questions or wanted to stop the interview or the recording at any time, they could. They were also informed that if they felt uncomfortable about participating at any stage, they were free to withdraw at any point and their data would be destroyed.

A topic guide (rather than an interview schedule) was developed based on findings from existing literature and key results from the previous quantitative phase. All interviews were conducted by one researcher (VC). Notes were taken during the interview by the researcher to highlight pertinent points for probing later in the interview. In addition, notes were taken at the end of the interview to reflect on the discussion.

5.3.5 Statistical analysis

Continuous variables were tested for normality using two methods. Firstly, histograms were plotted to provide a visual representation of the distribution of the data. Secondly, the Shapiro-Wilk test was used. Non-normally distributed variables were log-transformed before analysis. Continuous variables were summarised as means and standard deviations, and categorical variables were summarised as frequencies and percentages.

To explore correlations between dietary pattern scores and micronutrient intakes, Pearson's correlation coefficients were then calculated. Correlation was defined as very strong if >0.80, strong if

0.60 – 0.79, moderate if 0.40 – 0.59, weak if 0.20 – 0.39, and negligible if >0 - <0.20 (Dancey & Reidy 2004).

Univariate analyses were conducted to explore relationships between dietary pattern scores, micronutrient intakes, alcohol consumption and socio-demographic characteristics. Analyses of variance (ANOVA) were used to explore differences between dietary pattern scores and micronutrient intakes across categories of age, parity, marital status, ethnicity, education, occupation, smoking status and alcohol consumption.

To explore relationships between dietary intake and folic acid supplement use, dietary pattern scores were categorised into quartiles and folic acid supplement use was dichotomised into 'yes/no' categories. Logistic regression models were used to explore the odds of taking folic acid supplements during pregnancy according to dietary pattern adherence scores. FA supplement use was treated as a binary, dependent variable and quartiles of dietary pattern scores were treated as categorical independent variables.

Linear regression models were used to explore associations between micronutrient and antioxidant intakes (folate, folate + folic acid, choline, betaine, vitamin C and carotenoids) and alcohol consumption categories, before and during pregnancy. Micronutrient and antioxidant intakes were treated as continuous, dependent variables and alcohol consumption categories as independent variables. Six, separate linear regression models were run for all micronutrient and antioxidants. Regression models were then run again with the addition of potentially confounding variables, which included maternal age, parity, education, occupation, ethnicity, IMD score and marital status.

To explore relationships in a more 'real world' context (Hu 2002), this process was repeated to explore associations between dietary pattern scores and alcohol consumption patterns, before and during pregnancy, using a further two linear regression models. Dietary pattern scores were treated as continuous, dependent variables and alcohol consumption categories as independent variables. Separate linear regression models were run for each dietary pattern. Multivariate linear regression models were used to explore these relationships further, adjusting for maternal age, parity, ethnicity, education, occupation, IMD score and marital status.

A sensitivity analysis was conducted to explore if dietary intake was associated with the timing of alcohol abstinence. To highlight the frequency and quantity of alcohol consumption by women who stopped drinking alcohol upon recognition of their pregnancy, a two-by-two table was constructed. Linear regression models were then used to explore the intakes of micronutrient, antioxidants, and dietary pattern scores by timing of alcohol abstinence.

All unadjusted and adjusted regression models are presented with 95% confidence intervals and significance levels at 5%. All data was analysed using Stata 13.1.

5.3.6 Missing data

Women who completed paper-based questionnaires were excluded from the present analysis if 10 or more food and drink items were missing from the FFQ. In cases where less than 10 were missing, these items were coded as 0 to indicate they were not regularly consumed (Northstone et al. 2008).

In order to submit the questionnaire online, women had to complete every question on the FFQ section of the questionnaire; trying to proceed would produce a message on screen and prompt women to provide missing data. Women who started an online questionnaire, but did not submit it were excluded from the analysis, as consent could not be implied.

5.3.7 Qualitative analysis

All interviews were transcribed by an external, professional transcriber and then checked by the interviewer. Transcripts were managed and coded using Nvivo 10 (QSR 2012). Interview data were thematically analysed following the guidelines developed by Braun & Clarke (2006). This method of analysis was chosen due its flexible and pragmatic approach (Braun & Clarke 2006). Analysis involved five stages (Braun & Clarke 2006):

- The researcher became familiar with the data set by reading and re-reading the transcripts and keeping notes about initial patterns that are appearing and how the data will be coded. This first stage was conducted by two researchers, independently (VC and a supervisor). Initial patterns and codes were compared, and any discrepancies were discussed. The remaining stages were all conducted by VC and discussed with both supervisors.
- Initial codes were developed.
- These codes were then compared and broader over-arching themes were identified.
- Themes were reviewed and refined.
- Themes were defined and then named. This involved describing each theme and assessing how they related to the overarching research question.

5.4 Ethical approval

Ethical approval for route A was provided by NRES Committee South Central – Southampton A (REC: 12/SC/0402) in August 2012. R&D approval was obtained from Gloucestershire Hospitals NHS Foundation Trust in October 2012 (R&D: 12/069/GHT). The addition of substance misuse antenatal clinics as recruitment sites for route B required a Research Passport (NIHR 2010), which was obtained from Gloucestershire Hospitals NHS Foundation Trust. Letters of access were then provided by Brighton and Sussex University Hospitals NHS Trust in September 2013 and Northumbria Healthcare

NHS Foundation Trust in October 2013. To set up recruitment route C, ethical approval for the online questionnaire was obtained from Oxford Brookes University Research Ethics Committee (UREC) in July 2013 (Ref: 130733). Please see Appendix C for all relevant ethical approval letters.

5.5 Results

5.5.1 Recruitment

A total of 350 pregnant women were recruited across three routes of recruitment. The number of women recruited through each recruitment route are discussed below.

1) Antenatal clinics (December 2012 – June 2013)

In phase 1, approximately 600 screening questionnaires were distributed to pregnant women attending a 12-week scan at antenatal clinics in Gloucestershire; 490 (82%) were completed and returned. Of those who completed the screening questionnaire, 56 (11%) reported any alcohol consumption during their pregnancy. A total of 44 were invited to take part in phase 2: 32 (73%) returned completed questionnaires and of those, 3 participated in an interview.

2) Substance misuse antenatal clinics (September 2013 – February 2014)

At the substance misuse antenatal clinics, a total of eight women were given information about the study (5 by the researcher and 3 by a clinician) and were invited to participate in phase 2: one (13%) submitted a completed paper-based questionnaire and also took part in an interview.

3) Online recruitment (July 2013 – March 2014)

A total of 472 pregnant women were recruited through social media platforms (Facebook and Twitter). Of those, 155 (33%) women did not submit completed questionnaires and were excluded from the analysis. The remaining 317 (67%) submitted completed online questionnaires, and of those, 2 participated in an interview.

Phase 1: Quantitative study results

5.5.2 Sample characteristics

Socio-demographic characteristics of women are presented in Table 5.1. The majority of women were over 30 years old (n=224, 64%), white (n=334, 95%), educated to degree level (n=239, 68%), non-smokers (n=342, 98%), in professional occupations (n=298, 83%), and took folic acid supplements on a regular basis during their pregnancy (n=267, 76%). Univariate analyses indicated there were no significant differences in age, ethnicity, and education, smoking status, occupation and dietary

supplement use, between women who were recruited online, compared to those recruited in antenatal clinics.

Table 5.1 Socio-demographic characteristics of sample population (n=350)

	n	%
Age		
≤25	39	11
26-30	87	25
31-35	140	40
>35	84	24
Parity		
Primiparous	186	53
Multiparous	164	47
Marital status		
Married	246	70
Not married	104	30
Ethnicity		
White	334	95
Non-white	16	5
Education		
<Bachelor's degree	111	32
≥Bachelor's degree	239	68
Occupation		
Managerial/Professional	180	51
Non-managerial/professional	109	31
Unemployed	61	17
Smoking		
Non-smoker	342	98
Smoker	8	2

5.5.3 Alcohol consumption levels

Alcohol consumption levels, before and during pregnancy, are presented in Table 5.2. Approximately 95% of women reported any alcohol consumption prior to pregnancy and 20% reported consuming 5 or more units per drinking occasion. The majority (75%) of women reported binge drinking before they were pregnant, with 17% doing so on a weekly basis. However, during pregnancy, alcohol consumption patterns markedly changed, with 67% of women reporting they did not drink alcohol during their pregnancy.

Table 5.2 Frequency and percentages of alcohol consumption before and during pregnancy in sample population (n=350)

	n	%
Frequency of alcohol consumption before pregnancy		
<Monthly	99	28
2-4 times/month	106	30
2-3 times/week	145	41
Units of alcohol consumed per occasion before pregnancy		
≤1-2	154	46
3-4	115	34
5+	65	20
Frequency of binge drinking before pregnancy		
Never	82	25
<Monthly	144	43
≥Monthly	108	32
Frequency of alcohol consumption during pregnancy		
Never	236	67
<Monthly	45	13
2-4 times/month	69	20

A total of 114 (33%) women reported that they continued to drink alcohol during pregnancy. Of those who did report alcohol consumption, all women reported drinking no more than 1-2 units per drinking occasion. A total of two women reported at least one episode of binge drinking (6 or more units per occasion) on a less than monthly basis whilst pregnant.

Of those who drank alcohol prior to pregnancy, the time points at which they either reduced their intake or stopped drinking differed (Figure 5.1). A total of 236 (67%) women reported to not drink alcohol whilst being pregnant; however, 78 (33%) of these women only stopped drinking upon recognition of their pregnancy. It is possible these women have also had an alcohol-exposed pregnancy and may be incorrectly categorised as 'non-drinkers'.

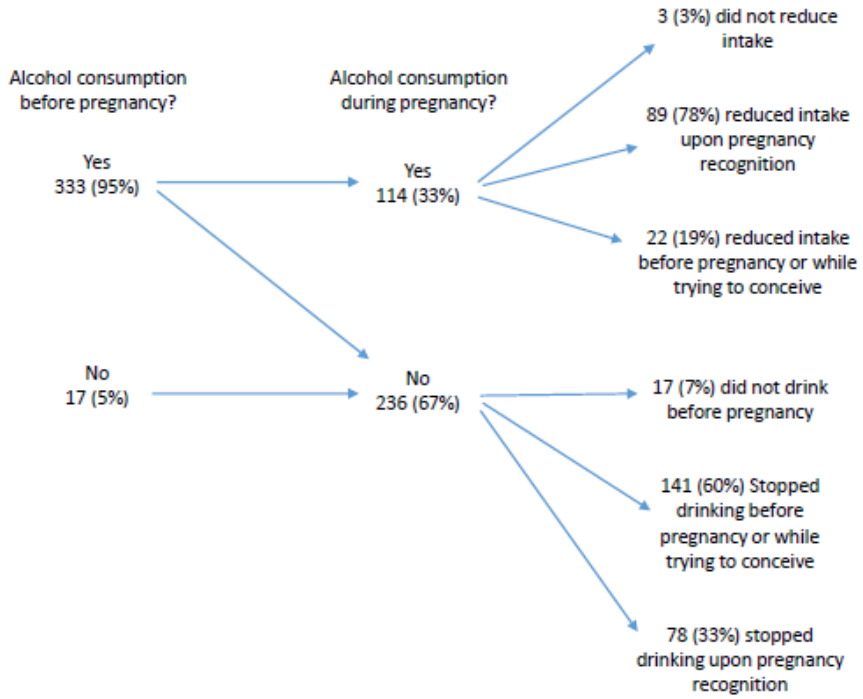

Figure 13 Flow diagram of women's alcohol consumption status before and during pregnancy

5.5.4 Maternal micronutrient intakes

Unadjusted β-coefficients of micronutrient and antioxidants intakes from linear regression models are presented in Table 5.3. As the frequency of alcohol consumption increased, maternal intakes of folate (β=77.89, 95% CI=8.68, 147.09; p=0.028), choline (β=51.29, 95% CI=18.47, 84.12; p=0.002) and carotenoids (β =4416.85, 95% CI=571.82, 8261.88; p=0.024) also significantly increased, compared to women who reported drinking on a less than monthly basis during that period.

Table 5.3 Unadjusted β-coefficients of micronutrient and antioxidant intakes by frequency of alcohol consumption prior to pregnancy (n=350)

Model	β	95% CI		p-value
1: Folate				
<Monthly				
2-4 times/month	-10.16	-84.35	64.03	0.788
2-3 times/week	77.89	8.68	147.09	0.028
2: Choline				
<Monthly				
2-4 times/month	12.42	-22.78	47.61	0.488
2-3 times/week	51.29	18.47	84.12	0.002
3: Betaine				
<Monthly				
2-4 times/month	3.29	-39.81	46.40	0.881
2-3 times/week	17.92	-22.28	58.13	0.381
4: Vitamin C				
<Monthly				
2-4 times/month	-32.28	-72.26	7.70	0.113
2-3 times/week	5.44	-31.85	42.73	0.774
5: Carotenoids				
<Monthly				
2-4 times/month	1356.32	-2765.73	5478.37	0.518
2-3 times/week	4416.85	571.82	8261.88	0.024

When maternal age, education, parity, marital status, ethnicity, occupation and smoking status were added to the models as covariates, drinking 2-3 times per week prior to pregnancy, remained significantly associated with increased choline (β =55.55, 95% CI= 20.61, 90.48; p=0.002) and carotenoid (β =4253.92, 95% CI= 231.58, 8375.26; 0.043) intakes, compared to women who drank less than monthly (Table 5.4).

Table 5.4 Adjusted β-coefficients of micronutrient and antioxidant intakes by frequency of alcohol consumption prior to pregnancy (n=350)

Model	β	95% CI		p-value
1: Folate				
<Monthly				
2-4 times/month	-17.58	-94.11	58.94	0.652
2-3 times/week	67.83	-6.56	142.23	0.074
2: Choline				
<Monthly				
2-4 times/month	8.43	-27.51	44.36	0.645
2-3 times/week	55.55	20.61	90.48	0.002
3: Betaine				
<Monthly				
2-4 times/month	3.59	-41.14	48.33	0.875
2-3 times/week	18.25	-25.23	61.74	0.410
4: Vitamin C				
<Monthly				
2-4 times/month	-32.62	-73.93	8.69	0.121
2-3 times/week	11.82	-28.34	51.99	0.563
5: Carotenoids				
<Monthly				
2-4 times/month	1266.78	-2972.47	5506.03	0.557
2-3 times/week	4253.92	132.58	8375.26	0.043

Micronutrient and antioxidant intakes were then compared by the typical number of units consumed per drinking occasion prior to pregnancy. Women who typically consumed 5 or more units per drinking occasion had significantly lower intakes of folate during pregnancy (β=-127.97, 95% CI=-2.06.35, -49.596; p<0.0001), compared to women who reported drinking 1-2 units or less per typically drinking occasion. The relationship remained significant after the addition of socio-demographic variables as covariates (β=-123.68, 95% CI=-204.29, -43.08; p=0.003) (Figure 5.2). The maternal intakes of choline, betaine, vitamin C and carotenoids did not differ by the typical number of units consumed per drinking occasion, prior to pregnancy. Micronutrient and antioxidant intakes by number of binge drinking episodes were also explored, but no relationships were observed.

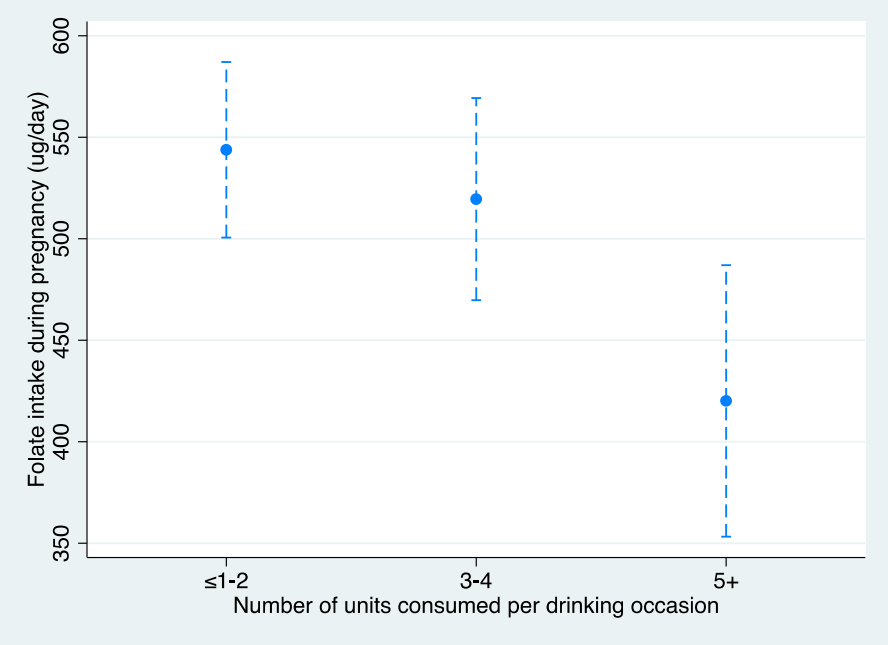

Figure 5.3 Adjusted estimates of daily folate intake (dietary + folic acid supplements) by the number of units typically consumed per drinking occasion prior to pregnancy

Micronutrient and antioxidant intakes by frequency of alcohol consumption during pregnancy were then explored. Unadjusted β-coefficients and 95% CIs are presented in Table 5.5. The results of the linear regression models indicate that as the frequency of alcohol consumption increased, the intake of choline significantly increased, compared to women who did not reported any alcohol during that period (β=54.56, 95% CI=20.13, 88.99; p=0.002). After the addition of socio-demographic covariates, this relationship remained significant (β=54.25, 95% CI=18.883, 89.61; p=0.003). No other micronutrients or antioxidants were associated with frequency of alcohol consumption during pregnancy.

Table 5.5 Unadjusted β-coefficients of micronutrient and antioxidant intakes by frequency of alcohol consumption during pregnancy (n=350)

Model	β	95% CI		p-value
1: Folate				
Never				
<Monthly	28.34	-58.92	115.60	0.523
2-4 times/month	24.07	-49.35	97.49	0.519
2: Choline				
Never				
<Monthly	40.07	-0.85	81.00	0.055
2-4 times/month	54.56	20.13	88.99	0.002
3: Betaine				
Never				
<Monthly	17.33	-32.86	67.53	0.498
2-4 times/month	-1.96	-44.19	40.27	0.927
4: Vitamin C				
Never				
<Monthly	15.93	-30.83	62.69	0.503
2-4 times/month	-11.49	-50.83	27.85	0.566
5: Carotenoids				
Never				
<Monthly	1431.64	-3378.84	6242.13	0.559
2-4 times/month	3933.01	-114.31	7980.33	0.057

5.5.5 Maternal dietary patterns

Two dietary patterns were identified in the data. The first was labelled the 'Prudent' dietary pattern and accounted for approximately 8% of variation within the data; it was characterised by high intakes of fruit, vegetables, fish, salad and pulses. The second was labelled the 'Cafeteria' dietary pattern and accounted for approximately 5% of the variation in the data; it was characterised by high intakes of fried food, chocolate, sweets, pudding and cakes. The factor loadings for both dietary patterns are presented in Tables 5.6. The 'Prudent' dietary pattern was associated with high educational attainment (β=0.15, p=0.039), and the 'Cafeteria' dietary pattern was associated with lower maternal age (β=-0.012, p=0.11), higher parity (β=0.29, p=0.001), and not being of white ethnic origin (β=0.48, p=0.012).

Table 5.6 Factor loadings from principal components analysis with varimax rotation for maternal dietary patterns*

Dietary patterns		
	Prudent (8%)	Cafeteria (5%)
Bread/pasta/rice		
White bread	-0.14	0.10
Other bread	0.07	0.05
Rice	0.27	0.22
Pasta	0.17	0.16
Breakfast foods		
Breakfast cereals	0.19	-0.02
Cereal bars	0.02	0.00
Animal produce		
Sausages	-0.13	**0.67**
Burgers	-0.04	**0.37**
Bacon	-0.16	**0.43**
Steak	0.05	**0.57**
Chicken	-0.14	**0.40**
Pork/Lamb	0.07	0.28
Minced beef dishes	0.03	**0.71**
Fish	0.36	0.03
Prawns	0.24	0.02
Tinned fish	0.25	0.13
Eggs	**0.36**	0.02
Vegetables		
Potatoes	0.18	**0.30**
Sweet potatoes	0.20	0.11
Leeks	**0.44**	-0.02
Onions	**0.49**	0.07
Courgettes	**0.52**	-0.10
Beetroot	**0.42**	-0.04
Butternut Squash	**0.42**	-0.04
Cauliflower	**0.36**	**0.37**
Red/white cabbage	**0.44**	0.03
Frozen mixed vegetables	0.13	0.27
Coleslaw	0.26	0.24
Tinned tomatoes	**0.60**	0.11
Salad vegetables		
Lettuce	**0.56**	-0.04
Peppers	**0.63**	0.05
Cucumbers	**0.51**	-0.09
Tomatoes	**0.65**	-0.11

*(Factor loadings in bold are ≤-0.3 or ≥0.3)

Table 5.6 (cont.) Factor loadings from principal components analysis with varimax rotation for maternal dietary patterns*

Dietary patterns		
	Prudent (8%)	Cafeteria (5%)
Green vegetables		
Broccoli	**0.50**	0.13
Green cabbage	**0.47**	-0.05
Spinach	**0.59**	-0.12
Brussel sprouts	0.24	0.04
Green beans	**0.54**	0.16
Mange tout	**0.54**	0.04
Peas	**0.45**	**0.31**
Fruit/fruit juice		
Fresh fruit	**0.63**	-0.10
Tropical fruit	**0.47**	-0.07
Citrus fruit	**0.31**	0.02
Fruit juice	0.10	0.18
Pulses/nuts		
Baked beans	**0.31**	0.18
Chickpeas	**0.58**	-0.12
Nuts	**0.36**	-0.13
Convenience foods		
Pizza	-0.05	0.17
Chips	-0.19	**0.42**
Pies	-0.03	0.24
Crisps	-0.19	0.22
Savoury snacks/sauces/spread		
Quiche	0.11	0.11
Soup	0.14	-0.07
Tomato pasta sauce	0.34	0.20
Ketchup	0.02	0.19
Mayonnaise	0.12	0.14
Peanut butter	0.18	0.01
Marmite	0.16	-0.02
Sweet foods		
Puddings	0.02	**0.66**
Cakes	0.05	**0.64**
Biscuits	-0.05	0.22
Chocolate	-0.08	**0.31**
Pastries	0.01	**0.66**
Drinks		
Tea/coffee	0.07	0.04
Milk	0.13	0.21

*(Factor loadings in bold are ≤-0.3 or ≥0.3)

Pearson's correlation coefficients between micronutrient intakes and dietary pattern scores are presented in Table 5.7. The 'Prudent' dietary pattern was strongly correlated with carotenoid intakes (r=0.70, p<0.0001), moderately correlated with folate (r=0.56, p<0.0001), choline (r=0.58; p<0.0001) and vitamin C (r=0.51; p=0.0001), and weakly correlated with betaine (r=0.21; p<0.0001). In contrast, the 'Cafeteria' dietary pattern was weakly correlated with all micronutrients, with the exception of choline (r=0.42, p<0.0001).

As the 'Prudent' dietary pattern adherence score increased, the odds of taking folic acid supplements during pregnancy also increased; however, the relationship was not significant. In contrast, adherence with the 'Cafeteria' dietary pattern was significantly associated with lower odds of folic acid supplements during pregnancy (Table 5.8).

Table 5.7 Pearson's correlation coefficients of micronutrient intakes and maternal dietary patterns during pregnancy

Dietary factor	Prudent	Cafeteria
Folate (diet only)	0.56*	0.13*
Folate (diet and supplements)	0.48*	0.12
Choline	0.58*	0.42*
Betaine	0.21*	0.17*
Vitamin C	0.51*	0.21*
Carotenoids	0.70*	0.11*

Table 5.8 Odds of taking folic acid supplements during pregnancy by quartiles of maternal dietary pattern scores

	β	95% CI		p-value
Prudent				
Q1				
Q2	0.79	0.41	1.51	0.478
Q3	1.57	0.77	3.18	0.214
Q4	1.96	0.93	4.10	0.076
Cafeteria				
Q1				
Q2	0.99	0.44	2.21	0.974
Q3	0.41	0.20	0.84	0.015
Q4	0.42	0.20	0.87	0.020

Unadjusted and adjusted estimates of dietary pattern scores by frequency of alcohol consumption prior to pregnancy are presented in Table 5.9. Women who reported drinking alcohol 2-3 times per week were significantly more likely to adhere to the 'Prudent' dietary pattern compared to women

who reported drinking alcohol on a less than monthly basis prior to pregnancy (β=0.46, 95% CI=0.20, 0.71; p<0.0001). This relationship remained significant once confounders were added to the linear regression model (β=0.38, 95% CI=0.11, 0.65; p=0.006). No relationships between the 'Cafeteria' dietary pattern and alcohol consumption were observed and no relationships were observed between maternal dietary pattern scores and the quantity of alcohol consumed per occasion.

Table 5.9 Estimates of dietary pattern scores by frequency of alcohol consumption prior to pregnancy (n=350)

	Unadjusted				Adjusted			
Model	β	95% CI		p-value	β	95% CI		p-value
1: Prudent								
<Monthly								
2-4 times/month	0.21	-0.06	0.48	0.14	0.18	-0.1	0.46	0.201
2-3 times/week	0.46	0.2	0.71	<0.0001	0.38	0.11	0.65	0.006
2: Cafeteria								
<Monthly								
2-4 times/month	-0.13	-0.41	0.14	0.34	-0.06	-0.33	0.21	0.668
2-3 times/week	-0.22	-0.48	0.04	0.09	-0.01	-0.27	0.26	0.963

CI = confidence intervals

Unadjusted and adjusted estimates of dietary pattern scores by frequency of alcohol consumption during pregnancy are presented in Table 5.10. Similar patterns were observed during pregnancy as those seen prior to pregnancy; women who reported drinking alcohol 2-4 times per month had significantly higher adherence to the 'Prudent' dietary pattern compared to women who reported no alcohol during pregnancy (β=0.44, 95% CI=0.17, 0.7; p<0.0001). This relationship persisted once adjusted for confounders (β=0.36, 95% CI=0.09, 0.63; p=0.009). No relationships were observed between the 'Cafeteria' dietary pattern and alcohol consumption during pregnancy.

Table 5.10 Estimates of dietary pattern scores by frequency of alcohol consumption during pregnancy (n=350)

	Unadjusted				Adjusted			
Model	β	95% CI		p-value	β	95% CI		p-value
1: Prudent								
Never								
<monthly	0.3	-0.01	0.62	0.06	0.2	-0.12	0.52	0.219
2-4 times/month	0.44	0.17	0.7	<0.0001	0.36	0.09	0.63	0.009
2: Cafeteria								
Never								
<monthly	0.06	-0.26	0.38	0.71	0.14	-0.18	0.45	0.396
2-4 times/month	-0.16	-0.43	0.11	0.24	0.01	-0.26	0.27	0.965

CI = confidence intervals

5.5.6 Sensitivity analysis: Alcohol exposed pregnancies

A sensitivity analysis was conducted to explore whether dietary intake (micronutrients, antioxidants and dietary patterns) differed by maternal alcohol exposure status. The two-by-two table (Table 5.11) indicated that 96 women were consuming alcohol 2-3 times per week and 35 women were typically drinking 5 or more units of alcohol per occasion up until they were aware of their pregnancy. Results from the linear regression models indicated that there were no relationships between dietary intake and potentially having an alcohol exposed pregnancy.

Table 5.11 Frequency and quantity of alcohol according to timing of alcohol behaviour change (n=350)

	Before pregnancy	Pregnancy recognition
Frequency		
Monthly or less	35	28
2-4 times/month	27	46
2-3 times/week	25	96
Quantity (units)		
1-2	35	66
3-4	23	63
5+	14	35

Phase 2: Qualitative study results

5.5.7 Sample characteristics

A total of 6 women participated in in the qualitative phase. Of these, 3 were recruited through antenatal clinics, 1 through a substance misuse antenatal clinic and 2 via the online questionnaire. The majority of women were married (83%), employed (67%) and were educated to degree level (83%). All women were of white, European descent.

Table 5.12 Sample characteristics of interview participants (n=6)

	n	%
Age		
<20	1	17
21-30	2	33
31-40	3	50
41+	0	0
Marital status		
Married	5	83
Partner, living together	0	0
Partner, not living together	1	17
Single	0	0
Education		
GCSE's	1	17
A-levels	0	0
Bachelor's degree	4	66
Postgraduate degree	1	17
Occupation		
Unemployed	1	17
Student	1	17
Caring, Leisure and Service	1	17
Skilled, Technical	0	
Professional, Managerial	3	50
Parity		
Nulliparous	3	50
Multiparous	3	50

5.5.8 Influences on women's patterns of eating and alcohol consumption during pregnancy

Six key themes were identified through the qualitative analysis of women's accounts of their approach to diet and alcohol consumption in pregnancy: 1) pregnancy as a time to change behaviour; 2) listen to your body – it will tell you what you need; 3) specific foods or alcohol as a 'treat' during pregnancy;

4) inconsistent or ambiguous information creates uncertainty; 5) social context important in shaping behaviour; and 6) more confident 'the second time around'

5.5.8.1 Pregnancy as a time to change behaviour

Most of the women described an interest in diet and nutrition prior to pregnancy. For some this meant they did not feel they had had to make major changes to their diet

> "I was always quite conscious of healthy eating before I got pregnant, so I didn't really have fast food or processed food too much… So I haven't really needed to change it too much to try and include other things that were missing before, so I haven't really needed to change, like calcium consumption too much or anything like that." (Participant 2)

> "OK, well generally we used to eat pretty healthily anyway, because my husband goes to the gym quite a lot, so we eat quite a lot of veg, meat and couscous and whatnot… But generally we were quite healthy before, so not a lot's really changed." (Participant 1)

Nevertheless, all the women also described how, as soon as they knew they were pregnant, they had at least modified their diet and alcohol consumption though the type and scale of changes varied between women.

All reported a change in their alcohol consumption: four women described a reduction in alcohol consumption and two abstained entirely once they discovered they were pregnant. While all women recognised the potential harm from heavy alcohol consumption, attitudes towards light to moderate consumption varied. Some women perceived no harm from low alcohol consumption and discussed using their common sense when making a decision about whether to drink or not.

> "Yeah, so it's definitely changed since I've become pregnant. The first three months I didn't have any alcohol at all. Didn't fancy it and obviously it's just not good for the baby. And then the last maybe four months I've let myself have I would say two glasses of wine a week...But I don't think, I don't know, personally I don't think it would do any harm in the last few months, to have a few glasses of wine. So yeah." (Participant 2)

> "Whereas before I would probably have like one or two glasses of wine a week. So it has changed…Made up my own mind really. Because I don't think that you should drink, but I think if it's like a very special occasion, I think one is fine, it's not going to do anything. That's my opinion." (Participant 5)

However, two women decided to cut out alcohol immediately because they felt it was "better to be safe than sorry". Interestingly, these women were the only two who perceived their consumption prior to pregnancy to be high.

> "Just before I found out, I was drinking quite a lot. Well, quite a lot, I don't binge drink, but we liked to open a bottle of wine and have a glass or two with dinner…Well I don't drink at all now. Because I mean I don't think there'd be anything wrong with having a glass of wine every now and then, but then I think, what's the point? It's a limited amount of time, there is no point taking that risk, might as well leave it for that period." (Participant 3)

> "It was a lot of drugs and alcohol involved. Like a very high extent. So yeah, weekly and weekends. And obviously since I found out I've been pregnant I've pretty much. I have stopped everything now. In the beginning I had a couple of struggles with certain things, but that's all been worked on now." (Participant 6)

Similarly, all women also reported eliminating certain foods from their diet during pregnancy: unpasteurised cheese, raw eggs, uncooked meats and seafood were the most consistently highlighted. These changes were recognised as reducing harm to their unborn child.

> "There are things that I'm not having that I would have had before, but my overall behaviours haven't changed. I don't feel like I've had to alter my whole diet, although choices, some choices, have come off the list as it were. So I've avoided completely brie and camembert for example, because the guidance was just so confusing" (Participant 4)

> "Yeah, I mean I miss brie and soft cheese and what not, like last night not having Parma ham, but it's no massive deal really, it's not worth risking it." (Participant 1)

In addition, some women also described a reduction in food items that were not recognised as directly reducing harm to the baby, but were perceived as improving their own or their baby's health and wellbeing. These foods included sugary foods and drinks, starchy carbohydrates and caffeine.

Three women also discussed this in relation to weight gain and their image and confidence once the baby was born; they identified a direct relationship between body image and mental health.

> "When I look at some of my friends who've had kids and put on so much weight, not just them having to work to get it off whilst looking after a child, but just not feeling good, feeling insecure. You can see relationship problems arise because they're not confident" (Participant 6)

5.5.8.2 Listen to your body – it will tell you what you need

Five women described feeling a craving for particular foods during their pregnancy, which they interpreted as their body telling them what they needed to eat to compensate for nutrients they were missing. They felt confident that, because it was their body telling them to do so, it was appropriate to satisfy their craving, which was commonly for starchy or sugary foods, fresh juicy foods and milk.

> "I haven't made any massive changes, apart from maybe the iron. . . . And probably just drinking more milk, just because I was craving it, so I guess it was a reasonable craving." (Participant 2)

> "Every now and then I get a real craving, like I feel like I've got to stop straight away and buy chocolate, so I have to pull into a garage or something, and I've never done that before. So that's obviously the sugar rush or something isn't it? You're lacking something" (Participant 1)

> "Because I don't, I'm not a big meat eater, but I think I am at the moment, because my body is craving it, because I don't eat enough of it." (Participant 5)

Similarly, some women also described avoiding specific food or alcohol during pregnancy because their body told them to do so.

> "I think the first months I definitely didn't want anything and I think maybe that might be my body's way of actually telling me it's not a good idea to have any alcohol now." (Participant 2)

5.5.8.3 Specific foods or alcohol as a 'treat' during pregnancy

Five women referred to certain food and drink choices as a source of enjoyment and a way maintaining a balance in life.

> "I think probably not restricting yourself in everything, because I know there's lots of legislation about things you can and can't have and there's not always a lot of research to back it up… I think otherwise you can make yourself really miserable, denying yourself everything for nine months." (Participant 2)

Some women described drinking alcohol as a 'treat' and felt it was acceptable to have a glass of wine now and again, as a reward or as part of a celebration such as a wedding.

> "Maybe for somebody else it would be chocolate or a dirty burger or something, but for me it's a glass of wine. I think it probably is the whole treat you know, I think that's what it is. I don't really want it or need it I think, it's just part of a habit and probably a naughty treat as well." (Participant 1)

> "I don't drink now. Well I had one glass of wine on our wedding day [last month}. . . I thought 'I'm allowed one on that day.' And I had one on Christmas Day, but that's it." (Participant 5)

5.5.8.4 Inconsistent or ambiguous information creates uncertainty

A common theme in all interviews concerned inconsistencies in the advice and guidance given to women during pregnancy. Women described contradictory advice from a variety of sources, including friends, relatives, the internet and also healthcare professionals.

> "Yeah, I have looked into research about the alcohol, and I think obviously the NHS guidelines, I think not enough research has really been done on it, so they generally will advise you to have none whatsoever. Midwives will say it's fine, have a couple of glasses a week, it's not going to do any harm. And then you get opinions from everyone else on whether you should or shouldn't" (participant 2)

Five women highlighted how guidelines from healthcare professionals on alcohol and certain foods, including seafood and nuts, have changed over the past few years. For a number of women this undermined their confidence in the advice and resulted in them seeking guidance from other sources, including family and friends.

> "I think generally the quality is just very poor, the message is mixed. I think it should be much more decisive. Even if we then change our minds as a medical body or as a nation about something in a few years' time, I would rather have been given definite advice now, even if in 2016 there was a revision to the definite advice." (Participant 4)

> "That you have to exercise a little bit of common sense. So I think that kind of personal filter. Also my mum, just because comparing 30 or 40 years of difference in guidance, I felt was a good way to contrast. So have they always said it? Or is it another new fad, new thing that people are saying but actually in five years' time they will change again. So I really valued her opinion as well on some of the topics." (Participant 4)

5.5.8.5 The importance of social context in shaping behaviour

While they recognised the inconsistencies and contradictions in the advice they were given, most women were also sensitive to the views and expectations of those around them regarding what was 'appropriate' for a woman to eat or drink during pregnancy. Several described concerns that their 'reputation' as a good mother was at risk if they did not conform to the often conservative expectations of others.

> "Yeah, I think it would actually, because I think when you're pregnant you feel like vulnerable; you don't want people to think you're a bad mum I suppose, so yes it would" (Participant 5)

> "But yeah, definitely benchmarked against my mum and the rest of my family is a key. If they were against it I don't, I don't see how I could have drunk alcohol because it just would have felt wrong. If my, if the grandmother of my child was really against that, I think I would have been really self-conscious." (Participant 4)

An interesting variant on this theme is illustrated by one participant who was concerned that her partner might tempt her into excessive drug use and alcohol consumption and so left him and stopped seeing a number of their friends in their social network in order to resist engaging in negative health behaviours.

> "one, it's fair on me, because when I'm around him, I've tried to separate myself from that whole group of people and start a new life…I'm still too fragile to be around, and still too vulnerable to be around you right now. And so I have explained that to all my friends." (Participant 6)

In keeping with their sensitivities to views and expectations of those around them, women who continued to drink alcohol during pregnancy were particularly interested in what other women in their social network thought was appropriate and were reassured by their responses.

> "I did feel quite guilty the first time I had a glass of wine. I thought, 'Oh god, what if it has an effect on the baby' and stuff like that. So I did do quite a lot of research around that. . . . I've had a chat with friends who've had babies and asked 'What did you do? Did you have any alcohol at all while you were pregnant? ' . . . [And] generally they would just say they had a few glasses here and there and they didn't think it did any harm. Their babies were all fine. So yeah, I made my own decision on it but it was kind of nice just to find out what other women had done." (Participant 2)

5.5.8.6 More confident the second time around

Three women had already had children and their attitude towards alcohol and dietary intake differed to those who were first time mothers. They also acknowledged that their attitude had changed considerably compared to their first pregnancy. Women described feeling more relaxed about their approach to food and drink in their current pregnancy, and referred to using their 'common sense' as a way to interpret guidance. Women recalled feelings of anxiety about whether they were following the right guidance during their first pregnancy. Having a healthy baby gave them confidence in their choices and ability to have another healthy pregnancy.

> "I think I have a pretty balanced diet this time round. And I don't worry. I think I worried with my first too much, I think I could have enjoyed it a bit more, and had more food that I enjoyed, instead of worrying. So I've definitely enjoyed this pregnancy more, because I just haven't worried too much about having like coke and crisps and stuff." (Participant 5)

> "I found it much harder to deal with that time, because of course first time round you don't really know. I mean I'm anxious now too, but first time around I was even more anxious, because I didn't know" (Participant 3)

In contrast, negative experiences in previous pregnancies were associated with stricter behaviour and more anxiety about choices regarding food and drink. One women had previously been pregnant and experienced a traumatic miscarriage at 5 months gestation and this had resulted in her feeling very protective and fearful of harm for her baby.

> "Yeah, I've become extremely protective really. I'm constantly looking things up online, am I doing things right, am I being good? Driving the midwife crazy with a million and one questions. Maybe a little bit too much, but it's all like healthy, it's all, because I just want the best." (Participant 6)

Other first time mothers also discussed negative experiences of their friends and how this has resulted in them having different attitudes towards diet and alcohol in their pregnancies.

> "I have a friend who's pregnant at the moment and she's had three miscarriages prior to now becoming pregnant with this one, so she is obviously a lot more aware of everything and she'll just kind of not have any alcohol, not have anything that says that you shouldn't, for the whole nine months. So I don't know if maybe I'm a little bit more relaxed because I have been so lucky." (Participant 2)

Second time mothers also discussed a lack of advice about diet and alcohol from healthcare professionals compared to their previous pregnancies.

> "I think they kind of think you know, when it's the second. But the first one, they do try and push it on you a lot with the diet." (Participant 5)

5.6 Discussion

The aim of Study 1B was to describe maternal dietary patterns and micronutrient intakes during pregnancy, explore their relationships with alcohol consumption patterns before and during pregnancy and gain insight into how these choices are made in a sample of pregnant women across the UK. This is the first study use a mixed method approach to do this.

Reported antenatal alcohol consumption was low in Study 1B, with women reporting to typically drink no more than 1-2 units per drinking occasion. This meant it was not possible to address all of the study's original aims. However, data was available on alcohol consumption prior to pregnancy, which enabled exploration of different patterns during this period.

There were a number of new findings from Study 1B. Firstly, prior to pregnancy, women who reported drinking more frequently also had significantly higher intakes of folate, choline and carotenoids. However, when quantity of alcohol was assessed, the intake of folate significantly decreased in women reporting 5 or more units per typical drinking occasion. Similar relationships were reported in a study conducted in the US with healthy, non-pregnant adults. Breslow et al (2006) explored alcohol consumption in relation to individual's Healthy Eating Index (HEI) score; a composite score based on the intakes of fruit, vegetables, red meat and dairy; a higher score typically indicates a better quality diet. The study found that as the frequency of alcohol consumption increased, so did peoples HEI scores, but when quantity of alcohol consumed increased, HEI scores decreased (Breslow et al. 2006).

The frequency of binge drinking prior to pregnancy was not associated with micronutrient and antioxidant intakes. This was unexpected, based on the current published evidence from investigations in non-pregnant populations. As discussed earlier, a number of studies have reported associations between binge drinking and poorer quality diets (Ruidavets et al. 2004; Nelson et al. 2009). One study conducted in Spain observed lower intakes of fruit and vegetables and higher intakes of meat in individuals that reported binge drinking, compared to those who did not (Valencia-Martin et al. 2011). This was particularly interesting as women in Study 1B who reported typically drinking 5 or more drinks per drinking occasion had lower intakes of folate. One possible reason for this is that some women may report drinking 5 or more drinks per occasion, but not consider themselves to be

binge drinking. The label is synonymous with drunk and raucous behaviour (Berridge et al. 2007); therefore, many women may not identify with this term.

While reported alcohol consumption decreased markedly during pregnancy, the prevalence was similar to what has been reported in national surveys; the 2010 infant feeding survey reported 64% of women in England did not drink alcohol during their pregnancy (Mc Andrew et al. 2012). Nevertheless, similar patterns did persist during pregnancy. Higher frequencies of alcohol consumption remained significantly associated with higher choline intakes, compared to women who reported no alcohol consumption. Rich sources of choline include meat, fish and eggs, and previous research has indicated that alcohol consumption is associated with an increase in these food groups. The results from Study 1A (Chapter 4) indicated there was good agreement between the FFQ and 24-HRs when measuring choline, suggesting this could be a genuine relationship. However, there are currently no UK food composition tables that report the usual choline content of food and drink items in the UK. Therefore, the values for choline and betaine are from US tables, which means concentrations may differ due to different animal breeds, ingredients and growing conditions.

When dietary patterns were explored to gain a broader picture of dietary intake, two dietary patterns were derived: the 'Prudent' and 'Cafeteria'. Studies with similar sample populations have derived comparable dietary patterns within their data. A study in the UK (n=198) derived a 'Prudent' dietary pattern score, which was characterised by high vegetable, fruit, wholegrain bread and pasta, cheese and fish intakes (Cole et al. 2009). Another study conducted in the UK (n=12,572) derived two very similar dietary patterns; the 'Prudent' dietary pattern, which was characterised by high fruit and vegetable intakes, and the 'High energy' dietary pattern, by high intakes of cakes, puddings and processed foods (Crozier et al. 2006).

One of the key findings from Study 1B included the relationship between increased frequency of alcohol and adherence to the 'Prudent' dietary pattern, before and during pregnancy. The 'Prudent' dietary pattern was characterised by high intakes of egg and fish, which may account for the correlation with choline intake. Women who were in the most frequent drinking category during pregnancy (2-3 times/week) were only drinking 1-2 units of alcohol per occasion, which is a relatively low level of alcohol consumption. Based on the evidence from animal models of FASD and choline supplementation (Thomas et al. 2004; Thomas, 2010), it may be plausible to suggest that the associations between low to moderate alcohol consumption during pregnancy and increased child IQ scores (Kelly et al. 2010) may be linked with maternal intakes of choline and folate.

No relationships were observed between the 'Cafeteria' dietary pattern and alcohol consumption, before or during pregnancy. This was unexpected, as a number of studies in non-pregnant populations have reported significant associations between alcohol consumption and diets characterised by high

intakes of processed foods and low intakes of fresh fruit and vegetables (Valencia-Martin et al. 2011). The lack of relationship could be attributable to a number of reasons. Firstly, the reported levels of alcohol consumption were low, both before and during pregnancy. Secondly, the 'Confectionery' dietary pattern was not strong and accounted for only 5% of variation in the data. Thirdly, this study recruited a small and homogenous sample population; a high proportion of women were well educated, married and in higher or managerial occupations.

Of particular concern was the association between increased adherence to the 'Cafeteria' dietary pattern and lower odds of folic acid supplement use. The dietary pattern was also weakly correlated with dietary folate intake. If women with low intakes of folate are also less likely to take folic acid supplements during pregnancy, this may increase the risk of children being born with NTDs (Wolff et al. 2009). Another interesting finding was the number of women who may have had alcohol-exposed pregnancies and be incorrectly categorised as non-drinkers. While the regression models revealed no relationships between these levels of exposures and dietary intakes, the descriptive two-by-two table did indicate that many women might be incorrectly categorised as non-drinkers, despite alcohol consumption during the early stages of pregnancy, prior to recognition. This finding highlights the challenges of accurately measuring alcohol exposure during pregnancy based on self-reported estimates.

The findings from Phase 2, the qualitative study, elaborated on some of the findings from the questionnaire. All women reported cutting down or stopping alcohol consumption once they found out they were pregnant. While all women accepted there was a risk of harm from heavy consumption, those who continued to drink low amounts believed there was little risk of harm from doing so. This has also been reported in previous studies. Two studies conducted in Australia, for example, reported that women who continued to drink low levels of alcohol during pregnancy perceived there to be very little or no harm from low levels of antenatal alcohol exposure (Meurk et al. 2014; Anderson et al. 2014).

One of the key findings from the interview phase was the important role that social context played in women's decision making process regarding diet and alcohol consumption during pregnancy. While all women reported changing their diet when they found out they were pregnant, none described extensive changes and most reported limited changes that were specific to their pregnancy. This was commonly attributed to an interest in nutrition prior to pregnancy or healthy eating habits learned at home while they were growing up or through a partner's interest in cooking. Those that continued to drink alcohol also described similar patterns of behaviour during pregnancy in their family and friends, although some also indicated that they refrained from drinking alcohol when they perceived this would not be acceptable to those present. The role of social and cultural context of health behaviours is well supported by a body of evidence (Hernandez & Blazer 2006; Burke et al. 2009; Taylor et al.

2009) and the influence of friends and family in decisions around diet and alcohol in pregnancy has been highlighted in several other qualitative studies (Raymond et al. 2009; Reyes et al. 2013; HPA 2014).

Another interesting finding was how previous experience of a successful pregnancy influenced behaviour around diet and alcohol consumption. Compared to participants in their first pregnancy, second time mothers were more relaxed in the way they interpreted guidance and advice and more confident in using their 'common sense' in making decisions about what to eat and drink. The relationship between parity and perceived risk during pregnancy has been reported in a number of other studies. Two qualitative studies conducted in the US and UK found that women who had previously given birth were more likely to drink alcohol during pregnancy (Testa & Reifman 1996; Raymond et al. 2009). The perception of risk may also account for the relationship observed between adherence to the 'Cafeteria' dietary pattern and increased parity. Similar findings have been reported in a number of other studies. A study conducted in Brazil found that as parity increased, adherence to dietary patterns characterised by either high intakes of processed meat, potatoes and soft drinks, or crisps and confectionery (Coelho et al. 2015). Another study conducted in Norway reported that nulliparous women were more likely to adhere to a dietary pattern characterised by high intakes of fruit, vegetables and whole grain cereals (Englund-Ögge et al. 2014).

All women reported receiving inconsistent advice from many different sources. This often resulted in women questioning the quality of the evidence or seeking out their own advice from family member or close friends with experience of previous pregnancies. Inconsistent advice has been reported as a barrier to alcohol abstinence in other studies. A study conducted in Australia reported similar findings and women described a need for clear, consistent guidance regarding alcohol consumption (Anderson et al. 2014). In line with Study 1B results, a qualitative study conducted in the UK reported that because of the contradictory guidance often given by healthcare professionals, women reached out to friends and family for advice about what message to trust (Raymond et al. 2009). This highlights the need for additional training to provide healthcare professionals with the support and skills in order to relay a clear and consistent message to women during pregnancy.

5.6.1 Strengths and limitations

This is the first study to explore relationships between maternal dietary patterns, micronutrient intakes and alcohol consumption during pregnancy and how these relationships develop. There are a number of strengths to this study. Firstly, the antenatal alcohol and dietary pattern data were collected contemporaneously, using the same reference period. Secondly, dietary data was collected using an FFQ that was purposefully designed to estimate the intake of folate, choline, betaine, vitamin C and carotenoids. The results from the previous chapter also provide details of the validity of the FFQ,

which is crucial to interpreting the relationships observed in this analysis. Thirdly, women were asked in detail about their dietary supplement use, as this can be a significant contributor to folate intake during pregnancy (Hodgetts et al. 2015). Finally, the qualitative data that has been collected compliments the dietary data by exploring why these choices are made.

However, there are also limitations. Some of the general limitations have already been addressed in Chapter 3. Firstly, the main limitation to this study is the low variance in reported quantities and frequencies of alcohol consumption during pregnancy, which means it was not possible to address some of the original aims of this study. Secondly, the small and homogenous sample consisting of only 114 women who reported any drinking during pregnancy means the study may not have statistical power to detect relationships between dietary pattern scores, micronutrient intakes and alcohol consumption. It is also important to note that the sample population in this study were highly educated, in professional occupations and lived in areas of the UK with relatively low levels of social deprivation. A systematic review found that high income and social class were often predictors of antenatal alcohol consumption (Skagerström et al. 2011) and a number of reviews have also reported a positive relationship between SES and diet quality during pregnancy (Hulshof et al. 2003; Mayen et al. 2014). Thirdly, the dietary patterns that have been derived only accounted for a small amount of the variation within the data. This may be due to the small sample size and could be responsible for the lack of relationships with alcohol consumption patterns. Finally, the sample population for the qualitative element of the study was very small and homogenous, meaning the results are not generalizable.

5.7 Conclusions

Overall, this study has highlighted several new and important findings that may provide insight when designing interventions to improve maternal health and fetal development. Understanding relationships between diet quality and alcohol consumption during pregnancy may highlight populations of women who are at increased risk of harming their unborn child, and understanding how these behaviours form may provide insight into how to design and deliver interventions. The results have suggested that low levels of alcohol consumption during pregnancy may be associated with better quality diets compared to women who report no alcohol consumption. The findings have also indicated that, prior to pregnancy, as frequency and quantity of alcohol consumption increase, intakes of particular micronutrient increase and decrease, respectively. However, the low levels of reported drinking mean it is not possible to draw any additional conclusions regarding other patterns of alcohol consumption. Findings from the qualitative phase to the study have suggested that decision making relating to diet and alcohol is complex and influenced by social context, previous pregnancy experience and consistent, clear advice is necessary for delivering a public health message in pregnancy. The study has also demonstrated the continued challenges of recruiting women who are

from ethic minority and low-income backgrounds. However, the results are a reminder that health behaviours often cluster and addressing diet or alcohol separately may not be the most effective method of improving health behaviours during pregnancy. The limitations of this study mean that further investigation is required to explore relationships between patterns of dietary intake and alcohol consumption during pregnancy before the potential clinical implications can be addressed.

Chapter 6. Exploration of dietary patterns and alcohol consumption in a sample of pregnant women in West England (Study 2A)

6.1 Introduction

The previous chapter presented the findings from Study 1B, an exploration into eating and drinking patterns in a sample of pregnant women from the across the UK. The results suggested that there might be an association between low levels of alcohol consumption during pregnancy and diets characterised by high intakes of fresh fruit, vegetables and low intakes of processed meats and confectionery. While Study 1B provided some interesting findings, the small sample size and low reported drinking levels meant it was not possible to address all of the original aims, which included an exploration of the relationships between maternal dietary intake and varying frequencies and quantities of alcohol consumption during pregnancy.

It is important that higher frequencies and quantities of antenatal alcohol consumption are explored in relation to diet, as evidence from non-pregnant populations suggests high quantities of alcohol consumed over short periods are associated with diets characterised by low intakes of fresh fruit, vegetables, seafood and diary, and high intakes of processed foods (Barefoot et al. 2002; Breslow et al. 2006; Valencia-Martin et al. 2011; Touvier et al. 2014), which are associated with lower intakes of important micronutrients (Northstone et al. 2008). Understanding whether these relationships are present during pregnancy is crucial, as results from animal models of FASD have indicated that harm to the fetus from alcohol consumption can be exacerbated in the presence of inadequate maternal dietary intakes (Cohen-kerem & Koren 2003; Ballard et al. 2012; May et al. 2014).

To address the original objectives described in Study 1B, a secondary analysis of data from the ALSPAC cohort was conducted. The main limitations of using the ALSPAC dataset are that the alcohol and dietary exposures were not measured contemporaneously or using validated data collection methods. Nonetheless, the large sample size, varied alcohol consumption levels and extensive measures of exposure and confounding variables means that many of the limitations of Study 1B can be overcome in order to address the original aims set out. Using data from Studies 1B and 2A provides a more comprehensive exploration of the research aims under investigation.

6.2 Aims and objectives

The overall aim of this study was to explore relationships between the frequency and quantity of maternal alcohol consumption, dietary patterns and micronutrient intakes during pregnancy, in a sample of women in West England, using data gathered as part of the ALSPAC cohort.

Specific objectives of the study included the following:

- To describe patterns of alcohol consumption (frequency and quantity) during early pregnancy
- To explore relationships between the frequency and quantity of alcohol consumption and relative intakes of OCM micronutrients (folate, choline, betaine, methionine, vitamin B12 and vitamin B6) and antioxidants (vitamin C, vitamin E and carotenoids) during pregnancy
- To replicate maternal dietary patterns previously described by Northstone et al. (2008)
- To explore relationships between maternal dietary patterns and daily intakes of OCM micronutrients (folate, choline, betaine, methionine, vitamin B12 and vitamin B6) and antioxidants (vitamin C, vitamin E and carotenoids)
- To explore relationships between maternal dietary patterns and folic acid supplement use during pregnancy
- To explore relationships between maternal dietary patterns and the frequency and quantity of alcohol consumption during pregnancy

6.3 Methods

6.3.1 Study design

Study 2A is a secondary analysis using data from the ALSPAC cohort, an on-going, population-based study that followed pregnant women and their children from eight weeks gestation to the present day. The main objective of the ALSPAC study was to "determine ways in which the individual genotype combines with environmental pressures to influence health and development" (Golding, Pembrey, Jones, et al. 2001).

6.3.2 Sample population

Women were deemed eligible for inclusion if they resided in a pre-defined area within the county of Avon, and their estimated delivery date was between 1st April 1991 and 31st December 1992. Initially, 14,541 pregnant women were recruited into the study; a total of 647 women were excluded, leaving 13,761 unique women enrolled, and a total of 14,062 live births (Fraser et al. 2013a). All recruitment procedures and data collection was designed and conducted by the ALSPAC team (Golding, Pembrey, Jones, et al. 2001).

Women were eligible for inclusion in the current analysis if they had a live, singleton birth (n=13,678), provided details of alcohol consumption at 18 weeks gestation (13,197) and had completed the FFQ at 32 weeks gestation (12,190).

6.3.3 Measures

Alcohol consumption

Women completed a questionnaire at 18 weeks gestation about their health and wellbeing and were asked to report their alcohol consumption at two time points: 1) during early pregnancy (over the first three months) and 2) mid-pregnancy (consumption over the past month, reported at 18 weeks gestation). Two measures of alcohol consumption were explored in this analysis:

1. *Frequency and quantity of alcohol consumption during early pregnancy*

Women were asked about the frequency of their alcohol consumption during the first three months of pregnancy. Responses included 'Never', 'Less than 1 glass per week', 'More than 1 glass per week', '1-2 glasses everyday', '3-9 glasses per day', and '10+ glass per day'. Due to low numbers in higher frequency categories, the responses were recoded into four groups: 'Less than once per week', 'More than once per week' and '1+ per day'. One glass was defined as one pub measure of spirits, one half pint of lager or cider, or one small glass of wine, which equates to approximately one unit (10ml/8g ethanol).

2. *Binge drinking during the second trimester (14 to 18 weeks gestation)*

Women were also asked how many days in the past month that they had drunk the equivalent of two pints of larger or cider, four glasses of wine, or four pub measures of spirit, equating to approximately four units (40ml ethanol). Responses included 'None', '1-2days', '3-4 days', '5-10 days', 'More than 10 days' and 'Everyday'. Due to low numbers of women who reported more than 2 episodes of binge drinking during mid-pregnancy, the variable was dichotomized into 'binge drinkers', defined as a woman who reported drinking four or more units on at least one day during the past month, and 'non-binge drinkers'. An intake of four or more units in one day is referred to binge drinking throughout this chapter, and is consistent with other secondary analyses using data from the ALSPAC study (Sayal et al. 2009; Alati et al. 2013)

Dietary data

At 32 weeks gestation women completed an FFQ and reported how often they have eaten and drunk 44 commonly consumed foods and drink items during their current pregnancy. The FFQ was based on a previous questionnaire developed by Yarnell et al. (1983) and was adapted to include certain food items based on the results of a weighed dietary survey published by Emmett et al. (1992).

The majority of the food items had the following available responses: 'Never/Rarely', 'Once in 2 weeks', '1-3 per week', '4-7 per week' and 'more than 1 per day'. The FFQ also included some more detailed questions about the preparation and cooking methods i.e. are fats removed from meat or not. Additional questions about the consumption of regularly consumed basic food and drink items,

such as bread, coffee, tea, sugar and fat spreads. Responses for bread consumption included 'Less than 1 slice per day', '1-2 slices per day', '3-4 slices per day' and '5 or more slices per day'. Women reported the average number of cups, teaspoons or portions consumed per day of coffee, tea, sugar and fat spread.

The ALSPAC team allocated standard portion sizes to food and drink items in the FFQ using a UK reference guide (Food Standards Agency 1988). Weekly nutrient intakes were calculated using the method outlined by Rogers and colleagues (Rogers et al. 1998). Weekly intake frequencies were coded as follows: 'Never/Rarely'= 0, 'Once in 2 weeks'= 0.5, '1-3 per week'= 2, '4-7 per week'= 5.5 and 'More than 1 per day'= 10, and were then multiplied by the assigned portion size. The weekly frequency of bread intake was coded as follows: 'Less than 1 per day'= 0, '1-2 per day'= 10.5, '3-4 per day'= 24.5 and '5 or more per day'= 42, and multiplied by the standard portion size. The weekly intake of milk was calculated by summing standard intakes from tea, coffee, cereal, puddings and drinks. Tea, coffee, table sugar and cola intake were calculated by multiplying the daily number of cups, spoons or glasses by the assigned portion size. Estimates of daily nutrient intakes were calculated by multiplying weekly portion sizes by nutrient concentrations using UK dietary composition tables (see formula below) (McCance & Widdowson 2002). However, UK composition tables do not include the concentrations of choline, betaine or methionine, therefore, these values were calculated using US dietary composition tables (Patterson et al. 2008). Mean daily nutrient intakes were obtained by dividing weekly nutrient intakes by seven:

Daily intake = Concentration per g x (portion size (g) x weekly frequency) / 7

A dietary pattern is a group of food and drink items that, based on the data, are commonly consumed together by groups of people (i.e. people that eat a lot of broccoli may also be more likely to eat a lot of cabbage or carrots). Dietary patterns are not mutually exclusive; instead, they provide a score that indicates a woman's adherence to that particular pattern. Therefore, a woman may have a high adherence scores to both the 'Processed' and 'Confectionery' dietary patterns.

Socio-demographic characteristics

Details of potential confounders were self-reported at 8 and 18 weeks gestation:

- Age: Maternal age at delivery was calculated from the date of births of the mother and baby, and were grouped into the following four age categories: 'Less than 20', '20-24', '25-29' and '30 or more' years of age.
- Parity: Maternal parity was dichotomized as 'Primiparous', if this was the woman's' first pregnancy, and 'Multiparous', if women had given birth previously.

- Ethnicity: The vast majority of women were white British, so a mother's ethnic background was dichotomised as 'white' or 'non-white'.
- Smoking: Data on maternal smoking was reported at 8 weeks gestation, and women were categorised as 'Smokers', defined as a women reporting to smoke any number of cigarettes during pregnancy, or 'Non-smokers'.
- Education: Women's educational attainment was categorised as 'Vocational', 'O-level', 'A-level' and 'Degree level'.
- Single parent household: Women reported whether their partner lived with them or not.
- Housing tenure: Women reported whether they owned or rented their home. 'Own/mortgaged' if a woman reported owning or having mortgaged her home, 'Council, HA renting' if a women reported living in a home rented from the local council or Housing Association, and 'private renting' if a woman reported living in a home privately rented.
- House Crowding Index (HCI): HCI is calculated by dividing the total number of household residents by the total number of rooms (excluding kitchen and bathrooms). Categories included '<0.05', '≥0.5 - 0.75', '>0.75 - 1' and '>1'.
- Maternal depression: Assessed at 18 weeks using the Edinburgh Postnatal Depression Scale (EPDS), a validated and widely used method to diagnose women at risk of depression (Murray & Cox 1990). The EPDS scores range from 1-30, with a score of 13 or above indicating possible depression; therefore, EPDS scores were dichotomised as 'Not at risk', indicating a score below 13, and 'At risk', indicating a score of 13 or more.
- Folic acid supplement use: Assessed at 18 weeks gestation. Women reported if they had ever taken folic acid supplements during their pregnancy. Variable was binary, with 'Yes' and 'No' answer.

6.3.4 Statistical analysis

Participant characteristics are summarised as frequencies and percentages, and dietary pattern scores are summarised as means and standard deviations. Continuous variables with a non-normal distribution were log-10 transformed for the analysis and back transformed for presentation. Unadjusted and adjusted ordinal and logistic regression models were used to explore the relationships between socio-demographic characteristics and both frequency of regular alcohol consumption and binge drinking during pregnancy, respectively. Drinking patterns were treated as dependent variables and potential confounders as independent variables.

Intakes of OCM micronutrients and antioxidants were explored in relation to alcohol consumption patterns using linear regression models. Separate models were run for each micronutrient and antioxidant, and intakes were treated as continuous, dependent variables. Alcohol consumption patterns were entered as categorical, independent variables. To explore the influence of potential

confounders, maternal age, parity, ethnicity, smoking, education, HCI, home ownership status, EPDS score and whether women lived in a single parent household were added to the models as covariates.

Dietary patterns were replicated using the methods previously described by Northstone and colleagues (2008). Briefly, the frequency of food and drink item intakes were standardised by calculating z-scores (frequency of intake minus the mean and divided by the standard deviation). PCA was performed on the standardised food and drink items, and five factors were chosen to best represent the dietary data. A Varimax rotation was applied to the data in order to redistribute and maximise the variance, therefore, creating a simplified structure that is easier to interpret. Factor loadings represent the correlation between the original dietary variable and the factor (dietary pattern). Food items with a factor loading of ≥0.3 or ≤-0.3 suggested a strong positive or negative association, respectively, and were considered significant descriptive characteristics (principal components) of that dietary pattern. For each dietary pattern, a corresponding component score was calculated, which represented adherence to that dietary pattern. Dietary pattern scores have a mean of zero; a value above or below zero indicates stronger or weaker adherence to that dietary pattern, respectively, compared to the mean. The dietary pattern component scores were treated as continuous variables.

To assess the relationship between nutrient density and each of the five dietary patterns, Pearson's correlation coefficients were calculated for each dietary pattern scores and mean daily OCM micronutrient and antioxidant intakes. Correlation was defined as very strong if >0.80, strong if 0.60 – 0.79, moderate if 0.40 – 0.59, weak if 0.20 – 0.39, and negligible if >0 - <0.20 (Dancey & Reidy 2004).

Univariate analyses were initially conducted to explore the relationships between dietary pattern score and alcohol consumption. The relationships between dietary pattern scores and frequency of regular alcohol consumption during pregnancy were explored using ANOVAs. Relationships between dietary pattern scores and binge drinking during pregnancy were explored using t-tests.

Linear regression models were used to explore relationships between dietary pattern scores and patterns of alcohol consumption. Dietary pattern scores were treated as continuous, dependent variables and alcohol consumption patterns were entered as categorical, independent variables. Separate models were run for each dietary pattern score and to explore the influence of potential confounders, maternal age, parity, ethnicity, smoking, education, HCI, home ownership status, EPDS score and whether women lived in a single parent household were added to the models as covariates.

Complete case analyses presented in this chapter were conducted on 11,457 study women. All analyses were conducted using STATA 13.1.

6.3.5 Missing data

Women with more than 10 food and drink items missing were excluded from the present analysis. Women with 10 or less missing items were included, and the items were recoded as 'Never/rarely' (0).

6.3.6 Ethical approval

Ethical approval for the present analysis was obtained from the ALSPAC ethics committee in April 2014. (Ref: B2198)

6.4 Results

6.4.1 Sample population

The socio-demographic characteristics of women are presented in Table 6.1. Overall, women from lower socio-economic backgrounds and those of non-white ethnic origin were underrepresented in this study sample; 14% lived in council or Housing Association rented accommodation, 6% scored >1 on the HCI, and 2% were from ethnic minority backgrounds. Women were more likely to be older, with almost 80% of the sample aged 25 years or older. The majority of women were educated to at least O-level, and 13% held a university degree. The majority of women were non-smokers and lived in a household with two parents, owned or had mortgaged their own home, and lived in un-crowded conditions. A total of 966 (8%) reported taking folic acid supplements during the first three months of pregnancy. The EPDS scores indicate that 15% of women were at risk of depression, which was defined as scoring 13 or more; this is slightly lower than the current national average of approximately 22% for this age group (Beaumont & Lofts 2013).

Table 6.1 Socio-demographic characteristics of sample population (n=11,457)

		n	%
Age (years)	<20	427	4
	20-24	1,990	17
	25-29	4,516	39
	30+	4,523	40
Parity	Primiparous	5,073	45
	Multiparous	6,194	55
Ethnicity	Non-white	11,059	2
	White	274	98
Maternal smoking	Smoker	2,034	19
	Non-smoker	8,714	81
Education	Vocational	3,292	29
	O-level	3,990	35
	A-level	2,618	23
	Degree level	1,495	13
Single parent household	Yes	649	6
	No	10,596	94
Home ownership	Owner/occupied	8,697	76
	Council/HA rented	1,650	14
	Private rent/other	1,149	10
House Crowding Index	≤0.5	4,825	44
	>0.5-0.75	3,496	32
	>0.75-1	2,088	19
	>1	636	6

6.4.2 Maternal alcohol consumption during pregnancy

Table 6.2 presents the frequency and quantity of alcohol consumption during early pregnancy and binge drinking during mid-pregnancy. Approximately 45% of women reported never drinking alcohol during early pregnancy and 2% reported drinking one or more drinks per day during early pregnancy. A total of 859 (8%) women reported binge drinking on at least one day in the previous month during mid-pregnancy.

Results from logistic regression models indicated that any alcohol consumption in pregnancy was significantly associated with being 30 to 39 years old (OR=1.65, 95%CI=1.28, 2.12; p<0.0001), having a bachelor's degree (OR=1.34, 95%CI=1.16, 1.56; p<0.0001), being multiparous (OR=1.17, 95%CI=1.06, 1.30; p=0.003), white (OR=1.51, 95%CI=1.12, 2.04; p=0.007) and smoking during pregnancy (OR=1.01, 95%CI=1.00,1.01; p<0.0001). In contrast, binge drinking during pregnancy was significantly associated with being 30 to 39 years old (OR=2.06, 95%CI=1.27, 3.33; p=0.003), smoking during pregnancy

(OR=1.01, 95%CI=1.01, 1.02; p<0.0001), living in rented accommodation (OR=1.39, 95%CI=1.08, 1.80; p=0.011), living in crowding accommodation (OR=1.39, 95%CI=1.01, 1.91; p=0.044), and scoring 13 or more on the EPDS (OR=1.41, 95%CI=1.16, 1.70; p<0.0001).

Table 6.2 Frequency and quantity of alcohol consumption during pregnancy (n=11,457)

		n	%
Alcohol consumption during early pregnancy	Never	5,157	45
	<1 drink/week	4,508	39
	≥1 drink/week	1,591	14
	1+ drink/day	201	2
Binge drinking during past month	Yes	859	8
	No	10,509	92

6.4.3 Maternal alcohol consumption and micronutrient intakes during pregnancy

The energy adjusted coefficients presented in Tables 6.3, 6.4 and 6.5 indicate that as alcohol consumption increased, the mean daily intakes of folate (Figure 6.1) and vitamin B6 (Figure 6.2) decreased, compared to women who reported no drinking during early pregnancy. Binge drinking during mid-pregnancy was associated with significantly lower intakes of folate (Figure 6.3), vitamin B6, vitamin C and vitamin E, compared to women who did not report binge drinking. After maternal age, parity, ethnicity, education, smoking, single parent household, housing tenure, HCI and EPDS scores were added as covariates, relationships were no longer significant.

Table 6.3 Energy adjusted coefficients of maternal folate, choline and betaine intake by alcohol consumption during pregnancy

	Folate (ug)			Choline (mg)			Betaine (mg)		
	β	95% CI	p	β	95% CI	p	β	95% CI	p
Alcohol consumption during early pregnancy									
Never		(ref)			(ref)			(ref)	
<1 drink/week	-1.40	(-3.47, 0.67)	0.18	0.04	(-2.11, 2.20)	0.97	-0.06	(-4.01, 3.89)	0.98
≥1 drink/week	-5.85	(-8.76, -2.94)	0.00	-1.84	(-4.87, 1.20)	0.24	-10.44	(-16.00, -4.89)	0.00
1+ drink/day	-16.02	(-23.32, -8.72)	0.00	2.35	(-5.25, 9.95)	0.54	-5.16	(-19.08, 8.77)	0.47
Binge drinking during pregnancy									
No		(ref)			(ref)			(ref)	
Yes	-9.35	(-12.93, -5.77)	0.00	-0.70	(-4.44, 3.04)	0.71	6.83	(-0.02, 13.67)	0.06

β= beta coefficient, 95% CI= 95 % confidence intervals, p= p-value
Adjusted for mean energy intakes

Table 6.4 Energy adjusted coefficients of maternal methionine, vitamin B12 and vitamin B6 intake by alcohol consumption during pregnancy

	Methionine (g)			Vitamin B12 (ug)			Vitamin B6 (mg)		
	β	95% CI	p	β	95% CI	p	β	95% CI	p
Alcohol consumption during early pregnancy									
Never		(ref)			(ref)			(ref)	
<1 drink/week	0.01	(0.00, 0.02)	0.05	0.13	(0.03, 0.23)	0.01	-0.01	(-0.02, 0.00)	0.16
≥1 drink/week	-0.01	(-0.02, 0.01)	0.51	0.19	(0.05, 0.32)	0.01	-0.05	(-0.07, -0.03)	0.00
1+ drink/day	0.01	(-0.03, 0.05)	0.72	0.26	(-0.08, 0.60)	0.13	-0.15	(-0.20, -0.10)	0.00
Binge drinking during pregnancy									
No		(ref)			(ref)			(ref)	
Yes	-0.01	(-0.03, 0.00)	0.14	0.06	(-0.11, 0.22)	0.50	-0.05	(-0.08, -0.03)	0.00

β= beta coefficient, 95% CI= 95 % confidence intervals, p= p-value
Adjusted for mean energy intakes

Table 6.5 Energy adjusted coefficients of maternal vitamin C, vitamin E and carotenoid intakes by alcohol consumption during pregnancy

	Vitamin C			Vitamin E			Total carotenoids		
	β	95% CI	p	β	95% CI	p	β	95% CI	p
Alcohol consumption during early pregnancy									
Never		(ref)			(ref)			(ref)	
<1 drink/week	-0.02	(-1.33, 1.29)	0.98	0.07	(-0.07, 0.21)	0.34	-119.84	(-2.53.03, 13.36)	0.08
≥1 drink/week	-1.29	(-3.13, 0.55)	0.17	-0.04	(-0.24, 0.16)	0.68	-173.39	(-360.78, 14.00)	0.07
1+ drink/day	-1.36	(-5.99, 3.26)	0.56	-0.37	(-0.87, 0.12)	0.14	-205.89	(-675.75, 263.96)	0.39
Binge drinking during pregnancy									
No		(ref)			(ref)			(ref)	
Yes	-6.07	(-8.33, -3.80)	0.00	-0.46	(-0.71, -0.22)	0.00	-154.08	(-384.37, 76.22)	0.19

β= beta coefficient, 95% CI= 95 % confidence intervals, p= p-value
Adjusted for mean energy intakes

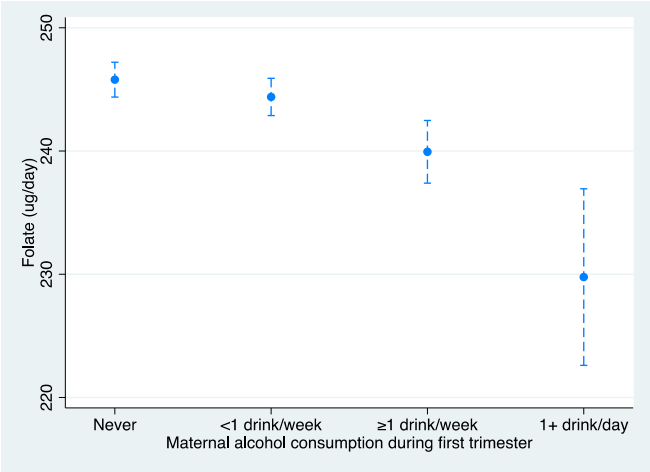

Figure 6.15 Energy adjusted daily intakes of maternal folate (ug) by alcohol consumption during early pregnancy

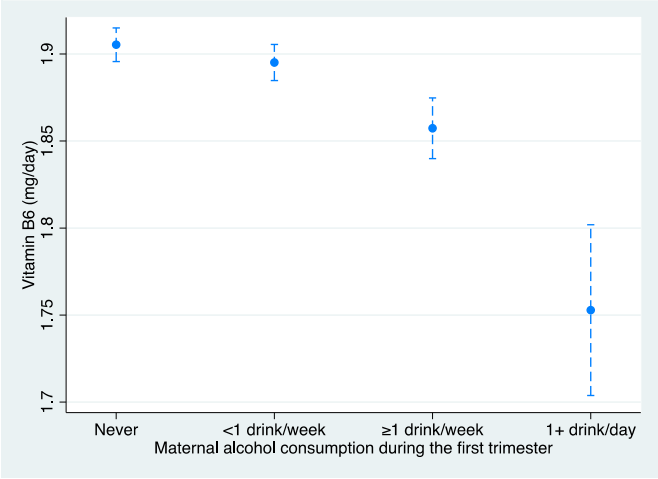

Figure 16.2 Energy adjusted daily intakes of maternal vitamin B6 (mg), by alcohol consumption during early pregnancy

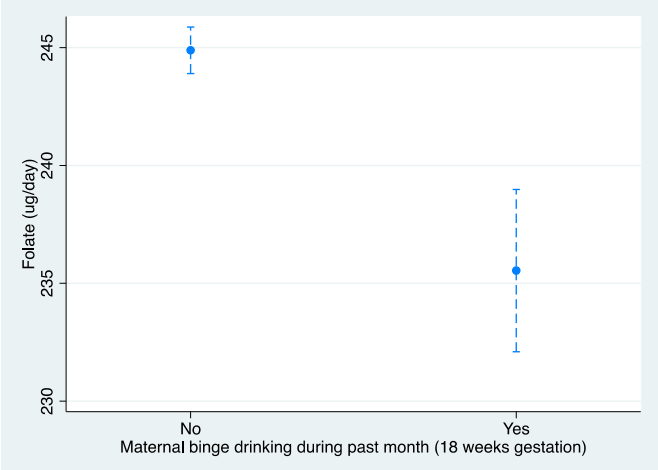

Figure 6.3 Energy adjusted daily intakes of maternal folate (ug) by binge drinking during mid-pregnancy

6.4.4 Maternal dietary patterns

The five dietary patterns derived from these data have been previously described (Northstone et al. 2008). The analyses were replicated here and the factor loadings for specific types off foods are presented in Table 6.6. The five replicated dietary patterns are characterised by the following:

- **Health conscious:** The 'Health conscious' dietary pattern accounted for 10.6% of variation and was characterised by in the intake of whole grains, fruits, vegetables, fish and a low intake of white bread.
- **Traditional:** The 'Traditional' dietary pattern accounted for 8.2% of variation and was characterised by a high intake of green vegetables and potatoes.
- **Processed:** The 'Processed' dietary pattern accounted for 4.9% of variation and was characterised by high intakes of white bread, fried foods, processed meats (sausage rolls, pies etc.) and low intakes of whole grains
- **Confectionery:** The 'Confectionery' dietary pattern accounted for 4% of variation and was characterised by high intakes of chocolate, sweets and biscuits
- **Vegetarian:** The 'Vegetarian' dietary pattern accounted for 3.6% of the variation and was characterised by low intakes of meat and high intakes of meat substitutes, nuts and pulses.

Table 6.6 Factor loadings from principal components analysis with varimax rotation for maternal dietary patterns*

	Dietary patterns				
	Health conscious	Traditional	Processed	Confectionery	Vegetarian
Bread/pasta/rice					
White bread	**-0.54**	0.08	**0.36**	0.09	-0.02
Other bread	**0.62**	-0.05	**-0.32**	-0.06	0.03
Rice	**0.54**	0.08	0.13	-0.12	0.06
Pasta	**0.58**	0.05	0.14	-0.07	0.12
Breakfast cereals					
Oat-based cereals	**0.30**	0.11	-0.04	0.05	0.14
Bran-based cereals	**0.37**	0.09	-0.12	0.00	0.00
Other cereals	-0.11	-0.01	0.14	0.22	-0.08
Animal produce					
Sausages	-0.09	-0.06	**0.56**	0.03	-0.17
Pies	-0.11	-0.03	**0.54**	0.09	-0.12
Red meat	0.15	0.22	0.17	0.10	-0.59
Offal	0.09	0.09	0.25	-0.07	0.09
Poultry	0.27	0.02	0.12	0.02	-0.54
Fish	**0.46**	0.16	0.14	-0.08	-0.02
Eggs	0.28	0.09	**0.40**	-0.03	-0.01
Cheese	**0.44**	0.08	0.05	0.12	0.03
Vegetables					
Boiled potatoes	0.25	**0.32**	0.11	0.07	-0.22
Roast potatoes	-0.27	0.22	**0.39**	0.15	-0.17
Baked beans	0.01	0.05	**0.41**	0.08	0.04
Carrots	0.18	**0.70**	-0.02	0.02	0.00
Peas	0.18	**0.35**	0.19	0.07	-0.10
Leafy green vegetables	0.04	**0.81**	0.01	-0.02	0.04
Root vegetables	0.09	**0.61**	0.02	0.00	0.10
Other vegetables	0.15	**0.80**	-0.04	-0.01	0.05
Salad vegetables	**0.42**	0.21	-0.08	-0.02	0.10
Fruit/fruit juice					
Fresh fruit	**0.52**	0.18	-0.23	0.09	0.00
Fruit juice	**0.49**	0.09	-0.09	0.09	0.06

Table 6.6 (Cont.) Factor loadings from principal components analysis with varimax rotation for maternal dietary patterns*

	Dietary patterns				
	Health conscious	Traditional	Processed	Confectionery	Vegetarian
Soy/pulses/nuts					
Meat substitutes (soy etc)	0.18	0.07	0.12	-0.03	**0.58**
Pulses	**0.35**	0.15	0.01	-0.06	**0.57**
Nuts	0.28	0.12	0.05	0.05	**0.53**
Fried foods					
Pizza	0.23	-0.11	**0.35**	0.10	0.11
Chips	-0.26	-0.06	**0.56**	0.24	-0.04
Fried foods	-0.10	0.00	**0.58**	0.17	-0.01
Crisps	-0.10	-0.04	0.29	**0.38**	0.01
Sweet foods					
Cake	0.20	0.00	0.08	**0.56**	-0.08
Crisp bread	0.22	0.08	-0.01	0.05	0.15
Biscuits	0.11	0.02	-0.01	**0.60**	-0.11
Chocolate bars	-0.08	-0.02	0.10	**0.75**	0.02
Chocolate	0.00	0.02	0.04	**0.72**	0.06
Puddings	0.26	0.06	0.12	**0.39**	-0.11
Sweets	-0.10	0.07	0.07	**0.51**	0.06
Drinks					
Tea	-0.10	0.07	0.16	0.03	-0.04
Coffee	-0.16	0.05	0.11	0.00	-0.04
Cola	-0.21	-0.08	0.23	0.14	0.06
Herbal tea	0.20	0.07	-0.09	-0.06	**0.31**

The associations between mean daily intakes of OCM micronutrients and antioxidants and dietary pattern scores are presented in Table 6.7. The 'Health conscious' dietary pattern was moderately positively correlated with the intake of folate (r=0.55) and vitamin C (r=0.56) and weakly positively correlated with remaining OCM micronutrients with the exception of betaine (r=-0.18), which was negligible. The 'Traditional' dietary pattern was strongly positively correlated with carotenoids (r=0.62) and moderately positively correlated with folate (r=0.50) and vitamin C (r=0.49). The 'Processed' and 'Confectionery' dietary patterns showed weak positive correlation with some OCM micronutrients and negligible correlation with dietary antioxidants. The 'Vegetarian' dietary pattern showed weak negative correlation with OCM micronutrients and negligible correlation with antioxidant intakes.

Table 6.7 Pearson's correlation coefficients of the relationship between maternal dietary patterns and mean daily nutrient intakes (n=11,457)

	Maternal dietary patterns				
	Health Conscious	Traditional	Processed	Confectionery	Vegetarian
Folate	0.55	0.50	0.17	0.24	0.01
Choline^	0.42	0.37	0.30	0.19	-0.13
Betaine^	-0.18	0.11	0.31	0.06	-0.02
Methionine^	0.46	0.24	0.22	0.15	-0.21
Vitamin B12^	0.38	0.20	0.29	0.01	-0.15
Vitamin B6^	0.48	0.37	0.30	0.28	-0.20
Vitamin C	0.56	0.49	-0.09	0.11	0.03
Vitamin E^	0.49	0.18	0.15	0.27	0.09
Total carotenoids^	0.27	0.62	-0.03	0.02	0.05

^log transformed

Results from the logistic regression model exploring relationships between maternal dietary patterns and folic acid supplements are presented in Table 6.8. Adherence to the 'Health conscious' dietary pattern was significantly associated with higher odds of folic acid supplement use before 18 weeks gestation; women in quartile four were almost twice as likely as those in quartile one to take folic acid supplements (OR=1.95, 95%CI=1.60, 2.37; p<0.0001). Women in quartile four of the 'Vegetarian' dietary pattern were almost 50% more likely to have taken folic acid supplements by week 18 compared to women in quartile one (OR=1.47, 95%CI=1.22, 1.78; p<0.0001). Adherence to the 'Traditional', 'Processed' and 'Confectionery' dietary pattern was not associated with folic acid supplement use in early pregnancy.

Table 6.8 Unadjusted odds of taking folic acid supplements during early pregnancy by quartiles of maternal dietary pattern scores

	OR	95% CI	
Health conscious			
Q1		(Ref)	
Q2	1.18	0.95	1.45
Q3	1.50***	1.22	1.83
Q4	1.95***	1.60	2.37
Traditional			
Q1		(Ref)	
Q2	1.01	0.84	1.22
Q3	1.09	0.90	1.31
Q4	1.01	0.84	1.22
Processed			
Q1		(Ref)	
Q2	1.17	0.98	1.40
Q3	1.15	0.95	1.38
Q4	0.90	0.74	1.09
Confectionery			
Q1		(Ref)	
Q2	0.98	0.81	1.18
Q3	1.03	0.85	1.24
Q4	1.07	0.89	1.29
Vegetarian			
Q1		(Ref)	
Q2	1.28**	1.05	1.55
Q3	1.08	0.88	1.33
Q4	1.47***	1.22	1.78

*p <0.05

**p <0.01

*** p<.0001

6.4.5 Maternal alcohol consumption and dietary patterns during pregnancy

In unadjusted linear regression models (Table 6.9), women who reported drinking less than one drink per day were significantly more likely to adhere to the 'Health conscious' dietary pattern, compared to women who reported they had never consumed alcohol during the first trimester (β=0.12, 95% CI=0.06, 0.17;p<0.0001). A similar trend was observed between alcohol consumption and 'Confectionery' dietary pattern scores (β= 0.10, 95% CI= 0.04, 0.16; p<0.0001). Alcohol consumption during the first trimester was significantly associated with adherence to the 'Processed' dietary pattern. This relationship was particularly apparent for women who reported drinking one or more drinks per day (β=0.17, 95% CI=0.03, 0.31; p<0.05). Women who reported drinking less than one drink

per week during the first trimester were less likely to adhere to the 'Vegetarian' dietary pattern compared to women who did not report drinking alcohol during the same period (β =-0.08, 95%CI=-0.12, -0.04; p<0.0001). Interestingly, women who reported drinking one or more drinks per day during the first trimester were significantly more likely to adhere to the 'Vegetarian' dietary pattern compared to women who did not drink during the same period (β =0.23, 95%CI=0.09, 0.37; p<0.001).

The results from the multivariate linear regression models are presented in Table 6.10. The relationship between frequency of alcohol consumption during the first trimester and adherence to the 'Health conscious' dietary pattern diminished once all other covariates were added to the model. Drinking one or more drinks per week remained significantly associated with increased adherence to the dietary pattern compared to never drinking during the first trimester; however, the difference in dietary pattern score was small (β=0.08, 95%CI=0.03, 0.14; p<0.001). Adherence to the 'Traditional' dietary pattern remained negatively associated with alcohol consumption during the first trimester, this was particularly apparent in women who reported one or more drinks per week during the first trimester (βt=-0.09, 95%CI=-0.15, -0.02; p<0.001). Drinking more than one drink per day during the first trimester remained significantly associated with adherence to the 'Processed' (β=0.19, 95%CI=0.03, 0.34; p<0.05) and 'Confectionery' (β=0.14, 95%CI=0.07, 0.20; p<0.0001) dietary patterns, after adjusting for confounders. Relationships between first trimester alcohol consumption and 'Vegetarian' dietary pattern scores diminished with the addition of all other covariates. Drinking less than one drink per week remained significantly associated with lower adherence to the dietary pattern compared to never drinking, however, this difference was small (β=-0.06, 95%CI=-0.10, -0.02); p<0.001).

The results from the unadjusted linear regression models in Table 6.9 indicate that at least one episode of binge drinking during pregnancy is associated with low adherence to the 'Health conscious' (β=-0.28. 95%CI=-0.35, -0.21; p<0.0001) and 'Confectionery' (β =-0.07, 95%CI=-0.14, -0.01; p<0.05) dietary patterns, compared to women who did not report binge drinking during the same period. The opposite trends were observed for the 'Processed' (β =0.23, 95%CI=0.17, 0.30; p<0.0001) and 'Vegetarian' (β=0.10, 95%CI=0.03, 0.16; p<0.001) dietary patterns. When all other covariates were added to the models, the relationships weakened, but similar trends remained (Table 6.10). At least one episode of binge drinking remained significantly associated with lower adherence to the 'Health conscious' (β=-0.10, 95%CI=-0.17, -0.03; p<0.001) and 'Confectionery' (β=-0.11, 95%CI=-0.20, -0.03; p<0.001) dietary patterns, and women who reported at least one occasion of binge drinking were more likely to adhere to the 'Processed' dietary pattern (β =0.12, 95%CI=0.04, 0.19; p<0.001), compared to women who did not report binge drinking.

Table 6.9 Unadjusted beta-coefficients of maternal dietary pattern score by alcohol consumption during pregnancy

	Health conscious			Traditional			Processed			Confectionery			Vegetarian		
	β	95% CI		β	95% CI		β	95% CI		β	95% CI		β	95% CI	
Alcohol consumption during early pregnancy															
Never		(ref)			(ref)			(ref)			(ref)			(ref)	
<1 drink/week	0.10***	0.06	0.14	-0.01	-0.05	0.03	0.01	-0.03	0.05	0.07**	0.03	0.11	-0.08***	-0.12	-0.04
≥1 drink/week	0.12***	0.06	0.17	-0.05	-0.11	0.00	0.08**	0.03	0.14	0.10***	0.04	0.16	0.02	-0.03	0.08
1+ drink/day	-0.10	-0.23	0.04	0.09	-0.05	0.22	0.17*	0.03	0.31	-0.07	-0.21	0.07	0.23**	0.09	0.37
Binge drinking during pregnancy															
No		(ref)			(ref)			(ref)			(ref)			(ref)	
Yes	-28***	-0.35	-0.21	0.05	-0.02	0.11	0.23***	0.17	0.30	-0.07*	-0.14	-0.01	0.10**	0.03	0.16

HC='Health conscious', T='Traditional', P='Processed', C='Confectionery', V='Vegetarian'

*p <0.05

**p <0.01

*** p<.0001

Table 6.10 Adjusted beta-coefficients of maternal dietary pattern score by alcohol consumption during pregnancy

	Health conscious			Traditional			Processed			Confectionery			Vegetarian		
	β	95% CI		β	95% CI		β	95% CI		β	95% CI		β	95% CI	
Alcohol consumption during early pregnancy															
Never		(ref)			(ref)			(ref)			(ref)			(ref)	
<1 drink/week	0.02	-0.02	0.06	-0.03	-0.07	0.01	0.04*	0.00	0.079	0.08**	0.03	0.12	-0.06**	-0.10	-0.02
≥1 drink/week	0.08**	0.03	0.14	-0.09**	-0.15	-0.02	0.06*	0.00	0.120	0.14***	0.07	0.20	0.01	-0.06	0.08
1+ drink/day	0.01	-0.13	0.16	-0.03	-0.20	0.14	0.19*	0.03	0.342	0.02	-0.15	0.20	0.07	-0.10	0.25
Binge during pregnancy															
No		(ref)			(ref)			(ref)			(ref)			(ref)	
Yes	-0.10**	-0.17	-0.03	0.08*	0.00	0.17	0.12**	0.04	0.191	-0.11**	-0.20	-0.03	0.05	-0.03	0.14

HC='Health conscious', T='Traditional', P='Processed', C='Confectionery', V='Vegetarian'
*p <0.05
**p <0.01
*** p<.0001

6.5 Discussion

This study was the first to explore relationships between varying levels of alcohol consumption and dietary patterns during pregnancy. The findings suggest that women who report drinking in potentially harmful patterns (one or more alcoholic drinks per day or at least one occasion of binge drinking in the past month) had increased adherence to dietary patterns that are associated with lower intakes of dietary antioxidants and micronutrients involved in OCM.

The dietary patterns analysed in this study have been previously described elsewhere (Northstone et al. 2008) and were consistent with the dietary patterns derived in Study 1B (Chapter 5) and throughout the wider literature (Crozier et al. 2006; Knudsen et al. 2008; Völgyi et al. 2013). A study in Southampton, UK, described two major dietary patterns within their study sample; the 'Prudent' dietary pattern, which was similar to the 'Health conscious' pattern in this sample, and the 'High-energy' pattern, which appeared to be a mixture between the 'Traditional' and 'Confectionery' patterns in this cohort (Crozier et al. 2006). Both patterns described by Crozier and colleagues were also similar in terms of nutrient density. The 'Prudent' dietary pattern was positively correlated with folate (r=0.59) and vitamin C (r=0.51) intake, whereas the correlation between the 'High-energy' dietary pattern scores and intakes of folate (r=0.26) and vitamin C (r=0.27) were much weaker (Crozier et al. 2006).

The reported levels of any antenatal alcohol consumption in the current sample (55%) were slightly lower than other published figures from the same time period. Results from the 1995 infant feeding survey report that approximately 67% of pregnant women in the UK drank alcohol during the antenatal period (Foster et al. 1997). It is likely that this difference is due to the measurement period. The infant feeding study reports any alcohol consumption, during the entire antenatal period, whereas ALSPAC measured alcohol consumption during particular time points during the pregnancy. It is possible that some women may be categorised incorrectly as non-drinkers or non-binge drinkers because the ASLPAC questionnaire did not capture alcohol consumption during particular time points during the pregnancy.

Potentially harmful patterns of drinking (one or more drinks per day or binge drinking) were associated with lower intakes of micronutrients, compared to women who did not report alcohol consumption. The absolute difference in reported intakes are very small, however, the FFQ only provides only a crude estimate of intake; it is possible that the differences in micronutrient intakes are much greater. A number of studies exploring micronutrient intakes and alcohol consumption in the general population reported similar relationships. A study conducted in France analysed FFQ dietary data from approximately 73,000 adult women. The authors reported that drinking more than 8g of ethanol per day – which is the equivalent to drinking one or more alcoholic drinks per day – was associated with

significantly lower mean daily intakes of vitamin C and β-carotene compared to women who drank lower amounts (Kesse et al. 2001).

When evaluating the relationships between dietary patterns and alcohol consumption, the results indicated that pregnant women who were drinking one or more alcoholic drinks per day, or reported at least one occasion of binge drinking, were more likely to adhere to the 'Processed' dietary pattern, compared to women who reported lower alcohol consumption levels. Similar findings have been presented in the current available literature on adult, female populations. The intakes described in the study by Kesse et al. (2001) are very similar to the 'Processed' dietary pattern analysed in this study, which was characterised by high intakes of meat and eggs, and lower intakes of fresh fruit. Another study conducted in France also reported similar relationships. Touvier et al. (2014) reported that women consuming alcohol were less likely to eat 400g of fruit and vegetables and more likely to eat 500g or more of red meat per week, compared to non-drinkers.

A number of other studies have also looked at heavy episodic patterns of drinking and found similar results. A study conducted in the USA (Breslow et al. 2006) explored the drinking habits and Healthy Eating Index (HEI) scores of 772 women (HEI scores represent an individual's adherence to various recommended dietary guidelines in the US). The mean HEI score decreased by 5.6 points in women who consumed 3 or more drinks per occasion, compared to women who reported one per occasion, after adjusting for socio-demographic characteristics. A study conducted in Madrid, Spain, explored binge drinking in a random sample of approximately 12,000 adults and found that people who reported heavy episodic drinking were more likely to consume less than three portions of fruit and vegetables per day, and more than one serving of meat per day, compared to never drinkers, after adjusting for all socio-demographic characteristics (Valencia-Martin et al. 2011).

Negative health behaviours often cluster in populations (Chiolero et al. 2006; French et al. 2008; Shankar et al. 2010) and a previous study explored relationships between the five dietary patterns described in the ALSPAC cohort study and socio-demographic characteristics. The 'Health conscious' dietary pattern was indicative of higher social affluence; adherence was associated with older age, higher educational attainment, living in an owned or mortgaged property, lower parity, being white, not smoking and having fewer financial difficulties. In contrast, adherence to the 'Processed' dietary pattern was associated with the opposite trends, indicating lower social affluence (Northstone et al. 2008). Evidence also suggests that patterns of alcohol consumption during pregnancy are related to socio-demographic characteristics. A systematic review reported that five studies found higher income or social class to be associated with alcohol consumption during pregnancy, but not with binge drinking (Skagerström et al. 2011). A study conducted in Sweden found similar results in a non-pregnant population; binge drinking was associated with lower social affluence (Backhans et al. 2012). The clustering of risky health behaviours increases the risk of adverse fetal development (Lanting et al. 2009), and may also provide an explanation for why some studies have published findings that

suggest women who drink low to moderate amounts during pregnancy have children with better cognitive outcomes: diet may be an overlooked confounder.

Folic acid supplement use was significantly associated with adherence to the 'Health conscious' and 'Vegetarian' dietary patterns. Taking folic acid supplements during the early stages of pregnancy has been shown to be effective in preventing Neural Tube Defects (NTD) (MRC 1991). This is particularly promising for women in this sample who adhere to the 'Vegetarian' dietary pattern, as this dietary pattern had very low correlation with folate intakes and was associated with both drinking one or more drinks per day and binge drinking during pregnancy. However, these predictions must be treated with caution due to the method of assessment. Women reported 'yes' or 'no' to a question asking if they had taken folic acid supplements during this pregnancy (reported at 18 weeks gestation). Although this is informative, it does not provide any details about the timing or dose of folic acid consumption. Demand for folate or folic acid is highest during the first few weeks of gestation, when the neural tube is closing. If women are not taking supplements during this period, and are binge drinking and consuming an inadequate diet, they may be putting their unborn child at increased risk of NTDs.

6.5.1 Strengths and limitations

The main strength of Study 2A is the large sample size and the variation in reported levels of alcohol consumption provided an opportunity to address the original aims of this thesis. Because ALSPAC had collected data on binge drinking, it was possible to assess the relationships with irregular patterns of alcohol consumption, which may often go undetected when asking questions about average consumption. The wide variety of data that ALSPAC collected meant it was possible to adjust for important confounders, such as smoking, depression risk, and markers of socio-economic status.

However, the study also had a number of limitations. Firstly, the validity of the FFQ and alcohol assessment tools used in ALSPAC were not assessed before use. Therefore, it is difficult to determine the validity of the tools at estimating the intake of the selected micronutrients and alcohol consumption. This issue is discussed in more detail in Chapter 3. Secondly, the drinking categories were unbalanced, with few women reporting to drink one or more drinks per day during early pregnancy. This may increase the risk of attenuating relationships, particularly in multivariate regression models. Thirdly, despite the large sample, many groups were underrepresented; 98% of the sample population was white, and only 14% lived in property rented by the housing association or council. Low recruitment and retention rates of women from low socio-economic backgrounds are well documented in public health research. Sampling bias can lead to erroneous or non-generalisable results (Johnson et al. 2000). Fourthly, the descriptive terms given to dietary patterns in Study 2A may be slightly misleading. The label 'Vegetarian' does not necessarily mean that women with a high adherence to this dietary pattern are vegetarians; they may just consume a diet that is characterised

by low intakes of red meat and high intakes of pulses. Therefore, some caution must be taken when using dietary pattern analysis to convey a public health message. Fifthly, the lack of robust data on folic acid supplement intake means it is not clear what dose of folic acid supplements and when they were taken during pregnancy; women in the category for folic acid supplements may have only taken one or two during their pregnancy, compared to other women who took 400µg/day. Finally, the measure of binge drinking only refers to the previous month (approximately 14 to 18 weeks gestation); it is possible that some women may have consumed four or more units in one day during another period of their pregnancy, but have been incorrectly categorised as non-binge drinkers. The question also refers to the consumption of four units across one day, rather than on one occasion, as 'binge' drinking is typically defined as. Some women may have consumed many units in a short time period, and others four units over an entire day. This has very different consequences for blood alcohol levels and the potential harm to the fetus.

6.6 Conclusions

The results from study 2A have provided several new and important findings regarding the clustering of two important health behaviours during pregnancy; alcohol consumption and dietary intake. Of particular concern is the relationship between potentially harmful drinking patterns (one or more drinks per day during the first trimester and heavy episodic drinking during the past month) and adherence to the 'Processed' and 'Vegetarian' dietary patterns, which were weakly correlated with micronutrients and antioxidants. OCM micronutrients and antioxidants play important roles in fetal development and evidence suggests that alcohol inhibits the absorption of micronutrients, and with a greater demand during pregnancy, low intakes and potentially harmful patterns of alcohol consumption, it is possible that some women may put their baby at increased risk of adverse fetal development. The findings were also in agreement with those in Study 1B; light to moderate alcohol consumption was associated with adherence to the 'Health conscious' dietary pattern, which was characterised by high intakes of fresh fruit and vegetables and was correlated with micronutrient intakes.

Overall, Study 2A has indicated that the relationships between diet and alcohol that have been previously reported in the general population persist into pregnancy. The findings also highlight the need to tackle groups of health behaviours, rather in isolation. Health behaviour interventions may be more successful if alcohol consumption during pregnancy is tackled as a broader goal, along with diet, by working with women at risk on other health behaviours and lifestyle factors in conjunction with education and support. In order to explore this further, the next chapter will build on these findings and explore whether dietary intake is a modifying factor in fetal development in an ethanol-exposed pregnancy.

Chapter 7. Exploration of alcohol consumption, dietary intake and fetal growth (Study 2B)

7.1 Introduction

Small for Gestational Age (SGA) is defined as a birth weight below the 10th percentile for gestational age. Being born SGA is associated with adverse offspring outcomes, including an increased risk of neonatal morbidity and mortality (Ho 2001) and of type-2-diabetes, cardiovascular disease (Nafee et al. 2008; Waterland 2009) and mental health problems later in adult life (Schlotz et al. 2010). Findings from the literature presented in Chapter 2 suggested an increased risk of women having an SGA infant if they were drinking 10g or more of ethanol per day during pregnancy, and that a dose-response relationship was observed beyond that threshold (Patra et al. 2011). However, the evidence for a relationship between lower intakes of maternal alcohol consumption and fetal growth restriction are not as consistent (Henderson et al. 2007; Cooper et al. 2013; Pfinder et al. 2013). One proposed mechanism to account for these inconsistencies is that antioxidants and micronutrients involved in One Carbon Metabolism (OCM) modify the relationship between alcohol and fetal growth (Grange et al. 1999; Cohen-kerem & Koren 2003; Gutierrez et al. 2007; Thomas et al. 2009; Wang et al. 2009).

The previous two Chapters (5 & 6) presented findings from Studies 1B and 2A; investigations into relationships between dietary patterns, micronutrient intakes and alcohol consumption during pregnancy. Overall, the results indicated that women who reported potentially harmful drinking patterns (one or more alcoholic drinks per day or at least one episode of binge drinking) were more likely to consume diets characterised by high intakes of processed foods and low micronutrient intakes. This is of particular concern, as low maternal intakes of dietary antioxidants and OCM micronutrients are associated with fetal growth restriction (Matsubasa et al. 2002; Kim et al. 2005; Bergen et al. 2012; Fekete et al. 2012; Dwarkanath et al. 2013; Weber et al. 2014; Hodgetts et al. 2015).

Evidence from animal models provide a sound rationale to suggest that diet modifies the relationship between alcohol and fetal growth; however, stark physiological and environmental differences mean results may not always translate to other species. Observational studies with human women are far more complex: environmental exposures, health behaviours and physiological characteristics can often have synergistic effects, altering the level of risk in certain populations. This chapter will present the findings of the first study in humans to explore whether maternal dietary intake modifies the relationship between antenatal alcohol consumption and the odds of fetal growth restriction.

7.2 Aims and objectives

The overall aim of this study was to explore whether particular aspects of maternal dietary intake modify the relationship between antenatal alcohol consumption and fetal growth, using data from the ALSPAC study.

Specific objectives of the study were:

- To describe the prevalence of SGA in sample of women from the ALSPAC cohort
- To explore relationships between maternal alcohol consumption during pregnancy and fetal growth restriction
- To explore relationships between maternal dietary intake (OCM micronutrients and antioxidants) and fetal growth restriction
- To investigate whether low maternal intakes of OCM micronutrients (folate, choline, betaine, methionine, vitamin B12 and vitamin B6) increase the odds of having an SGA infant in women who report:
    - Maternal alcohol consumption during early pregnancy
    - Maternal binge drinking in mid-pregnancy
- To investigate whether low maternal intakes of dietary antioxidants (Vitamin C, vitamin E and carotenoids) increase the odds of having an SGA infant in women who report:
    - Maternal alcohol consumption during early pregnancy
    - Maternal binge drinking in mid-pregnancy
- To explore relationships between maternal dietary patterns and fetal growth restriction
- To investigate whether adherence to a particular maternal dietary pattern increase the odds of having an SGA infant in women who report:
    - Maternal alcohol consumption during early pregnancy
    - Maternal binge drinking in mid-pregnancy

7.3 Methods

7.3.1 Study design

This study comprises of a secondary analysis of data from ALSPAC (Golding, Pembrey, Jones, et al. 2001); an observational cohort study conducted in the West of England (Full description in Chapter 3).

7.3.2 Sample population

Women were included in the present analysis if they had a live, singleton birth (n=13,678), provided details of alcohol consumption at 18 weeks gestation (n=13,197), completed the FFQ at 32 weeks

gestation (n=12,190) and their baby's birth weight (n=12,016) and gestational age at birth (n=12,173) were available from hospital records.

7.3.3 Measures

Main outcome measure: Small for gestational age

SGA is defined as a birth weight below the 10th percentile for gestational age. Standard, unadjusted cut offs for <10th percentile may increase the incidence of false-positive diagnoses of SGA infants. Some low birth weights are considered in the normal, healthy range for certain pregnancies due to genetic influences, rather than pathologies (Gardosi 2006). Therefore, customized birth weight centiles were used in order to better reflect adverse pregnancy events. Customised birth weight centiles were calculated using computer software, developed by the Perinatal Institute Birmingham (version 6.7.5, 2014). Centiles were customised based on the following data:

- Maternal ethnicity: obtained by self-report at 18 weeks gestation.
- Parity: calculated by ALSPAC team by subtracting number of miscarriages, abortions and terminations from number of previous pregnancies (gravidity).
- Maternal height (cm) and pre-pregnancy weight (kg): self-reported in questionnaire at 12 weeks gestation.
- Gestational age at delivery (weeks): Estimated using date of last menstrual period and findings from routine antenatal scans (obtained from hospital records).
- Child gender: Obtained from hospital records
- Birth weight (g): Obtained from hospital records

The above characteristics are used to derive the Term Optimal Weight (TOW), which is the birth weight predicted based on being born full term and without any pathological influences. The software then plots a proportionality curve which produces gestation related optimal weights (GROW), which are used to calculate the 10th percentile cut off to identify SGA infants (Gardosi 2006). If any of the above maternal and infant characteristics were missing – with the exception of birth weight and gestational age at delivery – the software imputed default values (see Section 7.3.5).

Alcohol consumption

Antenatal alcohol consumption was self-reported at 18 weeks gestation. The same alcohol variables were explored in Study 2A and full descriptions have been provided in the Chapter 6 (Study 2A, section 6.3.3). The following measures of maternal alcohol consumption were explored in relation to fetal growth in Study 2B:

- Frequency and quantity of alcohol consumption during early pregnancy
- Binge drinking during mid-pregnancy

Dietary intake

Women completed a FFQ at 32 weeks gestation. Full details of the FFQ and the preparation of dietary data have been provided in Chapter 6 (see section 6.3.3). The following measures of dietary intake are explored in relation to fetal growth in Study 2B:

1. Mean daily intakes of OCM micronutrients (folate, choline, betaine, methionine, vitamin B12 and vitamin B6) and dietary antioxidants (vitamin C, vitamin E and carotenoids). Intakes were categorised into quartiles. Being in quartile 1 (Q1) was consistently a predictor of being born SGA, compared to other quartiles. Quartiles 2, 3 and 4 (Q2-Q4) were combined and investigations compared Q1 vs. Q2-Q4. Folate intake was explored with and without the addition of folic acid supplements.
2. Maternal dietary pattern scores previously derived by Northstone and colleagues (2008), including 'Health conscious', 'Traditional', 'Processed', 'Confectionery' and 'Vegetarian'. Dietary pattern scores were categorised into quartiles. Quartile 4 (Q4) indicated the strongest adherence to each dietary pattern. Therefore, quartiles 1, 2 and 3 (Q1-Q3) were combined and investigations compared Q1-Q3 vs. Q4.

Socio-demographic data

Details of maternal socio-demographic characteristics were self-reported at 8 and 18 weeks gestation. Based on a review of the existing literature, maternal age (Clausson et al. 1998; Skagerström et al. 2011; Khalil et al. 2013), education (Raum et al. 2001), smoking (Mitchell et al. 2002; Van den Berg et al. 2013) and markers of SES, including housing tenure, house crowding index (HCI), and living in a single parent household (Rifas-Shiman et al. 2009; Shah et al. 2011; Skagerström et al. 2011; Chambers et al. 2014) were associated with risk of SGA, maternal alcohol consumption and dietary intakes, and were therefore, added to multivariable models as confounders. Full details of how these socio-demographic characteristics were categorised are included in Chapter 6 (Study 2A).

7.3.4 Statistical analysis

Cases of SGA infants were summarised as frequencies and percentages. Pearson's chi-squared tests were used to assess whether frequencies were significantly different by socio-demographic characteristics, alcohol consumption categories, quartiles of dietary pattern scores and micronutrient intakes during pregnancy.

Logistic regression models were used to explore the relationships between maternal alcohol consumption and fetal growth restriction. Separate models were used to explore frequency of alcohol consumption during early pregnancy and binge drinking during mid-pregnancy. SGA was treated as a binary, dependent variable and maternal alcohol consumption as categorical, independent variables.

Maternal age, ethnicity, parity, education, housing tenure, HCI, smoking status and single parent household were then added to the logistic regression models as covariates.

Logistic regression models were used to explore associations between maternal dietary intakes of micronutrients and antioxidants and fetal growth restriction. Separate regression models were used to explore each dietary factor due to multicollinearity (Williams et al. 2013). SGA was treated as a binary, dependent variable and maternal micronutrient intakes were categorised as quartiles and treated as categorical, independent variables. Multivariable models included maternal age, ethnicity, parity, education, housing tenure; HCI, smoking status and single parent household were then added to the logistic regression models as covariates.

Two methods were employed to assess whether maternal micronutrient and antioxidant intakes modified the relationship between patterns of alcohol consumption (frequency of alcohol consumption and binge drinking) and the odds of having a SGA infant:

1. Logistic regression models were used to assess the odds of giving birth to a SGA infant by maternal alcohol consumption during pregnancy and were stratified by quartiles of maternal dietary intakes.
2. Logistic regression models were run with main effect terms and an interaction term for each dietary factor and maternal alcohol consumption during pregnancy. The interaction term was then removed and the model was run again, with main effect terms only. The likelihood ratio test was used to assess the likelihood of the set of parameter estimates given the outcomes. The likelihood ratio test compared the fit of one model to another (with and without interaction term) and if the difference was significant, then the less restrictive model (with interaction term) was considered a better fit, indicating an interaction between dietary and alcohol variables (Kleinbaum 1994).

Models were run with dietary and alcohol predictors only and then again with all other covariates (maternal age, education, housing tenure, HCI and smoking status during pregnancy). Covariates were added to the model one-by-one to assess the change in OR with each addition.

Logistic regression models were then used to explore the relationships between each maternal dietary pattern described in Study 2A and fetal growth restriction. SGA was treated as a binary, dependent variable and maternal dietary pattern scores were categorised as quartiles and treated as categorical, independent variables. Maternal age, ethnicity, parity, education, housing tenure, HCI, smoking status and single parent household were then added to the logistic regression models as covariates.

To assess whether maternal dietary pattern scores modified the relationship between alcohol consumption (frequency of alcohol consumption and binge drinking) and odds of having a SGA infant,

the same two methods were carried out, as described previously, with maternal dietary pattern scores, instead of micronutrient and antioxidant intakes. Logistic regression models were run with alcohol and dietary predictors only, and then run again with the addition of maternal age, ethnicity, parity, education, housing tenure; HCI, smoking status and single parent household as covariates.

To aid the interpretation of interactions, plots of marginal means, calculated from logistic regression models with interaction terms, were generated. Significance was defined as $p<0.05$, and all statistical analyses were conducted using STATA 13.1.

7.3.5 Missing data

If maternal parity, ethnicity, height, weight or child gender were missing from the dataset, the customised birth centile software (version 6.7.5, 2014) imputed default values; the median values or characteristics associated with lowest risk of having an SGA infant. Values are as follows: parity (3+), ethnicity (white), height (163cm), weight (64kg) and child gender (average between male and female coefficients).

Women were excluded from the present analysis if more than 10 items were left blank on the FFQ. In cases where 10 or less FFQ items were missing, it was assumed women did not consume these items and they were coded as zero (Northstone et al. 2008).

7.3.6 Ethical approval

Ethical approval for the present analysis was obtained from the ALSPAC ethics committee in April 2014. (Ref: B2198)

7.4 Results

7.4.1 Sample population

The socio-demographic characteristics of women included in this analysis are presented in Table 7.1. Women who gave birth to babies in the 10th percentile of birth weight (SGA) were more likely to be younger, primiparous, of non-white ethnic origin, smoke during pregnancy, have lower educational attainment, live in rented accommodation, and live in crowded accommodation.

Table 7.1 Socio-demographic characteristics of sample population (n=11,715)

	Non-SGA (n=10,674)		SGA (n=1,041)		p-value
	n	%	n	%	
Age (years)					
15-19	395	3.7	58	5.6	
20-29	6,069	56.9	616	59.2	
30-39	4,084	38.3	346	33.2	
40+	126	1.2	21	2.0	<0.0001
Parity					
Primiparous	4,586	44.5	491	50.0	
Multiparous	5,725	55.5	492	50.1	0.001
Single parent household					
No	9,585	94.4	880	92.6	
Yes	571	5.6	70	7.4	0.027
Ethnicity					
Non-white	254	2.4	45	4.5	
White	10,305	97.6	967	95.6	<0.0001
Education					
Vocational	3,096	29.2	375	36.5	
O level	3,682	34.7	369	35.9	
A level	2,444	23.0	186	18.1	
Degree level	1,395	13.1	97	9.4	<0.0001
Smoking					
Never	8,024	80.5	580	59.8	
≤10/day	788	7.9	119	12.3	
>10/day	1,161	11.6	271	27.9	<0.0001
Home ownership					
Owner/occupied	7,932	76.6	8,587	75.6	
Council/HA renting	1,416	13.7	1,626	14.3	
Private renting	1,013	9.8	1,140	10.0	<0.0001
Crowding index					
<= 0.5	4,437	43.4	410	42.4	
>0.5 - 0.75	3,256	31.9	283	29.3	
>0.75 - 1	1,944	19.0	196	20.3	
> 1	582	5.7	78	8.1	0.011

7.4.2 Maternal alcohol consumption and fetal growth

Proportions of women who gave birth to SGA infants by alcohol consumption patterns during pregnancy are presented in Table 7.2. In unadjusted logistic regression models women who drank one or more alcoholic drinks per day during early pregnancy were approximately twice as likely to give birth to SGA infants compared to women who did not drink during the same period (OR=2.12, 95% CI=1.45-3.12; p<0.0001). No differences were observed between women who did not drink and women who drank less than one drink per day. In the multivariate model with all other covariates, drinking one or more alcoholic drinks per day in the first trimester was still associated with increased odds of giving birth to SGA infants compared to never drinking (OR=1.76, 95%CI=1.12-2.75; p=0.014).

A significantly higher proportion of women who reported at least one occasion of binge drinking during the second trimester gave birth to SGA infants. Results from the unadjusted logistic regression model suggested that odds of giving birth to SGA infants increased by 45% in women who reported at least one episode of binge drinking during the second trimester (OR=1.45, 95%CI=1.16-1.80; p=0.001). However, once potential confounders were added to the model, this relationship was no longer significant (OR=1.08, 95%CI=0.84-1.39; p=0.553).

Table 7.2 Proportions of women giving birth to SGA infant by maternal alcohol consumption during pregnancy

	Non-SGA (n=10,674)		**SGA** (n=1,041)		p-value
	n	%	n	%	
1st trimester drinking					
Never	4,693	45.1	449	45.3	
<1 drink/week	4,097	39.4	366	36.9	
≥1 drink/week	1,443	13.9	142	14.3	
1+drink/day	167	1.6	34	3.4	<0.0001
Binge drinking					
No	9,596	92.6	883	89.6	
Yes	767	7.4	102	10.4	0.001

7.4.3 Maternal micronutrient intakes

The proportions of women who gave birth to SGA infants by quartiles of micronutrient intakes at 32 weeks gestation are presented in Table 7.3. With exception of betaine and carotenoids, low intakes (Q1) of all micronutrients were significantly associated with higher proportions of women giving birth to SGA infants.

Table 7.3 Proportions of women giving birth to SGA infant by maternal micronutrient intakes during pregnancy

	Non-SGA (n=11,674)		**SGA** (n=1,041)		p-value
	n	%	n	%	
Folate					
Q1	2,602	24.5	310	30.1	
Q2-Q4	8,023	75.5	719	69.9	<0.0001
Folate (S)					
Q1	2,607	24.4	316	30.4	
Q2-Q4	8,067	75.6	725	69.6	<0.0001
Choline					
Q1	2,636	24.7	297	28.5	
Q2-Q4	8,038	75.3	744	71.5	0.006
Betaine					
Q1	2,661	24.9	255	24.5	
Q2-Q4	8,013	75.1	786	75.5	0.757
Methionine					
Q1	2,635	24.7	291	28.0	
Q2-Q4	8,039	75.3	750	72.1	0.02
Vitamin B12					
Q1	2,627	24.7	283	27.5	
Q2-Q4	7,998	75.3	746	72.5	0.049
Vitamin B6					
Q1	2,611	24.6	307	29.8	
Q2-Q4	8,014	75.4	722	70.2	<0.0001
Vitamin C					
Q1	2,594	24.4	327	31.8	
Q2-Q4	8,031	75.6	702	68.2	<0.0001
Vitamin E					
Q1	2,596	24.4	321	31.2	
Q2-Q4	8,029	75.6	708	68.8	<0.0001
Carotenoids					
Q1	2,650	24.8	281	27.0	
Q2-Q4	8,024	75.2	760	73.0	0.123

OCM micronutrients

The results from the stratified analysis and likelihood ratio tests indicated there was no evidence of an interaction between OCM micronutrient intakes and alcohol consumption during pregnancy. Low intakes of OCM micronutrients did not significantly increase the odds of having a SGA infant in women who reported alcohol consumption during pregnancy (exploring both frequency of alcohol consumption and binge drinking).

When maternal age, ethnicity, parity, education, housing tenure, HCI, single parent household and smoking status during pregnancy were added to the models as covariates. The stratified analysis revealed an interesting relationship (Table 7.4); drinking one or more drinks per day was significantly associated with increased odds of having an SGA infant, but only in women with higher intakes (Q2-Q4) of OCM micronutrients. No relationships were observed for binge drinking and OCM micronutrient intakes.

Table 7.4 Adjusted odds of giving birth to SGA infant by maternal alcohol consumption during early pregnancy, stratified by maternal intakes of folate, choline, betaine, methionine, vitamin B12 and vitamin b6^

Model	Never			<1 drink/week			≥1 drink/week			1+ drink/day			Interaction*
	OR	(95% CI)	p-value	OR	(95% CI)	p-value	OR	(95% CI)	p-value	OR	(95% CI)	p-value	
1: Folate													
Q1		(ref)		0.97	(0.72, 1.32)	0.87	0.83	(0.54, 1.27)	0.39	1.17	(0.53, 2.58)	0.69	
Q2-Q4		(ref)		0.96	(0.80, 1.15)	0.69	0.84	(0.65, 1.10)	0.21	1.91	(1.15, 3.19)	0.01	0.84
2: Folate (S)													
Q1		(ref)		0.94	(0.70, 1.27)	0.69	0.79	(0.52, 1.21)	0.28	1.37	(0.64, 2.93)	0.41	
Q2-Q4		(ref)		0.96	(0.80, 1.16)	0.70	0.86	(0.66, 1.12)	0.27	1.90	(1.16, 3.14)	0.01	0.94
3: Choline													
Q1		(ref)		0.95	(0.71, 1.29)	0.75	0.63	(0.40, 1.01)	0.06	1.19	(0.54, 2.66)	0.67	
Q2-Q4		(ref)		0.96	(0.80, 1.15)	0.64	0.92	(0.71, 1.19)	0.51	1.99	(1.22, 3.24)	0.01	0.23
4: Betaine													
Q1		(ref)		1.02	(0.75, 1.40)	0.89	0.95	(0.62, 1.45)	0.82	1.89	(0.90, 3.98)	0.09	
Q2-Q4		(ref)		0.94	(0.78, 1.12)	0.47	0.80	(0.62, 1.05)	0.11	1.70	(1.03, 2.83)	0.04	0.97
5: Methionine													
Q1		(ref)		0.90	(0.67, 1.22)	0.51	0.69	(0.43, 1.09)	0.11	1.08	(0.44, 2.64)	0.87	
Q2-Q4		(ref)		0.98	(0.81, 1.17)	0.79	0.90	(0.69, 1.16)	0.41	2.02	(1.26, 3.23)	0.00	0.38
6: Vitamin B12													
Q1		(ref)		0.83	(0.61, 1.12)	0.22	0.73	(0.47, 1.16)	0.19	0.73	(0.22, 2.47)	0.61	
Q2-Q4		(ref)		1.03	(0.86, 1.23)	0.79	0.89	(0.68, 1.15)	0.36	1.96	(1.24, 3.12)	0.00	0.16
7: Vitamin B6													
Q1		(ref)		1.06	(0.79, 1.44)	0.69	0.95	(0.63, 1.45)	0.82	1.32	(0.62, 2.81)	0.47	
Q2-Q4		(ref)		0.93	(0.78, 1.12)	0.444	0.80	(0.61, 1.04)	0.099	1.85	(1.10, 3.12)	0.02	0.56

OR=Odds ratio; 95% CI= 95% confidence intervals

*Results from likelihood ratio tests

^Adjusted for maternal age, parity, ethnicity, education, housing tenure, HCI, single parent household and smoking

Dietary antioxidants

Table 7.5 presents the results from the stratified logistic regression analyses and likelihood ratio tests assessing the odds of having a SGA infant by frequency of maternal alcohol consumption during early pregnancy, stratified by quartiles of dietary antioxidant intake. While the results from the likelihood ratio tests indicated that the interactions were not significant, the results from the stratified analysis did highlight that women who consumed one or more alcoholic drinks per day during early pregnancy were more likely to have a SGA infant if they also had low intakes (Q1) of vitamins C and E. However, the predicted estimates did not differ significantly between each strata of intake. The relationships diminished after the addition of confounders.

Interestingly, in both unadjusted (Table 7.5) and adjusted (Table 7.6) regression models, drinking one or more drinks per day was significantly associated with increased odds of having an SGA infant, but only in women with higher intakes of carotenoids (Q2-Q4).

Table 7.5 Unadjusted odds of giving birth to SGA infant by maternal alcohol consumption during early pregnancy, stratified by quartiles of maternal intakes of vitamin C, vitamin E and carotenoids

Model	Never			<1 drink/week			≥1 drink/week			1+ drink/day			Interaction*
	OR	(95% CI)	p-value	OR	(95% CI)	p-value	OR	(95% CI)	p-value	OR	(95% CI)	p-value	
1: Vitamin C													
Q1		(ref)		0.88	(0.67, 1.14)	0.33	0.83	(0.57, 1.20)	0.32	2.16	(1.14, 4.08)	0.02	
Q2-Q4		(ref)		0.99	(0.83, 1.18)	0.92	1.14	(0.90, 1.45)	0.26	1.91	(1.16, 3.15)	0.01	0.48
2: Vitamin E													
Q1		(ref)		1.01	(0.77, 1.32)	0.97	1.37	(0.97, 1.94)	0.08	2.68	(1.44, 5.00)	0.00	
Q2-Q4		(ref)		0.94	(0.79, 1.11)	0.44	0.93	(0.73, 1.18)	0.53	1.70	(1.02, 2.83)	0.04	0.26
3: Carotenoids													
Q1		(ref)		0.89	(0.67, 1.18)	0.42	0.85	(0.57, 1.28)	0.45	1.79	(0.78, 4.11)	0.17	
Q2-Q4		(ref)		0.96	(0.81, 1.13)	0.60	1.10	(0.88, 1.38)	0.41	2.26	(1.47, 3.48)	0.00	0.73

OR=Odds ratio; 95% CI= 95% confidence intervals

*Results from likelihood ratio tests

Table 7.6 Adjusted odds of giving birth to SGA infant by maternal alcohol consumption during early pregnancy, stratified by quartiles of maternal intakes of vitamin C, vitamin E and carotenoids^

Model	Never			<1 drink/week			≥1 drink/week			1+ drink/day			Interaction*
	OR	(95% CI)	p-value	OR	(95% CI)	p-value	OR	(95% CI)	p-value	OR	(95% CI)	p-value	
1: Vitamin C													
Q1		(ref)		0.94	(0.70, 1.26)	0.67	0.61	(0.39, 0.96)	0.03	1.58	(0.75, 3.34)	0.23	
Q2-Q4		(ref)		1.06	(0.88, 1.29)	0.51	0.92	(0.70, 1.21)	0.56	1.70	(1.00, 2.91)	0.05	0.49
2: Vitamin E													
Q1		(ref)		1.08	(0.79, 1.47)	0.64	1.11	(0.73, 1.68)	0.62	1.95	(0.96, 3.98)	0.07	
Q2-Q4		(ref)		1.00	(0.83, 1.20)	0.98	0.71	(0.54, 0.95)	0.02	1.46	(0.83, 2.54)	0.19	0.18
3: Carotenoids													
Q1		(ref)		0.98	(0.72, 1.33)	0.89	0.58	(0.35, 0.96)	0.04	1.03	(0.38, 2.79)	0.96	
Q2-Q4		(ref)		1.02	(0.85, 1.23)	0.80	0.89	(0.68, 1.16)	0.39	1.99	(1.24, 3.17)	0.00	0.35

OR=Odds ratio; 95% CI= 95% confidence intervals

*Results from likelihood ratio tests

^Adjusted for all other covariates (age, ethnicity, parity, education, housing, HCI and smoking)

Table 7.7 presents the results from the logistic regression analyses assessing the odds of an SGA birth by binge drinking during mid-pregnancy, stratified by quartiles of dietary antioxidant intakes. The findings from the stratified analysis revealed that women who reported binge drinking and were in Q1 for vitamin E intake were at increased odds of giving birth to SGA infants (OR=2.29, 95%CI=1.63-3.21; p=<0.0001), compared to women in other quartiles, who also reported binge drinking (OR=1.06, 95%CI=0.79-1.42; p=0.71).

Table 7.7 Unadjusted odds of giving birth to SGA infant by maternal binge drinking during mid-pregnancy, stratified by quartiles of maternal intakes of vitamin C, vitamin E and carotenoids

Model	No binge drinking			Binge drinking			Interaction
	OR	(95% CI)	p-value	OR	(95% CI)	p-value	
1: Vitamin C							
Q1		(ref)		1.35	(0.93, 1.95)	0.11	
Q2-Q4		(ref)		1.44	(1.10, 1.90)	0.01	0.777
2: Vitamin E							
Q1		(ref)		2.29	(1.63, 3.21)	0.00	
Q2-Q4		(ref)		1.06	(0.79, 1.42)	0.71	0.0009
3: Carotenoids							
Q1		(ref)		1.18	(0.75, 1.83)	0.48	
Q2-Q4		(ref)		1.55	(1.21, 1.99)	0.00	0.279

OR=Odds ratio; 95% CI= 95% confidence intervals
*Results from likelihood ratio tests

The results from the logistic regression model with main and interaction effects are presented in Table 7.8. The likelihood ratio test was performed to compare the fit of this model with the model with main effects only. The results indicated that the logistic regression model with main effects and an interaction term was a better fit, suggesting vitamin E may modify the relationship between binge drinking and odds of having SGA birth (X2 (1)=11.10; p=0.0009). The predicted marginal means are presented in Figure 7.1.

Table 7.8 Unadjusted odds of giving birth to SGA infant by maternal binge drinking during mid-pregnancy and quartiles of maternal vitamin E intake, with main and interaction effects

	OR	(95% CI)	p-value
Binge drinking			
No		(ref)	
Yes	1.06	(0.79, 1.42)	0.713
Vitamin E			
Q2-Q4		(ref)	
Q1	1.22	(1.05, 1.43)	0.012
Interaction			
Binge drinking x Q1 vitamin E	2.16	(1.38, 3.40)	0.001

OR=Odds ratio; 95% CI= 95% confidence intervals
*Results from likelihood ratio tests

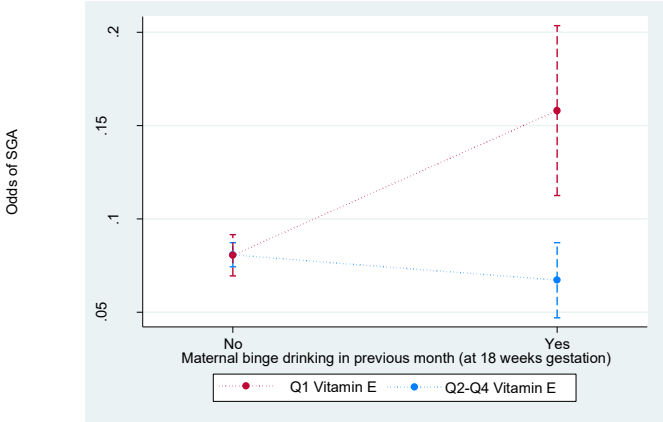

Figure 7.18 Odds of giving birth to a SGA infant by maternal binge drinking during mid-pregnancy, stratified by quartiles of maternal vitamin E intake, adjusted for all other covariates (age, ethnicity, parity, education, housing, HCI, single parent household and smoking)

When all other covariates were added to the logistic regression model, the relationship persisted. In the stratified analysis (Table 7.9), the odds of giving birth to SGA infants remained significantly higher for women who reported binge drinking, if they were in Q1 (OR=2.03, 95%CI=1.37,3.00; p<0.0001), compared to women in other quartiles (OR=0.83, 95%CI=0.59,1.17; p=0.31).

Table 7.9 Adjusted odds of giving birth to SGA infant by maternal binge drinking during mid-pregnancy, stratified by quartiles of maternal intakes of vitamin C, vitamin E and carotenoids^

Model	Never			Yes			Interaction
	OR	(95% CI)	p-value	OR	(95% CI)	p-value	
1: Vitamin C							
Q1		(ref)		1.17	(0.77, 1.78)	0.47	
Q2-Q4		(ref)		1.20	(0.88, 1.63)	0.25	0.92
2: Vitamin E							
Q1		(ref)		2.03	(1.37, 3.00)	0.00	
Q2-Q4		(ref)		0.83	(0.59, 1.17)	0.30	0.0002
3: Carotenoids							
Q1		(ref)		0.95	(0.56, 1.60)	0.84	
Q2-Q4		(ref)		1.28	(0.97, 1.69)	0.09	0.34

OR=Odds ratio; 95% CI= 95% confidence intervals
*Results from likelihood ratio tests
^Adjusted for all other covariates (age, ethnicity, parity, education, housing, HCI, single parent household and smoking)

Table 7.10 presents the results from the logistic regression model with main and interaction effects, adjusted for all other covariates. Smoking status during pregnancy remained the single strongest predictor of giving birth to SGA infants (OR=2.37, 95%CI=2.00-2.81; p<0.0001), with a z-score of 10.34. When the interaction term was removed and the model was compared, the results from the likelihood ratio test also remained significant (X2 (1) =12.62; p=0.0002), indicating a better fit. The predicted marginal means are displayed in Figure 7.2.

The likelihood ratio tests suggested there was no evidence of vitamin C (X2 (1)=0.00; p=0.92) or carotenoid (X2 (1)=1.04; p=0.34) intakes at 32 weeks gestation modifying the relationship between binge drinking at 18 weeks gestation and odds of giving birth to a SGA infant (Table 7.13).

Table 7.10 Adjusted odds of giving birth to SGA infant by maternal binge drinking during mid-pregnancy and quartiles of maternal vitamin E intake, with main and interaction effects^

	OR	(95% CI)	p-value
Binge drinking			
No		(ref)	
Yes	0.82	(0.59, 1.16)	0.26
Vitamin E			
Q2-Q4		(ref)	
Q1	0.99	(0.83, 1.18)	0.91
Interaction			
Binge drinking x Q1 vitamin E	2.63	(1.58, 4.37)	0.00
Age			
<20		(ref)	
20-29	1.13	(0.76, 1.67)	0.55
30-39	1.25	(0.82, 1.90)	0.30
40+	2.15	(1.07, 4.32)	0.03
Parity			
Nulliparous		(ref)	
Multiparous	0.68	(0.57, 0.82)	0.00
Ethnicity			
Non-white		(ref)	
White	0.55	(0.36, 0.83)	0.00
Education			
<O level		(ref)	
O level	1.07	(0.89, 1.28)	0.48
A level	0.86	(0.69, 1.08)	0.19
Degree level	0.87	(0.66, 1.14)	0.31
Housing tenure			
Home owner/mortgaged		(ref)	
Council/HA renting	1.34	(1.06, 1.68)	0.01
Private renting	1.15	(0.89, 1.48)	0.29
HCI			
≤0.5		(ref)	
>0.5 - 0.75	0.98	(0.81, 1.19)	0.84
>0.75 - 1	0.80	(0.63, 1.01)	0.06
> 1	0.99	(0.71, 1.38)	0.93
Smoker			
No		(ref)	
Yes	2.37	(2.00, 2.81)	0.00

OR=Odds ratio; 95% CI= 95% confidence intervals

*Results from likelihood ratio tests ^Adjusted for all other covariates (age, ethnicity, parity, education, housing, HCI, single parent household and smoking)

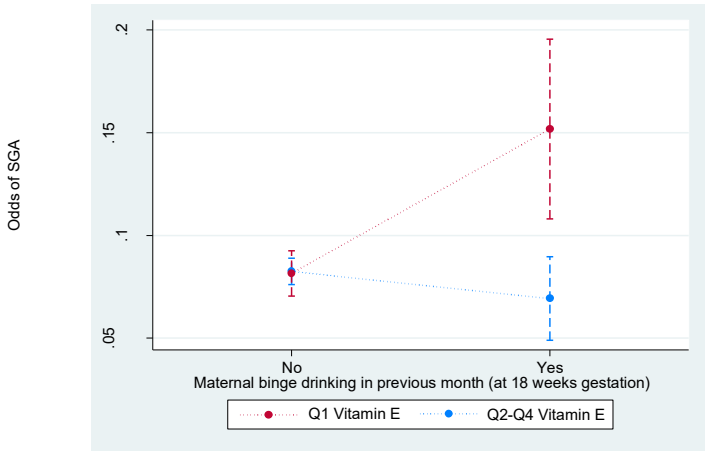

Figure 7.2 Odds of giving birth to a SGA infant by maternal binge drinking during mid-pregnancy, stratified by quartiles of maternal vitamin E intake, adjusted for all other covariates (age, ethnicity, parity, education, housing, HCI, single parent household and smoking)

7.4.4 Maternal dietary patterns

In unadjusted linear regression models, adherence (defined as being in Q4) to the 'Health conscious' dietary pattern was associated with significantly lower odds of having a SGA infant, compared to all other quartiles (OR= 0.77, 95%CI = 0.65, 0.90; p=0.001). The opposite trend was observed for adherence to the 'Processed' (OR= 1.39, 95%CI = 1.21, 1.60; p<0.0001) and 'Vegetarian' (OR = 1.19, 95%CI = 1.03, 1.37; p=0.019) dietary patterns. After adjustment for potential confounders, these relationships were no longer significant.

Investigations into the modifying effect of maternal dietary patterns on the relationships between alcohol consumption and fetal growth indicated that there was no evidence of an interaction between dietary pattern scores and alcohol consumption during pregnancy.

7.5 Discussion

The overall aim of Study 2B was to explore whether particular aspects of maternal dietary intake modify the relationship between antenatal alcohol consumption and fetal growth, using data from the ALSPAC cohort. The two patterns of alcohol consumption were frequency of alcohol consumption during early pregnancy and binge drinking during mid-pregnancy, and fetal growth restriction was defined as giving birth to a SGA infant. This is the first study to explore whether dietary intake modifies the relationship between antenatal alcohol consumption and risk of SGA in a human population.

The main finding from this study is that vitamin E appeared to interact with alcohol consumption, modifying the relationship between potentially harmful patterns of drinking and the risk of giving birth to a SGA infant. Women who reported at least one occasion of binge drinking in the past month and were in Q1 for vitamin E intakes were almost twice as likely to give birth to a SGA infant, compared to

women who also reported binge drinking, but were in other quartiles for vitamin E intake. This relationship persisted even after adjusting for all other covariates.

Vitamin E is a lipid soluble antioxidant that is made up of a number of different compounds; the most abundant found in the diet are α-tocopherol and γ-tocopherol. Vitamin E has the ability to scavenge free radicals, protecting against oxidative damage, and research studies investigating its effects on fetal development have reported a positive relationship between plasma concentrations of α-tocopherol and birth weight. A study in 1,231 pregnant women in the US reported a significantly lower risk of SGA in women who had higher plasma concentrations of alpha-tocopherol and dietary intake of vitamin E (O Scholl et al. 2006). A number of other studies have also investigated plasma or serum concentrations of α-tocopherol and reported a positive correlation between concentrations and birth weight (von Mandach et al. 1994; Dreyfuss et al. 2001; Lee et al. 2004). In contrast, one study conducted in Canada even reported that high doses of vitamin E were associated with significantly lower birth weights (Boskovic et al. 2005); however, vitamin E supplement intakes were self-reported, it was a small sample size (n=212), no details were provided on how they estimated intake, no measure of dietary intake was reported, and results were not appropriately adjusted for markers of socio-economic status.

Vitamin C and carotenoids are also potent scavengers of free radicals; however, there was no evidence of them modifying the relationship between alcohol consumption and fetal growth. This observation has been reported in other studies. A study conducted in Australia (n=299), found those with low intakes of vitamin E were at higher risk of hypertensive disorders compared to women with higher intakes, whereas no relationship was observed with vitamin C (Rumbold et al. 2005). A plausible mechanism for this association could be related to placental blood flow. Thromboxane (vasoconstrictor) and prostacyclin (vasodilator) are hormones involved in blood flow regulation to intrauterine tissues. Vitamin E enhances the release of prostacyclin, which inhibits platelet aggregation and increases blood flow and nutrient supply to the fetus (O Scholl et al. 2006). In contrast, ethanol has also been shown to increase thromboxane release, particularly on the fetal side of the placenta (Siler-Khodr et al. 2000). Therefore, low intakes of vitamin E, coupled with binge drinking during pregnancy may result in an imbalance in thromboxane and prostacyclin, increasing the risk of having a SGA baby.

An RCT designed to explore the effect of high doses of vitamin C and E during pregnancy found an increased risk of low birth weight in women who had been randomized to receive the antioxidants (Poston et al. 2006). Therefore, it could also be possible that vitamin E is actually acting as a proxy for some other factor that is associated with increased odds of having SGA birth in women who report binge drinking. It is feasible that smoking may be responsible for this observed relationship. Smoking also disrupts the balance between thromboxane and prostacyclin (Lynch et al. 2011), and in the

multivariable model it was the strongest predictor of giving birth to a SGA infant. While smoking during pregnancy was adjusted for in the analysis, it is possible that residual confounding may play a role.

There was no compelling evidence to suggest that low intakes of OCM micronutrients modified the relationship between maternal alcohol consumption and fetal growth. Results from the stratified logistic regression models and likelihood ratio tests indicated no significant differences between odds of giving birth to a SGA infant according to maternal alcohol consumption when stratified by quartiles of OCM micronutrient intakes. In contrast, in women who reported one or more alcoholic drinks per day during early pregnancy were significantly more likely to have a SGA infant compared to women who did not drink alcohol during the same period. But this was only significant if they had higher intakes of OCM micronutrients (Q2-Q4). This was unexpected, but it may be attributable to the small sample size in Q1; indicating a lack of power to detect differences (Button et al. 2013).

The current evidence has reported mixed findings regarding dietary intakes of OCM micronutrients and fetal growth (Kordas et al. 2009; Nilsen et al. 2010; Takimoto et al. 2011; Torres-Sánchez et al. 2014), and a study conducted in India concluded that it was in fact the ratio of folate to vitamin B12 intake that was associated with fetal growth restriction (Dwarkanath et al. 2013). It may be possible that because of the synergistic nature of micronutrients involved in OCM, ratios of micronutrient intakes need to be explored, rather than investigating the intake of individual micronutrients in isolation. Running separate logistic regression models for each micronutrient has meant it is not possible to detect these relationships. Dietary pattern analysis is one way of minimising this limitation; however, when maternal dietary pattern scores were assessed, there was also no evidence of effect modification.

There was no compelling evidence to suggest that maternal dietary patterns during pregnancy are associated with odds of having an SGA infant. This lack of a relationship may be attributable to a number of reasons. Firstly, the five dietary patterns explore accounted for only 31% of the variation in the data (described in detail in chapter 6). Secondly, the 'Health conscious' dietary pattern was only moderately correlated with vitamin E intake (r=0.49) and all other dietary patterns showed either weak or negligible correlation. However, this assumes the relationship between vitamin E and fetal growth is genuine and that vitamin E is not acting as a proxy for another variable.

Maternal smoking status during pregnancy was the strongest predictor of having a SGA birth, which is consistent with current evidence. One study has even suggested that smoking overrules all predictors of SGA births, including alcohol and dietary factors (Van den Berg et al. 2013). Poon et al (2013) investigated relationships between maternal dietary patterns and fetal growth and reported similar findings; once smoking was added to the model, adherence to the 'Mediterranean' dietary pattern and HEI scores were no longer associated with having a SGA birth (Poon et al. 2013). However, a study conducted in New Zealand reported that adherence to a 'Traditional' dietary pattern during the first

trimester, which was characterized by meat, fruit and vegetables, remained significantly associated with lower odds of SGA in multivariate logistic regression models that included smoking status (Thompson et al. 2010). The same relationship was not evident when adherence to the same dietary pattern was measured during the third trimester, suggesting that maternal dietary patterns may be significant predictors of SGA, potentially modifying the relationship between alcohol and fetal growth, but only during the early stages of pregnancy.

7.5.1 Strengths and limitations

The strengths pertaining to the ALSPAC study in general have previously been discussed in Chapters 3 and 6. However, there are a number of strengths that are unique to this study. Firstly, the use of customised birth centiles to define SGA births enables more accurate identification of fetal growth restriction (Gardosi et al. 1995). Secondly, exploring interactions using two different methods – logistic regression models, stratified by dietary intakes, and likelihood ratio tests - has provided a more comprehensive assessment of the potential effect modification of maternal dietary intake. Thirdly, research midwives and nurses collected birth weight in the hospital, at the time of birth, meaning birth weight data was available for the majority of the ALSPAC sample in order to calculate customised birth centiles.

However, there are also limitations to this study, which must be addressed (some limitations of this study have been discussed in more detail in Chapters 3 and 6). Firstly, the ALSPAC dataset was collected more than 15 years ago, between 1990 and 1991. Since then, government recommendations on alcohol consumption during pregnancy have changed (RCOG 2006), and reported antenatal alcohol consumption is much lower; a recent national survey, with only 3% of women reporting consuming two or more units of alcohol per week during their pregnancy in 2010 (Mc Andrew et al. 2012). Secondly, the definition of binge drinking in this study is at least one episode of four or more units of alcohol, across one day in the past month. This is a broad definition and does not provide any distinction between women who consume large amounts of alcohol in one sitting (e.g. 10 units over a number of hours) and women who drank four units across an entire day. The blood alcohol levels of women could vary greatly; meaning the risk of harm may not be consistent within this sub-sample of women. Typically, binge drinking is defined as more than five units in one occasion, and this difference may be responsible for the lack of relationship observed between this pattern of alcohol consumption and SGA in this study. Finally, multicollinearity can result in very sensitive and unstable models (Bland 2000); as micronutrient intakes are likely to be correlated, separate logistic regression models were run when exploring the relationship between each micronutrient, alcohol consumption and fetal growth. However, micronutrients also often act in a synergistic way, and by running separate models, the ability to assess combinations of intakes is lost; one micronutrient may be acting as a proxy for other dietary factors.

7.6 Conclusions

Overall, the results presented in this chapter suggest that low intakes of vitamin E during pregnancy may increase the risk of fetal growth restriction in women who engage in binge drink during the first 18 weeks of pregnancy. While the ALSPAC cohort study has provided a large, comprehensive data that has enabled a broad range of research to be conducted, the data collection methods were not designed specifically for the objectives of this study. Therefore, further research is required to further explore this relationship before the clinical implications of these findings can be fully considered.

Based on the findings of this study, there are a number of recommendations for future research. Firstly, dietary intake of vitamin E should be estimated using biomarker data and more detailed dietary assessment methods that enable absolute intake values to be calculated. Secondly, ratios of micronutrient intakes and status should also be explored; generating dietary patterns that are characterised by micronutrient intakes using methods, such as Reduced Rank Regression (RRR), may provide further insight into this issue. Until further research has been conducted to either refute or support the findings presented in this chapter, healthcare professionals should be made aware of the interactive nature of alcohol and particular micronutrients and asked to provide women with more guidance on the importance of abstaining and eating a varied diet during pregnancy.

Chapter 8. Exploration of maternal alcohol consumption, dietary intake and offspring cognitive outcomes (Study 2C)

8.1 Introduction

An Intelligence Quotient (IQ) is a measure of general intelligence that is derived from a number of tests and refers to an individual's mental agility. Existing evidence indicates a link between childhood IQ scores and mortality and morbidity in later life, with low childhood IQ scores associated with increased morbidity and shorter lifespans (Deary et al. 2004).

The findings from Chapter 2 highlighted the risks of heavy alcohol exposure during pregnancy on cognitive development (Jones & Smith 1973; Ouellette et al. 1977; Sokol et al. 1980), which can include lifelong devastating outcomes for affected individuals. However, the chapter also indicated that there are inconsistencies in the evidence surrounding low to moderate intakes of alcohol and the risk of cognitive deficits in later life (Alati et al. 2008; Falgreen Eriksen et al. 2012; Alati et al. 2013).

Findings from Chapters 5, 6 and 7 (Studies 1B, 2A & 2B) suggest that maternal diet may be an overlooked confounder that contributes to the inconsistencies within the published data. In Study 2A, women with greater adherence to dietary patterns characterised by high intakes of processed foods, and low micronutrient intakes, were more likely to report drinking one or more drinks per day during the first trimester and at least one episode of binge drinking during the past month. In addition to this, findings from Study 2B indicated that binge drinking, in the presence of low vitamin E intakes (Q1), might increase the odds of fetal growth restriction, compared to women with higher intakes of vitamin E (Q2-Q4).

Evidence from animal models of FASD have also suggested that maternal diet may modify the relationship between alcohol consumption and cognitive deficits in offspring (Cohen-kerem & Koren 2003; Ballard et al. 2012). A study by Thomas et al (2010) reported that choline supplements mitigated the cognitive and behavioural deficits that are attributable to ethanol exposure during pregnancy (Thomas et al. 2004; Thomas et al. 2010). Studies have also found that ethanol exposed, pregnant guinea pigs given vitamin C and vitamin E had offspring with higher brain weights compared to guinea pigs fed ethanol alone (Nash et al. 2007). Of the ethanol exposed rats, those treated with vitamin E showed fewer signs of hippocampal cell loss (Marino et al. 2004) and purkinje cell loss (Heaton et al. 2000).

A recent research study reported the findings from an RCT conducted in the Ukraine. Coles et al (2015) randomised pregnant women who reported moderate alcohol consumption to receive: 1) a multivitamin; 2) a multivitamin plus choline supplement; or 3) no supplements. Infants born to women in group 3 were more likely to show signs of cognitive deficits at 6 months of age (Coles et al. 2015).

However, the study did not report the composition of the multivitamin or randomize women to receive a choline supplement alone; therefore, it is difficult to understand whether choline alone is effective or if it is a synergistic effect between choline and a number of different compounds.

This chapter will now extend these findings by investigating the role of One Carbon Metabolism (OCM) micronutrients and dietary antioxidants in the relationship between antenatal alcohol consumption and child IQ scores.

8.2 Aims and Objectives

The overall aim of this study was to explore whether particular aspects of maternal dietary intake modify the relationship between antenatal alcohol consumption and child IQ scores, using data from the ALSPAC study.

The specific objectives of this study were:

- To describe childhood IQ scores at 8 years in sample of children born to women from the ALSPAC cohort
- To explore relationships between maternal alcohol consumption during pregnancy and childhood IQ scores at 8 years
- To explore relationships between maternal dietary intake (OCM micronutrients and antioxidants) and childhood IQ scores at 8 years
- To investigate whether low maternal intakes of OCM micronutrients (folate, choline, betaine, methionine, vitamin B12 and vitamin B6) are associated with childhood IQ scores at 8 years in women who report:
    - Alcohol consumption during early pregnancy
    - Binge drinking in mid-pregnancy
- To investigate whether low maternal intakes of dietary antioxidants (Vitamin C, vitamin E and carotenoids) are associated with childhood IQ scores at 8 years in women who report:
    - Alcohol consumption during early pregnancy
    - Binge drinking in mid-pregnancy
- To explore relationships between maternal dietary patterns and childhood IQ scores at 8 years
- To investigate whether adherence to a particular maternal dietary pattern are associated with childhood IQ scores at 8 years in women who report:
    - Alcohol consumption during early pregnancy
    - Binge drinking in mid-pregnancy

8.3 Methods

8.3.1 Study design

The present study comprises of a secondary analysis using data from ALSPAC (Golding, Pembrey, Jones, et al. 2001) (a detailed description of the observational, cohort study is included in Chapter 3). Women completed questionnaires at 8 and 18 weeks gestation, providing details of socio-demographic characteristics and measures of alcohol consumption up to that point in pregnancy. At 32 weeks gestation, women completed a Food Frequency Questionnaire (FFQ). When children were 7 years old, families were invited to attend annual assessments in ALSPAC clinics. The assessments involved a range of interviews, questionnaires and tests for both parents and children. Children completed a shortened version of the Wechsler Intelligence Scale test (3rd edition) (WISC III) for children when they were 8 years old, which was administered by a trained psychologist.

8.3.2 Sample population

A description of the whole ALSPAC sample population has been included in Chapters 3 and 6. Women and children were included in the present analysis if they had a singleton birth (n=13,678), provided details of alcohol consumption at 18 weeks gestation (n=13,197), completed the FFQ at 32 weeks gestation (n=12,190) and children participated in a WISC III assessment during an ALSPAC clinical visit at approximately 8 years of age (n=7,350).

8.3.3 Measures

Main outcome measure: Wechsler Intelligence Scale for Children (WISC III)

The WISC-III scale is a self-administered IQ test for children aged 6 to 16 years. It comprises of two scales (each one containing a number of items for children to respond to):

- Verbal IQ obtains measures of children's ability to understand instructions and their ability to respond appropriately. The verbal IQ is comprised of five subsets: Information; Similarities; Arithmetic; Vocabulary; and Comprehension.
- Performance IQ assesses the perceptual organisation of children using five subsets: Picture completion; Coding; Picture arrangement; Block design; and Object assembly.

Children who attended the focus clinical visits at 8 years completed a shortened version of the WISC-III scale, which comprised of alternative items for all subsets (with the exception of the coding subset, where children completed the full set of questions). Children also completed a digit span task, which tested short-term storage capacity by asking children to repeat listings of different numbers in varying orders. Members of the ALSPAC team prepared the raw scores so that the shortened version was comparable to the full scale. The methods have been described elsewhere (Joinson et al. 2007). The WISC-III has been compared with other tests of intelligence and cognitive ability, and results were

strongly correlated (Levinson & Folino 1994; Canivez 1995), indicating the WISC III is a valid tool for measuring child IQ scores.

Alcohol consumption

Antenatal alcohol consumption was self-reported at 18 weeks gestation. The same alcohol variables were explored in Study 2A and full descriptions have been provided in the previous chapter (Study 2A, section 6.3.3). Two measures of maternal alcohol consumption were explored in relation to childhood IQ in Study 2C:

- Frequency and quantity of alcohol consumption during early pregnancy
- Binge drinking during mid-pregnancy

Dietary data

Women completed a FFQ at 32 weeks gestation. Full details of the FFQ and the preparation of dietary data have been provided in Chapter 6 (Study 2A, section 6.3.3). Two measures of dietary intake are explored in relation to child IQ scores in Study 2C:

1. Mean daily intakes of OCM micronutrients (folate, choline, betaine, methionine, vitamin B12 and vitamin B6) and dietary antioxidants (vitamin C, vitamin E and carotenoids. Intakes were categorised into quartiles. Being in quartile 1 (Q1) was consistently a predictor of lower IQ scores at 8 years, compared to other quartiles. Quartiles 2, 3 and 4 (Q2-Q4) were combined and investigations compared Q1 vs. Q2-Q4. Folate intake was explored with and without the addition of folic acid (FA) supplements.
2. Maternal dietary pattern scores previously derived by Northstone and colleagues (K Northstone et al. 2008), including 'Health conscious', 'Traditional', 'Processed', 'Confectionery' and 'Vegetarian'. Dietary pattern scores were categorised into quartiles. Quartile 4 (Q4) indicated the strongest adherence to each dietary pattern. Quartiles 1, 2 and 3 (Q1-Q3) were combined and investigations compared Q1-Q3 vs. Q4.

Socio-demographic characteristics

Details of maternal socio-demographic characteristics were self-reported at 8 and 18 weeks gestation. Based on a the findings of the literature review in Chapter 2, maternal age, ethnicity, parity, education, smoking (Lawlor et al. 2005; Skagerström et al. 2011; Eriksen et al. 2013), breastfeeding, child gender, birth weight (Shenkin et al. 2004; Lynn & Kanazawa 2011) and markers of SES, including housing tenure, house crowding index (HCI), living in a single parent household (Skagerström et al. 2011; Hanscombe et al. 2012; Chambers et al. 2014), were added as confounders to the multivariable models. Full details of how these socio-demographic characteristics were categorised are included in Chapter 6 (Study 2A).

8.3.4 Statistical analysis

IQ score at 8 years was treated as a continuous variable and scores are presented as means and standard deviations. Socio-demographic characteristics are presented as frequencies and percentages and birth weights are presented as means and standard deviations. Two-by-two tables and chi-squared tests were used to compare the differences in socio-demographic characteristics between the sample population and women with missing data who were excluded from the present analysis. ANOVAs and t-tests were used to investigate the relationship between IQ scores at 8 years and socio-demographic characteristics of the sample population.

Linear regression models were used to explore the relationships between maternal alcohol consumption and child IQ scores. Separate models were used to explore frequency of alcohol consumption during early pregnancy and binge drinking during mid-pregnancy. Child IQ score was treated as a continuous, dependent variable and maternal alcohol consumption as categorical, independent variables. Maternal age, ethnicity, parity, education, housing tenure, HCI, smoking status, living in a single parent household, birth weight and gender were then added to the regression models as covariates.

Linear regression models were then used to explore associations between maternal dietary intakes of micronutrients and antioxidants (folate, choline, betaine, methionine, vitamin B12, vitamin B6, vitamin C, vitamin E and carotenoids) and child IQ scores. Separate regression models were used to explore each dietary factor due to multicollinearity (Williams et al. 2013). Child IQ score was treated as a continuous, dependent variable and maternal micronutrient intakes were categorised as quartiles and treated as categorical, independent variables. Multivariable models included maternal age, ethnicity, parity, education, housing tenure; HCI, smoking status, living in a single parent household, birth weight and child gender.

Two methods were employed to assess whether maternal micronutrient and antioxidant intakes modified the relationship between alcohol consumption (frequency of alcohol consumption and binge drinking) and child IQ scores at 8 years:

1) Linear regression models were used to assess child IQ scores by maternal alcohol consumption during pregnancy and were stratified by quartiles of maternal dietary intakes.
2) Linear regression models were run with main effect terms and an interaction term for each dietary factor and maternal alcohol consumption during pregnancy. The interaction term was then removed and the model was run again, with main effect terms only. The likelihood ratio test was used to assess the likelihood of the set of parameter estimates given the outcomes. The likelihood ratio test compared the fit of one model to another (with and without interaction term) and if the difference was significant, then the less restrictive model (with

interaction term) was considered a better fit, indicating an interaction between dietary and alcohol variables (Kleinbaum 1994).

Models were run with dietary and alcohol predictors only and then again with all other covariates (maternal age, education, housing tenure, HCI, smoking status during pregnancy, living in a single parent household, birth weight and child gender). Covariates were added to the model, one-by-one to assess the change in β-coefficient with each addition.

Linear regression models were then used to explore the relationships between each maternal dietary pattern described in Study 2A and child IQ scores at 8 years. Child IQ score was treated as a continuous, dependent variable and maternal dietary pattern scores were categorised as quartiles and treated as categorical, independent variables. Maternal age, ethnicity, parity, education, housing tenure, HCI, smoking status, living in a single parent household, birth weight and child gender were then added to the regression models as covariates.

To assess whether maternal dietary pattern scores modified the relationship between alcohol consumption (frequency of alcohol consumption and binge drinking) and child IQ scores, the same two methods were carried out, as described previously, with maternal dietary pattern scores instead of micronutrient and antioxidant intakes. Unadjusted models were run, followed by multivariate models with the addition of maternal age, ethnicity, parity, education, housing tenure; HCI, smoking status, living in a single parent household, birth weight and child gender as covariates.

To aid the interpretation of interactions, plots of marginal means calculated from linear regression models with interaction terms were generated. Significance was defined as $p<0.05$, and all statistical analyses were conducted using STATA 13.1

8.3.5 Missing data

Women with more than 10 missing items from the FFQ were excluded from the analysis. Women with less than 10 missing items were included in the present analyses, and each missing item was assumed to be consumed 'never or rarely', and therefore coded as '0' (Northstone et al. 2008).

8.4 Results

8.4.1 Sample population

The socio-demographic characteristics of ALSPAC women are presented in Table 8.1. A total of 7,148 women and their children from the original ALSPAC cohort attended the Children in Focus (CiF) visit and participated in the WISC III assessment. The whole sample analysis included 5,557 women and

their children. The majority of women were between the ages of 20 and 39, white, lived with a partner, did not smoke, lived in a property that they owned or had mortgaged and breastfed their child.

Table 8.1 Socio-demographic characteristics of sample population (n=5,557)

	Sample (n=5,557)		Missing (n=6,317)		p-value^
	n	%	n	%	
Maternal age					
15-19	66	1	397	6	
20-29	2885	52	3892	62	
30-39	2527	45	1960	31	
40+	79	1	68	1	<0.0001
Maternal ethnicity					
Non-white	83	1	213	3	
White	5474	99	5955	97	<0.0001
Parity					
Nulliparous	2559	46	2593	44	
Multiparous	2,998	54	3,296	56	0.03
Maternal education					
Vocational	1113	20	2413	39	
O level	1968	35	2127	34	
A level	1534	28	1134	18	
Degree level	942	17	573	9	<0.0001
Single parent household					
No	5369	97	5237	92	
Yes	188	3	462	8	<0.0001
Maternal smoking					
No	4851	87	4053	74	
Yes	706	13	1438	26	<0.0001
Housing tenure					
Owner/occupied	4775	86	3932	66	
Council/HA renting	426	8	1224	21	
Private renting	356	6	794	13	<0.0001
House crowding index					
<= 0.5	2796	50	2121	37	
>0.5 - 0.75	1758	32	1826	32	
>0.75 - 1	826	15	1343	23	
> 1	177	3	493	9	<0.0001
Any breastfeeding					
Yes	4710	85	4012	72	
No	847	15	1563	18	<0.0001
Child gender					
Female	2792	50	2976	47	
Male	2765	50	3341	53	0.001
Birth weight (g) mean, sd	3457	511	3409	537.48	<0.0001

^Results from chi-squared analysis

ANOVAs were used to explore relationships between IQ scores and socio-demographic characteristics (Table 8.2). Results indicated that total IQ scores at 8 years increased with maternal age, education, birth weight and breastfeeding; and decreased with smoking, increasing parity, living in crowded conditions (HCI) and in rented accommodation (in particular council or Housing Association rented accommodation).

Table 8.2 Estimates of child IQ scores at 8 years by maternal socio-demographic and lifestyle characteristics (n=5,557)

	Mean IQ (sd)	p-value
Age		
<20	97 (16)	
20-29	102 (16)	
30-39	107 (17)	
40+	108 (16)	<0.0001
Ethnicity		
White	104 (16)	
Non-white	102 (20)	0.249
Parity		
Nulliparous	106 (16)	
Multiparous	103 (17)	<0.0001
Smoking		
Non-smoker	105 (16)	
Smoker	100 (16)	<0.0001
Education		
<O level	96 (15)	
O level	102 (15)	
A level	108 (15)	
Degree level	115 (15)	<0.0001
Housing		
Owner/mortgaged	106 (16)	
Private renting	103 (16)	
Council/HA renting	94 (16)	<0.0001
HCI		
<0.5	108 (16)	
≥0.5 - 0.75	104 (16)	
≥0.75 - 1	99 (16)	
> 1	97 (16)	<0.0001
Single parent household		
No	105 (16)	
Yes	97 (17)	<0.0001

8.4.2 Missing data

Chi-squared analysis was used to compare the whole sample population with those with missing data. Compared to those in the present analysis, women with missing data were more likely to be younger, non-white, less educated, live without a partner, rent their property, live in crowded conditions, smoke and not breastfeed their children. Women with missing data were also more likely to report binge drinking during mid-pregnancy and more frequent drinking during early pregnancy, compared to women included in the present analysis (Table 8.3). They were also more likely to adhere to the 'Traditional' (OR=1.42, 95% CI=1.31, 1.54; p<0.0001) and 'Vegetarian' dietary patterns (OR=1.23, 95% CI=1.04, 1.22; p=0.0003), and be less adherent to the 'Health conscious' (OR=0.53, 95% CI=0.49, 0.58; p<0.0001) and 'Processed' dietary patterns (OR=0.87, 95% CI=0.81, 0.95; 0.001). The odds of being in Q1 for folate (OR=1.22, 95%CI=1.07, 1.40; p<0.0001), methionine (OR=1.30, 95%CI=1.16, 1.46; p<0.0001), vitamin C (OR=1.33, 95%CI=1.20, 1.47; p<0.0001), vitamin E (OR=1.20, 95%CI=1.09, 1.32; p<0.0001) and carotenoids (OR=1.10, 1.00, 1.21; p=0.04) were significantly higher in women who did not attend the follow up clinic assessments at 8 years, compared to women who did attend.

Table 8.3 Frequency and quantity of reported maternal alcohol consumption during pregnancy by women with and without IQ scores at 8 years

	Sample		Missing		p-value^
	n	%	n	%	
Binge drinking					
No	5204 (94)	94	5,417 (91)	91	
Yes	353 (6)	6	529 (9)	9	<0.0001
Alcohol 1st trimester					
Never	2,438 (44)	44	2,770 (46)	46	
<1 drink/week	2,281 (41)	41	2,249 (38)	38	
>1 drink/week	743 (13)	13	861 (14)	14	
1+ drink/day	85 (2)	2	119 (2)	2	<0.0001

^Results from chi-squared analysis

8.4.3 Maternal alcohol consumption and child IQ scores at 8 years

The mean total IQ score at 8 years was 104 (16.5 SD), ranging from 45 to 151. Results from the Univariate analyses are presented in Table 8.4. Unadjusted linear regression models indicated that low (<1 drink/week) to moderate (≥1 drink/week) alcohol consumption during the first trimester was associated with significantly higher IQs in children born to mothers who reported no alcohol consumption during that period. However, when reported intake levels reached one or more alcoholic drinks per day, there was a slight reduction in child IQ scores (β=-2.27, 95% CI=-5.48, 0.93; p=0.164). Children born to mothers who reported binge drinking were significantly more likely to have lower IQ

scores at 8 years compared to children born to women who did not (β=-4.40, 95% CI=-6.01, -2.70; p<0.0001).

Table 8.4 Unadjusted β-coefficients of child IQ score at 8 years by maternal alcohol consumption during pregnancy

	Total IQ		
	β	95% CI	p-value
1st trimester drinking			
Never		(ref)	
<1 drink/week	0.95	(0.08, 1.83)	0.033
>1 drink/week	1.82	(0.56, 3.08)	0.005
1+drink/day	-2.27	(-5.48, 0.93)	0.164
Heavy episodic drinking			
No		(ref)	
Yes	-4.4	(-6.01, -2.70)	<0.0001

8.4.4 Maternal micronutrient intakes

In unadjusted linear regression models, with the exception of betaine, low intakes (Q1) for all micronutrient intakes at 32 weeks gestation were associated with significantly lower IQ scores at 8 years. Interestingly, being in Q1 for betaine intake was associated with higher IQ scores compared to children born to women in all other quartiles (β=2.71, 95% CI=1.81, 3.62; p<0.0001) (Table 8.5).

Table 8.5 Unadjusted β-coefficients of child IQ score at 8 years by quartiles of maternal micronutrient intakes during pregnancy (Q1 vs. Q2-Q4)

Model	β	95% CI	p-value
1:Folate			
Q1*	-3.75	(-4.73, -2.77)	<0.0001
2: Choline			
Q1*	-3.4	(-4.36, -2.44)	<0.0001
3: Betaine			
Q1*	2.71	(1.81, 3.62)	<0.0001
4: Methionine			
Q1*	-3.43	(-4.40, -2.46)	<0.0001
5: Vitamin B12			
Q1*	-2.8	(-3.75, -1.85)	<0.0001
6: Vitamin B6			
Q1*	-2.69	(-3.66, -1.73)	<0.0001
7: Vitamin C			
Q1*	-5.33	(-6.31, -4.35)	<0.0001
8: Vitamin E			
Q1*	-3.22	(-4.20, -2.25)	<0.0001
9: Carotenoids			
Q1*	-3.3	(-4.26, -2.34)	<0.0001

*Reference group = all other quartiles (Q2-Q4)

OCM micronutrients

The results from the stratified analysis of IQ, alcohol consumption and OCM micronutrient intakes are presented in Table 8.6. The results did not provide any evidence of OCM micronutrients modifying the relationship between alcohol consumption during early pregnancy and offspring IQ scores at 8 years. Moderate alcohol consumption during early pregnancy remained significantly associated with higher IQ scores, compared to women who reported never drinking, when stratified by folate, betaine and vitamin B6 intakes. This relationship diminished when adjusted for confounders. The results from the likelihood ratio tests suggested that there were no signs of an interaction between alcohol and OCM micronutrients.

The results from the linear regression models investigating maternal binge drinking as a predictor of offspring IQ at 8 years, stratified by maternal OCM micronutrient intakes are presented in Table 8.7. The results from the likelihood ratio tests indicated significant interactions between maternal binge drinking and intakes of folate (X2 (1)=12.49; p=0.0004) (Figure 8.1) and vitamin B6 (X2 (1)=10.74; p=0.001) (Figure 8.2). The interaction for folate remained significant with the addition of FA supplements (X2 (1)=10.98; p=0.0009) (Figure 8.3). While the results of the likelihood ratio tests were not significant for other OCM micronutrients, the results from the stratified linear regression models did provide some evidence of effect modification. Maternal binge drinking was a significant predictor

of lower child IQ scores at 8 years in women who were in Q2-Q4 for all OCM micronutrient intakes, compared to women who did not report binge drinking. In contrast, no significant differences in IQ scores were observed in women in Q1. These relationships remained after adjusting for confounders (Table 8.8).

Table 8.6 Unadjusted β-coefficients of child IQ scores at 8 years by maternal alcohol consumption during early pregnancy, stratified by quartiles of maternal folate, choline, betaine, methionine, vitamin B12 and vitamin B6 intake

	Never	<1 drink/week			>1 drink/week			1+ drink/day			Interaction
Model		β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value	
1: Folate											
Q1	(ref)	1.04	(-0.87, 2.95)	0.29	3.40	(0.77, 6.03)	0.01	-2.09	(-7.80, 3.63)	0.48	
Q2-Q4	(ref)	0.85	(-0.13, 1.84)	0.09	1.40	(-0.02, 2.82)	0.05	-1.85	(-5.68, 1.99)	0.35	0.413
2: Folate (S)											
Q1	(ref)	0.91	(-1.01, 2.83)	0.35	3.25	(0.60, 5.89)	0.02	-3.50	(-9.74, 2.74)	0.27	
Q2-Q4	(ref)	0.85	(-0.13, 1.83)	0.09	1.44	(0.02, 2.86)	0.05	-1.64	(-5.35, 2.08)	0.39	0.728
3: Choline											
Q1	(ref)	1.97	(0.10, 3.83)	0.04	1.97	(-0.70, 4.64)	0.15	-4.31	(-10.41, 1.78)	0.17	
Q2-Q4	(ref)	0.59	(-0.40, 1.58)	0.25	1.71	(0.29, 3.13)	0.02	-1.26	(-5.01, 2.49)	0.51	0.849
4: Betaine											
Q1	(ref)	2.69	(0.95, 4.42)	0.00	3.55	(1.20, 5.91)	0.00	-1.05	(-6.34, 4.24)	0.70	
Q2-Q4	(ref)	0.31	(-0.71, 1.32)	0.55	0.97	(-0.51, 2.46)	0.20	-3.31	(-7.34, 0.72)	0.11	0.395
5: Methionine											
Q1	(ref)	2.44	(0.55, 4.34)	0.01	2.07	(-0.73, 4.87)	0.15	-1.35	(-8.23, 5.52)	0.70	
Q2-Q4	(ref)	0.42	(-0.57, 1.41)	0.40	1.57	(0.17, 2.97)	0.03	-2.61	(-6.22, 1.00)	0.16	0.571
6: Vitamin B12											
Q1	(ref)	2.31	(0.47, 4.14)	0.01	1.29	(-1.44, 4.01)	0.35	-3.43	(-10.94, 4.08)	0.37	
Q2-Q4	(ref)	0.41	(-0.59, 1.41)	0.42	1.72	(0.31, 3.14)	0.02	-2.34	(-5.88, 1.19)	0.19	0.181
7: Vitamin B6											
Q1	(ref)	1.32	(-0.56, 3.21)	0.17	3.21	(0.52, 5.91)	0.02	-2.14	(-7.96, 3.67)	0.47	
Q2-Q4	(ref)	0.78	(-0.21, 1.77)	0.12	1.37	(-0.05, 2.79)	0.06	-2.03	(-5.85, 1.80)	0.30	0.519

95% CI= 95% confidence intervals

*Results from likelihood ratio tests

Table 8.7 Unadjusted β-coefficients of child IQ scores at 8 years by maternal binge drinking during mid-pregnancy, stratified by quartiles of maternal folate, choline, betaine, methionine, vitamin B12 and vitamin B6 intake

	No	Yes			Interaction
Model		β	95% CI	p-value	
1: Folate					
Q1	(ref)	0.78	(-2.34, 3.90)	0.62	
Q2-Q4	(ref)	-5.84	(-7.72, -3.97)	0.00	0.0004
2: Folate (S)					
Q1	(ref)	0.36	(-2.73, 3.45)	0.82	
Q2-Q4	(ref)	-5.79	(-7.67, -3.91)	0.00	0.0009
3: Choline					
Q1	(ref)	-1.99	(-5.25, 1.27)	0.23	
Q2-Q4	(ref)	-5.06	(-6.91, -3.21)	0.00	0.107
4: Betaine					
Q1	(ref)	-2.63	(-5.81, 0.55)	0.11	
Q2-Q4	(ref)	-5.03	(-6.89, -3.16)	0.00	0.197
5: Methionine					
Q1	(ref)	-1.82	(-5.38, 1.73)	0.32	
Q2-Q4	(ref)	-5.11	(-6.91, -3.30)	0.00	0.103
6: Vitamin B12					
Q1	(ref)	-1.76	(-5.24, 1.72)	0.32	
Q2-Q4	(ref)	-5.10	(-6.92, -3.28)	0.00	0.093
7: Vitamin B6					
Q1	(ref)	0.20	(-2.88, 3.29)	0.90	
Q2-Q4	(ref)	-5.84	(-7.73, -3.94)	0.00	0.001

95% CI= 95% confidence intervals
*Results from likelihood ratio tests

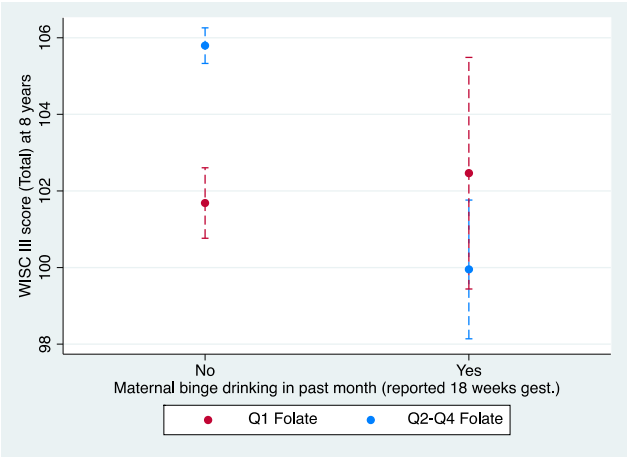

Figure 8.1 Unadjusted Child IQ scores at 8 years by maternal binge drinking during mid-pregnancy, stratified by quartiles of maternal folate intake

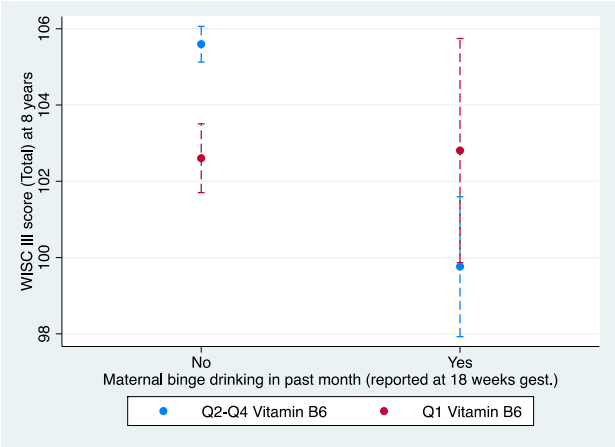

Figure 8.21 Unadjusted Child IQ scores at 8 years by maternal binge drinking during mid-pregnancy, stratified by quartiles of maternal vitamin B6 intake

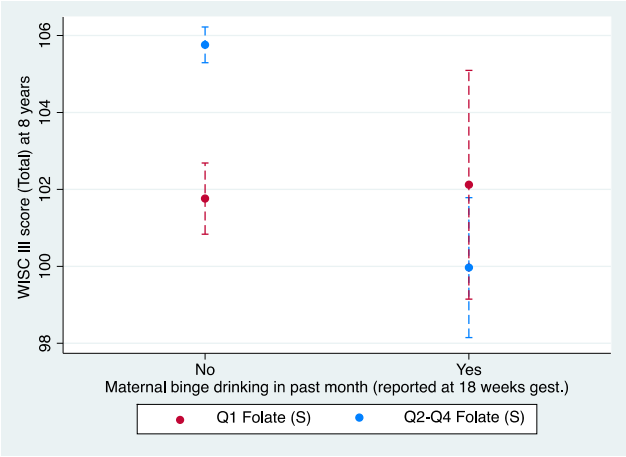

Figure 8.3 Unadjusted Child IQ scores at 8 years by maternal binge drinking during mid-pregnancy, stratified by quartiles of maternal folate intake (dietary folate + folic acid supplements)

Table 8.8 Adjusted β-coefficients of child IQ scores at 8 years by maternal binge drinking during mid-pregnancy, stratified by quartiles of maternal folate, choline, betaine, methionine, vitamin B12 and vitamin B6 intake^

	No	Yes			Interaction*
Model		β	95% CI	p-value	
1: Folate					
Q1	(ref)	2.69	(-0.38, 5.76)	0.09	
Q2-Q4	(ref)	-3.28	(-5.11, -1.45)	0.00	0.004
2: Folate (S)					
Q1	(ref)	2.95	(-0.08, 5.99)	0.06	
Q2-Q4	(ref)	-3.42	(-5.25, -1.58)	0.00	0.003
3: Choline					
Q1	(ref)	-0.17	(-3.42, 3.08)	0.92	
Q2-Q4	(ref)	-2.46	(-4.25, -0.66)	0.01	0.361
4: Betaine					
Q1	(ref)	-0.82	(-4.04, 2.40)	0.62	
Q2-Q4	(ref)	-2.22	(-4.03, -0.41)	0.02	0.644
5: Methionine					
Q1	(ref)	-0.53	(-3.99, 2.94)	0.77	
Q2-Q4	(ref)	-2.33	(-4.09, -0.56)	0.01	0.637
6: Vitamin B12					
Q1	(ref)	1.45	(-1.98, 4.89)	0.41	
Q2-Q4	(ref)	-2.81	(-4.58, -1.03)	0.00	0.232
7: Vitamin B6					
Q1	(ref)	1.70	(-1.31, 4.72)	0.27	
Q2-Q4	(ref)	-3.16	(-5.01, -1.32)	0.00	0.012

95% CI= 95% confidence intervals

*Results from likelihood ratio tests

^Adjusted for maternal energy intake, age, parity, ethnicity, smoking, single parent household, education, housing tenure, HCI, breastfeeding, child gender and birth weight.

The results from the fully adjusted linear regression models, with interaction terms, are presented as plots of the marginal means in Figures 8.4 and 8.5. In women who did not report binge drinking, child IQ scores at 8 years remained significantly lower in women in Q1 for folate and vitamin B6, compared to women in Q2-Q4. However, the confidence intervals for folate and vitamin B6 intakes were wide and overlapping in women who reported binge drinking.

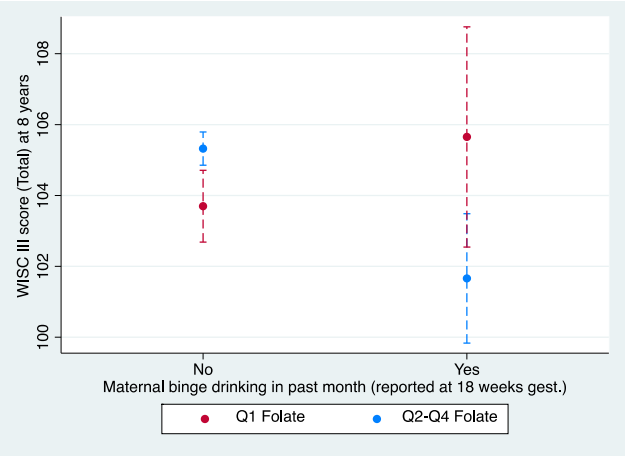

Figure 8.4 Child IQ scores at 8 years by maternal binge drinking during mid-pregnancy, stratified by quartiles of maternal folate intake, adjusted for all other covariates (energy intake, age, parity, ethnicity, smoking, single parent household, education, housing tenure, HCI)

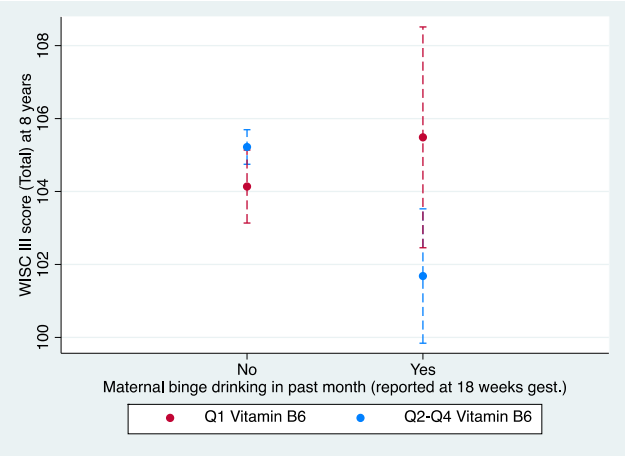

Figure 8.5 Child IQ scores at 8 years by maternal binge drinking during mid-pregnancy, stratified by quartiles of maternal vitamin B6 intake, adjusted for all other covariates (energy intake, age, parity, ethnicity, smoking, single parent household, education, housing tenure, HCI)

Dietary antioxidants

Table 8.9 presents the results from unadjusted linear regression models stratified by dietary antioxidant intakes at 32 weeks gestation. Moderate alcohol consumption during the first trimester was significantly associated with higher offspring IQ scores, but only in women who were in Q2-Q4 for vitamin C, E and carotenoid intakes. Results from the likelihood ratio tests provided no evidence of dietary antioxidants modifying the relationship between maternal alcohol consumption during the first trimester and offspring IQ scores at 8 years.

Table 8.9 Unadjusted β-coefficients of child IQ scores at 8 years by maternal alcohol consumption during early pregnancy, stratified by quartiles of maternal vitamin C, vitamin E and carotenoid intakes

	Never	<1 drink/week			>1 drink/week			1+ drink/day			Interaction
Model		β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value	
1: Vitamin C											
Q1	(ref)	0.77	(-1.13, 2.67)	0.43	1.23	(-1.38, 3.85)	0.36	-1.84	(-8.51, 4.84)	0.59	
Q2-Q4	(ref)	0.98	(0.00, 1.95)	0.05	2.09	(0.68, 3.51)	0.00	-2.33	(-5.94, 1.28)	0.21	0.817
2: Vitamin E											
Q1	(ref)	1.75	(-0.16, 3.66)	0.07	1.82	(-0.92, 4.57)	0.19	-2.56	(-8.80, 3.68)	0.42	
Q2-Q4	(ref)	0.68	(-0.31, 1.66)	0.18	1.74	(0.33, 3.15)	0.02	-1.98	(-5.70, 1.74)	0.30	0.363
3: Carotenoids											
Q1	(ref)	1.19	(-0.73, 3.11)	0.22	2.51	(-0.18, 5.20)	0.07	-4.17	(-12.56, 4.22)	0.33	
Q2-Q4	(ref)	0.78	(-0.20, 1.76)	0.12	1.59	(0.18, 3.00)	0.03	-2.29	(-5.73, 1.16)	0.19	0.959

95% CI= 95% confidence intervals

*Results from likelihood ratio tests

The unadjusted investigations into dietary antioxidants and binge drinking are presented in Table 8.10. The results from the likelihood ratio tests indicated that there is also a significant interaction between binge drinking and vitamin C intake on child IQ scores at 8 years (X2 (1)=4.26; p=0.039) (Figure 8.6). The results from the stratified analysis revealed that a similar relationship was observed with dietary antioxidants as with OCM micronutrients. Binge drinking was only a significant predictor of child IQ scores in women with higher intakes (Q2-Q4) of vitamin C, E and carotenoids. Once confounders were added to the linear regression model, the relationship diminished, but remained significant (X2 (1)=4.35; p=0.037) (Table 8.11) (Figure 8.7). Interaction terms were removed from linear regression models to also explore main effects. Once adjusted for confounders, low intakes (Q1) of vitamin C (β=-1.44, 95% CI=-2.47, -0.40; p=0.007) and carotenoids (β=-1.08, 95% CI=-2.06, -0.10; p=0.031) were significantly associated with lower child IQ scores at 8 years of age, compared to children born to women with higher intakes (Q2-Q4) at 32 weeks gestation.

Table 8.10 Unadjusted β-coefficients of child IQ scores at 8 years by maternal binge drinking during mid-pregnancy, stratified by quartiles of maternal vitamin C, vitamin E and carotenoid intakes

	No binge drinking	Binge drinking			Interaction*
Model		β	95% CI	p-value	
1: Vitamin C					
Q1	(ref)	-1.17	(-4.28, 1.95)	0.46	
Q2-Q4	(ref)	-5.03	(-6.90, -3.16)	<0.0001	0.039
2: Vitamin E					
Q1	(ref)	-2.12	(-5.43, 1.19)	0.21	
Q2-Q4	(ref)	-4.92	(-6.77, -3.08)	<0.0001	0.145
3: Carotenoids					
Q1	(ref)	-1.61	(-5.00, 1.79)	0.35	
Q2-Q4	(ref)	-5.19	(-7.01, -3.36)	<0.0001	0.062

95% CI= 95% confidence intervals
*Results from likelihood ratio tests

Table 8.11 Adjusted β-coefficients of child IQ scores at 8 years by maternal binge drinking during mid-pregnancy, stratified by quartiles of maternal vitamin C, vitamin E and carotenoid intakes^

	No binge drinking	Binge drinking			Interaction*
Model		β	95% CI	p-value	
8: Vitamin C					
Q1	(ref)	0.92	(-2.18, 4.01)	0.56	
Q2-Q4	(ref)	-2.84	(-4.66, -1.01)	<0.001	0.037
9: Vitamin E					
Q1	(ref)	0.83	(-2.50, 4.15)	0.63	
Q2-Q4	(ref)	-2.66	(-4.46, -0.87)	<0.001	0.11
10: Carotenoids					
Q1	(ref)	1.14	(-2.12, 4.40)	0.49	
Q2-Q4	(ref)	-2.83	(-4.63, -1.03)	<0.001	0.054

95% CI= 95% confidence intervals ^Adjusted for maternal energy intake, age, parity, ethnicity, smoking, single parent household, education, housing tenure, HCI, breastfeeding, child gender and birth weight.
*Results from likelihood ratio tests

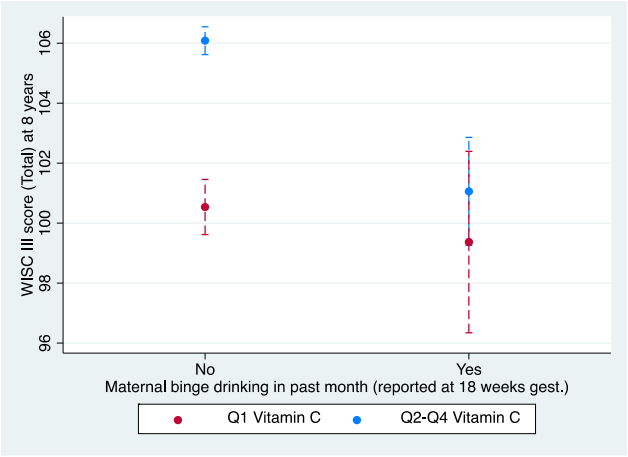

Figure 8.6 Unadjusted Child IQ scores at 8 years by maternal binge drinking during mid-pregnancy, stratified by quartiles of maternal vitamin C intake

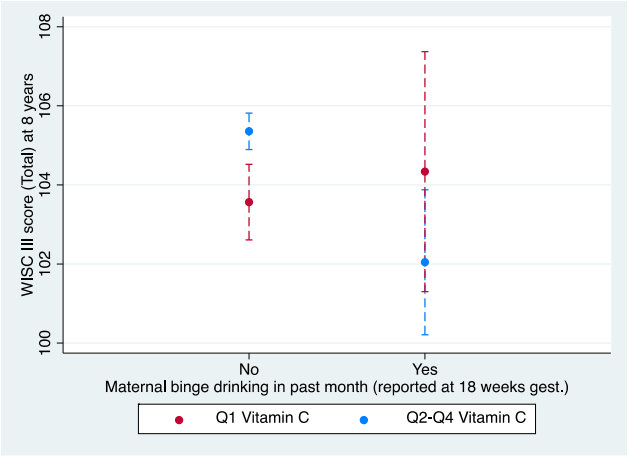

Figure 8.7 Child IQ scores at 8 years by maternal binge drinking during mid-pregnancy, stratified by quartiles of maternal vitamin C intake (energy intake, age, parity, ethnicity, smoking, single parent household, education, housing tenure, HCI)

8.4.5 Maternal dietary patterns

In unadjusted linear regression models, adherence (defined as being in Q4) to the 'Health conscious' dietary pattern was associated with significantly higher IQ scores in children at 8 years, compared to all other quartiles (β=8.14, 95% CI=7.30, 8.99; <0.0001). The opposite trend was observed for adherence to the 'Processed' dietary pattern at 32 weeks gestation (β=-4.26, 95% CI=-5.20, -3.33; <0.0001) (Table 8.12). After adjusting for potential confounders, adherence to the 'Health conscious' (β=3.37, 95% CI= 2.46, 4.30; p<0.0001) and 'Processed' (β=-1.79, 95% CI=-2.77, -0.81; <0.0001) dietary patterns remained significantly associated with child IQ scores at 8 years.

Table 8.12 Unadjusted β-coefficients of child IQ scores at 8 years by maternal dietary patterns during pregnancy (Q4 vs. all other quartiles)

Dietary pattern	β	95% CI	p-value
Health conscious			
Q4*	8.14	(7.30, 8.99)	<0.0001
Traditional			
Q4*	0.16	(-0.73, 1.05)	0.719
Processed			
Q4*	-4.26	(-5.20, -3.33)	<0.0001
Confectionery			
Q4*	-0.20	(-1.08, 0.69)	0.663
Vegetarian			
Q4*	1.00	(0.08, 1.93)	0.033

95% CI= 95% confidence intervals
*Reference category = all other quartiles (Q1-Q3)

Investigations into the modifying effect of maternal dietary patterns on the relationships between alcohol consumption and child IQ scores are presented in Tables 8.13 and 8.14. The results from the stratified linear regression models and likelihood ratio tests indicated that there was no evidence of an interaction between dietary pattern scores and alcohol consumption during pregnancy.

Table 8.13 Unadjusted β-coefficients of child IQ scores at 8 years by maternal alcohol consumption during early pregnancy, stratified by quartiles of maternal dietary pattern scores

	Never	<1 drink/week			>1 drink/week			1+ drink/day			Interaction*
		β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value	
Health conscious											
Q1-Q3	(ref)	0.68	(-0.34, 1.70)	0.19	1.00	(-0.51, 2.52)	0.19	-2.94	(-6.63, 0.74)	0.12	
Q4	(ref)	0.79	(-0.75, 2.34)	0.31	1.48	(-0.60, 3.56)	0.16	-0.55	(-6.37, 5.26)	0.85	0.906
Traditional											
Q1-Q3	(ref)	1.39	(0.37, 2.41)	0.01	2.34	(0.91, 3.78)	0.00	-2.05	(-5.95, 1.85)	0.30	
Q4	(ref)	-0.30	(-2.01, 1.41)	0.73	0.17	(-2.44, 2.78)	0.90	-2.97	(-8.58, 2.65)	0.30	0.302
Processed											
Q1-Q3	(ref)	0.88	(-0.11, 1.86)	0.08	1.78	(0.36, 3.20)	0.01	-3.00	(-6.85, 0.86)	0.13	
Q4	(ref)	1.45	(-0.38, 3.29)	0.12	2.31	(-0.29, 4.91)	0.08	1.08	(-4.53, 6.69)	0.71	0.684
Confectionery											
Q1-Q3	(ref)	0.90	(-0.13, 1.92)	0.09	1.70	(0.22, 3.18)	0.02	-1.03	(-4.93, 2.88)	0.61	
Q4	(ref)	1.14	(-0.58, 2.85)	0.19	2.19	(-0.21, 4.59)	0.07	-4.75	(-10.36, 0.85)	0.10	0.706
Vegetarian											
Q1-Q3	(ref)	0.62	(-0.37, 1.60)	0.22	1.39	(-0.05, 2.82)	0.06	-2.80	(-6.66, 1.07)	0.16	
Q4	(ref)	2.33	(0.41, 4.26)	0.02	2.95	(0.36, 5.54)	0.03	-1.61	(-7.41, 4.19)	0.59	0.413

95% CI= 95% confidence intervals

*Results from likelihood ratio tests

Table 8.41 Unadjusted β-coefficients of child IQ scores at 8 years by maternal binge drinking during mid-pregnancy, stratified by quartiles of maternal dietary pattern scores

	Non-binge drinking	Binge drinking			Interaction
		β	95% CI	p-value	
Health conscious					
Q1-Q3	(ref)	-3.51	(-5.29, -1.73)	0.00	
Q4	(ref)	-3.60	(-6.96, -0.25)	0.04	0.961
Traditional					
Q1-Q3	(ref)	-4.27	(-6.17, -2.38)	0.00	
Q4	(ref)	-4.74	(-7.83, -1.65)	0.00	0.802
Processed					
Q1-Q3	(ref)	-3.48	(-5.39, -1.58)	0.00	
Q4	(ref)	-5.40	(-8.35, -2.45)	0.00	0.291
Confectionery					
Q1-Q3	(ref)	-4.10	(-5.99, -2.20)	0.00	
Q4	(ref)	-5.21	(-8.30, -2.12)	0.00	0.548
Vegetarian					
Q1-Q3	(ref)	-4.04	(-5.88, -2.20)	0.00	
Q4	(ref)	-5.69	(-9.02, -2.37)	0.00	0.381

95% CI= 95% confidence intervals
*Results from likelihood ratio tests

8.5 Discussion

The overall aim of Study 2C was to explore whether particular aspects of maternal dietary intake modify the relationship between antenatal alcohol consumption and child IQ scores at 8 years, using data from the ALSPAC cohort. This is the first study to explore whether maternal dietary intakes modify the relationship between antenatal alcohol consumption and child IQ scores in a human population.

The findings presented in this chapter neither support nor refute that there is an interaction between micronutrient intakes and alcohol consumption during pregnancy that modifies the risk of adverse cognitive outcomes for children. The results from the stratified analyses indicated that the micronutrients explored in this thesis may have a significant effect on the cognitive performance of offspring later in childhood; women with relatively higher micronutrient intakes were significantly more likely to have children with higher IQ scores at 8 years of age, compared to women with lower micronutrient intakes. The relationship was significant for all micronutrients and remained after adjusting for all confounders.

However, this relationship was not observed in women who reported binge drinking during pregnancy. There appeared to be no significant difference between the IQ scores of children born to women with relatively high and low intakes of all micronutrients under investigation. It is possible that this relationship could result from alcohols inhibitory effects on micronutrient absorption. Ethanol is water

soluble, which means it is absorbed directly into the blood stream and can affect micronutrient status in a number of ways. Firstly, it can damage the gastrointestinal tract, which can result in a reduction in nutrient absorption (Bode & Bode 1997; Lieber 2000). Secondly, it can interact with nutrient carriers that are required to transfer nutrients across membranes and into the blood stream. For instance, a number of studies have indicated that alcohol interferes with the activity of two proteins that are important for folate absorption; Reduced Folate Carrier (RFC) and Glutamate Carboxypeptidase (GCPII) (Halsted et al. 2002; Villanueva & Halsted 2004). Thirdly, it can inhibit fat absorption, which can in turn impair the absorption of fat-soluble micronutrients, such as vitamin E and carotenoids (Boquillon 1976; Lieber 2000). However, these outcomes are typically associated with chronic alcohol exposure and it is unlikely that impaired micronutrient absorption would be attributable to moderate alcohol consumption in the majority of cases (Simonetti et al. 1993).

The results from the likelihood ratio tests suggest that folate, vitamin B6 and vitamin C interact with alcohol and modify the relationship with childhood IQ scores. Interestingly, the results appear to suggest that in binge drinkers, folate, vitamin B6 and vitamin C appear to have the opposite effect on the relationship between alcohol and child IQ scores; low micronutrient intakes appeared to be associated with higher child IQ scores. However, the results from the stratified analysis and plots of marginal means indicate that the interaction may be a result of the small numbers of women in the binge drinking category. Children's IQ scores born to women who reported binge drinking had wide confidence intervals that overlapped. This suggests that the interaction may be a spurious finding or a result of residual confounding (Button et al. 2013).

It is possible that this unexpected relationship may also be a result of residual confounding or measurement error. As discussed in Chapters 3 and 6, it is possible the estimates of micronutrient intakes are crude, as the FFQ used in the ALSPAC study was short and had not been validated beforehand. In addition to this, the definition of binge drinking differs in ALSPAC to that more widely used (see Chapter 1); defined as drinking four or more units of alcohol in one day, rather than in one occasion. Women were asked about binge drinking over the past month, which equates to 14 to 18 weeks gestation, so it is possible that some women may be incorrectly categorised as non-binge drinkers.

There was no evidence of maternal dietary patterns modifying the relationships between antenatal alcohol consumption and child IQ. However, in adjusted models with main effect terms only, adherence to the 'Health conscious' and 'Processed' dietary patterns were associated with higher and lower IQ scores in children, respectively. The 'Health conscious' dietary pattern is associated with higher maternal micronutrient intakes compared to the 'Processed' dietary pattern. However, it is also possible that maternal dietary intakes are acting as a proxy for dietary intake during childhood. Northstone and colleagues (2012) reported that adherence childhood dietary patterns characterized

by high fat, sugar and processed foods were related to small decreases in IQ scores at 8 years, while diets characterized by high intakes of fruits and vegetables were associated with small increases in child IQ scores at 8 years in the ALSPAC cohort. Future investigations should be carried out to evaluate the intricacies between the influence of maternal and childhood dietary intake on IQ.

8.5.1 Strengths and Limitations

This study has a number of strengths, including the large sample size, the variation in alcohol consumption frequencies and quantities, and the wide range of variables collected to enable adjusting for confounders, which have been discussed in more detail in Chapters 6 and 7.

However, there are also a number of limitations to this study, in addition to general the methodological considerations addressed in Chapter 3. Firstly, subcategories of IQ were not investigated (performance and verbal), only the total IQ scores were, which means a particular aspect of IQ may be related to diet and alcohol consumption during pregnancy but it has not been detected in this analyses. Further investigations should be conducted to explore the intricacies of the relationships between diet, alcohol and IQ. Secondly, the high attrition rate of the ALSPAC cohort meant that study 2C is vulnerable to selection bias; women with missing child IQ scores at 8 years were more likely to be younger, less educated, non-white ethnic origin, live in rented, and more crowded accommodation compared to women with outcome data. Women who were excluded due to missing data were also more likely to report binge drinking and also have low intakes of micronutrients (Q1). It is possible that the missing data may have attenuated relationships.

8.6 Conclusions

Overall, Study 2C has provided some interesting findings that raise a number of questions regarding future research in this area. While the findings presented in this chapter neither support nor refute that there is an interaction between micronutrient intakes and alcohol consumption during pregnancy that modifies the risk of adverse cognitive outcomes for children, they do emphasise the detrimental effects that binge drinking and low micronutrient intakes may have on the cognitive performance of children in later life. This chapter has also highlighted the methodological challenges that longitudinal cohort studies often face; an under-representative sample population and a high rate of attrition. Further work must be conducted to explore the relationships between folate, vitamin B6, vitamin C and binge drinking, in relation to measures of cognitive performance in childhood.

Chapter 9. General discussion and conclusions

9.1 Introduction

The overall aims of this thesis were to 1) explore the relationships between maternal dietary intake and alcohol consumption patterns during pregnancy, and 2) examine possible ways they may affect risks of adverse infant and child outcomes.

To address the first aim, a prospective study using a purposefully designed FFQ was carried out. The new FFQ was designed to measure the intake of folate, choline, betaine, vitamin C and carotenoids (α-carotene, β-carotene, lycopene, lutein and β-cryptoxanthin) and its validity was tested by comparing estimates with those from 24-hour dietary recalls and plasma folate concentrations (study 1A). This FFQ was then used in a sample of pregnant women in the UK, alongside a validated tool to estimate alcohol consumption (Study 1B). However, the study did not recruit sufficient numbers of women who reported alcohol consumption during pregnancy to enable meaningful comparisons.

This difficulty in recruiting sufficient numbers of women who report alcohol consumption during pregnancy has been reported in previous studies (Mc Andrew et al. 2012; Nykjaer et al. 2014; O'Keeffe et al. 2015). While it is possible that this reflects reductions in overall alcohol consumption, it is unlikely; another study conducted in the UK at a similar time (SCOPE) (2012) reported 82% of women drank alcohol at some stage during pregnancy and 45% reported at least one episode of binge drinking (O'Keeffe et al. 2015). The low reported rates are likely to reflect less willingness to report alcohol consumption due to the stigma attached to this (Ernhart & Morrow-tlucak 1988; Morrow-Tlucak et al. 1989) or an inability to accurately estimate unit intakes (Boniface & Shelton 2013). A number of studies have attempted to overcome these challenges in a variety of ways, including: face-to-face interviews with midwives at a number of time points during pregnancy (McCarthy et al. 2013); involving clinicians in the design and delivery of questions to potential participants (Pollak et al. 2006); and using more in depth methods, such as the Time-Line Follow Back (TLFB) method, in addition to the AUDIT-C questionnaire (Göransson et al. 2006).

The small and homogenous sample in Study 1B coupled with very low reported alcohol consumption meant that a second strategy was employed. This included a secondary analysis of data from the ALSPAC cohort (Study 2A). The ALSPAC cohort also provided childhood data, which made it possible to explore additional aims that were generated from the findings in Chapters 5 and 6. The aims of the thesis were extended to include birth and childhood outcomes (Studies 2B and 2C).

This final chapter begins with a summary of findings in relation to the objectives and existing literature, will be followed by a discussion of the general strengths and limitations, clinical and policy implications, recommendations for future research, and finally end with some concluding remarks.

9.2 Summary of findings

9.2.1 Exploration of the relationships between maternal dietary intake and alcohol consumption patterns during pregnancy

Studies 1B and 2A presented a number of key findings. Firstly, both studies (1B & 2A) indicated that self-reported, low levels of alcohol consumption during pregnancy are associated with better quality diets. While there were very few differences between estimates of daily micronutrient intakes, the findings from both studies did suggest that women who reported drinking less than once per week during pregnancy, were more likely to adhere to a dietary pattern that was characterised by relatively high intakes of fruit, vegetables, fish and whole grains, and correlated with micronutrient intakes. Similar relationships have been documented in the wider literature in non-pregnant populations. A number of studies have highlighted that individuals who report low to moderate amounts of alcohol consumption typically consume more vegetables (Barefoot et al. 2002; Sánchez-Villegas et al. 2009) and higher intakes of micronutrients compared to those who do not drink (Männistö et al. 1997; Walmsley et al. 1998; Fawehinmi et al. 2012).

Secondly, the findings indicated that as quantity per drinking occasion increases, the quality of diet typically decreases. It was not possible to explore quantity of drinking during pregnancy in study 1B due to the very low reported drinking levels. However, quantity per occasion prior to pregnancy was explored and women who reported drinking five or more alcoholic drinks per occasion before pregnancy, also reported lower daily intakes of folate, compared to women who were drinking low levels of alcohol (≤2 units per drinking occasion). The findings from Study 2A suggest that this trend persists during pregnancy; women who reported drinking four or more units per occasion during pregnancy also reported lower daily intakes of folate, vitamin B6, vitamin C and vitamin E, and were more likely to consume a diet that was characterised by high intakes of processed meats, fried foods and low intakes of fresh fruit, vegetables, fish and whole grains.

The relationship between quantity of alcohol consumed per occasion and diet quality has also been described elsewhere in the literature. A study conducted in the US explored frequency and quantity of alcohol consumption in relation to the Health Eating Index (HEI), which is a composite score based on the consumption of a number of indicator food groups; a higher score indicates a better quality diet. Low quantity coupled with high frequency was associated with the highest HEI scores, and high quantity coupled with low frequency was associated with the lowest HEI scores (Breslow et al. 2006).

A number of other studies have described similar relationships between binge drinking patterns and poor quality diets (Valencia-Martin et al. 2011; You et al. 2013).

It is likely that the relationships described above are attributable to the social patterning of health behaviours. Indicators of high SES have been linked with low to moderate patterns of drinking (Smith & Foxcroft 2009; Cerdá et al. 2011) and diets characterised by high intakes of fruit, vegetables and fish (Darmon & Drewnowski 2008; Kell et al. 2015). In contrast, a number of studies have reported the associations between negative health behaviours and markers of lower SES (Fone et al. 2013; Kim et al. 2013; Touvier et al. 2014), and more importantly, the increased risk of morbidity and mortality that is associated with them (Stringhini et al. 2010). The findings from this thesis highlight the persistent nature of these clusters of negative health behaviours into pregnancy.

Thirdly, the finding from Studies 1B and 2A both indicate that particular dietary patterns are associated with folic acid supplement use during pregnancy. Women in Study 1B who consumed a diet characterised by high intakes of processed and sugary foods were less likely to take folic supplements; and women in Study 2A who consumed a diet that was characterised by fruit, vegetables, fish and whole grains were more likely to take folic acid supplements during pregnancy. The exposure data collected as part of ALSPAC is from the early 1990s, around the same time the evidence was published for a relationship between folate and Neural Tube Defects (MRC 1991). However, the findings from Study 1B suggest that more than a decade later, there are still women who do not take folic acid supplements during their pregnancy. These patterns are of particular concern because if women are not obtaining folate from their diet, not taking folic acid supplements and are also drinking alcohol, they could putting their unborn baby at risk of NTDs (Pitkin 2007).

Finally, the results from Study 1B indicated that many women did not alter their alcohol consumption until they were aware of their pregnancy, meaning many women may have consumed alcohol while they were unknowingly pregnant and will therefore be incorrectly categorised as non-drinkers.

Overall, the findings from Studies 1A, 1B and 2A highlighted some of the challenges related to measuring alcohol and dietary intakes, and the clustering of potentially harmful patterns of eating and drinking before and during the antenatal period.

9.2.2 Examination of the possible ways they may affect risks of adverse infant and child outcomes.

The relationships between diet and alcohol consumption were then explored in relation to infant and childhood outcomes. To address this aim, secondary analyses of the ALSPAC dataset were conducted, exploring two outcomes; Small for Gestational Age (SGA) (Study 2B) and IQ scores at age 8 (Study 2C). Studies 1B and 2A provided evidence to suggest that potentially harmful patterns of alcohol

consumption are associated with lower micronutrient intakes compared to women who did not report drinking alcohol during pregnancy; therefore, the next aim of this research was to investigate what the potential effects these relationships may have on infant and childhood outcomes.

The evidence from Studies 2B and 2C, as limited as it may be, suggests that there may well be a modifying effect from micronutrients on the relationships between alcohol and offspring outcomes. Studies 2B and 2C provided two key findings. Firstly, the results from Study 2B suggest that vitamin E may interact with alcohol and modify the relationship between binge drinking and having a SGA infant. Even after adjusting for potential confounders, the odds of having a SGA infant were approximately double if a woman reported binge drinking and low intakes of vitamin E, compared to women who reported binge drinking but had higher intakes of vitamin E. Carotenoids and vitamin C, along with vitamin E, are also potent scavengers of free radicals; however, no relationships between vitamin C or carotenoids and binge drinking were observed in relation to fetal growth. This suggests that the observed interaction may be attributable to a different mechanism, such as blood flow regulation.

While ethanol may increase thromboxane release (vasoconstrictor), particularly on the fetal side of the placenta restricting blood flow and nutrient supply (Siler-Khodr et al. 2000), vitamin E enhances the release of prostacyclin (vasodilator), which inhibits platelet aggregation and increases blood flow and nutrient supply to the fetus (O Scholl et al. 2006). Therefore, binge drinking during pregnancy, coupled with low intakes of vitamin E, and may result in an imbalance in thromboxane and prostacyclin, increasing the risk of having a SGA baby. However, it is also possible that vitamin E is acting as a proxy for another factor. Smoking also disrupts the balance between thromboxane and prostacyclin, making it a strong predictor of fetal growth, and one study has suggested that it may overrule many other risk factors in women with lower SES (Van den Berg et al. 2013). Despite controlling for smoking in the analysis, there is a risk of residual confounding.

Secondly, the results from Study 2C indicated that folate, vitamin B6 and vitamin C may interact with alcohol and modify the relationship between binge drinking and childhood IQ scores at 8 years. Higher intakes (Q2-Q4) of folate, vitamin B6 and vitamin C were associated with higher childhood IQ scores at 8 years, compared to lower intakes (Q1), but only in women who did not report binge drinking. There was no difference in IQ scores by micronutrient intakes in women who did binge drink.

One plausible mechanism for this relationship could include the inhibitory effect of alcohol on micronutrient absorption. Folate, vitamin B6 and vitamin C are all water-soluble vitamins, which means they are absorbed directly into the blood stream and cannot be stored in the body, making them essential micronutrients. They are absorbed via specific carrier mediated processes and alcohol can inhibit this, resulting in lower concentrations of micronutrients (Said 2011). In contrast, vitamin E is fat soluble, which means it can be stored in the liver and fat within the body and may not interact

with alcohol in the same way. However, the large, overlapping confidence intervals around the estimates of IQ scores in women who reported binge drinking indicate that the results may be spurious findings due to the small sample sizes in the binge drinking categories. The high attrition rate in the ALSPAC sample means that Study 2C may not have enough power to detect the relationship between binge drinking, micronutrient intakes and child IQ scores (Button et al. 2013). While the regression models were adjusted for potential confounders, there is also a possibility that the relationships observed are due to residual confounding.

Interestingly, adherence to the 'Health conscious' and 'Processed' dietary patterns were associated with higher and lower child IQ scores, respectively; however, there was no evidence of an interaction in either Study 2B or 2C. This could be attributable to the small variation that each dietary pattern accounts for in the data; the five dietary patterns explored accounted for only 31% of the variation in the data. In contrast, a recent study conducted in Canada derived four dietary patterns using an FFQ; the patterns accounted for a total of 63% of the variation in the data (Slattery et al. 1998). This considerable difference may be a result of also having men in the sample population; previous results have shown men to have stronger adherence to dietary patterns than women, which may be a result of women having higher day-to-day variability in their dietary intake (Nelson 1989).

Overall, the results from Studies 2B and 2C provide limited evidence that micronutrients may interact with alcohol and modify the risk of adverse infant and childhood outcomes, and warrant further exploration in a sample of pregnant women with a greater proportion of binge drinkers.

9.3 Strengths and limitations

The strengths and limitations have been discussed in the relevant study chapters throughout. The general strengths and limitations pertaining to the whole thesis will now be discussed.

9.3.1 Strengths

One of the strengths relating to the initial body of work, comprised of Studies 1A, 1B and 2A, is the use of two different data sets to address the original aims of the thesis. One set of findings (1B) was based on a small, homogenous sample, but collected exposure data contemporaneously, using a purposefully designed FFQ and a validated tool to measure alcohol intake. The other set of findings (2A) was based on a study with a large sample size and relatively varied sample population; however, the alcohol and dietary data were collected using tools that had not been validated beforehand. The findings from Study 2A complement those from Study 1B, and by addressing the limitations of the data set, provide a more comprehensive investigation into the relationships between dietary habits and alcohol consumption during pregnancy.

Analyses of single nutrient exposures have been crucial for the understanding of many conditions resulting from nutrient deficiencies; for example, a vast number of birth defects in infants have been prevented since researchers identified the link between folate and neural tube defects (NTDs) (Pitkin 2007). However, because of the correlation between many nutrients it is difficult to examine their individual effects. Therefore, dietary pattern analysis has been identified as a method of overcoming these challenges. Dietary patterns provide a more 'real-world' approach to investigate the impact of diet on particular outcomes (Hu 2002). By using both measures, it has been possible to explore both aspects of dietary intake, providing a more detailed assessment of these relationships.

The main strength of using data from the ALSPAC cohort (Studies 2A, 2B and 2C) is the large sample size. The sample is also representative of the local population of pregnant women in the West of England, and with the whole of Great Britain, based on data from the 1990 census (Fraser et al. 2013). Large sample sizes provide more power to detect differences and reduce the risk of spurious findings (Button et al. 2013).

Another strength was the extensive measurement of potential confounders. The questionnaire used in Study 1B asked detailed questions about measures of SES, smoking, pre-pregnancy drinking and dietary supplement use during pregnancy. The timing and dose of dietary supplements can have considerable effects on pregnancy outcome (Hodgetts et al. 2015). The ALSPAC cohort also provided a wealth of additional data on a wide variety of confounding variables, enabling appropriate adjusting of multivariable models.

Finally, the wide range of outcome data that was available from the ALSPAC dataset enabled further investigation to provide a more complete picture of the potential risks associated with maternal dietary intake and alcohol consumption during pregnancy.

9.3.2 Limitations

The main limitations of Studies 1A and 1B were related to the small and homogenous sample populations, of which the majority of women were white, well educated, and non-smokers. This increases the risk of bias, threatening the external validity of the study. However, recruiting older women of higher SES is a common phenomenon in public health research (Warren-findlow et al. 2003). A study exploring barriers to minority and lower SES populations taking part in research reported that a lack of time, childcare and awareness as reasons for people from lower SES backgrounds not taking part in research (Withall et al. 2011). Women also reported very low levels of alcohol consumption in Study 1B, which meant it was not possible to address the original objectives of the thesis using the initial data set.

While ALSPAC recruited a large and fairly representative sample of women into the study, the number of women who reported drinking one or more drinks per day was still relatively low and therefore, some investigations may have lacked the statistical power to detect relationships, increasing the risk of type II errors. Recruiting heavy drinkers is a challenge commonly observed in epidemiological studies (Meiklejohn et al. 2012).

Another limitation of this study is the rate of attrition, with less than half of the original cohort attending the clinical assessment visits at 8 years of age. While it is acknowledged that multiple imputation of data is one way to adjust for attrition (Schafer & Olsen 1998), it was considered beyond the remit of this thesis.

There were also a number of areas vulnerable to measurement error throughout this thesis. Firstly, the measurement of dietary intake using an FFQ provides crude estimates that can only be used to assess relative intakes. This can also make it difficult to understand the implications of these findings; for example, it is not possible to understand the absolute difference in vitamin E intakes of women in Q1 compared to Q2-Q4. However, the findings provide an important first step in understanding the relationships between these two exposures in pregnancy. Secondly, the definition of binge drinking used throughout studies 2A, 2B and 2C refer to four units of alcohol being consumed in one day. This differs from the current definition, commonly used in the scientific literature, which is six or more units of alcohol on one occasion. The former definition may categorise women who consumed four units across an entire day, which may not result in the same blood alcohol concentration of four units on one drinking occasion. Finally, the questions used to measure folic acid intake during pregnancy in ALSPAC did not capture enough detail to understand the timing or dose of supplement use, which could have considerable implications on child IQ scores at 8 years of age (Hodgetts et al. 2015).

9.4 Clinical and policy implications

There are several important implications for clinical practice and public policy that stem from the findings of this thesis and will be discussed in relation to the wider literature in this field.

The key theme that has emerged from this thesis is of the clustering of potential harmful patterns of dietary intake and alcohol consumption in pregnancy and how these may increase the risk of adverse outcomes for offspring, from infancy through to childhood. This highlights the complex nature of health behaviours and while more research needs to be conducted, this does have a number of implications. The potential risks of engaging in multiple health behaviours need to be clearly communicated to healthcare professionals and to the public through public health campaigns, with an emphasis on women of reproductive age. The UK public health campaign ‘Change 4 Life’ promoted

changes to diet, exercise, smoking and alcohol consumption (DOH 2010). However, the campaign discussed these behaviours as separate entities, rather than acknowledging they often occur together.

In addition to this, clinicians should be given necessary training and support in order to provide advice on tackling multiple health behaviours. Evidence from health behaviour change interventions have indicated that when two or more health risk behaviours, such as diet, smoking, alcohol consumption or exercise, are approached in combination, individuals tend to have significantly better outcomes (Jepson 2000). Changing one health behaviour can function as a gateway to other behaviour changes; for example, research in Japan indicated that increased physical activity was associated with quitting smoking, and a decrease in exercise was associated with a smoking relapse (Nagaya et al. 2007). This is particularly important during pregnancy, as pregnancy confirmation is a key time for health behaviour change (Crozier et al. 2009). Due to a mothers motivation to protect her unborn baby, pregnancy is considered a teachable moment, which is defined as an event or circumstances that can result in behaviour change (Lawson & Flocke 2009). There are three underlying factors that define a teachable moment: heightened awareness of risks and outcome expectations; provokes an emotional response; changes self-concept or role (McBride et al. 2003). By understanding the potential for cognitive and behavioural changes during pregnancy, policy makers and health professionals can take advantage of this period.

The evidence presented in this thesis further supports the notion that there is no safe threshold for alcohol consumption during the antenatal period. Therefore, the current NICE recommendations should be amended and advise women to avoid alcohol completely, if planning to become pregnant and throughout the whole pregnancy[2]. The findings highlight the need for a clear, consistent public health message about alcohol in pregnancy. While the sample was very small in Study 1B, women who participated in an interview discussed receiving inconsistent advice about alcohol consumption between healthcare professionals, friends, relatives and messages in the media. Similar findings have also been reported in other studies. A qualitative study conducted in the UK with pregnant women found that there is considerable confusion regarding the interpretation of the current guidelines and advice that healthcare professionals give. Many women in the sample also reported they followed advice from family and friends rather than clinicians because of this unclear guidance (Raymond et al. 2009). Because many women only reduced intake or abstained once they were aware of their pregnancy, it is important that this message is also clear and consistent for women of reproductive age, even if they are not planning on becoming pregnant.

[2] At the time of examination the NICE guidelines advised pregnant women and women planning to become pregnant to avoid alcohol, particularly during the first trimester, but if they choose to drink alcohol, to drink no more than one to two units of alcohol, once or twice a week, and always to avoid binge drinking. After examination, these guidelines were amended and now women are advised to avoid alcohol during pregnancy.

Approximately one quarter of women did not report taking folic acid supplements during their pregnancy in Study 1B and those who did report taking it were more likely to have a better quality diet. In addition, the findings from Study 2B suggest that folate, vitamin B6 and vitamin C may interact with alcohol during pregnancy and modify the risk of lower cognitive performance in childhood. While these findings are very limited, they do provide a rationale for widely publicising the importance of folic acid supplements during the early stages of pregnancy and if women are planning on becoming pregnant, particularly if they are vegetarian, vegan or have other dietary restrictions. A systematic review reported that periconceptional use of folic acid is still fairly low in the UK, ranging between 21 to 48% of women reporting to take them before pregnancy (Stockley & Lund 2008). However, the factor that is most associated with not taking folic acid supplements is unintended pregnancy; therefore, a more widespread approach for all women of reproductive age may be a more effective method (Peake et al. 2013; Stockley & Lund 2008).

Finally, women should be advised about the importance of eating a varied diet. The current NICE guidelines recommend that healthcare professionals should discuss diet with women when they become pregnant. The recommendations state 'advice should include: eat five portions of fruit and vegetables a day and one portion of oily fish a week' (NICE 2014b). Findings from Study 1B indicate that this advice is not being passed on to women during pregnancy, with most women reporting to be told to simply cut out certain foods, such as unpasteurised dairy products and raw meat. A qualitative study exploring how midwives relay this advice to women in the UK found that the advice delivered varied greatly. The study also reported that midwives rarely explained the purpose of these dietary changes or set target goals for women (Brown et al. 2013). It is important that midwives have the skills and support in order to successfully implement this recommendation. Training courses and materials could be one practical way to introduce the importance of this advice to midwives and midwifery assistants.

9.5 Future research

This thesis makes an important contribution to the field of fetal alcohol research and also generates a number of potential areas for future research. This is the first large epidemiological study to explore the relationships between diet and alcohol in pregnant populations, and what influences women's choices about food and drink during pregnancy. The findings from this thesis highlight the need to explore the risk of adverse health outcomes in relation to clusters of health behaviours in pregnancy due to the potentially complex biological and behavioural interactions between substances. While the studies conducted are an important first step in understanding how diet and alcohol modify the risk adverse offspring outcomes, they are preliminary results and further research must be conducted in order to replicate these findings in other populations of pregnant women.

The findings from the qualitative interviews provided an interesting insight into what influences women's choices about food and alcohol during pregnancy. However, the very small sample size means results are not generalisable to all pregnant women in the UK. Additional interviews, with women from a range of socio-economic backgrounds who report a wide range of alcohol consumption before and during pregnancy should be undertaken. Once women's choices and what influences them are understood in greater detail, there is scope to use this information in order to inform multiple health behaviour change interventions in pregnancy.

As previously discussed, dietary patterns can vary greatly between studies due to differences in dietary assessment tools and sample populations. Therefore, further research is necessary to investigate these relationships in a number of different populations. In particular, a focus on recruiting women from socially deprived backgrounds may provide additional insight. However, the investigations reported in this thesis highlighted the challenges entailed in recruiting this hard to reach population. Thus, preliminary research may be required in order to understand the perceived barriers to participating in research studies while pregnant, and to find out potential ways of recruiting this hard to reach group.

While the findings from this thesis have provided evidence to suggest that vitamin E may interact with alcohol and modify the level of risk of adverse neonatal outcomes, further research to explore this relationship is imperative. An initial first step would be to explore this relationship using data from another large cohort study to assess whether similar results are found in other populations of pregnant women. The estimates of vitamin E intake and alcohol consumption collected as part of ALSPAC were measured using an FFQ and brief quantity-frequency tool, respectively. Therefore, the measures are crude and, ideally, future research studies should obtain a more accurate estimate of dietary vitamin E intake using a prospective dietary assessment tool, such as weighed food records, and a more detailed estimate of alcohol consumption; one such method would be to use the Time Line Follow Back (TLFB) procedure at a number of different time points throughout the pregnancy. In addition to this, biomarker data on maternal plasma vitamin E (α-tocopherol and γ-tocopherol) concentrations should also be analysed to provide an objective estimate of vitamin E status. Assessing both self-reported dietary intake and plasma vitamin E concentrations will enable researchers to obtain measures of absolute intake.

Dietary pattern analysis is becoming a more common method for exploring diet-disease relationships in nutritional epidemiology as the investigations into individual nutrients provide a number of challenges. Firstly, micronutrients are often correlated with each other, making it difficult to tease apart the individual effects. Secondly, the magnitude of individual nutrients may be too small in order to overcome confounding and crude estimates (Hu et al. 1999; Kant 2004; Nettleton et al. 2008). Throughout this thesis, a posteriori methods were employed to derive dietary patterns (e.g. PCA), as

they typically provide a more accurate reflection of how groups of foods are consumed together (Nettleton et al. 2008). However, using PCA means dietary patterns are not based on scientific literature, allowing for hypotheses to be more easily tested (Kant 2004). Future research using Reduced Rank Regression (RRR) to derive dietary patterns may help to overcome this. RRR is a data reduction technique that highlights correlations between a number of variables being explored, much the same as PCA; however, it is a more powerful technique, as it enables the user to choose disease/outcome-specific response variables. The dietary patterns are then derived by determining the combinations of food and drinks that explain the most variation in the response variables as possible (Hoffmann 2004). For example, future research exploring these methods may choose OCM micronutrients or dietary antioxidants are response variables and derive dietary patterns that associated with the intakes of these micronutrients (Sherafat-Kazemzadeh et al. 2010).

A posteriori methods also make it difficult to compare the findings from investigations into dietary patterns and particular outcomes. While dietary patterns are given similar descriptive names, such as the 'Prudent' or 'Western' dietary patterns, they can often be describing very different combinations of foods. Therefore, developing standardised UK diet quality scores during pregnancy may facilitate comparisons between studies of diet and neonatal and childhood outcomes more easily (Nettleton et al. 2008).

The relationships between micronutrient intakes, binge drinking and child IQ scores at 8 years are not clear, and warrant further investigation. A number of different approaches could be used to do this. One of the main challenges with the analysis in Study 2C was missing data; therefore, future research should explore the use of multiple imputations to increase the sample size and to reduce the likelihood of spurious results. In addition to this, Mendelian randomization may provide a potential way to explore causal relationships using observational data from birth cohorts (Smith & Ebrahim 2003). As discussed in Chapter 2, there are particular genotypes that are associated with ethanol metabolism. For example, the genetic variant rs1229984 (ADH1B) is associated with faster ethanol metabolism, but slower clearance, meaning individuals who carry this genotype are more likely to experience the negative effects of alcohol and subsequently, consume less alcohol. Genes are believed to be passed on from parents to offspring in a random fashion, which means genotypes associated with intakes of alcohol, can be used as a way of randomizing individuals into alcohol consumption categories. A number of studies have used similar methods to explore antenatal alcohol consumption and childhood IQ scores (Lewis et al. 2012; Zuccolo et al. 2013). There are also genotypes that are associated with micronutrient metabolism and certain genetic variants, which mean individuals may have lower micronutrient status because of an inability to metabolise a particular micronutrient effectively. Genotypes associated with vitamin B12 and folate have already been explored in this way (Schatzkin et al. 2009; Bonilla et al. 2012). Taking advantage of genotypes associated with micronutrients and

alcohol metabolism, could facilitate a more robust analysis and provide more meaningful findings that are not as vulnerable to the effects of confounding.

Finally, there is evidence to suggest that the cognitive deficits that result from antenatal alcohol consumption may not be detected until later life (Day et al. 2013); therefore, further research should be conducted using data from the ALSPAC cohort to explore diet and alcohol consumption in relation to other markers of cognitive performance that are collected later into childhood, such as Key Stage 2 and Key Stage 3 scores. (Alati et al. 2013; Zuccolo et al. 2013).

9.6 Concluding remarks

The investigations undertaken as part of this thesis have highlighted a number of new and important findings regarding the relationships between maternal diet, alcohol and fetal development. Firstly, women who reported potentially harmful patterns of alcohol consumption were more likely to adhere to diets that were characterised by low intakes of fresh fruit and vegetables and high intakes of processed meats. Secondly, binge drinking was associated with a significantly higher risk of having a SGA baby in the presence of low vitamin E intakes. Furthermore, there is weak evidence to suggest that folate, vitamin B6 and vitamin C may interact with alcohol and modify the relationship between binge drinking and child IQ scores at 8 years of age. While these findings may have implications for pregnant women and clinicians, the limitations of the datasets mean these are preliminary results and warrant further investigation.

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Appendices

Appendix A: Database search strings

SEARCH 1: MEDLINE	
1	*Pregnancy/ or pregnan*.mp.
2	Alcohol-Related Disorders/ or *Alcohol Drinking/ or alcohol*.mp.
3	*Ethanol/
4	fetal alcohol syndrome.mp. or Fetal Alcohol Spectrum Disorders/
5	Abortion, Spontaneous/
6	birth weight.mp. or Birth Weight/
7	Birth Weight/ or head circumference.mp. or Child Development/
8	pregnancy outcome.mp. or Pregnancy Outcome/
9	Infant Mortality/ or Risk Factors/ or Pregnancy Complications/ or still birth.mp.
10	Fetal Growth Retardation/ or "Embryonic and Fetal Development"/ or growth restriction.mp.
11	Premature Birth/ or premature.mp. or Infant, Premature, Diseases/ or Infant, Premature/ or Obstetric Labor, Premature/ or Infant, Extremely Premature/
12	preterm*.mp.
13	IUGR.mp. or Fetal Growth Retardation/
14	Infant, Low Birth Weight/ or Infant, Small for Gestational Age/ or SGA.mp. or Gestational Age/
15	IUGR.mp.
16	intrauterine growth.mp.
17	FAS.mp.
18	FASD.mp.
19	"Attention Deficit and Disruptive Behavior Disorders"/ or Attention Deficit Disorder with Hyperactivity/ or Learning Disorders/ or ADHD.mp. or Dyslexia/
20	Cognition/ or cognit*.mp. or Cognition Disorders/
21	Intelligence Tests/ or Memory Disorders/ or Intellectual Disability/ or Intelligence/ or Wechsler Scales/ or IQ.mp.
22	Neurobehavioral Manifestations/ or neuro?behav*.mp.
23	"behavior and behavior mechanisms"/ or perceptual distortion/ or social perception/ or space perception/ or visual perception/
24	executive function.mp. or Executive Function/ or Psychomotor Performance/ or Problem Solving/
25	memory.mp. or Memory, Short-Term/ or Memory/ or Memory Disorders/ or Spatial Memory/ or Memory, Long-Term/
26	motor skills.mp. or Motor Skills/
27	Neural Tube Defects/ or Congenital Abnormalities/ or birth defect*.mp.
28	2 or 3
29	4 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27
30	1 and 28 and 29
31	limit 30 to (english language and humans)
32	limit 31 to yr="1973 -Current"

SEARCH 1: EMBASE	
1	pregnan*.mp.
2	pregnancy/ or pregnant woman/
3	antenatal.mp. or prenatal period/
4	prenatal exposure/ or prenatal.mp.
5	alcohol*.mp. or alcohol consumption/ or alcohol/ or alcohol intoxication/ or "alcohol use disorder"/ or alcohol abuse/
6	ethanol.mp.
7	fetal alcohol syndrome.mp. or fetal alcohol syndrome/
8	fetal alcohol spectrum disorder.mp.
9	FASD.mp.
10	FAS.mp.
11	birth weight.mp. or birth weight/
12	fetal growth.mp. or fetus growth/
13	prenatal development/ or intrauterine growth retardation/ or gestational age/ or intrauterine growth*.mp. or prenatal growth/ or birth weight/
14	head circumference.mp. or head circumference/
15	pregnancy outcome.mp. or pregnancy outcome/
16	child development.mp. or child development/
17	infant mortality.mp. or infant mortality/
18	pregnancy complication.mp. or pregnancy complication/
19	premature.mp. or prematurity/
20	premature labor/ or preterm*.mp.
21	spontaneous abortion/
22	small for date infant/ or SGA.mp.
23	learning disorder/ or attention deficit disorder/ or hyperactivity/ or attention def*.mp. or autism/ or behavior disorder/
24	ADHD.mp.
25	cognitive defect/ or memory/ or cognition/ or cognit*.mp. or psychological aspect/
26	behavior disorder/ or neurobehavior/ or central nervous system/ or neuro?behav*.mp.
27	motor skills.mp. or motor performance/
28	nervous system/ or neural tube/ or neural tube.mp.
29	1 or 2 or 3 or 4
30	5 or 6
31	7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28
32	29 and 30 and 31
33	limit 30 to (english language and humans)
34	limit 31 to yr="1973 -Current"

SEARCH 1: PsychInfo	
1	pregnan*.mp.
2	exp Pregnancy/ or exp Adolescent Pregnancy/ or pregnant.mp. or exp Expectant Mothers/
3	exp Prenatal Care/ or antenatal.mp. or exp Prenatal Development/ or exp Perinatal Period/
4	alcohol.mp. or exp ALCOHOLS/
5	ethanol.mp. or exp ETHANOL/
6	exp Alcohol Abuse/ or exp Alcoholism/ or exp Binge Drinking/ or exp Alcohol Drinking Patterns/ or exp Alcohol Intoxication/ or exp Alcoholic Beverages/ or alcoholic drink.mp.
7	fetal alcohol syndrome.mp. or exp Fetal Alcohol Syndrome/
8	exp Prenatal Exposure/ or FASD.mp.
9	FAS.mp.
10	exp SPONTANEOUS ABORTION/
11	birth weight.mp. or exp Birth Weight/
12	pregnancy outcome.mp. or exp Pregnancy Outcomes/
13	exp Emotional Development/ or exp Mother Child Relations/ or exp Early Childhood Development/ or child development.mp. or exp Adolescent Development/ or exp Developmental Psychology/ or exp Childhood Development/ or exp Cognitive Development/
14	exp Brain Size/ or exp Cognitive Impairment/ or exp Cognitive Ability/ or exp Physical Development/ or head circumference.mp. or exp Infant Development/
15	exp Cognitive Ability/ or exp CONGENITAL DISORDERS/
16	congenital disorder.mp.
17	exp PREMATURE BIRTH/ or premature.mp.
18	exp Motor Development/
19	motor skills.mp. or exp Motor Skills/
20	SGA.mp.
21	small for gestational age.mp.
22	ADHD.mp. or exp Attention Deficit Disorder with Hyperactivity/
23	exp WECHSLER ADULT INTELLIGENCE SCALE/ or intelligence.mp. or exp INTELLIGENCE MEASURES/ or exp INTELLIGENCE/ or exp WECHSLER INTELLIGENCE SCALE FOR CHILDREN/ or exp INTELLIGENCE QUOTIENT/
24	memory.mp. or exp LONG TERM MEMORY/ or exp MEMORY DISORDERS/ or exp VISUAL MEMORY/ or exp MEMORY/ or exp MEMORY CONSOLIDATION/ or exp MEMORY TRACE/ or exp VERBAL MEMORY/ or exp "MEMORY AND LEARNING MEASURES"/ or exp SHORT TERM MEMORY/ or exp SPATIAL MEMORY/ or exp WECHSLER MEMORY SCALE/
25	exp Sleep Disorders/ or exp Eye Movements/ or exp Behavior Disorders/ or exp Motor Processes/ or behaviour disorder.mp. or exp Behavior Problems/
26	executive function.mp. or exp Executive Function/
27	exp Neuropsychology/ or neurobehavioural.mp.
28	exp Neural Development/ or exp Nervous System Disorders/ or neural tube defect.mp.
29	1 or 2 or 3
30	4 or 5 or 6
31	7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28
32	29 and 30 and 31
33	limit 32 to (human and english language and yr="1973 -Current")

SEARCH 2: MEDLINE	
1	pregnant.mp. or Pregnancy/ or Pregnant Women/ or Pregnancy, Animal/
2	Prenatal Care/ or antenatal.mp.
3	Alcohol-Induced Disorders/ or Alcohol Drinking/ or Alcohol-Induced Disorders, Nervous System/ or alcohol*.mp. or Alcohol-Related Disorders/ or Blood Alcohol Content/ or Fetal Alcohol Spectrum Disorders/
4	ethanol.mp. or Ethanol/
5	Fetal Alcohol Spectrum Disorders/ or f?etal alcohol*.mp.
6	FASD.mp.
7	FAS.mp.
8	folate.mp. or Folic Acid/
9	Choline Deficiency/ or Choline/ or choline.mp.
10	betaine.mp. or Betaine/
11	Betaine-Homocysteine S-Methyltransferase/
12	methionine.mp. or Methionine/
13	vitamin B12.mp. or Vitamin B 12/
14	vitamin B6.mp. or Vitamin B 6/
15	B vitamins.mp. or Vitamin B Complex/
16	Homocysteine/ or DNA Methylation/ or methyl donor.mp. or Dietary Supplements/
17	one carbon metabolism.mp.
18	Ascorbic Acid/ or vitamin C.mp. or Antioxidants/ or Vitamin E/
19	alpha-tocopherol.mp. or alpha-Tocopherol/
20	Tocopherols/
21	oxidative stress.mp. or Oxidative Stress/
22	Free Radical Scavengers/ or Zeaxanthins/ or Lutein/ or Vitamin A/ or Carotenoids/ or Xanthophylls/ or caroten*.mp. or beta Carotene/
23	1 or 2
24	3 or 4 or 5 or 6 or 7
25	8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22
26	23 and 24 and 25
27	limit 26 to (english language)
28	limit 27 to yr="1973 -Current"

SEARCH 2: EMBASE	
1	pregnancy/ or pregnant woman/ or pregnant.mp.
2	prenatal development/ or prenatal exposure/ or prenatal.mp. or prenatal growth/
3	antenatal.mp.
4	alcohol*.mp. or alcohol consumption/ or alcohol/ or alcohol intoxication/
5	ethanol.mp.
6	pregnancy/
7	experimental animal/ or animal/ or "dam (animal)"/ or animal.mp.
8	6 and 7
9	fetal alcohol syndrome.mp. or fetal alcohol syndrome/
10	fetal alcohol spectrum disorder.mp.
11	FASD.mp.
12	folate.mp. or folic acid/
13	choline/ or choline.mp.
14	betaine/ or betaine.mp.
15	methionine.mp. or methionine/
16	limit 15 to (english language)
17	limit 16 to yr="1973 -Current"

SEARCH 3: MEDLINE	
1	alcohol.mp. or exp ALCOHOLS/
2	exp Binge Drinking/ or exp Alcohol Drinking Patterns/ or exp Alcohol Intoxication/ or exp Alcoholic Beverages/ or alcoholic drink.mp.
3	ethanol.mp. or exp ETHANOL/
4	exp Food Intake/ or exp Food/ or exp Diets/ or exp Eating Behavior/ or exp Nutrition/ or dietary intake.mp.
5	dietary pattern.mp.
6	exp Nutritional Deficiencies/ or exp Vitamins/ or micronutrient.mp.
7	macronutrient.mp.
8	exp Food Preferences/ or food habit.mp.
9	nutrient.mp.
10	limit 9 to (human and english language and yr="1980 -Current")
11	1 or 2 or 3
12	4 or 5 or 6 or 7 or 8 or 9
13	11 and 12
14	limit 13 to (human and english language and yr="1980 -Current")

SEARCH 3: PschyInfo	
1	alcohol.mp.
2	Alcoholic Beverages/ or Alcohol Drinking/ or Alcoholic Intoxication/ or alcoholic drink.mp. or Alcoholism/
3	binge drinking.mp. or Binge Drinking/
4	Diet, Fat-Restricted/ or Diet, Paleolithic/ or Diet, Macrobiotic/ or Diet, Carbohydrate-Restricted/ or Diet, Protein-Restricted/ or Diet, Western/ or Diet, High-Fat/ or Diet, Vegan/ or Diet/ or Diet, Mediterranean/ or Diet Records/ or Diet Surveys/ or Diet, Vegetarian/
5	Food Habits/ or dietary pattern.mp.
6	Diet/
7	Nutrition Assessment/ or Nutrition Disorders/ or Nutrition Surveys/ or nutrition.mp.
8	nutrient.mp.
9	Food/ or food.mp.
10	vitamins.mp. or Vitamins/
11	minerals.mp. or Minerals/
12	food preference.mp. or Food Preferences/
13	Dietary Fats/ or Dietary Proteins/ or Dietary Carbohydrates/ or macronutrient.mp. or Energy Intake/
14	micronutrients.mp. or Micronutrients/
15	4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14
16	1 or 2 or 3
17	15 and 16
18	limit 17 to (english language and humans and "all adult (19 plus years)" and humans)

Appendix B: Publication

The contents of a published article (pp. 228-232) have been removed from this version of the thesis.
Coathup V, Wheeler S, Smith L. A method comparison of a food frequency questionnaire to measure folate, choline, betaine, vitamin C and carotenoids with 24-h dietary recalls in women of reproductive age. Eur J Clin Nutr. 2016 Mar;70(3):346-51. doi: 10.1038/ejcn.2015.159.

NHS
Health Research Authority
NRES Committee South Central - Southampton A
Bristol Research Ethics Committee Centre
Level 3, Block B
Whitefriars
Lewins Mead
Bristol
BS1 2NT

Telephone: 0117 342 1381
Facsimile: 0117 342 0445

22 August 2012

Miss Victoria Louise Coathup
Oxford Brookes University
Jack Straws Lane
Marston, Oxford
OX3 0FL

Dear Miss Coathup

Study title: **Exploring nutrient intake and alcohol consumption in pregnant women: a mixed methods approach**
REC reference: **12/SC/0402**

Thank you for your letter of 06 August 2012, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Vice-Chair.

Confirmation of ethical opinion

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

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Non-NHS sites

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

The Newcastle upon Tyne Hospitals NHS
NHS Foundation Trust

Royal Victoria Infirmary
Queen Victoria Road
Newcastle upon Tyne
NE1 4LP

Tel: 0191 233 6161
Fax: 0191 201 0155
www.newcastle-hospitals.nhs.uk

SS/JH

16 September 2013

Dr Kathryn Suzanne Jackson
Consultant Obstetrician
Women's Services
The Newcastle Upon Tyne Hospitals NHS Foundation Trust
Royal Victoria Infirmary

Dear Dr Jackson

Trust R&D Project:	**6781**
Title of Project:	***Exploring nutrient intake and alcohol consumption in pregnant women: a mixed methods approach***
Principal Investigator:	**Dr Kathryn Suzanne Jackson**
Number of patients:	**10**
Funder (proposed):	**Oxford Brookes University**
Sponsor (proposed):	**Oxford Brookes University**
REC number:	**12/SC/0402**

Having carried out the necessary risk and site assessment for the above research project, Newcastle upon Tyne Hospitals NHS Foundation Trust grants NHS Permission for this research to take place at this Trust dependent upon:

(i) you, as Principal Investigator, agreeing to comply with the Department of Health's Research Governance Framework for Health and Social Care, and confirming your understanding of the responsibilities and duties of Principal Investigators by signing the Investigator Responsibilities Document. A copy of this document will be kept on file within the Joint Research Office.

(ii) you, as Principal Investigator, ensuring compliance of the project with all other legislation and guidelines including Caldicott Guardian approvals and compliance with the Data Protection Act 1998, Health and Safety at Work Act 1974, any requirements of the MHRA (*eg* CTA, EudraCT registration), and any other relevant UK/European guidelines or legislation (*eg* reporting of suspected adverse incidents).

(iii) where applicable, you, as Principal Investigator, should also adhere to the GMC supplementary guidance *Good practice in research* and *Consent to research* which sets out the good practice principles that doctors are expected to understand and follow if they are involved in research – see http://www.gmc-uk.org/guidance/ethical_guidance/5991.asp

NHS Permission applies to the research described in the protocol and related documentation as listed on the favourable ethical opinion(s) from NRES Committee South Central – Southampton A Ethics Committee, dated 22 August 2012, 07 December 2012 and 20 May 2013. Specifically, the following versions of the key documents are approved:

Document	Version	Date
Protocol	6	08 April 2013
Participant Information Sheet: PIS Phase 1	6	08 April 2013
Participant Information Sheet: PIS Phase 2	6	08 April 2013
Participant Consent Form	2	08 April 2013
Invitation Letter	4	08 April 2013
Invitation Letter 2	4	08 April 2013

Northumbria Healthcare NHS
NHS Foundation Trust

Our Ref: KH/RP

17th October 2013

Miss Victoria Coathup
Oxford Brookes University
Jack Straws Lane
Oxford
OX3 0FL

Dear Miss Coathup

RE: Research Passport

Following satisfactory clearances, I am happy to confirm your starting date as 23rd October 2013.

Please complete the ID application form and bring along with you on your first day.

We have a Human Resources Representative available in the East Wing, HR office at Wansbeck General Hospital on Mondays, Tuesdays and Fridays from 8.30am – 5.00pm who will be able to issue you with your ID badge.

Enclosed are two copies of your written Terms and Conditions of Service **which you should sign.**

The copy marked 'FOR COMPLETION AND RETURN' should be returned to Human Resources Department, to signify that you accept the appointment as offered.

If you have any queries regarding your appointment, please do not hesitate to contact me.

Yours sincerely

H. Webster

Hannah Webster
Human Resources Assistant
Tel No: 0191 203 1415 option 2

Encs: Letter of Access/Honorary Research Contract
ID Form

Human Resources Department, Northumbria House, Unit 7/8 Silver Fox Way
Cobalt Business Park, Newcastle upon Tyne, NE27 0QJ
Tele: 0191 203 1412/1412 or Fax: 0191 203 1420

R:\Human Resources\HUMAN RESOURCES & ORGANISATION DEVELOPMENT\R&S\Research Passport\App Letter 1's, LAO & HRC's Issued 2013\LAO\LAO 1\Coathup, Victoria - Covering Letter.doc

17th September 2013

Brighton and Sussex University Hospitals NHS Trust

Private and Confidential

Miss Victoria Coathup
75 Southfield Road
OXFORD
OX4 1NY

The Princess Royal Hospital
Lewes Road
Haywards Heath
RH16 4EX

Tel: 01444 441881

Dear Miss Coathup

Letter of access for research

This letter confirms your right of access to conduct research through **Brighton & Sussex University Hospitals NHS Trust** for the purpose and on the terms and conditions set out below. This right of access commences on **17th September 2013** and ends on **31st December 2014** unless terminated earlier in accordance with the clauses below.

You have a right of access to conduct such research as confirmed in writing in the letter of permission for research from this NHS organisation. Please note that you cannot start the research until the Principal Investigator for the research project has received a letter from us giving permission to conduct the project.

The information supplied about your role in research at **Brighton & Sussex University Hospitals NHS Trust** has been reviewed and you do not require an honorary research contract with this NHS organisation. We are satisfied that such pre-engagement checks as we consider necessary have been carried out.

You are considered to be a legal visitor to **Brighton & Sussex University Hospitals NHS Trust** premises. You are not entitled to any form of payment or access to other benefits provided by this NHS organisation to employees and this letter does not give rise to any other relationship between you and this NHS organisation, in particular that of an employee.

While undertaking research through **Brighton & Sussex University Hospitals NHS Trust**, you will remain accountable to your employer **Oxford Brookes University** but you are required to follow the reasonable instructions of **Dr Neil Aiton, Consultant** and **Scott Harfield, Research & Development Manager** in this NHS organisation or those given on his behalf in relation to the terms of this right of access.

Where any third party claim is made, whether or not legal proceedings are issued, arising out of or in connection with your right of access, you are required to co-operate fully with any investigation by this NHS organisation in connection with any

Cont /……

Gloucestershire Hospitals NHS
NHS Foundation Trust
% Gloucestershire Research & Development Office
Leadon House
Gloucestershire Royal Hospital
Great Western Road
Gloucester
GL1 3NN
Telephone: 08454 225463
Facsimile: 08454 225469
Email: mark.walker@glos.nhs.uk

Our Ref: 12/069/GHT

Monday, 01 October 2012

Miss. Victoria Coathup
PhD Student
BSc Biology and Nutrition
MSc international health sciences
Oxford Brookes University
Jack Straws Lane
Marston
Oxford, OX4 0FL.

Dear Miss Coathup,

Study title:	**Exploring Nutrient intake and alcohol consumption in pregnant women**
REC reference:	**12/SC/0402**
R&D reference:	**12/069/GHT**

Thank you for forwarding information on the above study. I can confirm the approval of Gloucestershire Hospitals NHS Foundation Trust for this study to proceed. Your project will now be added to the Gloucestershire Health Community Research Register which will identify the following:

- Principle Investigator: **As above**
- Sponsoring Organisation: **Oxford Brookes University**
- Host Organisation: **Gloucestershire Hospitals NHS Foundation Trust**
- Type of Study: **PhD Student Project**

It is important that all research conducted with NHS patients and/or staff complies with the Research Governance Framework. In relation to this I would like to take the opportunity to remind you of some of your responsibilities under this framework.

1. **Health and safety:** You are reminded of your responsibilities for health and safety at work under the Health and Safety at Work Act 1974. You have a legal responsibility to take care of your own and other people's Health and Safety at work under the Health and Safety at Work ACT 1974 as amended and associated legislation. These include the duty to take reasonable care to avoid injury to yourself and to others by your work activities or omissions, and to co-operate with your employer in the discharge of its statutory duties. You must adhere strictly to the policies and procedures on health and safety.

2. **Codes of confidentiality/Data Protection:** Anybody who records patient information (whether on paper or by electronic means) has a responsibility to take care to ensure that the data recorded is accurate, timely and as complete as possible. It is vital that you conduct your research in accordance with the principles of the Data Protection Act 1998 and codes of confidentiality.

3. **Liability and Indemnity:** Indemnity for your study will be as described in any applicable Clinical Trial Agreement or other Research Contract.
   Where such an agreement is not available, the Trust will indemnify its employees and researchers holding NHS Honorary Contracts for the purposes of Negligent Harm.
   NHS Trusts cannot provide cover for No Fault or Non-Negligent claims. Where this is required, it is expected that the Research Sponsor will provide such indemnity.

4. **Intellectual Property:** Intellectual Property is defined as the tangible output of any intellectual activity that is new or previously undescribed. It can include the following:

    i. Inventions, such as new medical devices, software;
    ii. Literary works, such as software, patient leaflets, journal articles;
    iii. Designs and drawings, such as posters, leaflets;
    iv. Brand names, such as logos and trade marks; and
    v. Trade secrets, such as surgical techniques.

For projects originating from outside of the NHS Trust with which this agreement is made, Intellectual Property rights will remain with the Lead Site/Investigator unless developed from observations made outside of the scope and influence of the project. The rights to Intellectual Property generated in such a fashion will remain with the Host Trust unless an agreement to the contrary has been signed by both parties. Where a Clinical Trial Agreement or other Contract exists, this will take priority over this clause.

5. **Adverse Events/Incidents:** Any adverse events you witness or suspect to have happened *must* be reported to your supervisor or manager as soon as you know about them and dealt with as described in the research protocol.

6. **Fraud and Misconduct:** Any suspicions of active fraud or misconduct *must* be reported to your supervisor or manager immediately and will be treated in the strictest confidence. The monitoring of research will also seek to reduce incidents of research misconduct and fraud.

7. **Monitoring:** As part of the Research Governance Framework, during the course of your research you may be monitored to ensure that procedures in the protocol approved by the ethics committee are being adhered to. For locally sponsored studies this will be undertaken by the R&D Office. For externally sponsored studies this is likely to be arranged by the appropriate sponsor.

8. **Dissemination:** The Framework also requires the dissemination of research findings to the research subjects, NHS staff and the public. On completion of your research you will be expected to produce a summary of the project and an indication of how the results from the study will be disseminated. For studies where publication of research results is not the responsibility of the local Investigator, requests for such information will be made to the sponsor.

9. **Termination of Agreement:** The Trust also reserve the right to terminate the agreement for your research to proceed if, at any time, you are found to be in breach of the clauses in this Approval Letter or fail to adequately meet the requirements of the Research Governance Framework.

I wish you every success with your project.

Yours sincerely

Mark Walker
Senior Research & Development Manager
(Gloucestershire R&D Consortium)

CC: **Dawn Morrall- Assistant Divisional Director of Midwifery & Nursing, GHNHSFT**

OXFORD BROOKES UNIVERSITY

Dr Lesley Smith
Director of Studies
Department of Public Health
Faculty of Health and Life Sciences
Oxford Brookes University
Marston Road Site

5 July 2013

Dear Dr Smith

UREC Registration No: 130733
Exploring nutrient intake and alcohol consumption in pregnant women

Thank you for the recent correspondence outlining the response to the points raised in my previous letter about the PhD study of your research student Victoria Coathup and attaching the revised documents. I am pleased to inform you that, on this basis, I have given Chair's Approval for the study to begin.

The UREC approval period for this study is two years from the date of this letter, so 5 July 2015. If you need the approval to be extended please do contact me nearer the time of expiry.

In order to monitor studies approved by the University Research Ethics Committee, we will ask you to provide a (very brief) report on the conduct and conclusions of the study in a year's time. If the study is completed in less than a year, could you please contact me and I will send you the appropriate guidelines for the report.

Yours sincerely

Hazel Abbott
Chair of the University Research Ethics Committee

cc Mary Boulton and Simon Wheeler, Supervisory Team
Victoria Coathup, Research Student
Jill Organ, Graduate Office
Louise Wood, UREC Administrator

UNIVERSITY RESEARCH ETHICS COMMITTEE, FACULTY OF HEALTH AND LIFE SCIENCES

Headington Campus Gipsy Lane
Oxford OX3 0BP UK

Tel: 01865 482639
heabbott@brookes.ac.uk

Food Frequency Questionnaire

For use with the portion size photo booklet

0

Thank you for expressing an interest in completing this questionnaire.

The following pages consist of a food frequency questionnaire (FFQ) that measures your average intake of particular foods over the last three months. It is useful for us to understand what types of foods you have been eating so we can estimate your dietary intakes of folate, choline, vitamin C and carotenoids.

It is important that you answer each question as honestly as possible; otherwise we are unable to estimate your nutrient intake. All information provided will be confidential and used for research purposes only. Please read the instructions carefully on how to complete the questionnaire.

How to complete the questionnaire

This questionnaire is accompanied by a portion size booklet, containing photographs for many of the foods listed.

Food eaten	Frequency of consumption										Amount eaten	
	Never/ rarely	Once per month	Once per fortnight	Number of days per week							*Describe the amount you usually eat of a food by using the portion size photos and standard portions available. If you eat more or less than one serving per day, please write number of servings you eat/drink.*	
A	**B**	**C**	**D**	**E**							**F**	**G**
				1	**2**	**3**	**4**	**5**	**6**	**7**	**Standard portion**	**Amount I eat each time**
BREAD												
White bread								✓			*1 slice*	2/slice
Brown/wholemeal bread					✓						*1 slice*	2/slice
Granary/mixed seed bread	✓										*1 slice*	

Column A: Lists the foods/food groups of interest

Columns B to E: Describe how often you have eaten a particular food in the past three months. Please tick only one.

Column F: Describes a standard portion or includes a page number referring to a photograph in the portion booklet provided.

Column G: Within this column you will need to write how much you eat of that particular food when on days that you eat it. Use the standard portions and photographs to help you describe your typical portion.

If a standard portion is listed in column F, write down how many servings equal your typical portion. If it is a page number listed in column F, write down the corresponding picture code that best describes your typical portion. If you eat more than one typical portion per day, also write down the number of servings you will eat per day.

Some photographs of foods are used for other foods with a similar shape or texture. If the portion sizes listed do not represent your typical portion size you may also use standard household measures to describe a typical portion i.e. half pint, tablespoon, teaspoon, handful etc.

Please note: You do not need to describe a typical portion size if you 'never/rarely' consume that food.

EXAMPLES

If you usually eat a particular brand of ready-made food, then please write down the brand and type in the box on the right hand side.

E.g. I typically eat two chocolate hob nob biscuits every day in work

Food eaten	**Frequency of consumption**										**Amount eaten**	
	Never/ rarely	Once per month	Once per fortnight	Number of days per week							*Describe the amount you usually eat of a food by using the portion size photos and standard portions available. If you eat more or less than one serving per day, please write number of servings you eat/drink.*	
				1	**2**	**3**	**4**	**5**	**6**	**7**	**Standard portion**	**Amount I eat each time**
BISCUITS												
Chocolate biscuits								✓			*1 biscuit*	*2 (chocolate hob nobs)*
Cereal bars	✓										*1 bar*	

If you eat more than one portion per day of that particular food please write the number of portions you eat on a day when you eat that food.

E.g. twice a week I usually eat Kellogg's crunchy nut cornflakes for breakfast and lunch

CEREAL	**Frequency of consumption**									**Amount eaten**	
What brand and type of breakfast cereal do you usually eat?	Once per month	Once per fortnight	Number of days per week							Portion size	
			1	2	3	4	5	6	7	Standard portions	**Amount I eat each time**
Brand:……Kellogg's…… *Type:……Crunchy nut cornflakes……*				✓						page 7	53 X 2

If you eat less or more than a particular listed portion size, please describe it in the box provided on the right hand side.

E.g. I typically eat a small portion of yoghurt everyday

Food eaten	**Frequency of consumption**										**Amount eaten**	
	Never/ rarely	Once per month	Once per fortnight	Number of days per week							*Describe the amount you usually eat of a food by using the portion size photos and standard portions available. If you eat more or less than one serving per day, please write number of servings you eat/drink.*	
				1	**2**	**3**	**4**	**5**	**6**	**7**	**Standard portion**	**Amount I eat each time**
Courgette				✓							*¼ of a courgette*	Half of standard portion

Please describe your eating habits over the PAST THREE MONTHS by filling in the questionnaire on the following pages

Food eaten	Frequency of consumption										Amount eaten	
	Never/ rarely	Once per month	Once per fortnight	Number of days per week							*Describe the amount you usually eat of a food by using the portion size photos and standard portions available. If you eat more or less than one serving per day, please write number of servings you eat/drink.*	
				1	2	3	4	5	6	7	Standard portion	Amount I eat each time
BREAD												
White bread											*1 slice*	
Brown/wholemeal bread											*1 slice*	
Granary/mixed seed bread											*1 slice*	
CAKES												
Fruitcake											*page 8*	
Sponge/chocolate cake											*page 9*	
Carrot cake											*page 9*	
TEA BREADS												
Scone											*1 scone*	
Malt loaf											*page 8*	
SWEET & SAVOURY PASTRIES												
Doughnuts											*1 doughnut*	
Custard tarts											*1 tart*	
Croissants											*1 croissant*	
PUDDINGS												
Custard											*Page 11*	
Rice/milk puddings											*page 11*	
Cheesecake											*page 9*	
Bread and butter pudding											*page 10*	
Fruit crumble/pie/tart											*page 10*	
BISCUITS (Please write brands of biscuits and cereal bars eaten if possible)												
Oat biscuits											*1 biscuit*	
Chocolate biscuits											*1 biscuit*	
Cereal bars											*1 bar*	
CHEESE												
Cream cheese/cheese spreads											*page 12*	
Hard cheese (cheddar etc.)											*page 13*	
Soft cheese (Brie, camembert etc.)											*page 14*	
RICE/NOODLES												
Rice											*page 5*	
Egg noodles											*page 6*	
PIZZA/PASTA												
Pizza											*page 17*	
Wheat spaghetti/tagliatelle/noodles etc.											*page 6*	

Please describe your eating habits over the PAST THREE MONTHS by filling in the questionnaire on the following pages.

Food eaten	Frequency of consumption										Amount eaten	
	Never/ rarely	Once per month	Once per fortnight	Number of days per week							*Describe the amount you usually eat of a food by using the portion size photos and standard portions available. If you eat more or less than one serving per day, please write number of servings you eat/drink.*	
				1	2	3	4	5	6	7	**Standard portion**	**Amount I eat each time**
MEAT/CHICKEN												
Chicken											*page 20 or 21*	
Steaks											*page 22*	
Pork/Lamb											*Page 20*	
Liver											*page 20*	
Beef burger											*1 burger*	
MEAT DISHES												
Minced beef dishes (chilli con carne, Shepard's pie etc.)											*page 24*	
Meat stew/casserole											*page 24*	
Lasagne											*page 23*	
PROCESSED MEATS												
Sausage											*1 sausage*	
Bacon											*1 rasher*	
Liver Pate											*Page 12*	
FISH/SEAFOOD												
Fish											*page 26*	
Tinned tuna											*1 tin (175g)*	
Prawns											*page 27*	*size and number:* ....
POTATOES												
Boiled/mashed											*page 28*	
Chips											*page 29*	
Roast potatoes											*Page 28*	
Jacket potato											*page 28*	
Sweet potato											*page 28*	
ROOT VEGETABLES (Including vegetables in mixed dishes)												
Raw carrots											*page 30*	
Cooked carrots											*page 30*	
Beetroot											*page 30*	
GREEN LEAFY VEGETABLES (Including vegetables in mixed dishes)												
Green cabbage											*page 31*	
White/red cabbage											*Page 31*	
Spinach											*Page 31*	
Brussel sprouts											*5 sprouts*	
Broccoli											*Page 32*	
GREEN BEANS (Including vegetables in mixed dishes)												
Peas											*page 33*	
Green/french beans											*page 31*	
Mange tout peas											*Page 31*	

Please describe your eating habits over the PAST THREE MONTHS by filling in the questionnaire on the following pages.

Food eaten	Frequency of consumption										Amount eaten	
	Never/ rarely	Once per month	Once per fortnight	Number of days per week							*Describe the amount you usually eat of a food by using the portion size photos and standard portions available. If you eat more or less than one serving per day, please write number of servings you eat/drink.*	
				1	2	3	4	5	6	7	Standard portion	Amount I eat each time
SALAD VEGETABLES (Including vegetables in mixed dishes)												
Lettuce											*page 31*	
Cucumber											*page 34*	
Green/yellow pepper											*1 pepper*	
Red pepper											*1 pepper*	
TOMATOES												
Fresh tomatoes											*page 35*	
Tinned tomatoes											*1 400g tin*	
Tomato pasta sauce											*½ jar (250ml)*	
Tomato paste/puree											*1 tablespoon*	
OTHER VEGETABLES (Including vegetables in mixed dishes)												
Mixed vegetables (frozen)											*page 33*	
Butternut squash											*Page 28*	
Cauliflower											*Page 32*	
Onions											*1 onion*	
Leeks											*¼ of a leek*	
Courgettes											*¼ of a courgette*	
Vegetable pasty/pie											*Page 19 or 25*	
Coleslaw											*1 tablespoon*	
BEANS AND PULSES												
Chickpeas/kidney beans											*page 37*	
Baked beans											*page 37*	
CITRUS FRUIT												
Oranges											*1 orange*	
Satsuma/clementine/tangerine											*1 fruit*	
TROPICAL FRUIT												
Banana											*1 banana*	
Kiwi											*1 kiwi*	
Mango											*1 Mango*	
Melon											*1 slice*	
OTHER FRUIT												
Plums/apricots											*1 fruit*	
Apples/pears											*1 fruit*	
BERRIES												
Raspberries											*10 berries*	
Strawberries											*5 berries*	
EGGS												
Eggs											*1 egg*	
Quiche/savoury flan											*Page 18*	

Please describe your eating habits over the PAST THREE MONTHS by filling in the questionnaire on the following pages.

Food eaten	Frequency of consumption										Amount eaten	
	Never/ rarely	Once per month	Once per fortnight	Number of days per week							*Describe the amount you usually eat of a food by using the portion size photos and standard portions available. If you eat more or less than one serving per day, please write number of servings you eat/drink.*	
				1	2	3	4	5	6	7	**Standard portion**	**Amount I eat each time**
NUTS AND SEEDS												
Mixed nuts											*1 tablespoon*	
Peanut butter											*1 tablespoon*	
CRISPS/SAVOURY SNACKS												
Crisps											*1 35g packet*	
SPEADS/SAUCES/SOUPS												
Carrot soup											*Page 39*	
Tomato- based soup											*Page 39*	
Tomato ketchup											*1 tablespoon*	
Mayonnaise											*1 tablespoon*	
Bovril/marmite											*1 tablespoon*	
CHOCOLATE/SWEETS												
Milk chocolate											*4 squares*	
Dark chocolate											*4 squares*	
SOFT DRINKS												
Fresh orange juice											*half pint*	
Fresh apple juice											*half pint*	
TEA/COFFEE												
Tea (English breakfast/black)											*1 mug(250ml)*	
Coffee											*1 mug(250ml)*	
BEER/ALE												
Lager/light ale											*1 half pint*	
Dark beer/ale											*1 half pint*	
MILKY DRINKS												
Horlicks/Ovaltine/Complan etc.											*1 mug (250ml)*	

CONTINUED ON FOLLOWING PAGE…….

Please describe your eating habits over the PAST THREE MONTHS by filling in the questionnaire on the following pages.

What type and brand of breakfast cereals do you usually eat?

BREAKFAST CEREAL	**Frequency of consumption**								**Amount eaten**	
What brand and type of breakfast cereal do you usually eat? (including porridge and muesli)	Once per month	Once per fortnight	Number of days per week						Portion size	
			1	2	3	4	5	6	7	Standard portions	**Amount I eat each time**
Brand:..... *Type:*.....										page 7	
Brand:..... *Type:*										page 7	
Brand:..... *Type:*.....										page 7	
Brand:..... *Type:*.....										page 7	

Please list the fruits or vegetables that you normally always eat from frozen or from a tin. Please write down the name of the fruit or vegetable in the left hand column and then right down whether they are frozen or from a tin.

Fruit or vegetable	**Frozen/tinned/in jar**

How much milk do you drink each day, including milk with tea, coffee, cereals etc.?

None	
Quarter of a pint	
Half a pint	
Three quarters of a pint	
One pint	
More than one pint	

What type of milk do you usually use?

Skimmed milk	
Semi-skimmed milk	
Whole milk	
Channel Island (gold top)	

THANK YOU VERY MUCH FOR TAKING THE TIME TO COMPLETE THIS QUESTIONNAIRE.