Maternal folic acid supplementation throughout pregnancy

exploring the effect on children's psychological development

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Maternal folic acid supplementation throughout pregnancy: Exploring the effect on children's psychological development

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Submitted for the degree Doctorate of Philosophy

June 2023

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ACKNOWLEDGEMENTS

Firstly I would like to give a very special thanks to my supervisors, Prof Tony Cassidy and Dr Marian McLaughlin. Your patience, guidance and continuous support was appreciated more than you will ever know. You have not only seen me through my entire university career but you have been there during the most significant times of my life including two graduations, one wedding and two babies, so thank you from the bottom of my heart. All other Ulster University staff members have also been exceptional including the psychology lecturers who gave me all the opportunities I could ask for, the subject librarians for helping me navigate a systematic review and all the fantastic PhD students who travelled their PhD journey with me. I would like to give a special mention to Dr Eimear McDowell and Dr Carol-Ann Getty for all their words of encouragement and keeping me motivated during some of my most difficult times. I would also like to thank some of my closest friends Judith, Emma, Julie, Alex and Conal, just for being there through it all. You were a welcome distraction when I needed it most and an unwavering support all in one. Thank you to my family, my dad for allowing me to set up an office to work away from the chaos of home and my mum for all the babysitting, creche collections and hot dinners! But I want to give a very special thanks to my husband Noel and my three children, Matthew, Clara and Adam. Thank you for your patience, understanding and help. There was never a question when I needed to work late or start early. We made it through together, when at times it felt impossible. Finally, thank you to our colleagues in NICHE and Biomedical Sciences at Ulster. However, none of this would ever have been possible without the dedication and support of the mothers and children who have diligently taken part in this research for so many years, thank you.

THESIS ABSTRACT

Pregnancy is characterised by exponential fetal growth and development. During this period, optimal maternal nutrition is vital to provide the best possible health opportunities for both mother and child (King, 2000). A number of important nutrients have been identified and recommended by NHS (2020) to reduce the risk of adverse pregnancy and birth outcomes. There is clear evidence to support maternal consumption of 400µg/d of folic acid (FA) from preconception to at least 12 gestational weeks (GW), with deficiency during this critical window of development a known risk factor for Neural Tube Defects (NTD) occurrences (MRC, 1991). The link between maternal folate status during pregnancy and children's other developmental outcomes is less understood. There has been considerable investigation into the physical and cognitive implications for children whose mothers were FA deficient during pregnancy however, the effect on children's psychological, social, emotional and behavioural development is much less understood. This PhD sought to address this gap. However, rather than focusing on the negative effects of maternal folate deficiency on child developmental outcomes, this PhD applied a positive, resource-focused approach in order to identify the developmental benefits children could experience, psychologically, socially, emotionally and behaviourally in relation to typical (to 12GW) and continued (to at least 36GW) maternal FA use.

A systematic review was conducted to identify and evaluate all relevant literature and assess the quality and risk of bias, an abundance of evidence was uncovered relating to the developmental risks associated with maternal deficiency. Evidence has begun to consider the positive implications for children, particularly in terms of cognition and neurodevelopment, however the duration of use remains largely

aligned to the current recommendations. Findings from the review suggest FA dose, time of initiation and duration of use were all important factors requiring further investigation. Secondary data analysis was conducted using the Avon Longitudinal Study of Parents and Children (ALSPAC) to explore the effect of FA supplementation in late pregnancy on children's cognitive, motor, social, emotional, behavioural and language development using a large, independent study. Findings concurred with those found in the systematic review indicating a potential link between maternal nutrition and later child development. Finally an RCT was used to control FA dose and time of initiation and test the duration of maternal FA use. The developmental impact for children at ~10 years old could then be accurately assessed by comparing children whose mothers stopped supplementing at the recommended 12GW to those who continued to the end of their pregnancy. Key areas of psychological development were measured including their Trait Emotional Intelligence (TEI), Trait Psychological Resilience (TPR), peer attachment style and behaviour strengths and difficulties while testing for mediating effects of peer attachment and parenting style. Results found that continued use caused a significant increase in children's global TEI and TPR and creativity as measured by the Resiliency Skills and Attitudes Profile (RASP). The mothers of these children were also more likely to adopt a positive parenting style with folate level at 36GW, a significant predictor of TEI, prosocial behaviour and the creativity and values dimensions of TPR. Additionally, mediation analysis identified secure and anxious attachment as significant mediators between folate at 36GW and TEI at 10y.

This thesis recommended further investigation to explore play and children's language development as potential mechanisms of action. Play opportunities, type

and quality are important factors to consider as it fosters cognitive, psychological, social emotional and behavioural development in children. Similarly with language acquisition and interaction, relationships, attachments and emotional bonds can be buffered and nurtured by linguistic skill in the early years having a positive impact on children's developmental outcome. To conclude, this study indicates that all maternal folic use can provide developmental advantages for children, however continued supplementation promotes optimal development, particularly in the psychological, social, emotional and behavioural domains and recommends mothers continue to supplement for the duration of their pregnancy.

ABBREVIATIONS

Abbreviation	Description
µg	Microgram
µg/d	Microgram per day
AAFP	American Academy of Family Physicians
ADHD	Attention- deficit hyperactivity disorder
ALSPAC	Avon Longitudinal Study of Parents and Children
AMICS	Asthma Multi-centre Infants Cohort Study
ANT	Attention Networks Test
ASCQ	Attachment Style Classification Questionnaire
ASD	Autistic Spectrum Disorder
ASSIA	Applied Social Sciences Index and Abstracts
BMI	Body Mass Index
BSID	Bayley's Scales of Infant and Toddler Development
CASP	Critical Appraisal Skills Programme
CBCL	Child Behaviour Checklist
cm	centimetres
D-B	Double Blinded
DDST	Denver Developmental Screening Test
DF	Dietary Folate
DHA	Docosahexaenoic acid
DOHaD	Developmental Origins of Health and Disease
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4th Edition
EEG	Electroencephalography
EFA	Exploratory Factor Analysis
EI	Emotional Intelligence
ELSPAC	European Longitudinal Study of Pregnancy and Childhood
EMT	Electromagnetic tomography
EPA	Eicosapentaenoic acid
ERP	Electroencephalography/event-related potentials
EpiFASSTT	Epigenetic effects on children's development following maternal Folic Acid Supplementation during the Second and Third Trimesters in pregnancy
FA	Folic acid
FASSTT	Folic Acid Supplementation during the Second and Third Trimesters

Abbreviation	Description
	in pregnancy
Fconc	Folate concentration in blood
Fe	Iron
FO	Fish oil
g	grams
GW	Gestational weeks
HMRA	Hierarchical Multiple Regression Analysis
HSCT	Health and Social Care Trust
IBSS	International Bibliography of the Social Sciences
IFA	Iron and folic acid combined supplement
INMA	Infancia y Medio Ambiente project
IQ	Intelligence Quotient
JAMA	Journal of the American Medical Association
K-ABC	Kaufman Assessment Battery for Children
kg	kilograms
LCP's	Long chain polyunsaturated fatty acids
M	Mean
m	months
MeSH	Medical Subject Headings
mm	millimetres
MoBa	Norwegian Mother and Child Cohort Study
MTHF	Methylenetetrahydrofolate reductase
MV	Multivitamin
n	number
NHS	National Health Service
NHSCT	Northern Health and Social Care Trust
NI	Northern Ireland
NICE	National Institute for Health and Clinical Excellence
NIHR	National institute of Health and Care Research
NMIHS	National Maternal Infant Health Survey
nmol	nanomoles
nmol/L	nanomoles per litre
NR	Not reported
NTD	Neural tube defects
NUHEAL	Nutraceuticals for a Healthy Life Cohort

Abbreviation	Description
ORECNI	Office of Research Ethics Committees in Northern Ireland
PDG	Programme Development Group
PDSQ	Parenting Styles and Dimensions Questionnaire
PHA	Public Health Agency
PICOS	Population, Intervention, Comparison, Outcomes and Study Design (systematic review parameters)
PPVT	Peabody Picture Vocabulary Test
Pre	preconception
Preg	Pregnancy
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PROMs	Premature rupture of membranes
RASP	Resiliency Skills and Attitudes Profile
RBC	Red blood cell
RCF	Red cell folate
RCT	Randomised Control Trial
RDA	Recommended Daily Amount
SDQ	Strengths and Difficulties Questionnaire
SF	Serum folate
SR	Systematic Review
SSRI	Selective Serotonin Reuptake Inhibitor
TEI	Trait Emotional Intelligence
TEI-Que	Trait Emotional Intelligence Questionnaire
tHcy	Homocysteine
TPR	Trait Psychological Resilience
UK	United Kingdom
w	weeks
WHO	World Health Organisation
WPPSI-III	Wechsler Preschool and Primary Scale of Intelligence 3rd UK Edition
WRAVMA	Wide Range Assessment of Visual Motor Abilities

DECLARATION

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Chapter 1

General Introduction

1.1 Chapter Overview

Chapter one will provide an overview of the PhD in its entirety, presenting a brief background to the issues being addressed, identifying where the gap in knowledge exists and justifying the need for conducting each of the investigations. This PhD builds on an ongoing longitudinal study with a long history at Ulster University, the **F**olic **A**cid **S**upplementation during the **S**econd and **T**hird **T**rimesters in pregnancy (FASSTT) trial. The chapter will begin with some background information on important micro and macronutrients required by the body during pregnancy, including folic acid with some information on the effects of deficiency on pregnancy and birth outcomes including children's physical, cognitive and psychological development followed by the current guidelines for supplementing. A short description of the FASSTT trials will then provide a context for this thesis. The chapter continues with outlining the rationale for the PhD including identification of the outcomes of interest, the research aim and objectives, the research design, the significance of the study and concluding with a short overview of the thesis.

1.2 Background to the PhD

Pregnancy is a time when nutritional needs are increased due to the physiological changes of the mother and the metabolic demands of the embryo/foetus (King, 2000). During this period of growth, optimal maternal nutrition is vital for the best possible health for both mother and child. Evidence suggests that maternal health

status, lifestyle and history prior to conception strongly influences the pregnancy and outcome (Korenbrot *et al*., 2002), and parents who are fit and healthy at the beginning of pregnancy tend to have healthier babies (Department of Health, 2004). Furthermore, maternal nutrition and lifestyle throughout pregnancy, lactation, infancy and early childhood have been shown to induce long-term effects on later health of the child (e.g. Ballestin *et al*., 2021; Berner *et al*., 2014; UNICEF, 2013; Srivastava *et al.*, 2011; Ars *et al.*, 2019; Compan Gabucio *et al.*, 2021; Caffery *et al.*, 2018; Henry *et al.*, 2018). It is therefore important to develop preconception care that promotes health and prevents disease and maternal pre and perinatal nutrition plays an important role.

1.2.1 The importance of micronutrients for pregnancy and beyond

From preconception to birth a number of vitamins and minerals, referred to collectively as micronutrients have been identified as essential to maternal health and the developing child. Extensive research has shown that inadequate intake can have adverse effects on the mother including anaemia, hypertension, labour complications and death (e.g. Mousa *et al*., 2019; Bourassa *et al.*, 2019). The unborn child can also experience the negative effects of micronutrient deficiency increasing the risk of stillbirth, pre-term delivery, congenital malformations, abnormal organ development and immunocompetence (Ballestin *et al.*, 2021; Kanasaki & Kumagai, 2021). Micronutrient deficiency, particularly during pregnancy is complicated as deficiencies tend to co-exist and deficiency level of specific vitamins and minerals can vary due to a number of factors including stage of life, season, year, ethnic group, SES and place of residence (Black, 2001). This variability is typically due to differing diet content and bioavailability, in addition to individual disparity in losses

and requirements for micronutrients and their biological interactions. Methodological inconsistencies also impact on the reliability of the available evidence. Randomised Control Trials (RCT's) provide the most robust evidence to infer causation between micronutrient intake and pregnancy outcomes for mother and child. However, these tend to test the effects of an individual micronutrient, which may be problematic as in many cases these deficiencies co-exist. In these cases, the effect of adjusting a single micronutrient and their subsequent interactions is unclear. Furthermore, correcting multiple deficiencies through supplementation for example when diet remains inadequate will not stimulate optimal developmental outcomes. Despite these limitations RCT's provide important and relevant findings applicable around the globe.

Research confirms that women need an array of nutrients to promote healthy fetal development and maternal health during pregnancy. The National Health Service (NHS) (2020) lists adequate folate, vitamins B_{12}, C and D, iron and calcium as crucial to provide immediate and lasting positive effects for both mother and child. In most cases, with the exception of folate and vitamin D, adequate levels of these vitamins and minerals can be obtained through diet even while following vegetarian, vegan or a special diet in pregnancy. Due to individual differences with regards to bioavailability and interaction effects it is now commonplace in the UK for women to supplement with a multiple micronutrient to guarantee adequate intake and stores of each vitamin and mineral required for a healthy pregnancy, birth and later child development.

1.2.2 Iron deficiency during pregnancy

Iron deficiency is the most common micronutrient deficiency worldwide, particularly amongst pregnant mothers, infants and young children. This is due to the high demand required during periods of rapid growth (Stevens *et al.*, 2013). Iron deficiency occurs in stages typically due to inadequate dietary intake, compromised absorption or depletion in iron stores. Unresolved depletion due to demand outstripping supply can progress to deficiency when there is insufficient iron for normal functioning.

Mothers presenting with adequate levels of iron during pregnancy have been shown to have reduced risk of pregnancy complications such as pre-eclampsia (Wang *et al.*, 2018), preterm birth (Wang *et al*., 2018), low birth weight (Figueiredo *et al.*, 2018), gestational diabetes (Wang *et al.*, 2018) and maternal antepartum and postpartum depression (Kang *et al*., 2020). Conversely, maternal anaemia during pregnancy can result in the baby being iron deficient following the birth which can persist in infants for up to one year (Abu-Ouf & Jan, 2015). This can impact their neural functioning and metabolism which increases the risk of developmental delay and the occurrence of abnormalities. Research has linked iron deficiency to delayed language and motor development and difficulties in children's cognitive, socioemotional and adaptive functioning (Berner *et al.*, 2014).

A recent systematic review (McCann *et al*., 2020) found some evidence that prenatal iron deficiency was detrimental to early brain development in most of the included studies, however acknowledged the possibility that the impact of iron supplementation on development depended on the availability of other micro- and

macronutrients (Finklestein *et al*., 2018). Neither a critical window nor threshold was identified by authors. A large population-based study conducted by Wiegersma *et al*. (2019) tested if the gestational timing of maternal anaemia was associated with neurodevelopmental disorders and found that deficiency in early pregnancy (<30GW) as opposed to later pregnancy (>30GW) significantly increased the risk.

1.2.3 Vitamin C deficiency during pregnancy

Vitamin C, or ascorbic acid is vital for good health at every stage of life and recognised as particularly important during pregnancy for optimal development of mother and child. It is needed to make collagen; a structural protein that is a component of cartilage, tendons, bones and skin (DePhillipo *et al*., 2018). Moreover, Vitamin C enhances iron absorption (Triharini *et al*., 2018), low levels of which can decrease the absorption and utilisation of iron which promotes healthy pregnancy and fetal growth as previously discussed. Deficiency has been shown to increase the mothers risk of miscarriage, premature rupture of membranes (PROMs), placental abruption, impaired placental implantation, pre-eclampsia, preterm birth and gestational diabetes (Rumbold & Crowther, 2005; Casnanueva *et al*., 2005; Osaikhuwuomwan *et al*., 2011; Rumbold *et al*., 2015; Juhl *et al*., 2017) and could compromise intrauterine development (Schjoldager *et al.*, 2014). Research has shown that maternal vitamin C deficiency predisposes the newborn to oxidative stress (Casanueva & Viteri, 2003; Negi *et al*., 2011), posing serious implications for fetal brain development (Tveden-Nyborg, 2021) and subsequent development of the central nervous system (Paidi *et al*., 2014). These findings have been reinforced by animal research which found that interference to optimal vitamin C levels during pregnancy negatively affected developmental outcome in the offspring including low

birth weight (Dobbing, 2008), brain structure (Bedi, 1991) and cognitive development (Morley & Lucas, 1997) in rats. Vitamin C levels tend be lowest in the third trimester due to the physiological changes which can lead to haemodilution, increasing the risk of anaemia (Sharma & Mathur, 1995; Ugwa *et al*., 2016). However, vitamin C levels can be easily managed through diet. If fruit and vegetable consumption is low, then supplementation is recommended during pregnancy.

1.2.4 Calcium deficiency during pregnancy

Calcium levels affect many extracellular and intracellular processes and is a substrate for bone mineralisation therefore skeletal mass cannot be built or maintained if levels are not sufficient (Almaghamsi *et al*., 2018). Calcium is also a cofactor for hormonal secretion in the endocrine system (Kiran *et al*., 2021) and healthy production of thyroid hormones is necessary for healthy fetal development of the brain and nervous system particularly during the first 12 weeks gestation (Ahmed *et al.*, 2007). Some evidence suggests these hormones can impact on children's neurocognitive development during their early years (Pop *et al.*, 2003), however, this area of research needs more investigation.

Hypocalcaemia is a metabolic derangement caused by calcium deficiency either through loss of calcium from circulation or insufficient intake and often associated with advanced gestational age. Serum calcium typically falls throughout pregnancy, therefore maintaining sufficient levels is crucial for the mothers own nutritional needs and to support the growing foetus. A healthy, calcium rich diet reduces the risk of hypertensive disorders, pre-eclampsia, postpartum haemorrhage in the mother (Ritchie & King, 2000; Villar & Belizen, 2000), prevents preterm birth, jaundice and

congenital abnormalities in neonates (Kiran *et al.*, 2021) and improves maternal and infant bone health and density (Kumar & Kaur, 2017). For optimal bone health calcium works in conjunction with Vitamin D which encourages optimum absorption.

1.2.5 Vitamin D deficiency during pregnancy

Vitamin D deficiency is prevalent amongst the general population and pregnant women worldwide. Vitamin D production on the skin through the sun's ultraviolet-B (UVB) rays is the primary natural source of vitamin D for most, however many people have insufficient levels due to limited sun exposure or skin colour. Few foods contain vitamin D naturally therefore a supplement is advised if the Recommended Dietary Allowance (RDA) is not met. Inadequate vitamin D during pregnancy has been associated with a number of obstetric complications and outcomes including pre-eclampsia (Fogacci *et al.*, 2020), preterm birth (Qin *et al*., 2016), low birth weight (Khalessi *et al*., 2015) and gestational diabetes (Hu *et al.*, 2018). Research has also shown that maternal vitamin D deficiency could have a lasting negative impact on children's bone growth due to its interaction with calcium absorption (Javaid *et al.*, 2006) and in severe cases cause rickets (Mulligan *et al*., 2010).

A number of physical child health and developmental outcomes have been linked to vitamin D status including measures of fetal size, body composition and skeletal mineralization, in addition to later childhood outcomes, such as type 1 diabetes mellitus or asthma (Curtis *et al*., 2018; Korytko, 2020). Vitamin D also plays an important role in baby's brain development. The biological role of the vitamin can impact brain function and possibly influence later neurodevelopment (UNICEF,

2013; Bailey *et al*., 2015). This is in part due to the 'fine-tuning' effect that vitamin D has on the neuronal circuits which some have recognised is important in relation to cognition, behaviour and memory capabilities (Srivastava *et al.*, 2011). Despite this, investigations into the relationship between maternal vitamin D status and children's neurodevelopment have remained inconclusive (Pet & Brouwer-Brolsma, 2016; Veena *et al*, 2016).

A recent review (Janbek *et al*., 2019) examined these effects on neurodevelopment and found that maternal vitamin D status during pregnancy was associated with language and motor development in young children which persisted into adolescence. Another review concurred, concluding that deficiency could have a detrimental effect on mental, motor and language development (Villalobos *et al*., 2019). An experimental study by Lopez-Vicente *et al*.(2019) found social competence improved with increased maternal vitamin D offering further support. However, there is some inconsistency in the literature (e.g. Gale *et al.*, 2007) due to difficulties validating the optimal RDA required for the vitamin during pregnancy for maximum benefits for both mother and child in terms of both physical health and neurodevelopment (Korytko, 2020).

1.2.6 Vitamin B_{12} deficiency during pregnancy

Vitamin B_{12} is obtained through the intake of animal origin foods therefore deficiency is common in those with restricted diets such as vegetarians or vegans. Factors such as geographical area, religion, culture and poverty also contribute to the high prevalence of B_{12} deficiency (Behere *et al*., 2021). Currently there is no public health policies in place in relation to the consumption of this vitamin during

pregnancy. The metabolic role of B_{12} is closely related to folate as both participate in the one-carbon metabolism cycle, affecting cell growth and differentiation through DNA synthesis and epigenetic regulation thus regulating fetal growth (Kalhan, 2016). Children born to deficient mothers were at increased risk of Neural Tube Defects (NTD), congenital abnormalities (Neogi *et al.*, 2017; Malik *et al*., 2017) impaired infant growth, psychomotor function and potentially irreversible brain development (Finklestein *et al*., 2015) therefore impacting on cognitive function (Muthayya *et al.*, 2006; Behere *et al*., 2021). Some research has documented the negative impact B_{12} can have on child cognition, with maternal deficiency being associated with reduced mental and social development (Bhate *et al.*, 2012), executive function (Bhate *et al.*, 2008), expressive language and motor function (Thomas *et al.*, 2019) and intelligence (Bonilla *et al.*, 2012), however some inconsistency in relation to psychological functioning remains (e.g. Ars *et al.*, 2019)

Traditionally vitamin B_{12} deficiency is associated with pernicious anaemia (Behere *et al.*, 2021) which is a genetic autoimmune condition causing poor absorption of the vitamin in the digestive tract (NHS, 2019). However, insufficient levels of maternal B_{12} have also been associated with increased risk of a number of pregnancy complications including spontaneous abortion, low birth weight, intrauterine growth restriction and preterm birth (e.g. Sukumar *et al*., 2016; Rogne *et al.*, 2017). Furthermore, some evidence suggests that clinical B_{12} deficiency could be a cause of maternal infertility or recurrent spontaneous abortion (Puri *et al.*, 2013; Kaur *et al*., 2018).

1.2.7 Fatty acid requirements during pregnancy

Long chain polyunsaturated fatty acids (LCP's) are considered macronutrients and therefore not included in the NHS (2020) list of vitamins and minerals required during pregnancy. The benefits for maternal pregnancy and birth outcome and child developmental outcome however are well documented (e.g. Wadhwani *et al*., 2018; Miles *et al*., 2021; Gould & Smithers, 2019). These essential fatty acids are required for normal bodily function and can only be obtained through diet (Jensen, 2006). Oily fish and other seafood contain various essential nutrients, the most biologically active omega-3 fatty acids being eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and consuming two portions per week is recommended to all pregnant mothers. Omega-3 is critical during pregnancy for child retina and brain development and maturation; however, intake tends to be low due to mothers concerns regarding the presence of neurotoxins (Taylor *et al*., 2018) and confusion around the teratogenic effects of high doses of vitamin A contained in cod liver oil (Verbeke *et al*., 2005).

Research has linked maternal omega-3 fatty acid intake with a number of pregnancy and birth outcomes including healthy birth weight (Jensen, 2006) and bone health (Koren *et al.*, 2014). An increase in the average length of gestation and reduced risk of first instance and recurrent preterm birth has also been documented (Olsen *et al.*, 2004; Smuts *et al.*, 2003; Jensen, 2006; Makrides *et al.*, 2010) however, findings remain inconsistent (e.g. Ramakrishman *et al.*, 2010; Helland *et al.*, 2001; Harper *et al.*, 2010). Additionally, some evidence has demonstrated that maternal health and wellbeing could benefit from sufficient intake as the risk of antenatal and postnatal depression is reduced (Makrides *et al.*, 2010; Golding *et al.*, 2009).This is due to

healthier cell membranes which enables the serotonin to flow better thus reducing the risk of depressive symptoms (Golding *et al.*, 2010; Freeman *et al.*, 2008), however further investigation is required.

EPA and DHA have both been shown to have beneficial effects on child development if consumed by the mother during pregnancy. Requirements for the nutrient increase to above normal levels to accommodate the accelerated brain growth taking place during the second and third trimesters (Coletta *et al*., 2010). The association between omega-3 fatty acids and children's neurodevelopment has therefore understandably, been an area of much investigation. Research has discovered a plethora of benefits on child outcome including developmental milestones, problem solving, language development (Makrides *et al.*, 2010; Helland *et al.*, 2003), visual recognition memory (Oken & Bellinger, 2008) and verbal Intelligence Quotient (IQ) (Hibbeln *et al.*, 2007; Oken & Bellinger, 2004), performance IQ , behaviour, fine motor skills, communication and social development (Hibbeln *et al.*, 2007).

1.2.8 Folate deficiency

Folate is another vitamin recommended by the NHS (2020) and considered crucial for healthy development particularly during the periconceptional period (about one month before and one month after conception), providing a range of benefits for both the mother and child (Lassi *et al*., 2013) Similarly to vitamin B_{12}, folate is a naturally occurring B-vitamin (B_9) found in many foods and is an important co-factor in one-carbon metabolism, with an inverse relationship with homocysteine (tHcy). Adequate folate is difficult to achieve through di*et al*one because it is a water soluble

B vitamin, meaning that folate is metabolised quickly therefore it doesn't remain in the system long (Hayes *et al*., 2009). Folate is essential for the body to synthesize, repair and methylate DNA (Allen *et al.,* 1993). The demand increases during pregnancy to aid rapid cell division and growth which is vital for fetal, placental and maternal growth and development (Tamura *et al*., 2005).

Folic acid is the fully oxidised, synthetic form of folate and it is recommended that all women take at least 400µg daily from preconception to the end of the first trimester or 12GW (National Institute for Health and Care Excellence (NICE), 2008). Expectant mothers and women thinking of becoming pregnant therefore are advised to supplement to support optimal growth and development and help prevent pregnancy and birth complications. In many countries, health services have issued a recommendation that all reproductive-aged women should consume folic acid daily (Honein *et al*., 2001) which has led to mandatory food fortification programmes. This has only recently been introduced in the UK (Department of Health and Social Care, 2021; Haggarty, 2021).

1.3 Current guidance and policy

It is recognised that adequate folate and vitamin D is difficult to obtain from di*et al*one therefore a supplement is recommended. In the UK the NHS can provide folic acid and vitamin D on prescription or through government incentives such as the Healthy Start Programme which provides mothers who qualify with vouchers to buy vitamins, milk, fruit and vegetables to encourage healthy nutrition from early pregnancy to the child's fourth birthday (NHS, 2021). In recent years some mothers

have opted to supplement with a multivitamin containing all the nutrients required for a healthy pregnancy and birth. Some mothers cease supplementing at the end of the first trimester, while others continue for the duration of their pregnancy and in some cases extending past birth and into lactation.

1.3.1 Nutrition and maternal folic acid use before and during pregnancy

Preconception care is one aspect of primary care that is paramount for the health and wellbeing of prospective mothers and their children. It has been defined by WHO (2002) as accessible medical, psychological and social health interventions before pregnancy to improve health, promote positive behaviours and balance the effects of environmental influences. The aim is to enhance the health outcomes of both mothers and children throughout life primarily through knowledge and raising awareness provided as part of health and social care. Guided by NICE, effective preconception care is vital as many risk factors which can have a negative impact on pregnancy outcomes are present before conception (Van Der Zee *et al*., 2011). It provides an opportunity to address and manage any physical, mental or social risks prior to pregnancy (National Institute for Health and Care Research (NIHR), 2017) as often prenatal care is too late to change pregnancy outcome (e.g. Callegari *et al*., 2015). Women's pre and periconceptional health has significant impacts on their own maternal health and lifelong health of their children (NIHR, 2017).

Included in preconception but also prenatal care is the importance of nutrition, a healthy balanced diet and appropriate supplement use during pregnancy. The policy and guidance for maternal and child nutrition; Public Health Guideline 11 [PH11] was first published in 2008 and last updated in 2014 although remains under constant

review. Recommendation 2 in this guideline refers to folate and folic acid intake during pregnancy advising that women should supplement with 400µg/d of folic acid from preconception to 12GW and to encourage a folate rich diet in conjunction with supplementation (NICE, 2008). These guidelines are evidence based and supported by extensive literature.

According to the National Diet and Nutrition Survey (PHE, 2020) a fifth of UK females between 16 and 24 years (22.1%) are folate deficient with median blood levels about half of those in women from the US, a country which has a fortification programme in place. This deficiency rate was apparent in all age groups including women aged 25-34y (17.7%) and 35-49y (13.1%) which is a considerable proportion of women of childbearing age. Women from Northern Ireland (NI) had the highest proportion of women who had serum total folate (30.6%) and red blood cell folate (20.2) concentrations below the WHO thresholds in comparison to the UK as a whole and by UK country (Butriss, 2015).

1.3.2 Folic acid as a pregnancy supplement

Conclusive evidence has long existed that folic acid supplementation in early pregnancy prevents Neural Tube Defects (NTD) (Czeizel & Dudas, 1992; MRC, 1991). The relationship between other physical health issues such as low infant birth weight and preeclampsia (Vollsett *et al.*, 2000) and orofacial clefts (Wilcox *et al.*, 2007) has also been subject to investigation but remains inconsistent. The link between folate and adverse pregnancy outcomes could be explained, in part, by homocysteine. Homocysteine (tHcy) is an amino acid which plays a key role in the metabolic processes closely linked to folate such as methylation. Its presence

indicates an imbalance in the one-carbon metabolism and levels increase in instances of folate and vitamin B_{12} deficiency.

Folic acid is required by the body to help metabolize tHcy. Elevated levels of which have been associated with a number of pregnancy complications affecting both maternal and fetal health e.g. NTD's (Mills *et al*., 1995), preeclampsia (Cotter *et al*., 2001), intrauterine growth retardation (Lindblad *et al.*, 2005), placental abruption (Owen *et al*., 1997), preterm delivery (Ronnenberg, *et al*., 2002), low birth weight (Murphy *et al*., 2004), congenital heart defects (Wenstrom *et al*., 2001) and Downs syndrome (James *et al.*, 1999). Moreover, high levels of tHcy in conjunction with low folate status has been associated with recurrent miscarriage (e.g. Nelen *et al*., 2000; George *et al.*, 2002). Supplementing with folic acid, particularly in early and late pregnancy when tHcy levels naturally rise, could protect against a number of difficulties experienced during pregnancy.

Aside from the physical benefits for the child, several lines of research suggest that maternal folate intake could impact cognition and neurological development. Early animal research found that the offspring of folate deficient rats have an inferior maze learning ability and that a restriction in perinatal folic acid resulted in a reduction in brain weight and level of activity (Whitley *et al.*, 1951). Furthermore, deficiency during early gestation in mice reduced cell replication and increased apoptotic cells in the fetal forebrain indicating a negative impact on neurodevelopment in later life (Craciunescu *et al.*, 2004). The findings gained from human participation yielded similar results. Gross *et al*. (1974) identified abnormal child development and lower intellectual abilities in infants of folate deficient mothers and paved the way for

research to shift in focus to the potential effects of maternal folic acid use on other areas of health and development aside from the physical wellness of mother and child.

The evidence is expanding rapidly in relation to folic acid and children's cognitive and neurological development, and the prevention of neurodevelopmental disorders such as Autistic Spectrum Disorder (ASD) (Gao *et al.*, 2016). Research has shown that optimal folate use can improve many aspects of children's cognition and executive function for example, IQ, attention, memory (e.g. Villamor *et al.*, 2012; Veena *et al.*, 2010; Murphy *et al.*, 2007; Catena *et al.*, 2015) and more general neurodevelopment typically measured in younger children (e.g. Bhate *et al.*, 2012; Chatzi *et al.*, 2012; Julvez *et al.*, 2009; del Rio Garcia *et al.*, 2009; Li *et al*, 2009; Gross *et al.*, 1974).

An important feature of child cognition is language acquisition and development. Although limited, the evidence suggests that maternal folic acid use during pregnancy can have a positive impact on children's communication skills and vocabulary development. In most cases these were measured as sub-factors within a cognitive assessment, typically in young populations (e.g. Chatzi *et al.*, 2012; Villamor *et al.*, 2012; Julvez *et al.*, 2009). In another study conducted by Roth *et al.* (2011) results showed that adequate folic acid use could prevent language delay, however, extreme overuse of the vitamin or use in conjunction with other prescription medications such as SSRI's can hinder this development (Valera-Gran *et al*, 2017; Handel *et al.*, 2016).

Observational research investigating the association between maternal folate status and children's cognitive and neurological development continues. A study by Ars *et al.*, (2019) found that insufficient maternal folate in early pregnancy had a long-lasting global effect on brain development and in conjunction with tHcy it is associated with poorer cognitive outcomes. Additionally, a recent review concluded that routine maternal folic acid use significantly lowered instances of ASD (Iglesias *et al*., 2019). Findings are also addressing the possibility of the outcome being dose dependent. A large population-based study found that lower and higher than recommended doses negatively impacted children's attentional function at 4-5 years, particularly in males (Compan Gabucio *et al.*, 2021). Moreover, these findings are not limited by geographical location, Wang *et al.*, (2021) found that folate and tHcy was associated with the intellectual development of children under one year in rural China.

The evidence to date however has tended to focus on the negative impact of micronutrient deficiency during pregnancy on mother and child outcomes. This has been a useful approach for medicine to prevent ill-health but provides an unbalanced view, which has informed the current evidence-based recommendations for supplement use during pregnancy and the associated RDA's. In addition to this, researchers, health practitioners and the population need to know if sufficient maternal micronutrient use during pregnancy can improve children's brain and neurocognitive development, and if the vitamins and minerals required by the body during pregnancy, in particular folic acid, could be used to promote the health and wellbeing of mothers and their children.

This new direction for research has already had some investigation, offering support to the findings informed by the deficit model and extending the literature. A trial by Caffery *et al.* (2018) found that continued folic acid use could benefit children's neurocognitive development at 11 years. This research also identified DNA methylation as a potential biological mechanism linking maternal folate status and children's neurocognitive development (Irwin *et al.*, 2018; Caffery *et al.*, 2018). Folate is indirectly involved in DNA methylation through one-carbon metabolism, which in turn, is considered to be one of the epigenetic mechanisms controlling gene expression. Preliminary findings indicated that continued folic acid use throughout pregnancy could significantly affect DNA methylation of specific genes related to brain function in children (Caffery *et al.*, 2016). These pieces of research contribute to a series of studies conducted at Ulster University known as the Folic Acid Supplementation during the Second and Third Trimesters of pregnancy trial (FASSTT study; ISRCTN19917787).

1.4 History of FASSTT at Ulster

To date the research examining the impact of maternal folic acid use and subsequent cognitive development of the child has been limited by study design, relying heavily on observational studies. To address this, researchers at Ulster University implemented a Randomised Control Trial (RCT) to identify a causal link between maternal folic acid use during pregnancy and birth outcomes, and later child cognitive development.

1.4.1 FASSTT @ Birth

From September 2005 to December 2006 a Northern Ireland based Randomised Control Trial (RCT) investigated maternal folate and homocysteine responses and related effects in the newborn that resulted from continued folic acid use after the first trimester of pregnancy (McNulty *et al.*, 2013). A total of 119 women who reported taking folic acid during the first 12 weeks of pregnancy were recruited from an antenatal clinic and randomly assigned at the beginning of trimester 2 at the 14th GW (Gestational Week) to receive a daily supplement containing 400µg of folic acid (n=60) or a placebo capsule (n=59). Results showed that continuing supplementation during the second and third trimesters (>12GW) increased maternal and cord blood folate status and prevented the increase in homocysteine concentration observed in the women who were unsupplemented from 12GW. Additional investigations were recommended to confirm whether these effects have benefits for pregnancy outcomes and early childhood (Henry *et al.*, 2018; McNulty *et al.*, 2019)

1.4.2 FASSTT @ 3y

Three years later a follow-up study was conducted (McNulty *et al.*, 2019) on a sub-set of the original FASSTT study participants (FASSTT@3y). At the time the FASSTT@3y study was the first to explore the potential effect of maternal folate status on the child's cognitive, language and motor development, measured using the Bayley's Scales of Infant and Toddler Development 3rd Edition (BSID-III). A total of 39 mother-child pairs participated and results showed that the children born to mothers who continued supplementing with folic acid until the end of pregnancy (experimental group) scored significantly higher in the cognitive domain and in receptive communication compared to the children whose mothers stopped

supplementing at 12GW (control group), in line with the current guidelines and recommendations. No significant differences were observed between groups in children's weight, height, waist circumference, head circumference, BMI and body fat. This study suggested that folic acid use after 12GW could enhance the child's cognitive ability and warranted further investigation (McNulty *et al.*, 2019; Henry *et al.*, 2018; Caffrey *et al.*, 2019).

1.4.3 FASSTT @ 7y

A comprehensive follow-up was conducted when children were 7 years old (FASSTT@7y) to examine the potential cognitive benefits for children of maternal folic acid use beyond the first trimester (McNulty *et al.*, 2019). A total of 74 mother-child pairs participated and results showed that children in the experimental group scored significantly higher than the control group in the word reasoning subtest of the Wechsler Preschool and Primary Scale of Intelligence test 3rd UK Edition (WPPSI-III), no other significant differences were observed. However, when these findings were compared against the UK mean scores for 7-year old children, Verbal IQ and Full-Scale IQ scores were found to be significantly higher than the UK mean in the experimental group but not in the control group. The relationship between the WPPSI scores of the total cohort and maternal folate status at 36GW was examined further and maternal red blood cell (RBC) folate was found to be a significant predictor of Verbal IQ in the FASSTT sample. No significant differences were observed between groups in children's growth parameters (weight, height, waist circumference, head circumference, Body Mass Index (BMI) and body fat).

These differences observed in children's neurocognitive development and the surrounding literature raised questions as to how maternal folic acid use during pregnancy could affect other areas of development, namely children's psychological and emotional development. A sub-sample of 39 mothers completed a range of psychological measures on behalf of their child to assess behaviour, Emotional Intelligence (EI) and resilience. Findings from this pilot study showed that children tended to score higher on emotional intelligence and resilience when mothers had supplemented throughout the pregnancy in comparison to the placebo group. Further analysis identified folate level at 36GW as an important predictor of these scores indicating that continued folic acid use could enhance psychological development of 7-year old children (Henry *et al.*, 2018).

1.5 Rationale for this PhD

It is widely accepted that childhood development has significant impacts for subsequent adult health and wellbeing (Children's Research Network, 2017). According to the Developmental Origins of Health and Disease (DOHaD) theory, early life experiences, including those occurring during pregnancy can affect long-term health (Gillman *et al*., 2005). DOHaD research has typically focused on the health and lifestyle of mothers around the time of pregnancy including maternal exposures, and how these factors might impact childhood and later health and development. Consequently, identifying the factors beneficial to child development is a pertinent issue. Rapid and critical development occurs in utero and during the early years of life (National Scientific Council on the Developing Child, 2008) and as already reviewed, positive health behaviours such as good nutrition and supplement use; including folic acid, adopted by the mother antenatally can

dramatically improve the child's health and wellbeing, both in the interim and long-term (Ars *et al.*, 2019; Compan Gabucio *et al.*, 2021; Caffery *et al.*, 2018; Henry *et al.*, 2018; Phelan, 2010).

The concentration on the impact of folic acid on physical aspects of child development and the application of the deficit model has been useful for policy makers to ensure the best possible physical outcome for the child and has provided convincing evidence to support the use of folic acid for preventing ill-health. However, the evidence investigating the impact of maternal folic acid use on children's psychological, emotional, behavioural and social development is equivocal and inadequate to allow for any accurate conclusions to be drawn. This is due in part to limitations arising from research design with a notable lack of RCT's, instead relying on retrospective data from a small number of non-randomised or observational studies which by design are more susceptible to bias and are of lower quality than an RCT (Page *et al*., 2019).

In addition to the methodological limitations, there is a scarcity of evidence outside of FASSTT to explore the impact of maternal folic acid use beyond the recommended first trimester, and indeed comparing the benefit of maternal folic acid use in the first trimester only to continued use during trimesters two and three. Given the increase in mothers using pregnancy multivitamins (MV) containing folic acid after 12GW it is important to understand the benefits and risks to mother and child of doing so.

Another consideration is that psychological research, like biomedical research, typically implements a deficit model in an attempt to explain developmental problems or difficulties (e.g. Seligman & Csikszentmihalyi, 2000), as highlighted in this chapter. The use of folic acid for prevention and treatment of illness must now be balanced with benefits of supplement use for developmental improvement and facilitation of protective processes. Using an evidence-based approach informed by Masten (2001) and others (e.g. Seligman & Csikszentmihalyi, 2000; Cassidy *et al.*, 2013; Henry *et al.*, 2018) this PhD will employ a positive, resource-focused approach (Cassidy *et al.*, 2013), concentrating on the potential benefits of maternal folic acid use on children's developmental outcome.

1.5.1 A positive psychology approach

The positive psychology movement was pioneered in 1998 by Dr. Martin Seligman, the then American Psychological Association (APA) President, with a mission to unify practice and science so both can 'flourish' (Seligman & Csikszentmihalyi, 2000). This emphasis on flourishing was a somewhat novel approach in psychology and focused on building competency using psychological constructs such as creativity, optimism and happiness for example, to enhance life rather than repair damage. This would then 'make life worth living' by helping individuals, families and communities to thrive. However, it was not a new concept, and although driven by Seligman the roots of positive psychology can be seen in humanistic psychology (Froh, 2004) with views dating back to the work of William James. James (1906) argued that to comprehensively study human functioning the subjective experience of the individual must be considered. A revolutionary perspective in the time of psychoanalysts and behaviourists.

Positive psychology examines how individuals can prosper even when faced with adversity (Seligman & Csikszentmihalyi, 2000) through positive experiences (e.g. happiness), positive traits (e.g. gratitude, resilience, compassion and emotional intelligence) and positive institutions through their application of positive principles. Each will help nurture and develop an individual's character strengths, self-esteem, self-confidence, optimism, hope and wellbeing which in turn will buffer against any adversity they experience (Folkman & Moskowitz, 2000).

1.5.2 Wellbeing and its individual components

The importance of positive wellbeing for promoting mental and physical health and wellness is irrefutable. There are many descriptions of wellbeing available containing a number of components including mental, psychological, social and emotional wellbeing. Each has been recognised as fundamental for healthy growth and development by the World Health Organisation (WHO) who adopted a positive perspective in 1946, defining health as "a state of complete physical, mental and social wellbeing and not merely the absence of infirmity" (WHO, 1946, p. 1). A more recent definition states that mental health is "a state of wellbeing in which the individual realises their own abilities, can cope with the normal stresses of life, can work productively and fruitfully, and is able to make a contribution to their community" (WHO, 2002a, p. 1)

Subjective wellbeing is one aspect of psychological functioning referring to what people think and how they feel about their lives and the cognitive and affective conclusions they reach when they evaluate their existence (Diener *et al*., 2002). These evaluations include emotional responses to events, their moods, and

judgements they form about their life satisfaction, fulfilment and satisfaction with domains such as marriage and work (Diener *et al*., 2002). Subjective wellbeing is therefore the scientific study of what some might describe as happiness or life satisfaction. As this is typically an assessment of their feelings and emotions this thesis will therefore use the term emotional wellbeing (EWB), in line with current terminology (e.g. Robitschek & Keyes, 2009). This concept has been subject to extensive cross-cultural research (e.g. Diener, 2000) and is associated with pleasant emotions and moods, lower levels of negative emotions and moods alongside high life satisfaction, centring around the hedonic aspect of wellbeing (Chen *et al*., 2012).

Psychological wellbeing (PWB) on the other hand, is another perspective focusing on eudemonic wellbeing. This refers to a life of value and fulfilment which involves having a sense of purpose and pursing meaningful goals, having control over one's life, growing and developing as a person, experiencing positive relationships and thriving despite life's challenges (Huppert, 2009; Ryff & Singer 2008). This combination of positive functioning will help foster positive emotions highlighting the inter-related nature of EWB and PWB, however both are distinct constructs (Cicognani *et al*., 2014).

Evidence suggests that personality is a strong and consistent predictor of both EWB and PWB. Extroversion and neuroticism have received the most theoretical and empirical attention due to the correlations between EWB, PWB and extroversion, and neuroticism and negative affect (Diener *et al.*, 2002; Kokko *et al*., 2013). However, research indicates that focusing only on extroversion and neuroticism oversimplifies the complex relationship between personality and wellbeing (e.g.

DeNeve & Cooper, 1998). The evidence has since expanded to include all personality traits from The Big Five (Extraversion, Neuroticism, Conscientiousness, Agreeableness and Openness) due to its relatively high stability in adults, children and adolescents (e.g. Wortman *et al*., 2012; Shiner & Caspi, 2003; Markey *et al*., 2004). Kokko *et al.*(2013) found correlations between PWB and conscientiousness, openness to new experiences and agreeableness and concluded that personality traits were more strongly linked to PWB than EWB.

Other narrow personality traits or character strengths such as optimism (expecting that the future will provide the individual more good than bad), locus of control (the individuals belief that they control their own life) and self-esteem (how much the individual values themselves, their self-worth and their capabilities) have also been shown to significantly correlate with wellbeing (e.g. Diener & Lucas 1999). Social support and an active social life have also been associated with EWB (e.g. Chen *et al.*, 2013) and PWB (Diener & Oishi, 2005) but it remains unclear if they uniquely predict EWB or PWB (Cicognani *et al.*, 2014) after controlling for Extraversion and Neuroticism. Intervention studies have shown both PWB and SWB can be increased by fostering character strengths (e.g. Quinlan *et al*., 2012) and that some character strengths are more beneficial for wellbeing in general than others (Park *et al*., 2004).

Intricately related to both EWB and PWB is social wellbeing which expands on both of these models of wellbeing. Social wellbeing (SWB) has been defined as “a subjective evaluation of personal life circumstances and functioning in society” (Keyes, 1998 p. 122) and includes developing and maintaining positive relationships with family, peers and others in both local and global communities (Cicognani *et al.*,

2014).The positive psychology approach has contributed to the development of SWB through the study of prosocial emotions and behaviours which have both been shown to have a positive impact on SWB and consequently EWB and PWB.

1.5.3 Positive Psychology and wellbeing in the context of this PhD

Positive psychology in the context of this thesis is then to shift the focus from preventing child ill-health, to considering and testing the potential developmental benefits available for mother and child through good maternal health, nutrition and most importantly folic acid supplementation. Driven by evidence, a resource-focused approach (Cassidy *et al.*, 2013) will be applied to investigate the potential benefits of maternal folic acid use on children's psychological, emotional and social wellbeing. The literature asserts that a positive state of wellbeing can significantly improve mental and physical health in the short term and have longer lasting effects in both children and adults (e.g. Lamers *et al*., 2011; Howell *et al.*, 2007; Chida & Steptoe, 2008; Public Health England, 2013; Smedegaard *et al*., 2016). As previously discussed, positive wellbeing in general improves an individual's social connections which is influenced by emotions (e.g. emotional self-regulation and understanding the emotions of others) and in turn develops a host of psychological factors (e.g. autonomy and resilience). If optimal development in these three areas occurs, then the risk of internalising and externalising behaviours decreases, which further improves wellbeing. Conversely, if psychological, social and emotional development is suboptimal then the risk of problem behaviours increase which can have a negative impact on wellbeing and subsequent mental and physical health. This thesis will therefore consider each of these aspects of wellbeing and how they develop throughout childhood.

1.5.4 FASSTT@10y – the context of this PhD

The FASSTT@7y intervention found significant differences in the cognitive ability or IQ of children whose mothers continued supplementing throughout pregnancy and those who stopped supplementing at 12GW. This study raised questions about whether the benefits of maternal folic acid use could extend to other aspects of psychological development in children. Research has demonstrated that IQ alone does not determine life success or satisfaction (e.g. Bhoumick, 2018), only contributing to around 20% of the associated factors. Success or an individual's perception of personal success is directly related to the levels of satisfaction and happiness they experience (Lyubomirsky *et al*., 2005) which can have major implications on wellbeing. It is generally recognised that cognitive development goes hand in hand with psychological and socioemotional development in children and that both are built into brain development (National Scientific Council on the Developing Child, 2004). We can then speculate that the other factors accounting for the remaining 80% encompasses a range of other psychological, social and emotional factors, including but not limited to emotional intelligence, resilience and social quality with peers, or peer attachment. According to Bhoumick (2018), these factors would therefore have a more positive influence than IQ on an individual's level of success or perceived success impacting directly on their wellbeing.

One of the empirical studies in this thesis follows-up FASSTT participants now the children are approximately 10 years old (FASST@10y). Unlike in the previous FASSTT@7y study, where proxy measures were used and the mothers completed the psychological measures on behalf of their children, the children are now old enough to complete the measures themselves. Adopting a positive resource-focused

approach the children's emotional intelligence, resilience, behaviour and attachment will also now be evaluated. In addition to these self-reported measures, mothers will complete a questionnaire inquiring about their own health and lifestyle, as well as their parenting style. The aim of this phase of the study is to investigate if the children now aged 10y, experience any psychological, social, emotional or behavioural benefits if mothers supplemented with folic acid until the end of pregnancy rather than stopping at the current recommendation of 12GW and examine any mediating effects of parenting style and attachment.

Masten (2001) recognised that the greatest threat to child development was when the systems underlying adaptive processes were compromised which included brain development and cognition but also behaviour and emotion regulation, and family and peer relationships. These factors therefore will be used as measures of psychological, social and emotional development in this thesis. Internalising and externalising behaviours are a good indication as to how established each of these factors are, therefore, behaviour strengths (prosocial) and difficulties (internalising and externalising behaviour problems) will also be assessed. A brief review of each of these factors will now be provided.

1.5.5 What is Emotional Intelligence?

Emotional Intelligence (EI) has been identified as a construct that could improve all aspects of wellbeing (Di Fabio & Kenny, 2016) and complementary to IQ or cognitive ability (Kabir *et al*., 2021). Research has identified emotion as an individual's ability to regulate their own emotions and understand the emotions of others as a significant moderator of social and psychological development in

children. These are all important components of EI which has been defined as “the ability to perceive accurately, appraise and express emotions; the ability to access and or generate feelings when they facilitate thought; the ability to understand emotion and emotional knowledge; and the ability to regulate emotions to promote emotional and intellectual growth” (Mayer & Salovey, 1997 p. 10).

Since its theoretical inception in 1990 (Salovey & Mayer, 1990) there has been a growing body of evidence demonstrating how EI significantly predicts adaptive interpersonal and psychological development (Mayer *et al*., 2008). EI can be viewed as either an ‘ability’ similar to IQ which involves the cognitive processing of emotional information (Mayer *et al*., 2016) or an ‘enduring trait’ relating to a constellation of emotional self-perceptions positioned in the lower levels of personality hierarchies which integrate personality and affect (Petrides *et al*., 2016; Petrides *et al*., 2007). Despite the conceptual differences, both have substantial empirical support for predicting real-life outcomes (Extremera *et al*., 2020; Mayer *et al*., 2016; Petrides *et al*., 2016) and TEI in particular has often associated with a positive sense of wellbeing (Sanchez-Ruiz *et al.*, 2021; Salavera & Usan, 2020; Lekshmi *et al*., 2018; Sanchez-Alvarez *et al.*, 2016; Por *et al.*, 2011; Bhullar *et al.*, 2012). For example, a recent review by Petrides *et al.* (2016) found TEI to be a significant predictor of positive life outcomes. There is also further evidence linking TEI with happiness (Furnham & Petrides, 2003), life satisfaction (Austin *et al.*, 2005; Liu *et al.*, 2013), character strengths (Ros-Morente *et al.*, 2018) and prosocial behaviour (Mavroveli & Sanchez-Ruiz, 2011). Due to the extensive research available in relation to personality characteristics and psychological, emotional and social wellbeing this thesis will consider EI as an enduring trait or Trait EI (TEI).

There has been little research into the effects of maternal micronutrients during pregnancy and children's TEI. However, there is some evidence to suggest a relationship exists between maternal micronutrients and children's emotional development. A recent study found an inverse relationship between children's emotional symptoms and selenium zinc levels in cord blood (Gari *et al.*, 2022). This would suggest that a link exists between maternal nutrition during pregnancy and children's emotional development. Furthermore, Miyake *et al*. (2020) investigated the relationship between maternal B vitamins during pregnancy and children's behavioural outcomes and found that vitamin B_2 was inversely associated with emotional difficulties. This study provides further support for a potential link between maternal folic acid use (vitamin B_9) and children's emotional development and thus warrants further investigation.

1.5.6 What is Resilience?

Resilience is a complex concept and one aspect of psychological functioning which facilitates development which can have a positive impact on wellbeing alongside mental and physical health, enduring from childhood throughout life (e.g. van IJzendoorn *et al.*, 2011; Walsh *et al.*, 2010; Windle *et al.*, 2011). The ability to 'bounce back' from challenges (Bernard, 1993) or rise above adversity (Wolin & Wolin, 1993) is considered by many as resilience, and is represented by the interaction between risk factors and protective resources. It was defined by Masten (2011) as the "capacity of a dynamic system to withstand or recover from significant challenges that threaten its stability, viability or development" (p. 494) in a flexible and adaptive way. This means that recovery from negative emotional experiences is

possible despite the varying demands of subjective stressors (Block & Kremen, 1996).

The evidence in relation to resilience typically focuses on those who are currently or were previously at risk and the effect on their health and wellbeing. This includes children in care (e.g. Lou *et al*., 2018), those exposed to intimate partner violence (e.g. Fogarty *et al*., 2019; Callaghan *et al*., 2018), war (e.g. Bosqui & Marshoud, 2018) and more recently the COVID-19 pandemic (Tso *et al.*, 2020). This tendency to focus on children who are mainly at risk has led to a shortfall of investigations into children's resilience in general. There has however been some investigation into role of positive social relationships such as parents, educators and peers on the development of resilience and wellbeing in young people (e.g. Noble & McGrath, 2012). Strong relationships have been identified and led to the development of resilience models to be implemented in schools as an early preventative measure for later mental health difficulties, usually manifested in children as social, emotional and behavioural problems in school (e.g. Cefai *et al.*, 2014; Nicoll, 2014; Crenna-Jennings *et al*., 2021)

The benefits an individual can experience through being resilient is undisputed however there is much inconsistency in how it is operationally defined, resulting in measurement difficulties. Similarly to EI, resilience has been conceptualised as a constellation of individual resources, social conditions and the subjective stress stunting personal growth (Staudinger & Greve, 2015), and considered a personality trait that promotes adaptation by effectively moderating stress (Wagnild & Young, 1993). In addition, there is evidence to suggest that personal experiences can shape

personality traits, and that traits can influence exposure to adversity and function as either a vulnerability or protective influence (e.g. Shiner & Masten, 2012; Masten, 2014). Many factors can contribute to resilience incorporating social competence, problem-solving, autonomy and sense of purpose through character strengths such as optimism, self-esteem and cognitive flexibility (Bernard, 1993). Biological factors such as immunity (Masten & Barnes, 2018) and other external factors including family and peer relationship quality and belongingness (Cicognani *et al.*, 2014) can also promote resilience. For the purposes of this thesis resilience is also considered a personality trait, in the same vein as TEI.

1.5.7 What is Attachment

Historically attachment theory has focused on the parent-child attachment (e.g. Bowlby, 1969) and how this can influence an individual's experience in childhood and beyond. These first attachment relationships are established early with parents but as the child grows and develops enduring attachment forms with others outside of the child's family. Sroufe (2005) postulated that secure attachment is the most important capability that children can acquire in their first two years. Developing this style of attachment provides a context in which children feel safe and supported to explore their world, regulate their emotions and interact with others (Bowlby, 1969). Anxious or avoidant attachment styles on the other hand increase the risk of behavioural problems (Elicker *et al*., 1992; Greenberg *et al*., 1993) and impaired social relationships (Bost *et al*., 1998; Park & Waters, 1989).

However, achieving this attachment style is dependent upon a number of influential maternal and child factors such as maternal physical and mental health (e.g. Sroufe,

2005; Tomlinson *et al*., 2005; Martins & Gaffan, 2000), environment (e.g. Allen *et al*., 2004; Diener *et al*., 2003), maternal antenatal and postnatal nutrition (e.g. Frith *et al*., 2012; Wachs, 2009; Black & Ramakrishnan, 2009), mothers own attachment style with parents (e.g. Alhusen *et al*., 2013; Antonucci *et al*., 2004) and child temperament (e.g. Wachs *et al*., 2005). Research has shown that children's cognitive, social, emotional and behavioural development is built on high quality mother-child interactions (Alhusen *et al.*, 2013; Richter, 2004) and secure attachment in the early years (e.g. Murphy & Liable, 2013; Coyl *et al*., 2002; Tomlinson *et al.*, 2005). A lack of maternal nurturing and protective behaviours towards their children have been shown to impair these areas of development (Ainsworth, 1979; Bowlby, 1969, Bowlby, 1982) however, it is recognised that the quality of these parent-child interactions is bidirectional and is reflective of how each respond to the other (Wachs, 2009). Factors such as child's temperament in infancy and later emotional regulation and intelligence have been shown to have a significant impact on interaction quality and attachment style (e.g. Wachs *et al.*, 2005) with securely attached children tending to become more resilient and competent adults (Hong & Park, 2012) .

Adolescence is a key period where peer relationships begin to resemble adult attachment bonds (Gorrese & Ruggieri, 2012), with peers being influential sources of social and emotional support (e.g. Liable, 2007; Wilkinson, 2010) and have the capacity to determine an individual's trajectory for adjustment in later life. It is recognised that the newly forming peer relationships are closely interlinked with the early parent-child attachment bonds and the quality of these first bonds with parents can have serious implications for the quality of later attachments to peers (e.g.

Sroufe, 2021; Wilkinson, 2004; Liable, 2007) with the latter being improved by early feelings of security. Recent research has shown that early attachment security was strongly associated with peer interactions due to better social competence, and fewer internalising and externalising problems, outcomes often manifested in peer contexts (Groh *et al.*, 2017). Additionally, positive friendships have been shown to enhance psychological wellbeing and buffer against psychosocial stress with best friend relationships most important to psychological and social functioning in teenagers aged 13-19 years (Wilkinson, 2010). Secure peer attachment has also been shown to be significantly correlated to self-esteem (Gorrese & Ruggieri, 2012) whereas insecure peer attachment was associated with internalising problems, anxiety and depression (Gorrese, 2015).

1.5.8 Parenting style

Closely related to attachment is the parenting style construct. Like attachment, parenting style is a bidirectional, reciprocal process that varies greatly between families and societies (Sanvictores & Mendez, 2022). The style which parents adopt helps establish children's conduct, morals and principals (Sanvictores & Mendez, 2022) shaping later cognitive, social, emotional, psychological and behavioural development as they age (e.g. Joseph & John, 2008; Bornstein & Bornstein, 2007; Querido *et al*., 2002). The number and types of parenting styles vary across the literature but generally they fit into three categories: authoritative, authoritarian and permissive/ uninvolved parenting. Parents are usually categorised by one parenting style but at times display characteristics of another. Some reasoning has also indicated that style can be dependent on the situation (Sanvictores & Mendez, 2022)

Authoritative parenting is characterised by a close, warm and nurturing relationship between parent and child and is recognised as the ideal style for optimal child development (e.g. Baumrind, 1967; 1991). Parents tend to support their children whilst adhering to boundaries and there is frequent and effective communication. Children whose parents adopt this style of parenting grow in confidence and independence, increasing their self-esteem (e.g. Hong & Park, 2012) They are more able to self-regulate and manage their emotions, particularly negative emotions, which fosters better social development and emotional health (e.g. Sanvictores & Mendez, 2022). Research has shown that children of warm and responsive parents are more emotionally secure than those who are unsupported (Cooper *et al.*, 2009; Isabella, 1993; Valenzuela, 1990).

Other parenting styles include authoritarian and permissive/ uninvolved parenting. Authoritarian parents are less nurturing and inflexible with high expectations. They tend to impose strict rules and expect obedience with discipline and punishment used. Children of these parents tend to be shy with difficulty interacting socially and indecisive (Bornstein & Zoltnik, 2008). They can become aggressive as they are unable to effectively regulate emotions with low self-esteem (Sroufe, 2005; Rankin Williams *et al.*, 2009) and display feelings of inadequacy (Hong & Park, 2012). As they grow older, they are most likely to rebel against those they consider as authority (Rankin Williams *et al.*, 2009). Permissive or uninvolved parents tend to be warm and nurturing but do not provide the child with a supportive environment behaving more like a friend. These parents do not impose rules nor do they expect much in return from their children. This results in excessive freedom and a lack of parental guidance and although children appear to be confident and social, they can be

impulsive, demanding and unable to regulate effectively (e.g. Sroufe, 2005; Rankin Williams *et al.*, 2009).

1.5.9 Joining the dots

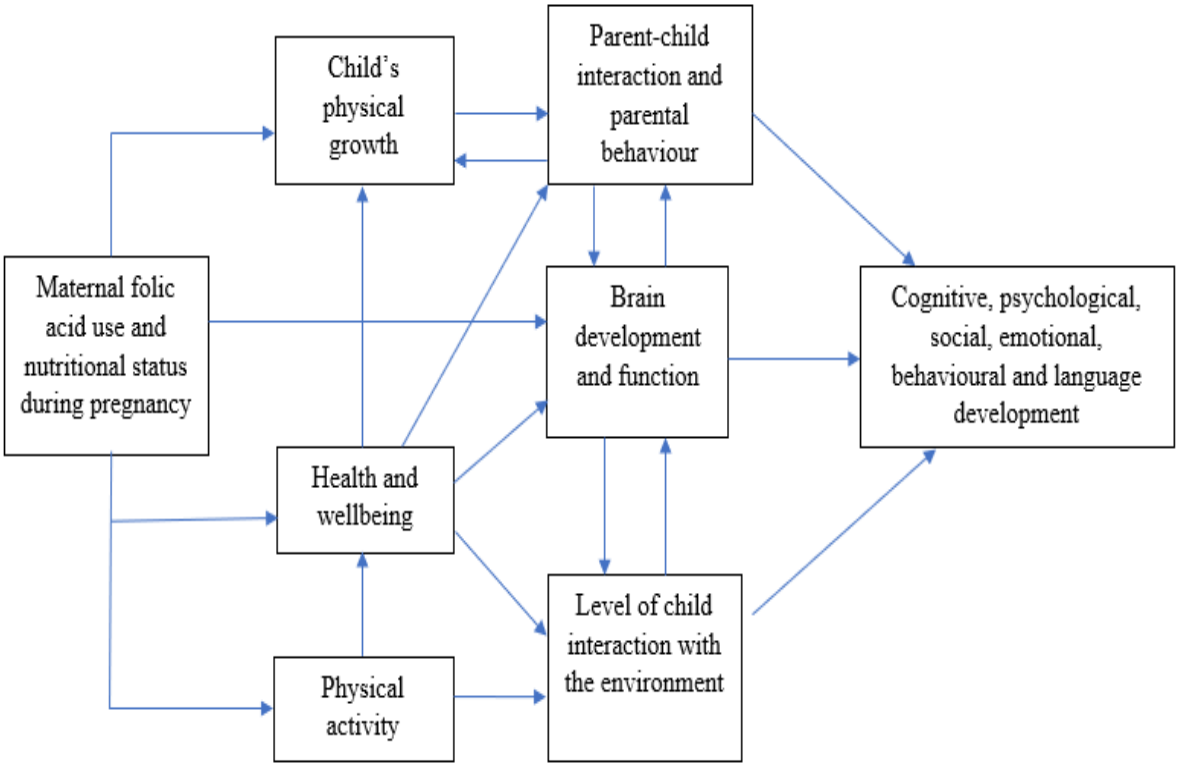
In an attempt to promote positive wellbeing, the literature has identified a number of developmental areas, namely psychological, social and emotional functioning that can help buffer both children and adults against ill-health. The links between personality and emotional and psychological wellbeing or development are already well established. As discussed, EI and resilience are traits that can directly impact on children's immediate and long-term wellbeing and protect them from internalising and externalising behaviours (Erikson *et al*., 2011) to grow their social identity (Diener & Oishi, 2005; Folkman & Moskowitz, 2000) through positive interactions and attachments. However, the question considering the effect of maternal folic acid supplement use on these areas of children's development remains. This review of the literature demonstrates that folic acid can improve children's physical development, cognitive and neurodevelopment from birth into adolescence.

Research included in this chapter highlighted that IQ only accounts for a small amount of children's brain development and therefore other factors including personality characteristics such as EI and resilience need to be examined. Theoretically these outcomes have been linked to attachment and behaviour (e.g. Filaire *et al*., 2011), therefore all four factors need careful consideration. It is also important to recognise the potential for mediating effects of children's attachment along with the mothers parenting style and how this impacts on the relationship

between maternal folic acid use and children's psychological, social and emotional development (See *Figure 1.1*).

The literature currently being used to develop guidance and recommendations for supplement use, support during pregnancy and fortification plans in the UK is not providing a complete picture. The application of the deficit model in research has resulted in a negative focus for existing reviews. However, for policy makers and health care personnel to make informed decisions there must be a balance in reporting not only the risks but also the benefits associated with taking folic acid. The shortfall in positive and/or resource-focused research has resulted in a notable lack of high-quality systematic reviews to explore the beneficial effects of supplementing. Conducting an acceptable review may not have been plausible due to the small number of available studies which are typically from the same cohorts of mother-child pairs. Nevertheless, this could have a detrimental effect on the official guidance being received by antenatal mothers in relation to their own health and their children's development. The evidence base is also lacking in RCT's specifically designed to examine the effect of individual micronutrients consumed by the mother during pregnancy on children's developmental outcomes. The FASSTT@10y trial has the capability to thoroughly investigate the relationship between continued maternal folic acid use on children's psychological, social, emotional and behavioural development. To scaffold the results for FASSTT@10y a systematic review and secondary data analysis of a large independent study is also warranted to provide context at a national level and explore any observations outside of the constraints of a RCT design.

Figure 1.1: Potential interaction effects between maternal folate status and child development investigated by this thesis.



1.6 Thesis Aims

In consideration of the aforementioned shortcomings and findings of previous research, the overarching aim of this PhD is to examine the role of maternal folate status during pregnancy on children's psychological, behavioural, social and emotional development. Each stage of the project will adhere to the positive, resource-focused approach and centers only on the beneficial developmental effects experienced by the children.

To achieve the research aim, a number of research objectives guided the study.

- To produce a high-quality systematic literature review to ascertain if maternal folic acid supplementation use during pregnancy impacts on the child's

cognitive, psychomotor, psychological, social, emotional, behavioural or language development during their first 12 years of life.

- To conduct a secondary data analysis in a large population-based cohort and provide a national context for folic acid use in the UK and examine children's cognitive, motor, social, emotional, behavioural and language development in relation to maternal folic acid use in late pregnancy.
- Use the FASSTT@10y RCT to examine the impact of maternal folic acid supplementation during trimesters two and three in comparison to supplement use during trimester one only on the children's psychological, behavioural, social and emotional development.

1.7 Thesis Methodology

A multi-method research design was applied to achieve the aims and objectives and address the issues outlined in the rationale. The first piece of research is a systematic review to identify all relevant research currently available in this area and assess the quality and risk of bias of each included article. The articles of interest had to meet strict inclusion criteria and were experimental or observational by design and with at least two participant groups to enable comparisons. Children's cognitive, psychological, social, emotional, behavioural and language development was measured throughout their first 12 years at various timepoints, and all included articles were subject to rigorous risk of bias and quality assessment, analysis and review processes.

The second research piece is a secondary data analysis using the Avon Longitudinal Study of Parents and Children (ALSPAC). This large, UK based population study is

ongoing and contains extensive clinical and psychological data on mother-child pairs from early pregnancy. This study will investigate if sufficient maternal folic acid use (400µg/d) during late pregnancy (32GW) is associated with any additional benefits to children's cognitive, motor, social, emotional, behavioural and language development in comparison to mothers who were considered folate deficient at 32GW. This analysis of existing data provides an opportunity to fully explore the impact of folic acid use in late pregnancy on many areas of children's development, which is often not feasible in primary research (Wickham, 2019). A large and diverse sample is captured without any challenges frequently associated with data collection such as recruitment, attrition or sample size. This approach is an important first step, providing context before testing for causation in an RCT.

The concluding study reports the follow-up of the FASSTT@10y study participants. Ten years after their initial recruitment, mother and child participants completed a series of psychological tests to measure the child's emotional intelligence (TEI), resilience (TPR) and behaviour. Attachment and the mothers parenting style were also tested as potential mediators. Proxy measures were no longer required unlike previous FASSTT studies as children were now old enough to reliably complete the measures on their own. This study examined the benefits of supplementing past the recommended 12GW and compared the children whose mothers supplemented >12GW to those who stopped at 12GW.

Integrating these research methods will increase scientific rigor and encourage the highest level of accuracy as each design is specific to the needs of each individual study.

1.8 Significance of the research

This thesis addresses a number of significant research gaps. Systematic reviews examining maternal folic acid use and associated beneficial effects for children are scarce as the literature tends to focus on non-use and the potential negative effects on children. There is growing interest in this new line of positive research, therefore a high-quality systematic review is imperative. The systematic review conducted as part of this PhD, to the author's knowledge will be the first examining the content and quality of the literature available in this area of interest and therefore this piece of research will address a significant gap in the literature.

The relationship between maternal folate status and children's psychological development has received little attention outside of FASSTT. Although this was a well-designed RCT, it was a relatively small-scale study based in Northern Ireland and therefore it was important to investigate if similar results were being found elsewhere. The ALSPAC study provides a unique opportunity to explore the same constructs in a large UK population. The significant gaps addressed by this research are twofold; Firstly, the data from this large observational study has never been used to analyse the positive impact of maternal folic acid use in relation to children's psychological outcomes. Additionally, the findings can support the capability and generalisability of the FASSTT@10y RCT.

The FASSTT study remains the only RCT to investigate the effect of maternal folate status beyond 12GW on the subsequent cognitive performance and psychological development of the children. Assessing the children who are now 10 years old and able to complete the measures themselves will provide a more accurate insight into

the potential benefits of maternal folate status on children's developmental outcomes. EI and resilience are two aspects of children's psychological development that have never been explored outside of FASSTT in relation to maternal folic acid consumption. This study will provide robust and reliable evidence, useful for informing policy on folic acid use during pregnancy and UK fortification programmes.

This research has the potential to have major implications, particularly in public health by providing the scientific evidence required to inform guidelines on maternal nutrition and folic acid use during pregnancy with a view to improving the health and wellbeing of both the mother and child. Moreover, it is recognised that late pregnancy (24-42GW) is a critical time for brain growth and development, identifying latent factors with the ability to promote child psychological and socioemotional development in utero and potentially through childhood could significantly improve later adult psychological health and wellbeing. This early intervention and prevention will allow health care resources to be allocated elsewhere. If findings indicate a significant positive impact on mothers and children, this research could potentially make a significant contribution to the evidence informing food fortification guidance which has only recently been introduced in the UK (Haggarty, 2021)

1.9 Overview of the thesis

This introductory chapter leads into the systematic literature review (*Chapter 2*) conducted to identify any gaps in the literature and where they exist. The research continues with secondary data analysis of the ALSPAC study (*Chapter 3*) in a larger

UK population followed by the FASSTT@10y experimental study (*Chapter 4*) which investigates the impact of maternal folic acid supplementation on children's psychological development in a Northern Irish population. A final discussion (*Chapter 5*) concludes this thesis providing an overview of the research project as a whole, detailing the limitations and suggesting the direction for any future research.

Chapter 2

Does maternal folate status during pregnancy impact on the child's development in the first 12 years of life? A systematic review of the literature.

2.1 Introduction

This chapter is comprised of a systematic review which aims to collect and evaluate existing evidence which examines the effect of maternal folate status during pregnancy on children's development. The reader will be presented with a short introduction detailing background information, the rationale for the review and the study aim and objectives. This section will be followed by an in-depth description of the method applied, providing information on the development of the search strategy, eligibility criteria for article inclusion, the review process, quality assessment including risk of bias and the data extraction process. The chapter continues to the results section where information is provided on article exclusions, the mother and child participant characteristics and intervention details and the risk and type of bias observed in included articles. Next the effect of folate interventions on developmental outcomes are presented in 4 categories: sufficient vs insufficient use, high levels vs normal levels, early vs late use and folic acid vs multivitamin. The chapter concludes with a discussion section containing a summary of results and discussion of the four intervention categories, followed by consideration of the completeness, applicability and quality of the evidence, the potential biases during the review process, strengths and limitations of the review process, the implications for future research and practice and a final conclusion.

2.1.2 Background to the study

The physical benefits of supplementing with folic acid during pregnancy are well established and have been subject to rigorous review in order to inform the policy and guidance followed by expectant mothers today. It is recommended that all women should supplement with folic acid preconceptionally and for the first 12 weeks of pregnancy in order to prevent Neural Tube Defects (NTD's) (Department of Health, 2002) and other physical abnormalities such as preeclampsia, preterm birth, low infant birth weight and orofacial clefts (e.g. Czeizel, 1996; Wilcox *et al.*, 2007; Scholl & Johnston, 2000; Vollsett *et al.*, 2000). Another popular research perspective investigates the impact of maternal folic acid use on children's cognitive (e.g. Murphy *et al.*, 2007) and neurological development (e.g. Gross *et al.*, 1974) and the prevention of developmental disorders such as ASD (Gao *et al.*, 2016).

The evidence examining folate exposure and the effect on other latent areas of child development such as psychological, social, emotional and language development is less consistent. Many factors are responsible for obtaining ideal folate conditions for optimal development including the time of initiation, dose and duration of use. Extensive research into the role of folic acid and the prevention of NTD's has meant the recommended time of initiation is at least 4 weeks preconception. This is to ensure adequate folate is available during embryogenesis to close the neural tube approximately 21-28 days after conception (Imbard *et al*., 2013).

The guidelines have produced an abundance of research focused on the role of adequate folic acid use from preconception to 12GW to prevent other developmental abnormalities, in line with the deficit model. Sufficient folate levels during early

pregnancy (<12GW) has been shown to reduce the risk of severe language delay (Roth *et al.*, 2011) and internalising and externalising problems (Roza *et al.*, 2010) in children while an improvement was also reported in gross motor development (Wehby & Murray, 2007). However, the paucity of research examining the effect of not supplementing in early pregnancy or delaying initiation presents conflicting findings. Whilst one study suggests these practices could negatively impact on the child's emotional development (Steenweg de-Graff *et al*, 2012), an earlier study found no significant associations between folate status in late pregnancy and children's neurodevelopment (Tamura *et al.*, 2005). Furthermore, it is unclear if supplementing for a longer duration could offer children any additional benefit. Existing research shows when mothers continue supplementing with sufficient folate (>400µg/d) from preconception into late pregnancy (28-34GW) children's motor, mental and social development (Bhate *et al.*, 2012) and neurodevelopment of older children (Veena *et al.*, 2010) is improved. An RCT (Catena *et al.*, 2016) reported a beneficial effect of late folic acid use on children's cognition after an earlier RCT found no significant effect (Campoy *et al.*, 2011).

Supplementing within the guidelines at 400µg/d has been shown to have a positive influence on children's development (e.g. Julvez *et al.*, 2009; Roza *et al.*, 2010; Steenweg-de Graff *et al.*, 2012). Whereas folate deficiency (<400µg/d) could potentially hinder development. Low folate levels in early pregnancy has been shown to reduce children's mental development (del-Rio Garcia *et al.*, 2009) and increase the risk of hyperactivity and peer problems in 3 year old children (Schlotz *et al.*, 2010). However, the question remains as to whether or not supplementing with a higher dose offers any additional benefits. Chatzi *et al.*'s (2012) study found that a

dose of 500µg/d was associated with better neurodevelopment in 18-month old children, specifically in the areas of receptive and expressive communication. In support of this Villamor *et al*. (2012) found a higher dose of 600µg/d improved receptive language in 3-year old children. However, consuming too much folic acid could have the opposite effect. Studies have shown that very high doses (>5000µg/d) and high doses (>1000µg/d) negatively affected children's neuropsychological development at 1 year and 4-5 years respectively (Valera-Gran *et al.*, 2014; Valera-Gran *et al.*, 2017).

2.1.3 Rationale

The physical benefits of folic acid supplementation are clear, and the neurocognitive benefits are also well documented. However, the evidence available exploring the impact of maternal folate status on other key areas of children's development including social, emotional, behavioural, motor and language development is limited. Children's development tends to occur simultaneously and therefore it is necessary to fully investigate the link between maternal nutrition in pregnancy and subsequent developmental outcomes. It is important to review all the evidence to date regarding folic acid intake during the prenatal and perinatal stages of pregnancy and explore the potential beneficial effects on children's development. Furthermore, research exploring the impact of folic acid use beyond the 12GW recommendation is scarce and conflicting. This review provides a unique opportunity to query if any additional benefits are associated with continued folic acid use beyond the first trimester by examining the available literature.

The evidence to date focuses on non-use, insufficient or over-use of folic acid during pregnancy and the possible detrimental effect to child development. Driven by this deficit model the focus has been on preventing developmental problems with sufficient folic acid use. Generally, this was a useful approach to guide research and inform policy on using folic acid to prevent negative aspects of development such as NTD's and child developmental disorders. However, in order for policy makers to make informed decisions the risks should be balanced with the potential benefits associated with supplementing. The recent shift to a more positive approach exploring the potential beneficial effect of sufficient maternal folic acid use on children's development has highlighted conflicting evidence. This review is the first, to the authors knowledge, to investigate the potential benefits associated with maternal folic acid use during pregnancy on children's development.

2.1.4 Study aim

This systematic review was designed to broadly search and find relevant literature aimed at addressing the question; *Does maternal folic acid supplementation during pregnancy impact on the child's psychosocial development in the first 12 years of life*?' The intention of this study is not to dispute the current recommendations developed for the prevention of NTD's but to collect and comprehensively evaluate the evidence investigating the impact of maternal folic acid supplementation during pregnancy on other areas of children's development from birth to 12 years. There needs to be clarity around when mothers should supplement, for how long and at what dosage. This review sought to address this gap by exploring how these factors can affect children's development.

2.4.1 Study objectives

To achieve this aim, a systematic review of electronic databases was conducted to determine the ideal conditions of folic acid or folate consumption to achieve the best developmental outcomes for the mother and child. A negative focus is expected however the positive resource-focused approach was applied where appropriate. The review was directed by a number of objectives:

1. Develop a comprehensive search strategy to include all research where children's cognitive, psychological, social, emotional behavioural or language developmental outcomes were assessed in relation to their mother's folate intake before and during pregnancy.
2. Systematically search all relevant databases and grey literature to identify relevant studies for inclusion through title and abstract reviews.
3. Conduct quality and risk of bias assessments on all included studies and extract all relevant data.
4. Synthesize and evaluate review findings.

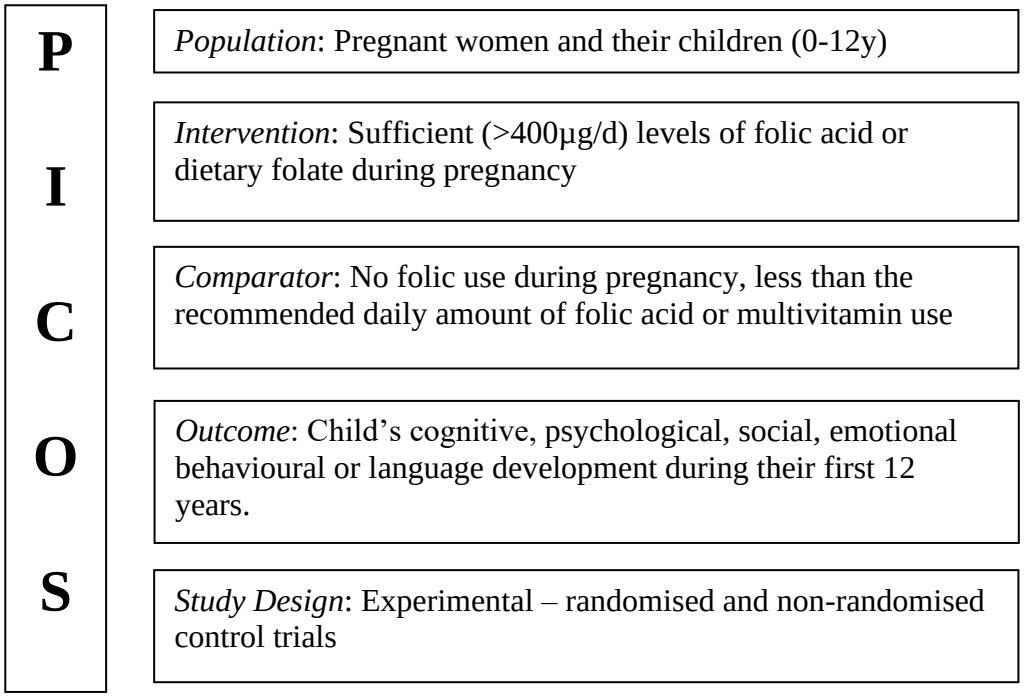
2.2 Methodology

2.2.1 Design

A protocol (*Appendix 2.1*) was developed for all reviewers to adhere to throughout each phase of the review. The PICOS framework (*Figure 2.1*) which considers the population of interest, the intervention and comparison used, and the outcomes observed was used to inform the protocol, develop the research question and facilitate the literature search. The application of PICOS for this systematic review was guided by the Centre for Reviews and Dissemination (Tacconelli, 2009) which

is recommended as a good source of practice for health care reviews and used by agencies including NIHR and NICE.

Figure 2.1: Integral information used to inform the systematic review using the PICOS Framework



2.2.2 Search Strategy Development

A list of potential search terms was identified during an initial scoping search of the literature. An Ulster University Research Librarian was consulted during development and refinement of the search strategy and to assist with the identification of potential databases. The search strategy was comprised of three key concepts: maternal folic acid use, prenatal and perinatal nutrition and child developmental outcomes. Medical Subject Headings (MeSH) and key words were used to search 13 Biomedical and Social Science electronic databases and relevant grey literature. Where advanced search options were not available key word searches were used.

Medline (OVID) example search

1. Folic acid (MeSH Term)
2. Vitamin B_9 (MeSH Term)
3. “folic acid” OR “folate” OR “vitamin B_9” (All fields),
4. 1 OR 2 OR 3
5. Pregnancy (MeSH Term)
6. Diet, food and nutrition (MeSH Term)
7. Prenatal care (MeSH Term)
8. Perinatal care (MeSH Term)
9. Preconception care (MeSH Term)
10. **6** OR **7** OR **8** OR **9**
11. "perinatal nutrit*" or "pregnan* nutrit*" or "preconception* nutrit*" or "maternal nutrit*" or "periconception* nutrit*" (All fields)
12. **10** OR **11**
13. **5** AND **12**
14. Resilience, Psychological (MeSH Term)
15. Adaptation, Psychological (MeSH Term)
16. Emotional adjustment (MeSH Term)
17. Social adjustment (MeSH Term)
18. Emotional Intelligence (MeSH Term)
19. Intelligence (MeSH Term)
20. Cognition (MeSH Term)
21. Child development (MeSH Term)
22. Language development (MeSH Term)
23. Social change (MeSH Term)
24. **14** OR **15** OR **16** OR **17** OR **18** OR **19** OR **20** OR **21** OR **22** OR **23**
25. “emotion* intelligen*” OR “EI” OR “emotion* quotient*” OR “EQ” OR “emotion* develop*” OR “emotion* matur*” or “intelligen* quotient*2 OR “IQ” OR “cogni* abilit*” OR “emotion* adjust*” OR “psycholog* develop*” OR “psychosocial* develop*” OR “language develop*” OR “verbal* IQ” OR “Verbal* intelligen* quotient*" or "emotion* adjust*" or "emotion* matur*" or "social* develop*" or cogniti* or wellbeing or "well being" or well-being or resilien* or coping (All fields)
26. **24** OR **25**
27. **4** AND **13** AND **26**

2.2.3 Systematic Search

One reviewer (LH) searched 13 electronic databases between May 25, 2016, and July 7, 2016, including CINHAL, Ovid (PsychINFO, Medline, AHMED, Embase), Nutrition and Food Sciences, ProQuest Complete, Web of Science, Scopus, TRIP, Applied Social Sciences Index and Abstracts (ASSIA), International Bibliography of the Social Sciences (IBSS) and Sociological Abstracts. An internet and grey literature search was also completed to find anything that may have been missed. This included Google Scholar, Science Direct, Sage Psychology, conference proceedings or other studies published in abstract form only, grant awards and theses or dissertations (Ethos). There was no publication year or language limits imposed however only articles with original data was included i.e. no reviews, editorials or retracted articles. In addition, reference lists of included articles and relevant reviews were also hand-searched to ensure all important studies had been included.

2.2.4 Study Eligibility

Inclusion Criteria

To be considered for review studies must have satisfied at least one criterion from each category as outlined by the PICOS framework. A brief overview can be viewed in *Figure 2.1*.

1) Studies must have been experimental in design.
2) Mothers must have supplemented with folic acid (FA) or consumed sufficient folate through diet during pregnancy and either ceased at the recommended 12GW or continued supplementing into the second or third trimester. Mothers could begin supplementing either preconceptionally or post-

conception but required detectable folate levels when first assessed for the trial by researchers.

3) A comparative group must have been present containing either mothers with no exposure to FA, mothers who had low levels (<400µg/d) or high levels (>800 µg/d) of FA or folate or mothers who supplemented with multivitamins (MV) (including multivitamins containing FA).
4) The offspring must have provided at least one measure of cognitive, language, psychological, social or emotional development before they reached 12 years. Multiple measures of development were acceptable.

Exclusion Criteria

Studies were excluded if the mother participant was undernourished or HIV infected due to the risk of nutritional disorders and the potential impact on the unborn child. To be comparable to the FASSTT study participants offspring over 12 years old were excluded. Papers where child participants had been diagnosed with any developmental disorder (e.g. ASD) were also excluded to keep studies as homogenous as possible. Studies with non-human participants or *in silico* or *in vitro* studies utilising organs, tissues, cell-lines or cellular components were also excluded. Furthermore, mothers who had supplemented with a multivitamin containing FA during pregnancy or within 4 weeks preconception were excluded due to the difficulty in separating the effects of multiple nutrients and subsequent bioavailability.

2.2.5 Title and Abstract Review

Article titles and study design was used to identify the most relevant articles to be included in the abstract review. Studies were not considered further when the title or abstract clearly indicated that the study did not meet the inclusion criteria. Three reviewers (LH, TC and MM) independently screened each title and abstract and those meeting the inclusion criteria were recorded for full text review. In the case of screening conflicts, reviewers independently screened the article again to confirm the inclusion/exclusion decision and discrepancies were discussed amongst the team. Any articles with unresolved screening conflicts were included for full text review.

2.2.6 Full Text Review: Quality Assessment and Data Extraction

Full text articles were retrieved for those studies that either clearly met the inclusion criteria or where eligibility to meet the inclusion criteria is unclear. After reviewing the full text of each study, 18 papers were excluded from the review for not meeting the inclusion criteria (*Table 2.1)*. See *Figure 2.2* for a study selection overview.

A data extraction form was developed specifically for this review by LH and agreed by the team. The form was piloted on 3 RCT and 3 cohort papers and adjusted as necessary to ensure all relevant key data was being recorded. Key data was extracted by one reviewer (LH) and included publication information, study and participant characteristics and exposure and outcome details including authors, publication year, journal of publication, study design, study location, mother participant age, number recruited, folate dosage, time spent supplementing, blood sample availability, length of follow up, number at follow up, outcomes observed, and measure used.

Table 2.1: Articles excluded during full-text review and reason for exclusion

Publication information	Reason for exclusion
Leventakou *et al.*2016	FA not measured in pregnancy
Duong *et al.*2015	FA not measured in pregnancy
Prado *et al.*2012	Mothers supplemented with MV including FA
Eilander *et al.*2010	FA not measured in pregnancy
McAfee *et al.*2012	FA not measured in pregnancy
Kumar *et al.*2007	FA not measured in pregnancy
Bhate *et al.*2008	FA not measured in pregnancy
Tofail *et al.*2008	Mothers supplemented with MV including FA
Siegel *et al.*2011	FA not measured in pregnancy
Kvestad *et al.*2015	FA not measured in pregnancy
Prado *et al.*2016	Mothers supplemented with MV including FA
Prado *et al.*2016	Mothers supplemented with MV including FA
Schmidt *et al.*2004	Mothers supplemented with MV including FA
Dobo & Czeizel 1997	Mothers supplemented with MV including FA
Goedhart *et al.*2011	Outcome not relevant
Mireku *et al.*2015	FA not measured in pregnancy
Forns *et al.*2012	Mothers supplemented with MV including FA
Christian *et al.*2010	Mothers supplemented with MV including FA

The Critical Appraisal Skills Programme (CASP, 2012) checklist was used to assess the quality of the evidence (Appendix 2.3). CASP is the most widely used appraisal tool, in use for over 25 years (CASP-UK.net). The core checklists (RCT and systematic review) were based on the 24 JAMA 'Users' guides to the medical literature (Guyatt et al., 1994). A total of 7 appraisal tools are now available to assess a range of research design: systematic review, RCT, cohort studies, qualitative research, case-control studies, diagnostic test studies and economic evaluations. The design of each included article therefore determined which version of the checklist to apply, for this review only the RCT and cohort tools were applicable. When appraising the literature each checklist challenged the reviewer to consider 3 issues;

"are the results of the study valid?", "what are the results?" and "will the results help locally?". The RCT checklist listed 11 questions each with several prompts to focus the reviewer and to help address these overarching issues which were responded to on a 3-point scale; Yes, Can't Tell or No. The cohort checklist addressed the same 3 broad issues and responses but contained 12 questions with prompts. For the purposes of inter-rater reliability, the CASP tool was completed independently by 3 reviewers and later discussed amongst the team. This also allowed for any discrepancies to be addressed before data extraction.

2.2.7 Risk of Bias

Incorporated in study quality is a risk of bias assessment. The questions posed encouraged the reviewer to consider a list of potential biases present in research and consider the effect bias could have on the evidence quality. Although CASP has the capability to explore bias it is unable to examine it thoroughly in a quantifiable manner. The National Institute for Health and Clinical Excellence (NICE) produced and included such a tool in the Guidelines Manual (2009). Both RCT and cohort checklists aimed to assess the internal validity of a study and contained a total 14 risk assessment items, 5 items examined detection bias and selection, performance and attrition bias were each assessed with 3 items. There were a total of 5 possible responses; Yes (✓) indicates that the study has been designed and conducted to minimize risk, Partially addressed/ unclear (✓✗) indicates that either the authors are unclear in their reporting or all potential bias had not been addressed in the design, No (✗) should only be used where significant bias is present, Not reported (NR), used in cases when authors failed to report how bias was considered and Not Applicable (NA), when the item is not applicable due to study design. The reviewer

could then draw informed conclusions on the likely risk of bias; low risk, high risk or unclear/ unknown risk. The NICE tools were completed in full by 1 reviewer (LH) as inter-rater reliability had already been confirmed with CASP. Other team members remained informed throughout the process.

1) **Selection Bias** – *systematic differences between baseline characteristics of the comparison groups*

It is possible that group differences rather than the treatment being measured could potentially lead to unexplained outcomes being observed and in turn giving inaccurate results. RCT's reduce the risk of selection bias through methods such as randomisation, concealment of allocation and baseline comparability. Non-randomised trials are particularly susceptible to selection bias therefore researchers need to ensure that allocation to groups was unrelated to potential confounding factors and that attempts were made within the design or analysis to balance the groups for confounding factors and that treatment groups were comparable at baseline including all major confounding and prognostic factors.

2) **Performance Bias** – *Systematic differences between groups in the care provided, apart from the intervention under investigation*

This can occur if intervention groups get treated differently from one another and is a major threat to internal validity. Performance bias is more likely to happen if investigators and other study personnel are aware of group allocation however participants can intentionally or unintentionally respond or behave differently if they are aware of their treatment group. Non-randomised studies are again more susceptible to performance bias due to the strict protocols, careful planning and

execution of RCT trials, which is often absent in non-randomised trials. If possible, blinding is a useful method to ensure that all groups receive the same care apart from the intervention under investigation. Additionally, the effect of performance bias is minimized as interference due to individuals' knowledge, experiences and beliefs is less likely.

3) **Attrition Bias** – *systematic differences between the comparison groups with respect to loss of participants*

Participants are allocated to a particular group based on a range of study characteristics to prevent confounding. Attrition bias occurs when participants drop out of a study causing a change in the sample during the trial which can weaken both internal and external validity. Attrition can relate to drop out after being allocated to a treatment group or differences being observed between participants lost and those who remained in a trial. To reduce the risk of bias drop-out rates and reasons for drop-out should be similar across groups and addressed in the study report. To reduce the risk of attrition bias investigators should follow all groups for an equal length of time or adjust the analysis to allow for differences in length of follow up. Additionally, investigators should record how many participants did not complete the treatment in each group and report how many participants in each group had no outcome data available and query were groups comparable, were inconsistencies present between groups in terms if those who did and those who did not complete the treatment? Statistically assessing the effect of drop-out is also helpful to observe the extent and impact of attrition. Researchers have questioned the threshold of when attrition results in bias which is still open for debate however an acceptable attrition rate appears to be unique to each study and the variables being measured.

4) **Detection Bias** – *bias in how outcomes are ascertained, diagnosed or verified*

This refers to systematic differences between groups in how outcomes are determined, therefore standardising the outcome assessment procedure is one method for reducing the risk of detection bias. Blinding of the investigator to the exposure and other important and prognostic factors is another method and is particularly important in the assessment of subjective outcomes (Cochrane, 2019). Detection bias can be present in some but not necessarily all outcomes measured in a trial therefore assessing each outcome individually is favourable. It is also important that investigators use valid and reliable measures to address precise definitions of outcomes and that participants are followed for an appropriate length of time.

2.2.8 Assessment of Homogeneity

Data extraction confirmed that studies were not adequately homogenous for meta-analysis due to the variety of statistical methods used therefore a narrative synthesis of the literature was the most conducive approach to address the research question posed by this systematic review.

2.3 Results

2.3.1 Description of included studies

A total of 1237 papers were identified during an initial systematic search of the databases and all search strategies were saved at the time to enable updated searches. A total of 73 articles were assessed for inclusion of which 19 were eligible (See *Figure 2.2*). Each included reference has been listed in *Table 2.2*. Following the title and abstract review, a total of 54 articles were excluded and 20 of these were excluded during the full text review, listed in *Table 2.1*. Throughout this section each article will be referred to using the allocated identification number also listed in *Table 2.2*.

Updated searches were performed on February 20, 2018, to search for articles recently published, or any other relevant material made available since the initial search. A total of 17 additional studies were identified, 10 of these were excluded based on information presented in the abstract and 7 progressed for a full-test review. All but one of the additional studies found during this updated search failed to meet the inclusion criteria and were excluded from the review.

2.3.2 Excluded studies

A total of 18 studies found during the first search were excluded at the full text review stage for failing to meet the inclusion criteria. See *Table 2.1*. Nine of these exclusions were due to maternal folic acid levels not being measured during pregnancy. Eight were excluded as mothers supplemented with a multivitamin (MV) including FA and therefore the outcomes observed could not be attributed to folic acid alone. One exclusion was due to the outcomes failing to meet the inclusion criteria detailed in the protocol and two articles were excluded due to study design

not meeting the inclusion criteria detailed in the protocol (*Figure 2.2*). During the updated search 16 studies were excluded. Six were excluded as maternal folic acid levels were not measured during pregnancy. Seven were excluded as mothers supplemented with a multivitamin (MV) including FA and therefore the outcomes observed could not be attributed to folic acid alone. Two exclusions was due to the outcomes failing to meet the inclusion criteria detailed in the protocol and one article was excluded due to study design not meeting the inclusion criteria detailed in the protocol (*Figure 2.2*).

2.3.3 Characteristics of included studies

Of the 19 included articles, 15 had a prospective cohort design (1; 2; 3; 4; 5; 6; 7; 8; 9; 10; 11; 12; 13; 14; 15), there were 2 individually randomised RCT's (16; 17) and 2 cluster RCT's (18; 19). Some articles were follow-up studies to established cohorts, these included the Generation R cohort (2; 3), the Norwegian Mother and Child Cohort Study (MoBa) (11; 13) and the Nutraceuticals for a Healthy Life (NUHEAL) project (16, 17), four were derived from established population cohorts and included the Rhea (1), Project Viva (4), AMICS (6), National Maternal Infant Health Survey (NMIHS) (7). Seven did not use established cohorts (5; 8; 9; 10; 14; 18; 19). Data were collected worldwide and countries included Greece (1), Netherlands (2; 3), USA (4; 7), UK (5), Spain (6; 12; 15; 16; 17), Mexico (8), India (9; 10), Norway (11; 13), Germany (16; 17), Hungary (16; 17) and China (18; 19). A total of 200,188 women and 115,627 children participated, some mothers and children may have been reported twice due to cohorts participating in more than one follow-up investigation. Study populations ranged in size from n=123 (9) to n= 108, 841 (13). All studies assessed at least one area of children's psychosocial or

cognitive development in relation to their mother's supplementation with folic acid (FA), dietary folate (DF) or both during pregnancy. See *Table 2.3* for the characteristics and further details of included studies.

Figure 2.2: PRISMA flow diagram (2009) to illustrate the flow of information through each phase of the systematic review

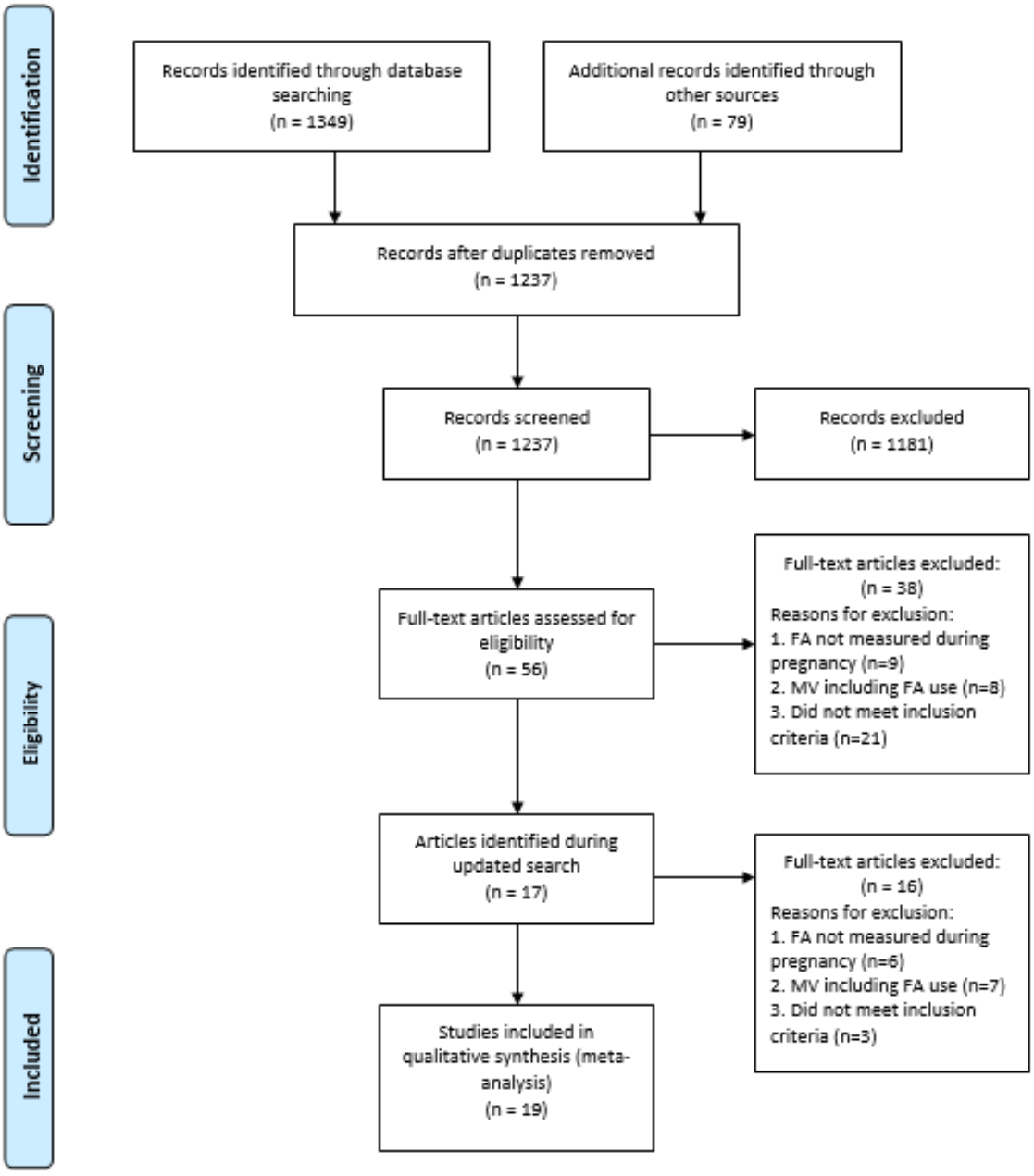


Table 2.2: Full reference for each eligible study included in the review and its Article ID.

Article ID.	Reference
1	Chatzi, L., Papadopoulou, E., Koutra, K., Roumeliotaki, T., Georgiou, V., Stratakis, N., ... & Kogevinas, M. (2012). Effect of high doses of folic acid supplementation in early pregnancy on child neurodevelopment at 18 months of age: the mother–child cohort 'Rhea'study in Crete, Greece. *Public Health Nutrition*, 15(9), 1728-1736.
2	Roza, S. J., van Batenburg-Eddes, T., Steegers, E. A., Jaddoe, V. W., Mackenbach, J. P., Hofman, A., ... & Tiemeier, H. (2010). Maternal folic acid supplement use in early pregnancy and child behavioural problems: The Generation R Study. *British Journal of Nutrition*, 103(3), 445-452.
3	Steenweg-de Graaff, J., Roza, S. J., Steegers, E. A., Hofman, A., Verhulst, F. C., Jaddoe, V. W., & Tiemeier, H. (2015). Maternal folate status in early pregnancy and child emotional and behavioural problems. *Prenatal Nutrition and Early Childhood Behaviour*, 59.
4	Villamor, E., Rifas-Shiman, S. L., Gillman, M. W., & Oken, E. (2012). Maternal intake of methyl-donor nutrients and child cognition at 3 years of age. *Paediatric and Perinatal Epidemiology*, 26(4), 328-335.
5	Schlotz, W., Jones, A., Phillips, D. I., Gale, C. R., Robinson, S. M., & Godfrey, K. M. (2010). Lower maternal folate status in early pregnancy is associated with childhood hyperactivity and peer problems in offspring. *Journal of Child Psychology and Psychiatry*, 51(5), 594-602.
6	Julvez, J., Fortuny, J., Mendez, M., Torrent, M., Ribas-Fitó, N., & Sunyer, J. (2009). Maternal use of folic acid supplements during pregnancy and four-year-old neurodevelopment in a population-based birth cohort. *Paediatric and Perinatal Epidemiology*, 23(3), 199-206.
7	Wehby, G. L., & Murray, J. C. (2007). The effects of prenatal use of folic acid and other dietary supplements on early child development. *Maternal and Child Health Journal*, 12(2), 180-187.
8	del Río Garcia, C., Torres-Sánchez, L., Chen, J., Schnaas, L., Hernández, C., Osorio, E., ... & López-Carrillo, L. (2009). Maternal MTHFR 677C> T genotype and dietary intake of folate and vitamin B12: their impact on child neurodevelopment. *Nutritional Neuroscience*, 12(1), 13-20.

Article ID.	Reference
9	Bhate, V. K., Joshi, S. M., Ladkat, R. S., Deshmukh, U. S., Lubree, H. G., Katre, P. A., ... & Yajnik, C. S. (2012). Vitamin B_{12} and folate during pregnancy and offspring motor, mental and social development at 2 years of age. *Journal of Developmental Origins of Health and Disease*, 3(2), 123-130.
10	Veena, S. R., Krishnaveni, G. V., Srinivasan, K., Wills, A. K., Muthayya, S., Kurpad, A. V., ... & Fall, C. H. (2010). Higher Maternal Plasma Folate but Not Vitamin B-12 Concentrations during Pregnancy Are Associated with Better Cognitive Function Scores in 9-to 10-Year-Old Children in South India–3. *The Journal of Nutrition*, 140(5), 1014-1022.
11	Handel, M., Skurtveit, S., Roth, C., Hernandez-Diaz, S., & Selmer, R. (2016). Prenatal Exposure to Folic Acid and Antidepressants and Language Development: A Population-Based Cohort Study. *Journal of Clinical Psychopharmacology*, 36(4), 333-339.
12	Valera-Gran, D., de la Hera, M. G., Navarrete-Muñoz, E. M., Fernandez-Somoano, A., Tardón, A., Julvez, J., ... & Rebagliato, M. (2014). Folic acid supplements during pregnancy and child psychomotor development after the first year of life. *JAMA Pediatrics*, 168(11), e142611-e142611.
13	Roth, C., Magnus, P., Schjølberg, S., Stoltenberg, C., Surén, P., McKeague, I. W., ... & Susser, E. (2011). Folic acid supplements in pregnancy and severe language delay in children. *JAMA*, 306(14), 1566-1573.
14	Tamura, T., Goldenberg, R. L., Chapman, V. R., Johnston, K. E., Ramey, S. L., & Nelson, K. G. (2005). Folate status of mothers during pregnancy and mental and psychomotor development of their children at five years of age. *Pediatrics*, 116(3), 703-708.
15	Valera-Gran, D., Navarrete-Muñoz, E. M., Garcia de la Hera, M., Fernández-Somoano, A., Tardón, A., Ibarluzea, J., ... & Julvez, J. (2017). Effect of maternal high dosages of folic acid supplements on neurocognitive development in children at 4–5 y of age: the prospective birth cohort Infancia y Medio Ambiente (INMA) study. *The American Journal of Clinical Nutrition*, 106(3), 878-887.
16	Catena, A., Muñoz-Machicao, J. A., Torres-Espínola, F. J., Martínez-Zaldívar, C., Diaz-Piedra, C., Gil, A., ... & Koletzko, B. (2015). Folate and long-chain polyunsaturated fatty acid supplementation during pregnancy has long-term effects on the attention system of 8.5-y-old offspring: a randomized controlled trial–3. *The American Journal of Clinical Nutrition*, 103(1), 115-127.
17	Campoy, C., Escolano-Margarit, M. V., Ramos, R., Parrilla-Roure, M., Csábi, G., Beyer, J., ... & Koletzko, B. V. (2011). Effects of

Article ID.	Reference
	prenatal fish-oil and 5-methyltetrahydrofolate supplementation on cognitive development of children at 6.5 y of age–. The *American Journal of Clinical Nutrition*, 94 (suppl_6), 1880S-1888S.
18	Li, Q., Yan, H., Zeng, L., Cheng, Y., Liang, W., Dang, S., ... & Tsuji, I. (2009). Effects of maternal multimicronutrient supplementation on the mental development of infants in rural western China: follow-up evaluation of a double-blind, randomized, controlled trial. *Pediatrics*, 123(4), e685-e692.
19	Li, C., Zeng, L., Wang, D., Yang, W., Dang, S., Zhou, J., & Yan, H. (2015). Prenatal Micronutrient Supplementation Is Not Associated with Intellectual Development of Young School-Aged Children–3. *The Journal of Nutrition*, 145(8), 1844-1849.

Table 2.3: *General characteristics of eligible studies included for review*

Lead Author	Study Design	Journal	Pub. year	Location	Year recruited	Established Trial	Follow-up
1. Chatzi	Cohort	Public Health Nutrition	2012	Greece (Crete)	2007-2008	Rhea	18m
2. Roza	Cohort	British Journal of Nutrition	2010	Netherlands (Rotterdam)	2002-2006	Generation-R	18m
3. Steenweg-de Graff	Cohort	American Journal of Clinical Nutrition	2012	Netherlands (Rotterdam)	2002-2006	Generation-R	3y
4. Villamor	Cohort	Paediatric and Perinatal Epidemiology	2012	USA (Massachusetts)	NR	Project Viva	3y
5. Scholtz	Cohort	Journal of Child Psychology and Psychiatry	2010	UK	NR	N/A	8.75y
6. Julvez	Cohort	Paediatric and Perinatal	2009	Spain (Menorca)	1997-1998	AMICS	4y

Lead Author	Study Design	Journal	Pub. year	Location	Year recruited	Established Trial	Follow-up
		Epidemiology					
7. Wehby	Cohort	Maternal and Child Heath Journal	2007	USA	1988	NMIHS	3y
8. Del-Rio Garcia	Cohort	Nutritional Neuroscience	2009	Mexico	2001	N/A	1-12m
9. Bhate	Cohort	Developmental Origins of Health and Disease	2012	India (Pune)	2004-2006	N/A	2y
10. Veena	Cohort	Journal of Nutrition	2010	India (Pune)	1997-1998	N/A	9-10y
11. Handel	Cohort	Journal of Clinical Psychopharmacology	2016	Norway	1999-2008	MoBa	3y
12. Valera Gran	Cohort	JAMA Pediatrics	2014	Spain	2003-2008	INMA	1y
13. Roth	Cohort	JAMA	2011	Norway	1999-2008	MoBa	3y
14. Tamura	Cohort	Pediatrics	2005	USA (Alabama)	1990-1993	RCT	5.3y
15. Valera-Gran	Cohort	American Journal of Clinical Nutrition	2017	Spain	2003-2008	INMA	4.5y
16. Catena	RCT	American Journal of Clinical Nutrition	2016	Europe (Germany, Spain Hungary)	2001-2003	NUHEAL	8.5y
17.Campoy	RCT	American Journal of Clinical Nutrition	2011	Europe (Germany, Spain Hungary)	2001-2003	NUHEAL	6.5y
18. Li	RCT	Pediatrics	2009	Western China	2002-2006	N/A	3, 6 + 12m
19. Li	RCT	The Journal of Nutrition	2015	Northwest China	2002-2006	N/A	7-10y

2.3.4 Mother participant confounding background characteristics

A total of 142,456 pregnant women were recruited between 1990-2008 with mean ages ranging between 22-34 years. Study populations in Mexico, India, China and Alabama (USA) reported lower mean age (<24y) than other US states and European populations (>25y). Mothers were supplemented with folic acid at varying gestational stages ranging from early preconception to 36GW. Reported maternal background characteristics included a variety of factors such as BMI during pregnancy, education level, marital status, parity, employment status, smoking and alcohol use during pregnancy and breastfeeding. In each case the data extracted was from the FA only group . All but 2 studies were comparable: Article 7 grouped participants according to ethnicity and Article 9 defined groups by gestational stage. Out of the 17 comparable studies, 16 controlled for maternal education (1; 2; 3; 4; 5; 6; 8; 10; 11; 12; 13; 15; 16; 17; 18; 19), 15 controlled for maternal BMI (1; 2; 3; 4; 5; 8; 10; 11; 13; 14; 15; 16; 17; 18; 19), 14 controlled for parity (1; 3; 4; 5; 8; 10; 11; 12; 13; 14; 15; 16; 17; 18) and smoking during pregnancy (1; 2; 3; 4; 5; 6; 8; 11; 12; 13; 14; 15; 16; 17), only 1 study controlled for ethnicity (12). See *Table 2.4* for full details on maternal confounders.

2.3.5 Child participant confounding background characteristics

A total of 115,627 children were assessed at various ages. The age of the children at the time of assessment and the associated articles are outlined in *Table 2.5* below. Reported child background characteristics included a variety of factors such as child's sex, gestational age, birthweight, birth length, birth head circumference,

Table 2.4: Maternal confounders controlled for in each study. Data is taken from the folic acid only group or folic acid sufficient at 400-500µg /d group

Background variables	**Paper ID**																
	1	**2**	**3**	**4**	**5**	**6**	**8**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
BMI (M)	24	24.2	24.1	24.6	adj		22.6	23.2	69% <25		65% <25	28.2	73.1% <25	25.3	25	20.8	20.9
Education >12y (%)	88.2	63.5	60.1	71.9	53	43.3	11.3/ 3.4 (m/sd)	32.9	69.8	35.6	83.8		37.2	40.7	53.1	11.9	11.4
Married (%)	92.2		92.5			93			96.4		93.6						
Parity <1 (%)	42.2		61.2	47.7		17.3	81.8	50.8	48	15.5	45.1	43.7	57.1	44.4	82.9	53.9	
Employed (%)	62.6					69.9	45.5		91.1	62.5							12.6
Non-smoker (%)	57.4	80	79	89.7	adj	82	91.3		89	71.2	93.3	84	71.4	89	81.1		
Alcohol use in pregnancy (%)	27.8	26	25.8		adj	20.5			51.3		11.2	13.5					
Breastfeeding >3m (%)		37.3				44.3					57.8			38.5	46.9		
White ethnicity (%)				73.8													

*Data not presented in articles 7 and 9; *adj = adjusted for in analysis but no details provided*

Table 2.5: Lists the age of children at the time of assessment in months and years and the corresponding Article ID. This demonstrates the range of children and developmental stages included in this review.

Age of child at time of assessment	**Article ID**
1, 3, 6, and 12 months	8
3, 6, and 12 months	18
11-22 months	12
18 months	1 and 2
2 years	9
3 years	3, 4, 7, 11, 13
4 years	6
4.5 years	15
5 years	14
6.5 years	17
8.5years	5, 16
7-10 years	19
9-10 years	10

caesarean section delivery and preterm birth occurrence. Follow-up characteristics include age, weight, height, head circumference, BMI, Apgar score (1 and 5min) and childcare attendance. In each case the data extracted was from the FA only group. All but 2 studies were comparable (5; 7). Three studies (6; 11; 13) had child participant details reported elsewhere but listed confounders controlled for during analysis. Out of the fifteen comparable articles with information available, twelve articles reported child's birthweight (1; 2; 3; 5; 8; 9; 10; 14; 16; 17; 18; 19), eleven articles reported the child's sex (1; 2; 3; 4; 9; 12; 15; 16; 17; 18; 29) and gestational age (1; 2; 3; 5; 8; 9; 10; 12; 14; 16; 17), six articles reported the child's head

circumference at birth (5; 9; 10; 14; 16; 17) and one (2) took this measurement at late pregnancy. Few studies took anthropometric measures at follow up; three measured head circumference and height (9; 10; 14), two measured weight (9; 14) and two measured the child's BMI (10; 17). See *Table 2.6* for full details on the child participant confounding characteristics.

2.3.6 Risk of bias in included studies

The methodological quality and risk of bias was assessed using CASP and NICE tools, respectively. The quality of included articles was generally satisfactory. In some cases, authors did not report some of the relevant information. It is also notable that some of the included articles are follow-up investigations using the same participants and interventions therefore there are many occurrences where the author/s did not report or clearly address some details; these statements were only marked as present if explicitly stated in the article. See *Table 2.7* and *Table 2.8* for the assessment of bias.

2.3.7 Selection Bias

In most cohort studies allocation to treatment group was unrelated to confounding factors with the exception of Articles 12 and 15 which derived their samples from the same cohort. In all but 1 article (14) researchers attempted to balance the treatment groups for potential confounders however only 6 cohort studies (8; 9; 10; 11; 13; 14) provided clear information on the comparability of treatment groups at

Table 2.6: Child confounding characteristics controlled during each study. Data is taken from the folic acid only group or folic acid sufficient at 400-500µg /d group

Background variables	**1**	**2**	**3**	**4**	**5**	**8**	**9**	**10**	**12**	**14**	**15**	**16**	**17**	**18**	**19**
Gestational age at birth (M weeks)	38.3	40.1 (median)	40.1		40	56.5	39.2	39.3	39.6	38.7		39.9	39		
BW (g)	3198	3493	3464		3470	3200	2710	2873		3187		3358	3420	3194	3200
Low BW (%)		3.6					25		9.7						
Length (cm)							48.2					514	512.6		
Head circumference (mm)		286.3 (preg)			351.4 (birth)		332	338		339		347	349.6		
Breastfeeding (months)	4.4					66.4 (>12w)						38.4%	46.9%		
Male (%)	198 (n)	48.7	49.4	49.7			61/123		51.2		50.8	48.2	45.7	63.1	58.8
C-section (n)	188					56.1	15%								
Incidences of preterm births (%)	36 (n)	5.1					6%					11.5	11.4		
Follow-up ages (m)	18	18.4	36.2	39.5	8.73 (y)		2.0 (y)	9.7 y	14.3	5.3 (y)	4.5y		80.6	12.2	8.8y
Weight at FU (kg)							10.3			21.8					
Head circumference at follow up							463	506		512					
Height (cm)							84.6	130.7		112					
BMI (kg/m)								14.5					16.42		

Data not presented in articles 6, 7, 11 and 13

Table 2.7: NICE quality Assessment and risk of bias for cohort studies

	Paper ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Selection Bias	The method of allocation to treatment groups was unrelated to potential confounding factors	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	×	✓	✓	×
	Attempts were made within the design or analysis to balance the comparison groups for potential confounders	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	×	✓
	The groups were comparable at baseline, including all major confounding and prognostic factors	×	×	×	×	×	×	✓×	✓	✓	✓	✓	×	✓	✓	×
Performance Bias	The comparison groups received the same care apart from the intervention(s) studied	NR	NR	NR	NR	NR	NR	✓×	✓×	NR	NR	NR	✓	✓	✓	✓
	Participants receiving care were kept 'blind' to treatment allocation	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
	Individuals administering care were kept 'blind' to treatment allocation	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Attrition Bias	All groups were followed up for an equal length of time (or analysis was adjusted for differences)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	>60% but <80% included in final analysis	✓	✓	✓	✓	<	>	NR	✓	✓	✓	<	>	✓	✓	NR
	Did all follow up participants have outcome data available?	✓	✓	✓	✓	×	✓	NR	✓	✓	✓	✓	✓	×	✓	✓
Detection Bias	The study had an appropriate length of follow-up	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	The study used a precise definition of outcome	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	A valid and reliable method was used to determine the outcome	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Investigators were kept 'blind' to participants' exposure to the intervention	NR	NR	NR	NR	NR	NR	NR	✓	NR	NR	NR	NR	NR	NR	NR

	Paper ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	Investigators were kept 'blind' to other important confounding and prognostic factors	NR	NR	NR	NR	NR	NR	NR	✓	NR	NR	NR	NR	NR	NR	NR
Overall Assessment		**8**	**8**	**8**	**8**	**6**	**8**	**6**	**11**	**9**	**9**	**8**	**8**	**9**	**9**	**7**

Table 2.8: NICE quality assessment and risk of bias for RCT studies

	Paper ID	16	17	18	19
Selection Bias	An appropriate method of randomisation was used to allocate participants to treatment groups	✓	✓	✓	✓
	Method of randomisation stated	NR	✓	✓	NR
	There was adequate concealment of allocation	NR	✓	✓	NR
	Method of allocation concealment stated	NR	NR	✓	NR
	The groups were comparable at baseline including all major confounding and prognostic factors	NR	NR	NR	NR
	The groups were comparable at follow up including all major confounding and prognostic factors	✓	×	✓	×
Performance Bias	The comparison groups received the same care apart from the intervention(s) studied	✓	✓	✓	✓
	Participants receiving care were kept 'blind' to treatment allocation	NR	✓	✓	NR
	Individuals administering care were kept 'blind' to treatment allocation	NR	✓	✓	NR
Attrition Bias	All groups were followed up for an equal length of time (or analysis was adjusted to allow for differences)	✓	✓	✓	✓
	How many participants did not complete treatment in each group?	×	×	✓	✓
	The groups were comparable for treatment completion	✓	✓	✓	✓
	For how many participants in each group were no outcome data available?	✓	✓	✓	✓
	The groups were comparable with respect to the availability of outcome data	✓	✓×	✓	✓
Detection Bias	The study had an appropriate length of follow-up	✓	✓	✓	✓
	The study used a precise definition of outcome	✓	✓	✓	✓
	A valid and reliable method was used to determine the outcome	NR	NR	✓	✓
	Investigators were kept 'blind' to participants' exposure to the intervention	NR	✓	✓	NR
	Investigators were kept 'blind' to other important confounding and prognostic factors	NR	✓	✓	NR

Paper ID	16	17	18	19
Overall Assessment	**9**	**13**	**18**	**10**

baseline: 1 study (7) contained some detail but lacked clarity. All RCT articles reported that an appropriate method of randomisation was used however, only two articles (17; 18) stated the method of randomisation. Articles 17 and 18 also reported utilising an adequate method of concealment but only Article 18 stated the method of allocation concealment. No articles using RCT design stated if the treatment groups were comparable at baseline and only 2 (16; 18) reported if they were comparable at follow-up.

2.3.8 Performance Bias

Each of the cohort studies failed to report any information on blinding of participants and individuals administering care. Two RCT's (17; 18) reported this information, the remaining two did not. Eight articles (12; 13; 14; 15; 16; 17; 18; 19) including the 4 RCT's clearly stated that all treatment groups received the same care except for the intervention; two articles (7; 8) indicated this but it was not explicitly stated in the article.

2.3.9 Attrition Bias

All RCT (16; 17; 18; 19) and the majority of cohort studies confirmed outcome data was available for all follow up participants with the exception of Articles 5 and 13; Article 7 did not report this information. Eleven articles (1; 2; 3; 4; 8; 9; 10; 13; 14; 18; 19) included 60-80% of participants in their follow up investigations, four articles (5; 11; 16; 17) recruited less than 60% and 2 studies (6; 12) recruited more than 80% of original participants. Two studies (7; 15) did not report this information.

Comparability for treatment completion was only applicable to RCT's and all four presented this information.

2.3.10 Detection Bias

In each of the cohort study articles information on blinding of investigators to participants exposure or confounding factors was not reported. Two of the 4 RCT's (17; 18) provided this information. All included studies used an appropriate length of follow up and a precise definition of outcome. All cohort studies and 2 RCT's (18; 19) used a valid and reliable method to measure outcome and presented supporting information in the article, the remaining RCT's (16; 17) did not provide this information.

2.3.11 Intervention

Dosage and deficiency level: Of the 19 included articles, eleven measured the effect of folic acid (FA) on children's developmental outcomes (1; 2; 3; 6; 7; 10; 11; 13; 14; 18; 19), four used dietary folate (DF) (8; 9; 16; 17) and the remaining 4 used a combination of both (4; 5; 12; 15). In cohort studies supplementing with folic acid, five articles had information available on dosage used approximating 400-500µg/d (1; 2; 3; 11; 14), all 4 RCT's used a dosage of 400µg /d (16; 17; 18; 19) and studies using a folic acid and dietary folate combination reported a dosage of 400µg/d (5), 600µg/d (4) and 400-5000µg/d (12). Three studies reported the folate deficient level used as <7nmol (3; 9; 10) while another used <11nmol as the deficient level (14). Others reported deficiency in terms of micrograms per day (µg/d), two articles referred to deficiency as <400mg/d (8; 15) when using a combination of FA and DF,

and another applied a DF deficiency level as <671µg/d (12). Eight articles failed to report any deficient level information (1; 2; 4; 5; 6; 7; 11; 13).

Timing: Nine articles investigated the impact of FA and/or DF during the first and second trimesters (0-28GW) (1; 2; 3; 4; 5; 6; 7; 8; 13), and 4 of these considered the first trimester only (<13GW) (6; 7; 8; 13). Five trials continued supplementing into the third trimester with Article 9 (DF only) stopping at 34GW, Article 10 continued to 32GW, Article 11 continued to 30GW and studies 12 and 15 had measured DF (32GW) and FA (28GW) separately. Article 14 differed in that folate concentration during late pregnancy was of interest and therefore folate level was measured from 19GW to delivery. Three RCT trials (16; 17; 18) continued supplementing until delivery with 2 only initiating supplementation at 20GW (16; 17). Article 19 did not report this information.

Duration: In many trials participants reported folate and FA use from preconception (ranging from -4months) or periconception (around conception) to early pregnancy (<10GW) (1; 2; 3; 4; 5; 7; 11; 12; 13; 15); the timing was irrelevant to the dietary folate studies (8; 9). Two cohort studies investigated the impact of FA during late pregnancy only (10; 14) with the introduction of supplementation from 24GW and 19GW, respectively. In Article 9 mothers were recruited at approximately 24GW with blood samples given at 30GW and therefore data is only available from this point. Two RCT's (16; 17) initiated supplementation in later pregnancy (>20GW), one RCT (18) introduced FA at 14GW and Article 19 did not report when women started or finished supplementing.

Fortification: Seven studies (1; 4; 6; 8; 9; 10; 14) reported that fortification was mandatory in their country however other studies took place in these same countries, but authors failed to address fortification in their report (7; 12; 15). The UK (5), Norway (11; 13) and Netherlands (2; 3) do not have fortification guidelines in place.

Blood samples: Ten cohort studies validated folate concentrations using either red cell folate (RCF) or serum folate (SF) levels in blood (1; 2; 3; 5; 9; 10; 11; 12; 13; 14). In six of these articles (3; 5; 9; 10; 12; 14) each participant provided a blood sample, in comparison to the remaining four articles (1; 2; 11; 13) where a sub-sample of participants were used. Three studies did not collect blood samples (4; 6; 7) and Articles 8 and 15 did not report if a blood sample was collected or used for validation of FA use. Two RCT's measured folate levels using cord blood samples (16; 17) and the remaining 2 RCT's (18; 19) did not use blood samples in either pregnancy or cord blood to confirm FA use.

Additional info: See *Table 2.9* for full details on exposure characteristics in cohort studies and *Table 2.10* for exposure characteristics in RCT trials.

Due to the wide range of developmental outcomes being included for review, the difference in operational definitions of outcomes and the measures used for assessment, comparisons were made based on the intervention. The comparison groups for this review included: sufficient FA use compared to deficient use, high levels of folate compared to normal levels of folate, early initiation compared to supplementing in later pregnancy and folic acid compared to multivitamin (without

FA) use. Some studies explored more than one comparison within the article, and each is discussed in detail below.

2.3.12 Comparison 1: Sufficient FA vs insufficient FA

Five cohort studies (2; 3; 8; 10, 14) and 2 RCT's (16; 17) explored the effect of sufficient levels of folate in comparison to deficient levels. Evidence suggests that children of mothers who used adequate FA during early pregnancy have a significantly lower risk of internalising and externalising behavioural problems (2) and at higher risk of experiencing emotional problems but not behavioural problems at 3 years (3). A non-significant reduction in psychomotor development and mental development was found in children of mothers who were folate deficient in early pregnancy when compared to folate sufficient mothers (8). Furthermore cognitive scores tended to be lower in the group of children whose mothers had low folate status (<7nmol), these findings were non-significant (10). Significant positive associations between normal maternal folate levels and the cognitive measures of learning ability, long-term storage and retrieval, visuo-spatial ability and attention and concentration were found (10). In contrast, other studies found no significant difference between the sufficient and deficient folate groups and concluded that folate status of mothers in late pregnancy had no impact on the neurodevelopment of children aged 5 years (14). When considering 5-MTHF (folate) use in comparison to a placebo a significant link was found between early maternal nutrition including FA use and children's brain development at 8.5 years (16). FA supplementation improved the children's ability to resolve conflicts and resulted in higher brain activity in the area responsible for executive function. In comparison, a long term effect on executive function was not found in children whose mothers supplemented

Table 2.9: Maternal characteristics and exposure details recorded during cohort studies

Study No.	N (enrol)	Maternal age	Supplement (S), dietary folate (D) or both (B)	Dosage (µ/d)	Timing start	Timing end	Folate in blood	Blood sub sample	Deficient level reported	No of Groups	Folic Acid only group?	Fortification
1	1388	30	S	500	Preg known	14-18w	RBC	Y	NR	3	Y	Y
2	6491	30	S	400	Pre-10	18w	Serum	Y	NR	2	Y (F/MV)	NR
3	4900	30	S	400-500	<10	18w	Fconc	N	<7nmol/L	2	Y (F/MV)	NR
4	2128	32	B	600	-3m	28w	N	-	-	1	Y	Y
5	453	NR	B	>400	-3m	199d	RBC	N	NR	1	Low/ High	N
6	482	NR	S	NR	N/A	13w	N	-	-	3	Y F/MV	Y
7	6774	29.2	S	NR	-3m	12w	N	-	-	2	N	NR
8	253	22	D	>400	-	12w	NR	NR	<400	3	N	Y
9	123	23	D	-	-	34w	Y	N	<7	2	N	Y
10	536	24	S	NR	<24w	32w	Y	N	<7	2	N	Y
11	58410	NR	S	400	-4w	30w/g	Y	Y	NR	5	Y	N
12	2644	30.8	B	>5000	F=Pre S= -3m	F= 32w S= 7m	Y	N	<671	4	All FA only	NR
13	108841	25-34	S	NR	-4w	8w	Y	Y	NR	4	Y	N
14	589	23.7	S	400	>19w	delivery	F, tHcy, EF	N	<11	2	Y NR	Y
15	2506	31	B	>1000	Pre	7m	-	-	<400	4	All FA only	NR

Table 2.10: Maternal characteristics and exposure details recorded during RCT studies

Study No.	N	Design	M age (mean)	No of Groups	Normal sample	Inter-vention	Comparison	Placebo used	FA only group	Dosage (µ/d)	Supp start	Supp end	Folate measured (preg/ cord)
16	312	D-B, placebo	31	4	Y	FO	5-MTHF	Y	N	400	>20w/g	delivery	Y/Y
17	270	D-B, placebo	31	4	Y	FO	5-MTHF	Y	Y	400	>20w/g	Delivery	Y/Y
18	5828	D-B, Placebo	25	3	Y	MV inc FA	IFA + FA	N	Y	400	~14w/g	Delivery	N/N
19	5828	D-B Cluster	34	3	Y	MV inc FA	IFA +FA	N	Y	400	NR	NR	N/N

with fish oil and when fish oil and folate were combined. Interestingly a reduction in the executive function advantage was observed meaning that the beneficial effects of FA could be dose dependent. In the same sample no significant differences in children's cognitive scores at 6.5 years were found (17).

Within these studies (2; 3; 8; 10; 14; 16; 17) various maternal lifestyle factors were shown to be associated with folic acid use. According to the evidence, women are more likely to supplement with folic acid if they are older, have higher educational attainment, with educated parents and of high SES. Supplement use was also associated with marital and employment status with married and working mothers reporting more FA use. These mothers also tend to breastfeed for a longer duration and following a period of maternity return to work and place their children in childcare. Those who take FA have significant differences in various lifestyle factors including smoking, drinking and BMI. Mothers who do supplement smoke less, consume less alcohol and have lower BMI than those who do not. Folate concentrations are also associated with lower parity. In addition, some anthropometric measures were identified as potential mediators between maternal folic acid use or folate status on children's outcomes at various stages of their development. Higher birthweight and longer gestation were positively associated with children's development whereas head circumference at birth was not.

Moreover, children whose mothers were folate deficient in early pregnancy had lower birthweight than children whose mothers had normal levels. Conversely, higher folate concentrations were associated with higher BW and weight in 9 year old children, and children's cognitive scores were strongly associated with their

current BMI, head circumference, educational level and current folate status. In some cases neither birthweight nor gestational age was related to maternal FA use in early pregnancy or intelligence scores.

2.3.13 Comparison 2: High vs normal levels

Three studies investigated the impact of high folate levels in comparison with normal levels (1; 12; 15). Significant positive associations were found between normal levels of FA and children's mental and psychomotor development at 18 months but found higher doses were not associated with any additional improvement (1). While other research found that high doses (>5000µg/d) of folate during pregnancy was associated with lower psychomotor development but not mental development in 1 year olds when compared with an average dose of 400-1000µg/d of FA (12). In the same sample high doses of FA (>1000) were associated with lower levels of cognitive development in children 4.5 years old and recommended that high doses be monitored by medical professionals (15).

2.3.14 Comparison 3: Early vs late use

Six cohort studies (3; 4; 5; 7; 9; 11) and 1 RCT (17) investigated if early FA use was more beneficial for child outcomes than supplementing in later pregnancy. Article 3 found that children of mothers who were folate deficient or reported inadequate FA use (starting later or not using) were at higher risk of emotional problems, children were also at higher risk if mothers did not supplement or started supplementing late indicating a potential window for optimal effect (3). Furthermore, lower folate concentrations in early pregnancy were associated with hyperactivity and peer

problems in the children but found no association with emotional or conduct problems (5). Total folate intake was measured in late pregnancy and no associations were found (4, 5). When considering motor development FA use in early pregnancy was positively associated with gross motor development in children aged 3 but found a weak negative effect in the personal/ social domain (7). Maternal folate use in later pregnancy (28GW and 34GW) was associated with motor , mental and social development (9). An RCT assessed the effect of folate supplementation during the second half of pregnancy on cognitive development of the children and found no significant associations (17). Atypical use of FA in conjunction with SSRI's was also considered and results showed that folic acid use only (with no SSRI) in preconception and the first 8 weeks of pregnancy resulted in the best language competency scores and lowest proportion of language delay (11).

Articles included in this review also identified some environmental factors as important covariates in maternal folic acid use. Mothers with higher folate intake in early pregnancy were slightly older and lower levels of smoking while those who supplemented with folic acid while taking SSRI's were more likely to have higher education level, were working and had planned their pregnancy (11). Unlike others, this study found no association between parental education and children's intelligence. SES, parental education and occupation, sex of child and children's schooling were also found to be strongly associated with children's intellectual development (19).

Maternal folic acid use and children's hyperactivity/ inattention and peer problems was mediated by fetal head growth but not by fetal body growth and found no

association with postnatal head growth (5). Mental and social development was positively associated with head circumference and negatively with BW, and social development was associated with the child's haemoglobin levels at 2 years (9).whereas motor development was not associated with current or postnatal measurements (9).

2.3.15 Comparison 4: FA vs MV without FA or prescribed medication

Three cohort studies (6; 7; 13) and 2 RCT's (18; 19) compared the effects of folic acid only to a multivitamin not containing folic acid (6; 7; 13). FA supplementation was shown to be associated with improvements in neurocognitive development specifically verbal, verbal-executive functions, social competence and inattention symptoms in 4 year old children (6); results were less consistent in the MV treatment group with the verbal subscale attaining the only significant association albeit weaker (6).

MV use had a more positive impact on child development when compared to FA alone (7). Positive associations were found between MV use and language and social developmental domains in comparison to FA positively affecting gross motor development and negatively affecting social development. FA was also shown to have a significant protective effect against risk in overall development in African American children (7). In addition, evidence demonstrated that beginning to supplement with FA within the first 8 weeks reduced the risk of severe language delay in 3 year old children, starting after this point did not lower the risk (13). Supplementing with MV without FA slightly increased children's odds of experiencing moderate or severe language delay when compared to the FA only

treatment. No association was found between FA supplementation and gross motor delay (13). Significant differences were also found between FA only and MV with the MV providing a higher mental development score at 12m (18) however this advantage equated to ~6days and therefore MV provided a limited contribution to mental development. No significant differences were found in psychomotor development (18). In the same sample no significant differences were found in cognitive scores in group comparisons (FA, MV +Fe) when children were between 7 and 10 years (19). The effects of FA, SSRI use and a combination of both were compared and a significant association between long-term use of SSRI's and delayed language competence was found in children aged 3 years, but only when taken simultaneously with folic acid (11). Using SSRIs without FA was not associated with increased risk of lower language competence.

Breastfeeding, maternal social class, education level, parity, smoking and area of residence were positively associated with FA use at 12GW (6). While maternal education was most strongly associated with FA use and severe language delay (13), but parity, BMI and marital status were also notable associations (13).

2.3.16 Overview of outcomes

The majority of studies reported a beneficial effect of adequate FA use on child development (1; 2; 3; 4; 5; 6; 7; 9; 10; 13; 16; 18). One study (8) found that the beneficial effect was only seen in those who were genetically susceptible and carrying the MTHFR-677C>T genotype. Article 1 described the beneficial effect of high doses of FA on mental and psychomotor development whereas others reported a detrimental effect on psychomotor development (12) and cognition (15). Although Article 18 found no significant effect, an improvement in children's mental

development at 1 year was reported when mothers supplemented with MV. Four studies explored the impact of FA deficiency on child development (2; 3; 5) and found a higher risk of behaviour problems and mental health problems (2), emotional problems (3), hyperactivity and peer problems (5) in children whose mothers had inadequate folate status. Article 11 observed delayed language competence in children but only when mothers combined their FA with SSRI use. One cohort study (14) and 2 RCT's (17; 19) found no significant effects.

Seven studies tested for a variety of unrelated secondary outcomes (1; 4; 6; 9; 13; 14; 16), no effect was found on socio-emotional development (1), visual-motor skills (4; 14) and gross motor delay (13). Some articles did report a positive effect on outcomes including social competence (6), social development (9) and brain activity in the areas associated with executive function (16). All details are listed in *Table 2.11* and *Table 2.12*.

Table 2.11: Child participant characteristics and outcomes recorded during cohort studies

Study No.	N final	Child age	Outcome 1	Measure 1	Outcome 2	Measure 2	Beneficial Effect?
1	553	18m	Mental/ Psychomotor	BSID-III	Socioemotional development	BSID-III	Y
2	4214	18m	Behaviour and Emotional problems	CBCL	-	-	Y
3	3209	3y	Behaviour and Emotional problems	CBCL	-	-	Y
4	1210	3y	Cognition	PPVT	Visual-motor skills	WRAVMA	Y
5	100	8.75y	Behaviour problems	SDQ	-	-	Y
6	420	4y	Motor and Cognitive abilities	MSCA	Social competence	CPSCS	Y
7	6774	3y	Child development	DDST	-	-	Y
8	253	1,3,6,12m	Mental/ Psychomotor	BSID-II	-	-	Y
9	123	2y	Psychomotor	DASII	Social development	VSMS	Y
10	536	9-10y	Cognitive function	K-ABC	Cognitive function	WISC-III	Y
11	51747	3y	Language competence	LGRS	-	-	N- FA +SSRI detrimental
12	2213	11-22m	Cognitive/ Psychomotor	BSID-I	-	-	N - detrimental
13	38954	3y	Severe Language delay	LGRS	Motor delay	ASQ	Y
14	355	5y	Cognitive function	PPVT	Psychomotor	WRAVMA	No effect
15	1682	4-5y	Motor and Cognitive abilities	MSCA	-	-	N - detrimental

Key: BSID (Versions I, II and III): Bayley Scales of Infant and Toddler Development, CBCL: Child Behaviour Checklist, PPVT: Peabody Picture Vocabulary Test, WRAVMA: Wide Range Assessment of Visual Motor Abilities, SDQ: Strengths and Difficulties Questionnaire, MSCA: McCarthy Scales of Children's Abilities, CPSCS: California Preschool Social Competence Scale, DDST: Denver Developmental Screening Test, DAS-II: Developmental Assessment Scale for Indian Infants, VSMS: Vineland Social Maturity Scale, K-ABC: Kaufman Assessment Battery for Children, WISC (III, IV) Wechsler Intelligence Scale for Children, LGRS: Language Grammar Rating Scale, ASQ: Ages and Stages Questionnaire.

Table 2.12: Child participant and outcome details recorded during RCT studies

Study No.	N (Final)	Child age	No of males	Children supplemented	Outcome 1	Measure 1	Outcome 2	Measure 2	Beneficial Effect?	Effect size
16	136	8.5y	67/130	Y to 6m	3 attention networks; executive, alerting and orienting	ANT/ EEG	ERP data	Collected during ANT	Y	NR
17	154	6.5y	80/154	Y to 6m	Cognitive function	K-ABC	-	-	N	-
18	995	3, 6, 12m	762/1305	N	Mental development	BSID-I	Psychomotor development	BSID-I	Y- Mental development only	~0.22 points at 12m
19	1744	7-10y	1045/1744	N	Cognitive ability	WISC-IV	-	-	N	-

Key: ANT: Attention Network Test, EEG: Electroencephalography, ERP: Event Related Potentials, K-ABC: Kaufman Assessment Battery for Children, BSID-I: Bayley Scales of Infant and Toddler Development, WISC-IV: Wechsler Intelligence Scale for Children

2.3.17 Synthesis of results

A total of fifteen studies (11 cohort and 4 RCT) compared sufficient and insufficient folate use on child development which included recommended use vs deficiency, late use only, overuse or in conjunction with other medications. Seven studies reported a beneficial effect when mothers were folate sufficient; lowering the risk of internalising and externalising behaviour at 18m (2), improved general development (7) and neurodevelopment (6, 9), language competence (13) and enhanced executive function (16) at 8.5y and cognition (10) at 9-10y.

In these studies mothers supplemented with either FA (400µg/d) (2, 13, 10, 16) or DF (9) and the time of initiation and duration of use differed between studies ranging from preconception (-12 to -4GW) to 8GW (13), to mid-pregnancy (2, 7) or continued use to through late pregnancy (9, 10) and onto delivery (16) resulting in various durations of use including 1-12 weeks (13), 8-22 weeks (2) or up to 40 weeks (16). In some cases, information on dose, time of initiation, duration and pattern of use was not available (6, 7) or it was not applicable due to dietary folate consumption in a largely vegetarian population (9). Cord blood samples were used to confirm folate level present (16), or maternal blood samples were provided during pregnancy to validate self-reported folic acid use (2, 13). Two studies defined sufficient use by using a deficient folate level of <7nmol/L present in maternal blood (9, 10), however results from the earlier study (10) conducted in the same geographical area suggest that this concentration used to define deficiency may be too low. Two studies did not collect blood samples (6, 7) however this was recognised as a limitation by authors.

Evidence indicates that maternal folate deficiency in pregnancy can put many areas of children's development at risk. Three cohort studies assessed the impact of low folate status or deficiency during early pregnancy and reported a higher risk of child emotional problems (3), hyperactivity and peer problems (5) and reduced psychomotor and mental development (8). Furthermore, maternal folate deficiency in the first half of pregnancy increased children's risk of behaviour problems (2). Authors concluded that sufficient use protected children from internalising and externalising problems.

Mother participants in these studies supplemented with either folic acid (400µg/d) (3), dietary folate (8) or a combination of both (5). Deficiency was defined as <400µg/d (8) or <7nmol/L (3) present in mothers blood and initiation of supplement use differed between studies ranging from preconception (-12GW) to 28GW (5), a maximum duration of 40 weeks and <10GW to 18GW (3), a duration of 8-22 weeks. Mothers who consumed dietary folate only (8) were measured at 12GW however, the time of initiation and duration of use is unclear due to fortification in Mexico. Combined folate intake (FA and DF) was measured at ~13GW and again ~28GW (5). In two studies folate concentrations were measured in all maternal blood samples (3, 5), this information was not reported by authors in Article 8.

The impact of sufficient/insufficient maternal folate on developmental outcome accounted for the majority of the evidence, other important considerations included MV not containing FA in comparison to folic acid only (18/19) and the impact of high levels (1, 4) or very high levels (12, 15) of folate during pregnancy on children's development.

Two RCT's investigated the effect of multivitamin (containing FA) use on the mental development children under 1 (18) and the intellectual development of 7-10 year olds (19) using folic acid only as a reference. Results showed that MV use provided the most benefit to infant's mental development in comparison to folic acid alone or folic acid + iron (Fe) supplements (18) when participants received 400µg/d from study enrolment to delivery. no additional benefits were found in relation to intelligence (19) in older children (7-10y) when mother participants supplemented from ~14GW to delivery. Moreover, no significant improvements were observed in relation to psychomotor development (18). It is therefore important to consider if the benefits gained from supplementing during pregnancy reduce over time. Both studies were conducted in China where pre and periconceptional folic use is recommended, therefore the duration of use ranges from 26-52 weeks.

Other studies compared the beneficial effects of supplementing with folic acid to other vitamins or multivitamins (MV) not containing FA. Significant differences were observed in verbal, motor and verbal-executive function (6) and teacher ratings of social competence (6) however, it is important to note that during analysis the folic acid group was divided into two subgroups, folic acid only (n=143) and folic acid with other vitamins (n=101). These data or results were not published and the results provided by authors were for combined folic acid-containing supplements. Conclusions should therefore be drawn with caution. In the MoBa cohort (13) the risk of severe language delay was assessed using 4 conditions, no supplement use (reference group), supplements not containing folic acid, folic acid only and supplements containing folic acid and found the risk of having a child with severe language delay was lower under two conditions, when mothers supplemented with

folic acid only (OR 0.55; CI, 0.35-0.86) and supplements containing folic acid (OR 0.55; CI 0.39-0.78). Further research using the MoBa sample (11) demonstrated a potential risk of supplementing with folic acid in conjunction with prescribed SSRI's and found that long-term SSRI use delayed children's language competence, but only when used in conjunction with folic acid.

When examining the impact of high levels or very high levels of folate during pregnancy on children's development, a slight increase in dose was associated with improved communication (1) and cognitive development (4). Mothers supplemented with doses described as high, 500µg/d from preconception or when pregnancy was known to ~18GW, a maximum duration of 22 weeks (1). Red Blood Cell (RBC) concentrations in cord blood were used to validate self-report measures in a sub-sample of mothers (1). A marginal increase in dose during early pregnancy was associated with improved receptive and expressive communication in 18m children. No additional improvement was observed with larger doses (>500µg/d). In another study, mother participants supplemented with combined folic acid and dietary folate (600µg/d) from -12GW to 28GW with duration of use ranging from 18-40 weeks (4). Blood samples were not collected in this instance. Findings suggest that higher folate levels in early pregnancy is associated with improved cognitive function at 3 years (4).

The effect of very high doses of folate on children's neuropsychological development at 1-2y (12) and later at 4-5y (15) was investigated using Infancia y Medio Ambiente (INMA) project participants. The impact of using a combination of folic acid and dietary folate totalling >5000µg/d and found this excessive dose had a

significant negative effect on toddlers (1-2y) psychomotor development scores and increased their risk of developmental delay in this area when compared to children whose mothers supplemented with 400-1000µg/d (12). The long-term effects of very high doses of folate was also examined (exceeding >1000µg/d) at an older age (4-5y) and found that very high doses during early pregnancy was associated with lower levels of cognitive development (15). In both studies the time of initiation was reported as preconceptional use (-12GW to -4GW) and supplementation continued to ~32GW, a duration of approximately 36-44 weeks. Mothers folate level was confirmed using blood samples during pregnancy provided between 12-16GW.

2.3.18 Evidence relating to supplementing past 12GW?

The literature considering the potential benefits of continued supplementation is sparse and conflicting. A number of articles (3, 4, 5) tested the effect of continued supplementation on children's developmental outcomes and found no additional benefit. Inadequate use, defined as non-use or initiating use in late pregnancy increased the risk of emotional problems in 3-year old children (3). A significant improvement in children's cognitive development was recorded when mothers supplemented during early pregnancy but found no additional benefit to supplementing to the end of trimester 2 (4). Children were at higher risk of hyperactivity and peer problems when mothers presented with low folate status in early pregnancy but not in late pregnancy (5) concluding that low folate status in early pregnancy could damage children's brain development increasing the risk of behaviour difficulties. Additionally, one cohort study observed no effect on development when mothers supplemented in late pregnancy (14) however these findings are limited due to the lack of folate measures and information available in

early pregnancy. Two RCT's reported no cognitive benefits for children when mothers were folate sufficient throughout pregnancy to delivery (17, 19). One study identified continued folic caid use as potentially damaging to children's language development but only when supplementation was simultaneous with long-term SSRI use. More research is needed to further investigate the interaction effects between folic acid and prescription medication like SSRI's due to frequent use by women of childbearing age.

Some articles reported contrary to the evidence above and recommended continued supplementation through late pregnancy (9, 10) and to delivery (16, 18). Improvements were seen in cognition (10), attention (16), psychomotor (9), social (9) and mental development (9, 18). However, it is important to consider the confounding effect of other variables in these studies and question if the benefits observed are truly and entirely due to folic acid use.

2.4 Discussion

The purpose of this review was to investigate if *maternal folic acid supplementation during pregnancy impacts on children's development in the first 12 years of life* by identifying and evaluating the relevant evidence to date. This evidence examined the impact of maternal folic acid use during pregnancy on key areas of children's development including cognitive, motor, language, psychological, social, emotional and behavioural development. This section will begin with a short summary followed by a discussion of the results. The relevance, completeness and quality of the included articles will then be considered. Next, potential biases in the review

process and limitations of this review will be acknowledged and discussed. This section concludes with implications for practice and directions for future research and conclusion.

2.4.1 Summary of main results

Nineteen articles from various geographical locations and populations were reviewed and the majority (13/19) reported a beneficial effect of adequate folate (FA or DF) on child development. Others studied the impact of folate deficiency and found children were at higher risk of experiencing developmental problems or difficulties (4/19), a small number of studies reported no significant effect on development (3/19) and three reported a detrimental effect (11, 12, 15), however, two of these examined the adverse effects associated with high doses of folate (12, 15) and in the other study mothers were long-term SSRI users simultaneously supplementing with folic acid during pregnancy (11).

A number of intervention factors appeared to be important to children's developmental outcomes. These included a combination of folate type (folic acid/ dietary folate), time of initiation, duration of use and dose administered and the balance to achieve optimal benefit on development which is still not understood.

Many of the studies included in this systematic review measured folic acid use during early pregnancy, from preconception to 12GW, in line with current recommendations and consequently resulted in a larger volume of experimental research assessing these conditions. There was general consensus amongst these articles that sufficient FA use during this period improved children's developmental

outcomes with many concluding that children's development could benefit from adequate and sufficient periconceptional and early prenatal folic acid use. Optimal FA dose and risks of deficiency are also well evidenced in the literature due to the substantial research into the association between FA and NTD's and the risks associated with very high doses have also received some attention. However, the potential benefits of marginally higher or lower doses, initiating use after 12GW and supplementing for a longer duration, are areas that are yet to be fully explored.

2.4.2 Measurement of developmental outcome

The broad nature of this review dictated that comparisons between studies would be drawn on intervention/exposure rather than outcome. Seven key areas of children's development were under review with varying operational definitions, and subsequently, the measures applied to assess these outcomes. It is therefore important to consider how authors defined the construct they were measuring, their chosen measure and who completed it (i.e. child, parent or teacher completed) and the variation amongst studies in relation to construct measurement across studies in children under 12y as it could impact the synthesis of included evidence.

2.4.3 Neurodevelopment

Articles included in this review used a total of six instruments to measure children's neurodevelopment, three of which were different editions of BSID (I, II, III). The BSID are used extensively worldwide to assess young children's neurodevelopment and detect developmental delay in clinical and research settings. Since its inception in 1969 these scales have evolved through 4 editions with the most recent being

published in 2019. Two studies included in this review used the early edition (12, 18) which assessed motor and mental development in children aged 3-28 months (Bayley, 1977). The participants included were Spanish (12) and Chinese (18) with ages ranging from 3-22m in both studies. It is unclear why the 1st edition was administered rather than a more recent edition.

One included article (8) used BSID-II (Bayley, 1993). In this edition the age range was widened to include children ages 1-42m, however has received some criticism in relation to a lack of subscale-standardised scores for assessing language and cognitive development (Johnson & Marlow, 2006). Again it is unclear why this edition, rather than the updated version was used during this study. One explanation could be that it had already been used and standardised in a Mexican population (Gomaa *et al.*, 2002).

The BSID-II was revised and reconstructed to separate the assessment of cognition from language in the BSID-III (Bayley, 2006) and restandardised following Johnson and Marlow's (2006) criticism. The Mental Developmental Index (MDI) was divided into cognitive, receptive language and expressive language subscales and the Psychomotor Developmental Index (PDI) was divided into fine and gross motor subscales. The BSID-III was applied in one included study (1) and is considered a useful tool to identify any developmental delay in young children. However, recent research has shown that the capabilities of this edition for this purpose may be limited due to the potential risk of overestimating children's development thus identifying fewer children with delay (Yi *et al.*, 2018).

The psychomotor development of Indian children (9) was assessed using the Developmental Assessment Scale for Indian Infants (DASII) (Phatak, 1997). This scale is frequently used to assess the development of severely malnourished children in India (e.g. Dwivedi *et al*., 2018) and is an Indian adaptation of the BSID-II (Bayley, 1993). Roopesh (2019) recently reported difficulty in obtaining accessible user manuals resulting in administration and scoring problems however, issues such as these were not reported by authors in this study (9).

The MSCA (McCarthy, 1972) was completed by two study populations (6, 15) and found an improvement in children's verbal and motor performance, executive function and instances of inattention. Both assessments occurred at ~4y in Spanish populations and although adapted and standardised for use with Spanish participants (Julvez, *et al.*, 2009), evidence demonstrating validity and the applicability of the MSCA in recent years is scarce. It was a popular and psychometrically sound instrument but has become outdated, and as it has never been re-normed it has fell out of favour for professional use (Levin, 2001; Bracken, 1991). Authors failed to justify the reason for choosing this scale above others.

2.4.4 Cognitive development

Nine different scales and measures were used to test children's cognitive development or function including their IQ, executive function, attention and visual-motor skills. These included the Kaufman Assessment Battery for Children (K-ABC), Peabody Picture Vocabulary Scale (PPVT), Koh's Block Test, Wechsler Intelligence Scale for Children (WISC-II, II, IV), Attention Network Test (ANT) and ERP data (Event Related Potentials).

The K-ABC (Kaufman & Kaufman, 1983b) was used to assess cognitive development at 2 different ages; 6.5y (17) and 9-10y (10). The K-ABC is a versatile instrument used frequently by educational psychologists in everyday practice to measure cognition in children aged 3-18 years and it has been shown to have good psychometric abilities in various study populations (Drozdick *et al.*, 2018), despite receiving some criticism in a special edition of the *Journal of Special Education* devoted to the K-ABC (Miller & Reynolds, 1984). A combination of the K-ABC, Koh's Block Design Test (Kohs, 1923) and the Wechsler Intelligence Scale for Children (WISC-III) (Wechsler, 1991) was administered to 9-10y children (10), each instrument was adapted and validated for the sample (Transler *et al*., 2008) and assessed different facets of cognition. The WISC is used widely used to identify strengths and weaknesses in cognitive function, particularly in educational settings to inform educational and intervention planning. Developed specifically for children aged 6-16y, the WISC has been translated or adapted to many languages and norms have been established for a number of countries and therefore a popular choice amongst researchers. A Chinese version of the WISC-IV (Wechsler, 2007) was utilised to measure children's intellectual ability (IQ) in an RCT (19). This was standardised and shown to be culturally appropriate with good levels of reliability and validity (Chen *et al*., 2010).

The PPVT (R, III) (Dunn & Dunn, 1981; Dunn & Dunn, 1997) and WRAVMA (Jastak & Wilkinson, 1984) were used to assess cognitive function and visual motor skills at 3y (4) and 5y (14). Both instruments are well-standardised and psychometrically sound (Beres *et al*., 2000; Adams & Sheslow, 1995). No significant effect was found at 5y (14) however, a more recent edition of the PPVT

was administered at 3y (4) which could have contributed to its significant findings. The instruments outlined in this section are frequently used by health professionals. This is usually to assist with diagnosing delay or abnormal development in children and most often applied when children are experiencing difficulties in school. As each of these instruments were developed for recognising potential developmental difficulties there is a risk that they are less sensitive to pinpointing any developmental improvements.

To test children's attention and executive function an RCT (16) used EEG to objectively measure participants reaction times and response accuracy while completing the child version of the ANT (Fan *et al*., 2002). In addition, electromagnetic tomography (EMT) was used to estimate the brain areas involved (Pascual-Marqui, 2002).

2.4.5 Language development

Language competence (11) and delay (13) was assessed in the Norwegian Mother and Child Cohort Study (MoBa) using a measure designed specifically for this cohort. The Language Grammar Rating Scale was contained in the MoBa specific 3-year Questionnaire and completed by parental self-report. The scores of a subsample of children being assessed for language delay (13) were compared to the communication domain of the Vineland Adaptive Behaviour Scale (Sparrow *et al*., 2005) to ensure parental reports were a reliable measure. Other research shows that mothers and children included in the MoBa study had adequate plasma folate level at 17GW (Nilsen *et al.*, 2010) and 30GW (Handel *et al.*, 2016) therefore it is possible

that mothers continued to supplement which could have improved language development at another developmental stage in pregnancy.

2.4.6 Motor development

Gross motor delay was assessed as a secondary outcome using the Ages and Stages Questionnaire (Squires *et al*., 1997) to further typify the children displaying severe language delay (13), however authors found that the large majority of children were within the normal range.

2.4.7 Emotional and behavioural development

The Child Behaviour Checklist (CBCL/1.5-5) assesses a broad range of behaviour and emotional problems in children (Achenbach & Rescorla, 2000) and was utilised in two studies (2, 3). Good reliability and validity for the CBCL were reported, both studies used the same established cohort (Generation-R) and norm scores applicable to this cohort have been established (Tick *et al*., 2007). The instrument manual reports that emotional problems are more stable in comparison to behaviour problems in toddlers (Achenbach & Rescorla, 2000) and as children get older stability increases (Bilancia & Rescorla, 2010). This could explain why the problems observed in behaviour reduced and emotional problems became more evident as children aged in Article 3 (3y) in comparison to Article 2 (18m). External factors such as socioeconomic status (SES) and poverty can also have a more profound impact on behaviour and emotional problems and must be considered (Costello *et al*., 2003), however, authors adjusted for many important covariates statistical

analysis. This instrument has been shown to have strong psychometric properties (Stone *et al*., 2010) particularly in the older children.

2.4.8 Social development

The assessment of social development in children was typically a secondary outcome in included studies. Various measures were used including the Social-Emotional scale of the BSID-III (1), the California Preschool Social Competence Scale (CPSCS), both adapted and standardized for the Spanish population (6) and the revised Vineland Social Maturity Scale (9) (Malin & Raj, 1992).

As discussed in *Section 2.4.2*, the range of scales used by researchers in combination with the number of constructs measured makes it difficult to draw definitive conclusions. This review highlights the need for succinct operational definitions in order to enable accurate measurement. Scales are a manifestation of latent constructs, developed to measure what cannot be measured directly such as neurodevelopment, cognition, language, emotional, behavioural, social and motor development. They typically consist of multiple items designed to isolate and measure underlying latent constructs, underpinned my psychological theory. However, scales often measure different dimensions within a construct which is problematic for systematic review. Generally, all researchers implemented valid and reliable measures in included studies, but were they comparable? To accurately compare results, studies using the same instruments should be pooled for review as recommended by Olsson *et al*., (2009) who reported that although scales claim to measure the same construct they are not interchangeable.

2.4.9 Considering the effect of how mothers supplement

The evidence included in this review indicates that folic acid dose, time of initiation and duration of use are important factors to consider, each having different effects on different areas of development. The majority of evidence supports the use of 400µg/d from preconception to 12GW with this dose and time of initiation being subject to considerable research. With more women choosing to supplement with folic acid or MV containing folic acid past 12GW research needs to focus now on the effects of continued use 12GW, dependent upon early uptake and adequate adherence to the vitamin. It is important to determine the ideal conditions required for optimal development as folic acid use during pregnancy could provide the foundation for children and parents to build upon.

2.4.10 Folic acid dose

The dose of folic acid consumed alongside folate in its nutritional context are important factors to consider. Findings indicate that too much or too little folate during pregnancy is potentially damaging to children's development, corresponding to the available guidelines. Using folic acid as recommended is evidenced by some to improve children's neurodevelopment and later cognitive development however this is not conclusive support. Using higher doses (>500µg/d) does not provide any additional benefit and very high doses (>5000µg/d) were found to have a negative impact on both cognition and psychomotor development in children under 2y and at 4-5y suggesting that the negative effects of very high doses are lasting. Dose could not be considered for motor, social or emotional development as this information was not reported.

It is important to consider not only how much folate is consumed, but also how it interacts with other medications or vitamins being taken by the mother. Research has shown that although folic acid in early pregnancy can reduce the risk of severe language delay, however, when taken in conjunction with SSRI's language delay was reported. This highlights the potential for interacting medications to negatively impact on children's language development. This is especially important as SSRI's are the primary antidepressant prescribed to mothers with antenatal depression and warrants further investigation as SSRIs are generally considered safe to use in pregnancy. Conversely, language development could be enhanced beyond what was observed with folic acid with maternal MV use and the other micronutrients they contain.

Just as adequate folic acid use can affect pregnancy, birth and developmental outcomes, inadequate use must also be considered. Low maternal folate until 28GW was associated with internalising and externalising behavioural difficulties at 8y (Schlotz *et al.*, 2010). High levels were also shown to negatively affect psychomotor development only at 1y (Valera-Gran *et al.*, 2014) and cognition at 5y (Valera-Gran *et al.*, 2017) but not mental development at 1y (Valera-Gran *et al.*, 2014). These are somewhat contradictory findings. Children's performance ability, memory and verbal learning was assessed at 1y while verbal, perceptive-performance, quantitative, memory and motor functions were measured at 5 years in the same sample and under the same intervention conditions. A possible explanation for this discrepancy in results is language development. Language ability at 1y is limited in comparison to 5y which can affect the quality of interactions a child experiences. Language competence can also shape the parenting style adopted and the style of

attachment a child develops. These could be mediating and therefore improving children's cognitive development in those intermediary years. Findings from these non-randomised studies suggest that adequate (400µg/d) supplementation beyond 12GW could have a positive impact on children's cognitive, language, behavioural, psychomotor and neurodevelopment at various ages throughout childhood.

2.4.11 Time of initiation

The evidence suggests that when mothers begin supplementing could also impact on their children's development. Children's cognition and neurodevelopment can benefit if mothers began supplementing <12GW whereas folate deficiency reduces psychomotor and mental development in children, but only if genetically susceptible (8). Preconceptional and very early use (<8GW) was optimal for language (11) competence and protected against language delay (13). Initiating after 8GW did not protect against severe language delay. This suggests that although children may benefit from preconceptional and very early use of maternal FA use, this is a small window of opportunity which is problematic for children of unplanned pregnancies. Time of initiation appeared to be irrelevant to motor development as a positive impact was reported regardless of when mothers began supplementing.

Few studies have investigated the effect of delayed initiation of maternal folic acid on children's social, emotional and behavioural outcomes however this would be an interesting line of research. Analysis of secondary data could provide an opportunity to explore these outcomes in mothers who did not supplement in early pregnancy for reasons such as unplanned pregnancy. Although psychological developmental outcomes were typically secondary to cognition and neurodevelopment in the

included literature there is evidence to suggest that delayed initiation (>10GW) could increase the risk of emotional problems in young children. The contrary was found in older children therefore it is possible the effects of deficiency lessens as children age, perhaps children develop emotionally as they progress through school, growing in independence and building peer relationships.

Similar findings were reported for social development with folic acid being attributed to better social competence at 4y but not in younger children. It is then possible that supplementing with folic acid in early pregnancy could provide a foundation for children's social emotional development on which they build themselves through their own experiences. Folate deficiency in early pregnancy but not in late pregnancy was shown to increase levels of hyperactivity and peer problems in older children but not younger children. Folic acid intake in early pregnancy was shown to reduce the risk of children internalising and externalising behaviour problems and was attributed with better social competence at 4y but not in younger children.

2.4.12 Duration of use

For how long mothers supplement is closely associated with time of initiation. Continued use beyond the recommended 12GW is an important factor but generally not addressed in the literature. Research indicates that the brain develops rapidly during the last trimester from 28GW therefore having sufficient nutrients to support brain growth and development during this time would be advantageous. Those who did investigate found sufficient folate in late pregnancy resulted in cognitive improvements at 2y however no effect was observed in older children. Sufficient

folic acid supplementation in very early pregnancy was shown to assist with children's language development in the MoBa sample (13). Other studies have reported these mothers to have adequate plasma folate at 17GW (Nilsen *et al.*, 2010) and 30GW (Handel *et al.*, 2016) therefore it is possible that mothers continued to supplement which could have improved language development at another developmental stage in pregnancy.

The majority of included studies (14/19) reported supplementing beyond 12GW with participants of five studies supplementing to delivery, all but three of these reported a beneficial effect. Without thorough investigation, assumptions cannot be made as to whether or not these improvements would have been observed if mothers had stopped at 12GW.

2.4.13 Limitations of included studies

The evidence included in this review is not without limitations at various stages in the research. In terms of the exposure, the time of initiation was not clearly defined or described in any article therefore making it difficult to accurately report and consider the impact of duration of use on children's development. Furthermore, some studies did not have information on the dose consumed by mothers therefore investigating the dose/response relationship or dose threshold (maximum or minimum) was not possible, resulting in a lack of evidence for a critical exposure time window. Many of the studies relied on mothers self-reporting their folic acid use in a questionnaire, in some cases mothers were recalling use up to 4 months prior to recruitment, increasing the risk of bias. In order to counteract this some studies used folate concentrations in mother's blood during pregnancy or cord blood

samples to verify and validate self-report data in either a sub-sample or all participants.

The operational definitions of developmental outcomes varied greatly between articles therefore the choice of instrument to measure the outcome differed. Many of the instruments used did not measure development per se but are used to screen for developmental risks. Again, some studies relied on parental or teacher ratings which could be of less value in comparison to testing the children themselves. Additionally, in many cases authors did not justify the choice of instrument used to measure development. It is possible that outdated instruments provided results that could differ from the latest editions and measures.

Cohort studies in particular are at risk of residual confounding. Supplement use is related to socioeconomic status (SES), lifestyle factors such as dietary habits and adverse health behaviours including smoking (Iglesia *et al.*, 2014; Shi *et al*., 2014). Many researchers attempted to reduce the effect of confounding within the limits of their project however some studies did not collect data on important extraneous variables and others did not adjust for it during statistical analysis. Additionally, many of these studies used large population-based cohorts, conducting follow-up assessments at varying ages, attrition did occur but the effects were acknowledged and discussed in most cases. A reduced number of participants particularly in the smaller experimental groups could introduce problems with study power, cautionary notes were provided in these instances.

Other limitations include the age of datasets used and generalisability of results. Some exposure information was collected at recruitment during the 1980's, before the WHO recommended supplementing with folic acid to prevent NTD's and before fortification programmes were put in place. Nevertheless, the majority of included evidence did follow current guidelines and is comparable and applicable to today. In some cases, generalisability is limited outside of the sample used or geographical area where the research was conducted, however the value of the evidence is not lessened and only adds to the external validity. These limitations were met and offset by the many strengths of the research projects due to the satisfactory and largely low risk of bias present in the articles.

2.4.14 Completeness and applicability of evidence

An extensive literature search with no publication year or language limitations identified studies conducted over the last 20 years, and recruitment of participants of included trials began as early as 1988. A combination of randomised and non-randomised experimental studies met the inclusion criteria each with their own strengths and limitations. The prospective cohort studies were typically large population-based studies and their longitudinal design meant the risks or benefits associated with a folic acid intervention could be assessed at regular intervals but were prone to bias and confounding (Sedgwick, 2013). In contrast, the design of an RCT study minimizes the effect of bias and confounding and is the gold standard for studying causal relationships. However, their applicability is limited due to low external validity (Dans *et al*., 1998). Despite their differences they can be used as complementary forms of research, helping the results of clinical trials translate into tangible benefits for the general population (Booth & Tannock, 2014).

In line with WHO recommendations all countries included in this review have policies in place for antenatal folic acid use alongside fortification in some countries. All included trials were conducted in high or middle (lower/ upper) income countries Maternal age was representative of women of child-bearing age (22-34y) and mean age was lower in populations with less economic growth. Children's developmental outcomes were assessed at various stages of development (1m-10y). Maternal and children's background characteristics were comprehensive, and populations were homogenous. In terms of the folate intervention, time of initiation ranged from preconception to 19GW, the stage of pregnancy when supplementation ceased also varied from the recommended 12GW to delivery and therefore the duration of the intervention differed between studies. This was to provide a complete picture of the impact of folate intake on child development at different stages of pregnancy and help determine if dose, time of initiation or duration of intervention individually affect development or if it is subject to a combination of all three factors.

2.4.15 Quality of the evidence

Quality in relation to research has two complementary aspects, methodological and reporting quality (Harrison *et al*., 2017). Methodological quality considers a study's design, conduct and analysis and is an integral factor to promote confidence in its findings and help understand the results. Reporting quality describes how well a scientific article is written. Paying careful consideration to the information provided and would it allow others to replicate the study. Poor reporting quality is common (Pocock *et al.*, 2004) and can make assessing the methodological quality difficult (Chan & Altman, 2005). Various approaches have been proposed and tools developed to improve the 'quality' of research including those used in this review.

The methodological quality of the evidence was assessed using the CASP checklist and all articles were found to be of satisfactory or high quality. Risk of bias was measured using the NICE assessment tool. Most of the studies did not report the information required to accurately evaluate the risk of detection or performance bias in both cohort and RCT designs. Selection bias was considered low in cohort studies but much of this information was not reported in the RCT's and the risk of attrition bias was low in both cohort studies and RCT's. As many of these trials were follow-up investigations and established datasets it is possible the information was presented elsewhere. Each item was marked as either present or not if the information was explicitly stated in the article. Effort was made to source this information but finding it in some cases proved difficult.

In terms of reporting quality, most articles appeared to be accurate and thorough however achieving high reporting quality in longitudinal research is difficult as there are no standard reporting guidelines to assist authors (Tooth *et al*., 2005). As all of the included studies were longitudinal in design it is possible the quality of reporting was low which can lead to misinterpretation of the results. Transparency was another issue highlighted during the risk of bias assessment. Many details were not reported or reported elsewhere but this was attributed to the follow-up design of large established datasets.

2.4.16 Strengths, limitations and potential biases in the review process

A strength of this review was its objectivity, clearly defined aims and objectives and explicit inclusion and exclusion criteria. The aim of this thesis and systematic review was to investigate how maternal folic use impacts on child development therefore the

review team listed MV containing folic acid as a criterion for exclusion. This could be considered problematic by some due to the large numbers of women who supplement with MV during pregnancy, particularly when MV use such as Pregnacare is being advised by the Department of Health and antenatal teams across the UK. However, the biological processes and interaction effects of nutrients are complex, it is difficult to determine which outcomes are related to which nutrients and for now, the interests of this research lies in the effects of folic acid alone on developmental outcomes and identifying causal relationships between exposure and outcome. When a better understanding is gained between individual relationships then research can progress to consider how nutrients interact and the result on child development.

Ahn and Kang (2018) describe a systematic review is an "objective, reproducible method to find answers to a certain research question by collecting all available studies related to that question and reviewing and analysing their results" (p. 104). This systematic review followed Cochrane review guidelines and principles to reduce the likelihood of bias as much as possible throughout the review process. In order to yield reliable results each included article was subject to a rigorous quality and risk of bias assessment. Inclusions of low quality, at risk of bias or improperly assessed studies could provide misleading results and lead to inappropriate or incorrect conclusions being drawn.

In order to improve the reporting quality of systematic reviews and meta-analyses in 2009 the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement was published (Liberati *et al.,* 2009) which aims to standardise

review processes and reporting (Willis & Quigley, 2011) and is particularly useful for evaluating interventions. During planning certain parameters central to evidence-based research are required for study selection, these include Population, Intervention, Comparison, Outcomes and Study Design (PICOS). This review examined the effects of folic acid as an intervention in evidence-based research and to ensure quality this review adhered to both the PICOS parameters and PRISMA framework utilising the checklist (Appendix 2.3) and flow diagram (*Figure 2*) as recommended (Page *et al.*, 2021).

A scoping search identified a notable lack of research in the areas of social, emotional, behavioural, motor and language development. It is understood that all areas of children's development are linked (National Scientific Council on the Developing Child, 2016) and occur simultaneously as part of an intricate network therefore broadening the search to include cognitive development at this early stage of the review process was justified. The research question was formulated based on the lack of clear evidence available but followed the principle of simultaneous development. Maternal folic acid use in pregnancy has been shown to be beneficial with some consistency to children's neurodevelopment (e.g. Bhate *et al.*, 2012; Chatzi *et al.*, 2012; Julvez *et al.*, 2009; del Rio Garcia *et al.*, 2009; Li *et al*, 2009; Gross *et al.*, 1974) and cognitive functioning (e.g. Villamor *et al.*, 2012; Veena *et al.*, 2010; Murphy *et al.*, 2007; Catena *et al.*, 2015), in theory we can then hypothesise that the other areas of development listed above would also experience a positive effect.

A detailed protocol was developed and approved by the research team prior to starting the review. This ensures the review process was transparent, reproducible and replicable (Moher *et al.*, 2015). The protocol guiding this review was not registered and although the integrity of the study was not affected it remains a limitation. Registering a protocol enables others to see ongoing research in their area of interest, the research plan is described in detail and in the event of a change those following the study can observe when, why and how the process was altered.

A broad and extensive literature search was conducted to source all published studies in relevant databases, in addition grey literature such as unpublished work including abstracts, ongoing research, studies awaiting publication and theses with no language or date limits. This was to ensure all relevant material meeting the inclusion criteria was being included to improve the accuracy of review results and to reduce the risk of publication and inherent bias. The difficulty with including unpublished work is that it has not been subject to peer review, however no unpublished research met the criteria for inclusion. Studies to be included in the review based on the inclusion/exclusion criteria were identified during the title and abstract review and later during the full-text review. Following the method described in the protocol these stages were completed independently by three members of the research team in order to maintain objectivity and transparency. An updated search was conducted before writing on this chapter commenced to check for and include all new research meeting the inclusion criteria. Similarly, the title, abstract and full-text reviews were completed independently by three reviewers. This adds further strength to this review however it is possible new evidence has emerged since the updated search took place in 2018.

After careful planning to eliminate as many potential biases and limitations as possible it is important that high quality studies are included for evaluation to obtain accurate results. Assessing their quality and risk of bias using valid and reliable tools is therefore imperative. The articles in this review were assessed using two tools, the Critical Appraisal Skills Programme (CASP, 2012) checklist to check study quality and National Institute for Health and Clinical Excellence (NICE, 2009) to gauge the risk of bias.

Three independent reviewers applied the CASP checklist to all included articles for inter-rater reliability, adding to the strength of this review. The JAMA 'Users' guides (Guyatt *et al.*, 1994) on which these checklists are based provide evidence based strategies to help clinicians interpret published material and integrate it into their patient care (Guyatt *et al.*, 2000) and therefore the basis of the CASP checklist is a strong one. These were designed to be used as educational tools in a workshop setting (Harrison *et al*., 2017) which is a potential limitation of using CASP to assess quality for this review. However, their purpose is to encourage subjective thought and facilitate discussion around methodological quality which proved useful in the research team.

The Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach (Guyatt *et al*., 2008) is considered the gold standard, used by Cochrane and informs NICE guidelines. Unfortunately, the application of GRADE was not possible for this review due to the inclusion of prospective cohort studies. Instead the NICE risk of bias tool was implemented by one reviewer, which could be considered a potential weakness. However, inter-rater reliability had been

established during CASP quality assessments, and the other two reviewers were kept informed throughout the process. This tool examined the risk of bias and internal validity present in a study in a quantifiable manner and was sufficient for the purposes of a narrative review. It is accepted that a certain degree of subjectivity is involved in implementing these tools however incorporating them in this review ensured a reproducible and transparent framework was being used for estimating the risk of bias in included articles.

Key data was extracted by one reviewer and extracted data was reviewed by the team. The difference in outcome variables, the use of different measures and different evaluation timepoints meant the data could not be combined for meta-analysis and was limited to a narrative systematic review (Egger *et al*., 1998). Extracting some important maternal and child background characteristics also proved difficult. The variables viewed as important differed across studies with some collecting limited information, potentially influencing the results.

As many of these study populations were already established it is possible that the range of data collected at the time of recruitment was broad. It is unclear how many of these studies were specifically designed to examine the impact of folic acid on child development. Moreover, a small number of studies failed to validate maternal folate levels using blood samples (6/19) which is a limitation. In the remaining studies blood samples were collected from either all women in the study or a sub-sample. These were collected either during pregnancy or from cord blood. In order to counteract the potential impact of this in the review process participant adherence was considered if reported. Another important weakness is the variability in folate

type (FA or DF), dose, time of initiation and duration of use across studies. It is difficult to ascertain from this review at what stage of gestation or for how long does FA affect development and what dose produces maximum benefit.

2.4.17 Implications for future research and practice

In recent years, researchers have begun to adopt a more positive approach to investigating the potential benefits of optimal maternal folate status on children's development. However, the literature is awash with the potential risks of inadequate use of the vitamin. This review is the first to the authors knowledge to examine the impact of folic acid or folate on children's psychological and socioemotional development, areas of development typically underrepresented in the literature. It is the first step in helping to balance the risks and benefits associated with FA use, essential in the decision making process for the development, updating and implementation of new guidelines for folic acid use during pregnancy.

This review highlighted issues associated with construct definitions and subsequent measurement. This made the review process more difficult and results were not easily synthesised or compared. Nevertheless, articles included in this review reported similar findings generally, with evidence to support mothers supplementing with an optimal dose of between 400-500µg/d with other doses having potentially a detrimental effect to children's development. Other important factors identified during this review was the importance of early initiation and a longer duration of use. Development begins at conception, occurring rapidly and exponentially through each of the trimesters. The research suggests that folate can support and possibly improve many aspects of development including physical health (NTD), brain

development, cognition, language and psychological development including social, emotional and behavioural development. For this reason supplementing for longer and from earlier in the pregnancy would be justified. Additionally, no risks have been identified for adequate and sufficient use of the vitamin during pregnancy for either mother or child however, problems associated with deficiency and overuse could become apparent as the child grows and develops. Included evidence would indicate that mothers would benefit from improved preconception care to increase the rates of uptake and adherence, particularly in the Northern Ireland population where rates considerably lower than the rest of the UK(PHE, 2020; Butriss, 2015). Although this review was broad in scope, it provided informative and meaningful results. Research now needs to focus, preforming reviews and meta-analyses on homogeneous studies to develop the literature from this positive perspective.

Further experimental research in the area is required using a combination of randomised and non-randomised designs due to the strengths and weaknesses already discussed. All evidence must be subject to review with the incorporation of a meta-analysis. To strengthen the review process utilising the GRADE system to assess quality of effectiveness would be beneficial as it is the only approach recognised by NICE to inform guidelines. Another improvement would be to review the impact of adequate and inadequate use separately and conduct two meta-analyses if the data permits. This current study combined all FA use which was difficult to synthesize in an applicable and meaningful way.

2.4.18 Conclusions

The results from the SR suggests that adequate and sufficient prenatal maternal folic acid use, particularly during early pregnancy could benefit children's cognitive, motor, psychomotor and language development. The evidence for social, emotional and behavioural development was less consistent demonstrating the need for further exploration. Studies included in this review indicate that sufficient folic acid supplementation during pregnancy can protect toddlers against behaviour and emotional problems (2, 3) and improve cognitive (10) and executive function in older children (16). Infant psychomotor and mental development could also benefit from sufficient use (8). Adequate use in late pregnancy (>19GW) did not appear to offer any additional benefit to children in two studies (14, 17), however, one study (10) did report a beneficial effect when mothers supplemented after 30GW. Sufficient folic acid use could also have a positive impact on children's mental development but only in those who are genetically susceptible, born to mothers carrying the *TT* genotype (8). This finding was not supported by other studies (3) but further investigation is required.

Evidence suggests that optimal benefit for children could be dose dependent. Two articles reported the positive effects of a small increase in folic acid dose (1, 4) however consuming more that the tolerable level (>1000µg/d) is classed as excessive use and could damage the long-term health and development of the child (12, 15). Furthermore, using folic acid in conjunction with a multivitamin may help support development in the short-term (18) but these effects may not be lasting (19). This was not the case in the development of children's language where the positive effects

were markedly better in those who consumed folic acid only when compared to multivitamin use (13).

This is an important area of research as healthy childhood development could promote positive mental health in later life and recognising how maternal nutrition and supplement use during pregnancy is an important step in improving public health. It is therefore important to determine if a critical window of exposure exists and when that might be. Other important factors such as dose, time of initiation and duration all need to be considered and tested under rigorous experimental conditions in order to identify causality. It is also possible that these factors can affect each area of development differently with certain conditions potentially being more important than others to a particular area of development. The combination of factors is still unknown to obtain the maximum positive impact on children's development.

Chapter 3

The impact of maternal folic acid supplementation during pregnancy on children's wellbeing and development: Secondary analysis of Avon Longitudinal Study of Parents and Children (ALSPAC) data.

3.1 Introduction

This chapter contains a secondary analysis of The Avon Longitudinal Study of Parents and Children (ALSPAC). The ALSPAC study is introduced in the first instance and includes information about the trial and a selection of research that has been derived from the data. This is followed by an overview of what and how ALSPAC data contributes to this thesis, leading to the research aim and hypotheses to be tested. The methodology section provides a summary of the data acquisition process, the variables selected for secondary data analysis including the selection process details and all relevant information relating to ALSPAC design, recruitment, sample, intervention and outcome measures. The results section presents the findings in relation to the hypotheses being tested followed by a discussion of the findings which includes several strengths and limitations of both this current study and the ALSPAC study. The chapter concludes with a short summary.

3.1.1 Background to the ALSPAC study

'The Avon Longitudinal Study of Parents and Children' (ALSPAC), known to its participants as 'Children of the 90's', was a transgenerational prospective

observational study exploring different influences on health and development across the life course (Boyd *et al*., 2013). This population-based cohort study with methodology designed by Professor Jean Golding at Bristol University, UK (Golding, 1989), was one of seven independent studies contributing to the 'European Longitudinal Study of Pregnancy and Childhood' (ELSPAC) initiated by the World Health Organisation (WHO) in 1985 (ELSPAC, 2019). The aim of which was to recruit children across Europe from centres based in Avon, Isle of Man, Czech Republic, Slovakia, Ukraine, Greece and Russia to explore the effects of biological, genetic, epigenetic, psychosocial, economic and environmental factors on pregnancy, delivery and later development and health of the children, mothers and family units as a whole.

During the study's infancy the ALSPAC acronym represented the 'Avon Longitudinal Study of Pregnancy and Children' which reflected the focus of the original study. As ALSPAC evolved and data collection continued into and throughout childhood the study was renamed 'The Avon Longitudinal Study of Parents and Children' to indicate the importance of continued parental involvement in conjunction the children. The expanse and depth of both biological and behavioural data for the quantity of participants is unrivalled and has inspired and guided the design and implementation of other birth cohorts such as the MoBa study which is tracking over 100,000 participants in Norway (Norwegian Institute of Public Health, 2016). Additionally, data linkage has also been completed for education, death and cancer records, clinical practice research databases and other hospital statistics including health care provider records. Linkage to other resources

such as primary health information, employment and social records held electronically is ongoing (Boyd *et al.*, 2013).

ALSPAC is a large and dynamic cohort, followed intensively now for over 20 years and is world-leading in its detail and richness for investigating environmental and genetic factors that can affect health and development. Researchers in various disciplines including health, education and social sciences have access to a wealth of data, and through research the aim of the project is to inform policy and practice to provide a better life for future generations. 'Children of the 90's' participants are now young adults and due to the transgenerational and family-based nature of the project the intention is for the follow-up to be long-term, even lifelong, and consolidated through the 'Focus on Mothers' and 'Focus on Fathers' studies. In order to enhance the projects capability to understand the transmission of health and wellbeing through generations, approval was recently obtained for a follow-up of the next ALSPAC generation (ALSPAC-G2) known as 'Children of Children of the 90's' (COCO90's) which will provide information for three generations and is the first of its kind. The UK Medical Research Council (MRC), the Wellcome Trust and the University of Bristol have provided the core funding and support for ALSPAC since 2000. Early funding for the project was obtained from various sources including WHO, UK based Research Councils, Government Departments, charities and several American funders (ALSPAC, n.d).

3.1.2 Notable findings from ALSPAC

Since 1990 the results from ALSPAC have generated more than 1,500 scientific publications, details and links to which can be found on the study website (ALSPAC,

n.d). In 2010, Golding published a summary of some of the key findings many of which have been used to inform health and social policy. One of these key pieces of research was the ‘Back to Sleep’ policy change, where ALSPAC data provided evidence, reassuring health professionals and policymakers to recommend mothers place babies on their back while sleeping to reduce the risk of cot death and while not increasing the risk of any developmental delay (Hunt *et al*., 1997; Dewey *et al*., 1998). ALSPAC findings have further influenced UK and US guidelines by demonstrating that oily fish consumption during pregnancy was associated with benefits to child behaviour and verbal IQ (Hibbeln *et al*., 2007), early development (Daniels *et al*., 2004) and visual stereoacuity (Williams *et al*., 2001). Additionally, ALSPAC research identified that the application of peanut oil-based skin creams to broken skin sensitized children to peanut allergy (Lack *et al*., 2003; Khakoo & Lack, 2004), an important revelation due to the emerging epidemic of peanut allergy in Western countries. This association prompted manufacturers to alter the composition of baby lotions and to identify the ingredients used on labels.

More recently, investigations have recognized the influence of particular genetic factors on eczema (Henderson *et al*., 2008) and obesity (Frayling *et al*., 2007; Timpson *et al*., 2008). While other studies have explored how environmental factors such as prenatal maternal anxiety, paracetamol use, cleaning products and excessive hygiene can influence the development of child asthma (Cookson *et al*., 2009; Shaheen *et al*., 2002; Shaheen *et al*., 2005; Sherriff *et al*., 2005; Henderson *et al*., 2008; Sherriff & Golding, 2002). Research derived from ALSPAC also associated maternal weight during pregnancy with child adiposity and cardiovascular risk factors (Fraser *et al*., 2010) but identified that maternal age, diet and smoking did not

contribute to the child's blood pressure (Leary *et al*., 2005; Roberts *et al*., 2005; Brion *et al*., 2007; Brion *et al*., 2008).

3.1.3 ALSPAC measures of interest applicable to this study

The short research summary outlined above illustrates the vast data collected and stored by ALSPAC researchers and its applicability to current health and social research. Since its inception ALSPAC researchers have collected an extensive range of data and samples from the mother, her partner and child including phenotype, genetic and biological samples and environmental, health and wellbeing data and were collected at multiple time points through both self-report and clinical assessments. The method of data collection was determined by type of data and when it was collected. Only a small selection of data were relevant to this study (listed in *Appendix 3.1, Table 1*) and typically were collected using self-report, parental proxy measures or clinical assessments.

Self-report measures were used to collect information on maternal and child background characteristics including but not limited to child sex, birthweight, head circumference at birth, breastfeeding status, height, weight and waist circumference at 10 years, folic acid use in early and late pregnancy, mother's pre-pregnancy age, weight and height, delivery type, SES, lifestyle factors, parenting style and family size. Outcome measures provided by proxy or self-reported by children included motor development, social development, cognitive development, communication and difficult behaviours.

Alternatively children's anthropometric measurements (length/ height, weight, waist and head circumferences), hearing and vision tests, blood samples and video recorded parenting observations were taken and children's cognition was assessed during 'Children in Focus' (CiF) clinical assessments. During these sessions children provided data by answering important clinical questions and providing validation for self-report responses in a controlled setting. A 10% random subsample of all ALSPAC infants were invited to the four initial CiF clinical assessments and this information was included in the current analysis. Clinical outcome variables relevant to this study and any scales used are listed in *Appendix 3.1, Table 2.* This selection and availability of ALSPAC data demonstrates its suitability for secondary data analysis in the context of maternal folic acid use and children's cognitive, psychological, social, emotional, behavioural and language development.

There were 68 data collection time points between birth and 18 years which included 25 questionnaires about the child completed by the mother (MCQ), 34 children completed questionnaires (CCQ) and 9 'CiF' clinical assessments. Each assessment fell into a specific growth phase; infancy (>4 weeks <2 years), early childhood (>2 years <7 years), childhood (~7 years), late childhood (>7 years <13 years), adolescence (>13 years <16 years) and transition to adulthood (>16 years and <18 years). Furthermore, the schools attended by these children were given questionnaires, completed by the child's teacher who also provided information on educational performance. For the purposes of this study only the questionnaires, clinical assessments and educational data during pregnancy, infancy and early childhood (up to 3y) were considered relevant.

3.1.4 The potential contribution of ALSPAC to this PhD

As highlighted in the systematic review presented in *Chapter 2,* few studies have examined the association between maternal folic acid use during pregnancy and its beneficial effect on children's cognitive, psychological, social, emotional, behavioural and language development. Of the few that are available, results show that sufficient and adequate folic acid use has a positive impact on children's development, with further investigation using more rigorous methods recommended. ALSPAC is a large prospective population-based cohort study based in the UK and designed to explore genetic and environmental factors that affect the health and development of both mothers and children. A mixed methods approach was applied using a combination of quantitative surveys, clinical assessments and biological measures completed and provided by participants every six months for approximately 20 years to date. Information on maternal folic acid use in both early and late pregnancy was available, and a wide range of psychological measures were completed by both parents on behalf of their children and by the children themselves via self-report and clinical assessments at various ages and developmental stages. These included measures of children's cognitive, psychological, social, emotional, behavioural and language development. Additionally, information on a vast number of potential confounders were also collected which allows for a more controlled statistical analysis. Secondary data analysis of ALSPAC data adds significantly to the folic acid literature and provides context and justification for further analysis using more rigorous designs such as RCT.

3.1.5 Research Aim

The aim of this study was to conduct a secondary data analysis on ALSPAC data to investigate if sufficient maternal folic acid use (400µg/d) in late pregnancy (32GW) produced any additional benefits to children's cognitive, motor, social, emotional, behavioural and language development in comparison to mothers who were considered folate deficient at 32GW. Existing research in the area, included in the systematic review, informed the constructs and variables chosen for investigation including cognitive, psychological, social, emotional, behavioural and language development. For this investigation mother participants from the ALSPAC study were grouped as either folate sufficient (>400µg/d) or folate deficient (<400µg/d) depending on their reported FA use at 32GW. The children of these mothers could then be compared by developmental outcome at 3y to examine if 3-year old children only experience developmental benefits from sufficient maternal use of FA (>400µg/d) during late pregnancy or could any level of FA intake provide a benefit even if classified with either mild (300-399µg/d) or moderate (<299µg/d) folate deficiency.

The focus on the detrimental effects of no folic acid use or folate deficiency on child development has resulted in little investigation into the potential benefits of supplementation for both mothers and children therefore these findings will be a useful addition to the ALSPAC research bank.

3.1.6 Research objectives and hypotheses

The beneficial effect of sufficient maternal folate levels in late pregnancy (32GW) on children's cognitive, psychological, social, emotional, behavioural and language

developmental outcome in the ALSPAC sample was assessed using three comparisons.

1. Sufficient (>400µg/d) vs deficient (<400µg/d) levels of folate at 32GW
2. Sufficient (>400µg/d) vs mildly deficient (300-399µg/d) levels of folate at 32GW
3. Mildly deficient (300-399µg/d) vs moderately deficient (<299µg/d) levels of folate at 32GW

The hypotheses to be tested using ALSPAC data are as follows:

1. Children born to mothers who report taking sufficient levels of folic acid in late gestation will obtain higher scores of IQ, social development, motor development, resilience, prosocial behaviour and language and have lower instances of behavioural problems at 3 years old in comparison to children born to mothers who reported taking insufficient levels of folic acid at 32GW.
2. Children whose mothers were folate sufficient at 32GW would achieve better scores in IQ, social development, motor development, resilience and language and have less behavioural problems than children whose mothers reported mild or moderate deficiency at aged 3 years. Furthermore, children whose mothers reported being mildly deficient would score better in these developmental areas than children whose mothers were moderately deficient.

3.2 Methodology

3.2.1 Data acquisition

A detailed proposal was developed and submitted to ALSPAC for approval. This included relevant background to the study, aims and objectives including anticipated outcomes, proposed methods and overview of statistical methods, exposures, outcomes and confounders to be considered and justified as necessary, reasons for using ALSPAC and likely impact of the research. Once approved, access to the ALSPAC data catalogue was provided for variable selection and costing. Each variable was carefully selected by the author (LH) and the de-identified raw data was delivered in a built SPSS file. The dataset consisted of 1118 variables in total for 15445 participants and required extensive cleaning before analysis could begin. Data was carefully managed throughout the project in line with ALSPAC and Ulster University regulations.

3.2.2 ALSPAC Recruitment

ALSPAC recruitment used a convenience sampling method and aimed to enrol women as early in pregnancy as possible. Contact was made with eligible women through poster displays in various locations, considerable media coverage and recruitment staff visiting various community locations to encourage participation. Routine antenatal and maternity services were also used to promote the study and to distribute ‘expression of interest’ cards. Women returning the card could request further information on the study or decline participation. Completed cards contained sufficient information to allow researchers to determine eligibility (information included name, address, DOB, her last menstrual date and expected delivery date). Study consent was ‘opt-out’ therefore women who returned a completed card would

be included in future data collection follow-up until they declined participation. Those who requested further information were sent a study brochure and provided a telephone number if parents had a query or requested help. Participants recruited during Phases II and III were not included for reasons stated earlier in the chapter. See *Figure 3.1* for an illustration of ALSPAC recruitment.

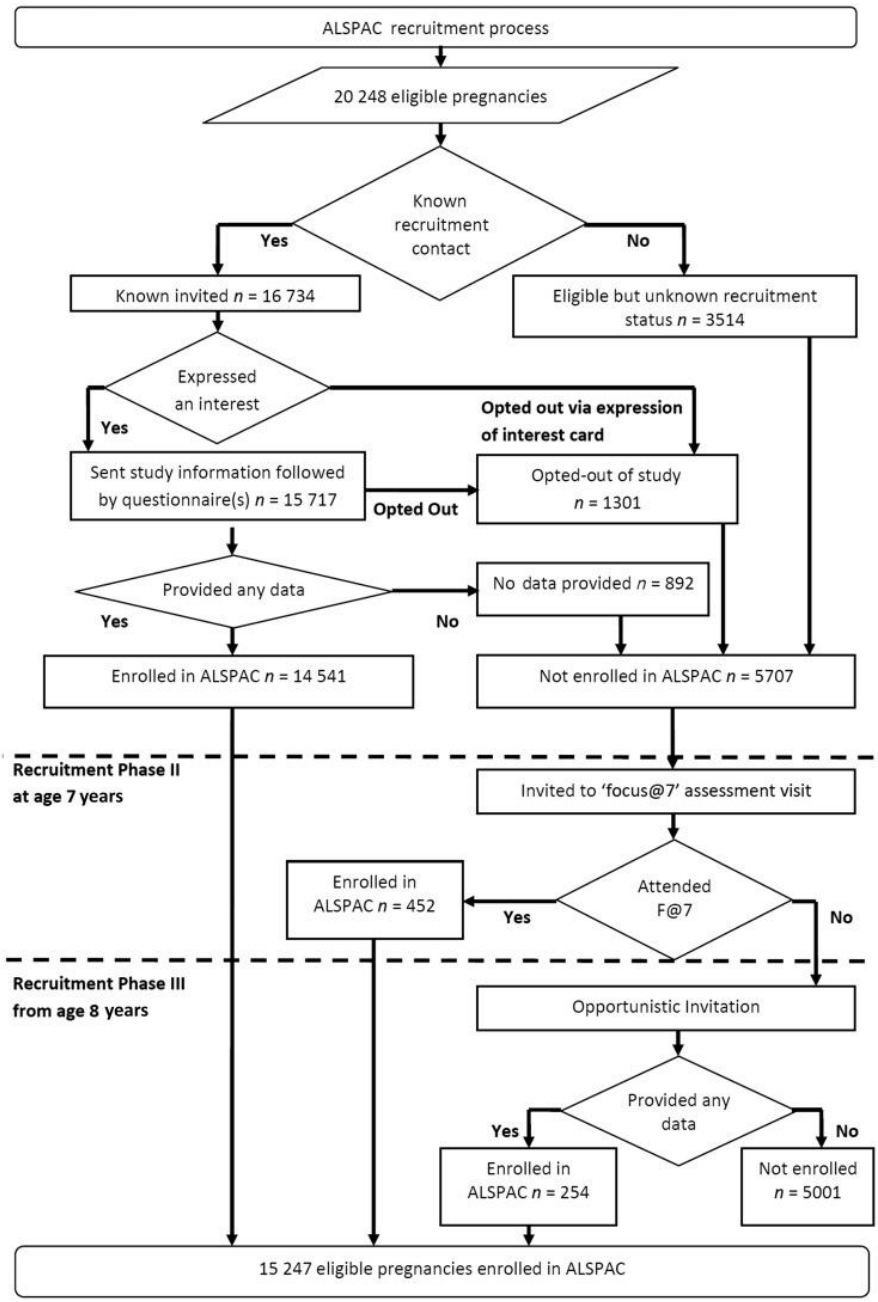
3.2.3 Participant Information

The ALSPAC eligible study area centred on the city of Bristol and County of Avon in the South-West of England, which was comprised of three distinct NHS Health District Authorities; Southmead, Frenchay and Bristol and Weston. At the time of recruitment this catchment area (population of ~0.9 million) included the City of Bristol (~0.5 million) and surrounding urban and rural areas. All pregnant women living in these areas with an estimated delivery date between 1st April 1991 and 31st December 1992 inclusive were able to participate, as was each child born from these pregnancies. Any pregnant women who moved out of the catchment area remained eligible for inclusion if they had completed the 32GW questionnaire otherwise they were excluded.

3.2.4 Recruitment Rates

The cohort was a large and dynamic population and was defined retrospectively by comparing ALSPAC recruitment records with maternity, birth and child health records (Boyd *et al*., 2011). It is unknown how many eligible participants were missed during recruitment or were non-responders during Phase I. The number of women declining using the expression of interest cards is known however the *Figure*

3.1: ALSPAC recruitment flow diagram detailing Phases I, II and II of enrolment. Extracted from (Boyd et al., 2013)



number of cards distributed is unknown. The number of individuals invited during recruitment Phases II and III is also unknown. The recruitment rates for enrolled and non-enrolled for both pregnancies and children are outlined in *Table 3.1*.

Table 3.1: *Recruitment rates and pregnancy outcome for core pregnancies (Phase I), total pregnancies enrolled (Phases I-III) and non-enrolled pregnancies*

	ALSPAC Core Pregnancies (*n*=14,541)	**Total Enrolled Pregnancies (*n*=15,247)**	**Non-Enrolled Pregnancies (*n*=5,001)**
Eligible pregnancies (n=20,248)			
Pregnancy outcome (n)			
Singleton	14 273	14 971	4909
Twins	195	204	43
Triplets	3	3	1
Quads	1	1	0
No known outcome	68	68	48
Eligible children/ foetuses (n=20,390)			
Child outcome (n)			
Fetal loss <20GW	547	547	138
Fetal death >20GW	67	67	37
Neonatal death <7days	45	45	11
Neonatal death 7-27 days	8	8	2
Post-neonatal death >28 days	21	21	15
Alive >1 year	13 988	14 701	4797
Total	14 676	15 390	5000

The number of eligible pregnancies and foetuses recorded was 20,248 and 20,390 respectively. A core sample of 14,541 mothers (71.8%) enrolled during Phase I. Of these pregnancies 68 had no known outcome, 199 were multiple birth pregnancies, and the remaining 14,273 were singleton pregnancies. This resulted in 14,676 known children and of these 14,062 were live births, 13,988 were still alive at 1 year old. Phase II (~7y) provided a non-core sample of 456 children and Phase III (8-18y) added a further 257 children giving a total of 15,247 (75.3%) pregnancies and 14,775 (75.7% of eligible sample) live-born children of which 14,701 were alive at 1 year

old. Those who were deemed eligible remained so regardless of participation history or migration from study area; invitations to contribute to questionnaires and assessments were sent worldwide. Those who withdrew were still eligible and able to re-enrol at any time.

3.2.5 Outcomes of Interest

The ALSPAC data catalogue containing 59,608 variables was searched by the author in May 2017 for measures of children's cognitive, psychological, social, emotional, behavioural and language development when children were approximately 3 years old. A total of 40 variables were obtained from ALSPAC and used during this analysis, these included 22 outcome variables (*Appendix 3.1, Table 1*) and 18 background variables (*Appendix 3.1, Table 2*).

3.2.6 Questionnaire administration during pregnancy

Four questionnaires were administered to mother participants during pregnancy and the one she received depended on her gestational week at the time of enrolment. Two of these were sent at a fixed time point - 'Having a Baby' (B) at 18GW and 'Your Pregnancy' (C) at 32GW. If the mother enrolled before 14GW then 'Your Environment' (A) was sent immediately after enrolment and was designed to identify particular features of early environment that could be responsible for effects on the foetus. The fourth questionnaire 'About Yourself' (D) documented details on the mother's past medical, social, and environmental history and consequently the timing of administration is relatively unimportant. If necessary, this questionnaire was sent out after the baby had been born. If mothers had not responded within 7

days a reminder letter was sent and after a further 10 days of no response a second reminder was sent. If no response was received after 4-weeks then a researcher made contact and encouraged or helped the mother to complete the questionnaire.

A questionnaire titled ‘Your Home & Lifestyle’ was developed for mothers who enrolled long after 18GW and was a combination of ‘Your Environment’ and ‘Having and Baby’. Many of the original items concerned with attitudes, activities and emotional wellbeing at that particular point in pregnancy were invalid however a certain amount of information concerning their environment and lifestyle was validly collected. There were some instances that mothers did not receive the questionnaire at 32GW which asked for information on ethnicity, education, social and occupation levels and early sexual experiences. These items non-specific to the third trimester were included in the ‘Filling the Gaps’ questionnaire and administered to these mothers when the child was 12 months. For details on questionnaire content see *Appendix 3.2, Tables 1-5*.

3.2.7 Infancy measures

Maternity services or parents notified the study centre of the child’s DOB, birthweight, sex and singleton or multiple birth pregnancy. This information was entered into the database and determined when mothers, partners and children were sent questionnaires and invited for assessments. In the occurrence of multiple births, a child specific questionnaire was sent out for each child. *Table 3.3* below lists the questionnaires relevant to the thesis.

Table 3.2: Questionnaires completed by mother participants from their children's birth to ~4years old

Time-point	Questionnaire type	Filename	Questionnaire Title
4 weeks	Child specific	KA	My Young Baby Boy/ My Young Baby Girl
8 weeks	Mother and partner	E	Me and My Baby
6 months	Child specific	KB	My Son/ My Daughter
8 months	Mother and partner	F	Looking After the Baby
21 months	Mother and partner	G	Caring for a Toddler
30 months	Child specific	KF	My Study son/ Daughter
38 months	Child specific	KG	My Three Year Old Girl/ Boy
42 months	Child specific	KJ	My Son/ Daughter's Health and Behaviour

Mothers received the 'Me and My Baby' (E) questionnaire 8 weeks after the birth of the child. A total of 11,689 (83.5%) questionnaires were completed and returned. 'Looking after the Baby' (F) was sent to mothers approximately 8 months after the child was born. A total of 11,213 (80.1%) were completed and returned. Mothers received 'Caring for a Toddler' (G) when the child was 21 months. A total of 10,323 (74%) questionnaires were completed and returned. Mothers were invited to return blank questionnaires if they did not wish to participate in the corresponding follow-up. Information on included content can be seen in *Appendix 3.2, Tables 6-8.*

A number of child specific questionnaires were also completed by proxy. These included the 'My Young Baby Boy/ Girl' questionnaire (KA) was administered to the mother about 4 weeks after delivery and was to be completed if the baby was home or in the Special Care Baby Unit. A total of 12,344 (89.14%) were returned to the study centre. 'My Son/ My Daughter' (KB) was sent to mothers 6 months after the child was born, 11,478 (82.89%) were completed and returned. Mothers received the 'My Study son/ Daughter' (KF) questionnaire when the child was 30 months

(2.5y), 10,340 (75.47%) were returned to the centre. ‘My Three Year Old Girl/ Boy’ (KG) administered at 38 months (3y), 10,137 (73.99%) questionnaires were completed. ‘My Son/ Daughter’s Health and Behaviour’ (KJ) sent out at 42 months (3.5y), 10,053 (73.38%) were completed and returned. Mothers could participate in all or some of follow-up investigations, if mothers had not responded or returned their blank questionnaire within 3 weeks a reminder letter was sent. After a further 3 weeks of no response a second reminder was sent. If no response was received after 3 weeks, then the case was flagged as being eligible for a visit by a member of the research team. For full details on questionnaire content see *Appendix 3.2, Tables 10-14.*

3.2.8 Developmental measures considered relevant for secondary data analysis

Children’s development was measured using a variety of scales applicable to the sample and outcomes of interest for this study. Specific outcomes of interest included cognitive, social, motor and language development, resilience and behavioural difficulties in children ~3y. The scales measuring each of these developmental outcomes are each discussed below.

3.2.9 WPPSI-R

Children’s cognitive ability was assessed using the Wechsler Preschool and Primary Scale of Intelligence-Revised (WPPSI-R; Wechsler, 1989) when ALSPAC participants were ~4y. This scale which was designed for use with children aged 3y to 7y 3m and measures global or Full-Scale IQ by combining scores from Verbal and Performance scales. Full-Scale IQ provides the most reliable and is most

representative of general intellectual functioning. Verbal IQ relates to acquired knowledge, verbal reasoning and comprehension, and attention to verbal stimuli. Performance IQ on the other hand, assesses fluid reasoning, spatial processing, attention to detail and visual-motor skills. The Verbal and Performance IQ subtests are listed in *Table 3.4* below. Authors stipulated that all scales were administered to ALSPAC participants except the two optional tests; not included in this list.

Table 3.3: The WPPSI-R scales and sub-tests which provided a measure of cognitive ability in 4y ALSPAC participants

Scale and dimensions	**Sub-tests**
WPPSI-R	
Verbal IQ	Comprehension
	Arithmetic
	Vocabulary
	Similarities
	Sentences
Performance IQ	Object Assembly
	Block Design
	Mazes
	Picture Completion
	Animal Pegs
Full-scale IQ	Scores of all 10 sub-tests are combined

Each of the three scales are standard scores with the mean set at 100 and SD set at 15 with a possible score range of 41-160 for Full-Scale IQ (Colom *et al*., 2004). The WPPSI-R is reported to be a highly reliable and valid measure with a large normative sample based on U.S. census data (Beres *et al*., 2007), and widely used worldwide in clinical and educational settings.

3.2.10 Milestones Survey – developed for ALSPAC use

This proxy measure was completed by ALSPAC mothers on behalf of their children at 2.6y and 3.6y. The Milestones survey was adapted from the Denver Developmental Screening Test (DDST) (Frankenburg & Dodds, 1967) and designed by ALSPAC researchers for use in the cohort. A total development score was provided through the measurement of fine and gross motor development (example questions provided in *Table 3.5*) and social and language development by considering children's social and communication skills (see *Table 3.6*). The full scales containing all included items are listed in *Appendix 3.2.*

Table 3.4: Scales and individual dimensions that measured motor development in ALSPAC

Scale and dimensions	**Example**
Milestones (2.6y)	
Fine motor	Can copy a circle and draw it more or less
	Will turn pages of a book
	Can thread beads on a string
Gross motor	Can balance on one foot for at least 1 second
	Can walk on tiptoes
	Can hop
Milestones (3.6y)	
Fine motor	Can copy a square and draw it more or less
	Can undo/fasten big buttons
	Can build a tower or 8 bricks
Gross motor	Can walk up steps – one foot on each step
	Can balance on one foot for at least 4 seconds
	Can jump over an obstacle (e.g. toys on floor)
EAS Temperament Scale	
Activity (3.2y)	When child moves about, he/she moves slowly
	Is always on the go

Table 3.5: Scales and individual dimensions that measured social development in ALSPAC

Scale and dimensions	Example
Milestones (2.6y)	
Social development	Is able to drink from a cup
	Indicates what he/she wants without crying for it
	Can take clothes off with help
Milestones (3.6y)	
Social development	Is able to drink from a cup without spilling it
	Asks for what he/she wants without crying for it
	Can get dressed without help
EAS Temperament Scale	
Sociability	Likes to be with people
	Finds people more stimulating than anything else
Shyness	Makes friends easily
	Takes a long time to warm up to strangers

3.2.11 MacArthur CDI

ALSPAC mothers also completed proxy measures to assess their children's language development at 3.2y. An adaptation of the MacArthur Communicative Development Inventories (CDIs) was developed for ALSPAC researchers to use specifically for this sample. CDIs were originally developed as cost-efficient but effective instruments to assess a range of communicative skills in infants and toddlers (Fenson *et al.*, 2000), utilising the parents experience of their children's language. CDIs are some of the most widely used tools which evaluate early language development (Mayor & Mani, 2018). The form was developed for children aged 16-30 months and contains 100 items of vocabulary and mothers were asked to mark the words that their child has a sound understanding of, says or both (example words are provided in *Table 3.7*). Mothers were also asked if their children use verbs, plurals and past tense and if so, how often on a 3-point scale ('not yet' 'sometimes', 'often').

Table 3.6: Scales and individual dimensions that measured language development in ALSPAC

Scale and dimensions	Example	
McArthur Communicative Development Inventories (CDIs) (3.2y)		
Saying	Hello	Coat
	Bear/Teddy	Strawberry
Understanding	Nose	Fork
	Door	Star
	Turn on/off	Farm
ALSPAC Talking and Listening		
Intelligibility	Can you/ your family understand what your child says?	
Language	Use of vocabulary, plurals, past tense, word combinations	
Communicative	Does your child talk a lot, stay mainly silent, avoid looking at people's faces when talking or echo what has been said to them?	

3.2.12 Talking and Listening – developed for ALSPAC use

ALSPAC researchers developed a list of 13 questions which gathered information on children's talking and listening ability. These questions were completed by mothers when children were 3.2y who responded using a 3-point scale ('no', 'sometimes', 'often'). The dimensions measured and example questions are provided in *Table 3.7*.

3.2.13 EAS Temperament Scale

The temperament of ALSPAC children was assessed at 3.2y using the EAS Temperament Survey for Children: Parental Ratings (Buss & Plomin, 1984). This scale was designed for use with children aged 1-9 years and measures four dimensions of temperament including Emotionality (*Table 3.8*), Activity (*Table 3.5*), Sociability and Shyness (*Table 3.6*), and is comprised of 20 items, 5 items per temperament dimension and parents responded on a 5-point scale ((1)'not at all like

my child' to (5) 'exactly like my child'). This scale is reported to have moderately high levels of reliability and stability (Mathiesen & Tambs, 1999).

Table 3.7: *Scales and individual dimensions that measured emotional development in ALSPAC*

Scale and dimensions	**Example**
EAS Temperament Scale	
Emotionality	Cries easily
	Reacts intensely when upset

3.2.14. The Revised Rutter Parent Scale for Preschool Children

The Revised Rutter Parent Scale for Preschool Children are established scales to assess behaviour difficulties in children (Rutter *et al*., 1970). The measure consists of two scales, Scale A, the parental questionnaire and Scale B, the teachers questionnaire which assesses four dimensions of behaviour; emotional, conduct and hyperactive difficulties and prosocial behaviour. Mothers completed the parental questionnaire consisting of 31 behaviour descriptions. Mothers were required to indicate on a 3-point scale the degree to which their child displayed the listed behaviours (1 does not apply to 3 definitely applies). This measure was originally developed for 9-13 year old children (Elander & Rutter, 1996) but valid for children aged 6-16y. Ketende and Jones (2011) cited acceptable inter-rater (r = 0.64) and retest reliability (r =0.74), albeit lower than the teachers scale (Rutter *et al*, 1970).

3.2.15 'Focus' Assessments

Children were invited to attend various clinical assessments. As discussed earlier in the chapter, a 10% random subsample was used in the CiF assessments. A total of

1,241 children (8.96% of those eligible for follow-up) attended CiF at 12 months and 1,032 (7.53%) participated at 49 months

Table 3.8: Scales and individual dimensions that measured behavioural development in ALSPAC

Scale and dimensions	Example
Revised Rutter Parent Scale for Preschool Children	
Emotional difficulties	Is worried or worries about many things
	Gives up easily
Conduct difficulties	Destroys own or other's belongings
	Fights with other children
Hyperactivity difficulties	Has poor concentration or attention span
	Is restless, runs about or jumps up and down
Prosocial behaviour	Is considerate of other people's feelings
	Is kind to animals

3.2.16 Intervention details

At approximately 18GW mothers were asked to report if they had been taking folic acid/ folate during their pregnancy and later at 32GW mothers were asked to report if they had taken folic acid/ folate in the past 3 months. No information was available on when supplementation was initiated by the mother (preconception or postconception) or if FA was taken via multivitamin. Current analysis compared folate sufficient (>400µg) and deficient (<400µg) mothers. Plasma folate samples were not available at the time of analysis therefore folate level in the blood was unable to be confirmed at this stage. Biological data is in the process of being catalogued by ALSPAC researchers and will be available in the future.

3.2.17 Statistical Analysis

Data was accessed through IBM SPSS Statistics (Version 25), cleaned and coded. Normality of participant background information and psychological outcomes were assessed and did not meet the assumptions for parametric tests therefore non-parametric alternatives were utilised. Chi-square was used to test for significant differences between categorical background characteristics and a combination of Independent Samples Mann-Whitney U tests and Kruskal-Wallis tests were used to check for significant differences in all scale variables including background characteristics and developmental outcomes between those who were folate sufficient and insufficient at 32GW. For further analysis considering the impact of folate deficiency level at 32GW mothers who were folate deficient were categorised into two groups: mild or moderate deficiency at 32GW.

3.3 Results

Results of this secondary data analysis are presented as they are listed in the Research objectives and hypotheses section earlier in this chapter. To address hypothesis 1, mother-child pairs were grouped by folate status; FA sufficient (>400µg/d) vs. FA deficient (<400µg/d) at 32GW. When considering hypothesis 2, the level of deficiency (<299µg/d or 300-399 µg/d) in comparison to FA sufficient (>400 µg/d) at 32GW on child development outcomes at 3y was examined.

3.3.1 Maternal folate status at 32GW: FA sufficient vs FA deficient

Of the 14,541 Phase I participants, a total of 8494 child participants and 241 mothers (Mean age, 28.29 SD; 5.15) were considered folic acid sufficient with an intake of more than 400µg/d at 32GW whereas 8540 mothers (Mean age; 28.50 SD; 4.75) were folic acid deficient with an intake of less than 400µg/d at 32GW. Other relevant background characteristics were evaluated and are listed in *Table 3.10*.

Table 3.9: *General characteristics of Phase I ALSPAC participants who were folate sufficient (>400) and folate deficient (<400) at 32GW.*

	FA Sufficient at 32GW (>400 µg/d)	**FA deficient at 32GW (<400 µg/d)**
Maternal characteristics		
Age at birth (y)	28.29 ± 5.15	28.50 ± 4.75
Pre-pregnancy weight (kg)	60.18 ± 9.67	61.65 ± 10.82*
Height (cm)	164.56 ± 6.92	164.06 ± 6.71
BMI (kg/cm^2)	22.23 ± 3.40	22.90 ± 3.78*
Smoking during pregnancy %	21.94	23.36
Alcohol use during pregnancy %	49.57	55.17
Married during pregnancy %	72.46	75.12
Homeowner at 1y %	75.24	78.82
Child Characteristics		
Sex (male) %	52.70	52.22
Sex (female) %	47.30	48.78
Ethnicity (white) %	92.58	95.09
Birthweight (g)	3350.46 ± 580.37	3379.41 ± 564.61
Born by caesarian section (%)	12.03	10.46
Age breastfeeding stopped (wks.)	11.88 ± 8.63	10.06 ± 7.65
Head circumference birth	34.88 ± 2.20	34.62 ± 1.76

**Significant differences exist between the groups. Data are presented as mean ± SD or %. Data analysed using Independent Samples Mann-Whitney U Test. Results considered significant if P<.05. Data collected from self-report questionnaires completed by the mother.*

As illustrated in *Table 3.10*, both groups were relatively similar to each other in most aspects of their background characteristics with the exception of a significant difference between groups for the mother's pre-pregnancy weight and subsequent BMI calculation. Although not significantly different, the mothers classified at FA deficient at 32GW were more likely to smoke and use alcohol during their pregnancy but also more likely to be married and a homeowner. The children born to these mothers were less likely to be born by caesarean section and were breastfed for a shorter duration. These were not statistically significant differences.

3.3.2 Maternal folic acid status and children's cognitive outcomes at 4y.

Children's cognitive ability was assessed during CiF at 4 years using the WPPSI test. Children whose mothers were FA sufficient at 32GW scored slightly lower in all domains of the WPPSI when compared to children with mothers classified as FA deficient at 32GW (*Table 3.11*), again these were not statistically significant findings.

Table 3.10: *Differences observed in WPPSI cognitive outcomes between ALSPAC children whose mothers were FA sufficient (>400µg) at 32GW and those who were considered FA deficient (<400µg) at ~4 years.*

	FA Sufficient at 32GW (>400 µg/d)		**FA deficient at 32GW (<400 µg/d)**		**U-value**	**P**
	N	Mean Rank	N	Mean Rank		
WPPSI – 4y						
Performance IQ	18	341.75	858	440.53	5980.500	.101
Verbal IQ	18	407.67	857	438.64	7167.000	.607
Full-scale IQ	18	364.97	857	439.53	6398.500	.215

Data are presented as means ± SD. Data analysed using Independent Samples Mann-Whitney U Test. Results considered significant if P<.05. Data was child completed and collected during CiF session.

3.3.3 Psychological developmental outcomes

Other aspects of child emotional, social, behavioural and language development were measured in the ALSPAC study and are listed in *Table 3.12*. Children whose mothers were FA sufficient at 32GW scored significantly higher in social development **(U = 801650, p = .035, r = 0.02)**, fine motor **(U = 752282.5, p = <.001, r = 0.03)** and total development **(U = 755590.5, p = .001, r = 0.04)** scores at 2.6y and intelligibility **(U = 780732, p = .013, r = 0.03)** and prosocial behaviour **(U = 810138.5, p = .047, r = 0.02)** at 3.2y than children whose mothers were FA deficient at 32GW. No other significant differences were observed between groups, however the same children also scored slightly higher in social development, emotionality, activity, shyness, language and communication at 3.2y.

3.3.4 Maternal folate level at 32GW: FA sufficiency vs level of deficiency (mild/moderate)

To further investigate the effect of less than adequate maternal folate levels at 32GW on children's development, ALSPAC mothers were categorised into 3 groups: folate sufficient (>400µg), mild folate deficiency (300-399µg) and moderate folate deficiency (<299µg). Using these categories, the optimum level of folate could then be established using post-hoc tests. The same developmental outcomes tested in both analyses enables a more in-depth examination of each condition in order to identify the level of folate most beneficial to children's development.

Table 3.11: *Differences observed in psychological developmental outcomes of interest between ALSPAC children whose mothers were FA sufficient (>400µg) at 32GW and those who were FA deficient (<400µg) at ~3 years.*

	FA Sufficient at 32GW (>400 µg/d)		**FA deficient at 32GW (<400 µg/d)**		**U-value**	**P**
	N	Mean Rank	N	Mean Rank		

	FA Sufficient at 32GW (>400 µg/d)		FA deficient at 32GW (<400 µg/d)		U-value	P
2.6y						
Social development	215	4519.40	8140	4168.98	801650	.035
Fine motor score	215	4772.01	8163	4174.16	752282.5	<.001
Gross motor score	215	4365.79	8154	4180.23	837684.5	.259
Total development score	215	4722.63	8129	4157.95	755590.5	.001
3.2y						
Social development	213	4548.30	8256	4226.92	812530.5	.056
Fine motor score	213	4384.60	8260	4233.19	848251	.369
Gross motor score	213	4426.54	8267	4235.71	840808	.258
Emotionality	210	4014.70	8199	4209.87	820932.5	.249
Activity score	210	4322.38	8198	4201.48	836035.5	.472
Shyness score	210	4027.38	8198	4209.04	823595	.283
Sociability score	210	4245.04	8194	4201.41	851437.5	.769
Language score	203	4221.03	7965	4081.02	780732	.403
Intelligibility score	208	3888.83	8156	4189.99	780732	.013
Communicative score	209	4441.44	8154	417.35	787141.5	.099
Emotional difficulties	213	4300.91	8263	4236.89	866715.5	.701
Conduct difficulties	213	4311.59	8263	4236.62	864441	.656
Hyperactivity	213	4265.79	8263	4237.80	874197	867
Prosocial score	213	456653	8263	4230.04	810138.5	.047
Total behavioural difficulties score	213	4338.29	8263	4235.93	858755	.546

Data are presented as means ± SD. Data analysed using Independent Samples Mann-Whitney U Test. Results considered significant if p<.05. Data collected from child-specific questionnaire completed by the mother.

Again, children's cognitive ability was tested using the WPPSI and a Kruskal-Wallis test to compare distributions showed that folate level had a weak but significant effect on children's performance IQ **($\chi^2(2, n = 876) = 13.11, p = .001, \varepsilon^2 = .02$)** and full-scale IQ **($\chi^2(2, n = 875) = 9.20, p = .010, \varepsilon^2 = .010$)** (*Table 3.13*). with children whose mothers reported being folate deficient at 32GW scoring better in these cognitive assessments than those whose mothers were folate sufficient. A post hoc

test with Bonferroni correction showed significant differences between moderate (Mean Rank = 426.02) and mild (Mean Rank = 494.05) folate deficiency (p = .004) and moderately deficient and sufficient (Mean Rank = 341.75) folate levels (p = .044) for Performance IQ. With regards to Full-Scale IQ, the post hoc test identified a significant difference between moderate (Mean Rank = 427.09) and mild (Mean Rank = 485.38) deficiency (p = .017).

Table 3.12: *Differences observed in cognitive ability using the WPPSI, between ALSPAC children whose mothers were FA sufficient (>400µg) at 32GW and those who were considered mildly (<299 µg/d) or moderately FA deficient (300-399µg/d) at ~3 years.*

	Moderately FA deficient at 32GW (<299 µg/d)		**Mildly FA deficient at 32GW (300-399 µg/d)**		**FA Sufficient at 32GW (>400 µg/d)**		**K-W value**	**p**
	N	Mean Rank	N	Mean Rank	N	Mean Rank		
4y - WPPSI								
Performance IQ	675	426.02	183	494.05	18	341.75	13.11	.001
Verbal IQ	674	432.29	183	462.10	18	407.67	2.27	.321
Full-scale IQ	674	427.09	183	485.38	18	364.97	9.20	.010

Data are presented as means ± SD. Distributions compared using the Kruskal-Wallis Test. Results considered significant if P<.05. Data was child completed and collected during CiF session.

Using the Kruskal-Wallis test to compare the distributions of the other outcome variables showed that folate level also had a significant effect on various aspects of children's development at ~3years (*Table 3.14*) including children's social development **(χ^2(2, *n* = 8355) = 9.19, *p* = .010, ε^2 = .02)**, fine motor **(χ^2(2, *n* = 8378) = 31.43, *p* = <.001, ε^2 = .02)** and total development **(χ^2(2, *n* = 8344) = 23.89, *p* = <.001, ε^2 = .02)** at 2.6y, and for social development **(χ^2(2, *n* = 8469) = 13.85, *p* = .001, ε^2 = .02)** and fine motor scores **(χ^2(2, *n* = 8473) = 14.48, *p* = .001, ε^2 = .02)** at

3.2y. Children in the folate sufficient group scored significantly better than those in the deficient groups with exception to fine motor development at 3.2y with children whose mothers reported being mildly deficient achieving the highest means scores.

Table 3.13: *Differences observed in various other psychological developmental outcomes of interest between ALSPAC children whose mothers were FA sufficient (>400µg) at 32GW and those who were considered mildly (<299 µg/d) or moderately FA deficient (300-399µg/d) at ~3 years.*

	Moderately FA deficient at 32GW (<299 µg/d)		**Mildly FA deficient at 32GW (300-399 µg/d)**		**FA Sufficient at 32GW (>400 µg/d)**		**K-W value**	**p**
	N	Mean Rank	N	Mean Rank	N	Mean Rank		
2.6y								
Social development	6587	4140.82	1553	4288.41	215	4519.40	9.19	.010
Fine motor score	6603	4118.37	1560	4410.29	215	4772.01	31.43	<.001
Gross motor score	6596	4180.51	1558	4179.04	215	4365.79	1.28	.529
Total development score	6578	4112.38	1551	4351.22	215	4722.63	23.89	<.001
3.2y								
Social development	6669	4185.27	1587	4401.92	213	4548.30	13.85	.001
Fine motor score	6673	4184.97	1587	4435.95	213	4384.60	14.48	.001
Gross motor score	6679	4216.84	1588	4315.06	213	4426.54	3.38	.184
Emotionality	6636	4211.18	1563	4204.35	210	4014.70	1.34	.551
Activity score	6636	4237.69	1562	4047.66	210	4322.38	8.41	.015
Shyness score	6636	4212.34	1562	4195.01	210	4027.38	1.22	.554
Sociability score	6631	4213.06	1563	4151.98	210	4245.04	.88	.645

	Moderately FA deficient at 32GW (<299 µg/d)		Mildly FA deficient at 32GW (300-399 µg/d)		FA Sufficient at 32GW (>400 µg/d)		K-W value	p
Language score	6441	4022.47	1524	4238.46	203	4221.03	21.47	<.001
Intelligibility score	6606	4155.40	1550	4337.40	208	3888.83	19.92	<.001
Communicative score	6598	4187.30	1556	4137.38	209	4441.44	3.24	.198
Emotional difficulties	6676	4250.53	1587	4179.54	213	4300.91	1.27	.531
Conduct difficulties	6676	4242.03	1587	4213.85	213	4311.59	.37	.830
Hyperactivity	6676	4251.44	1587	4180.40	213	4265.79	1.14	.566
Prosocial score	6676	4197.65	1587	4366.32	213	4566.53	10.09	.006
Total behavioural difficulties score	6676	4253.30	1587	4162.86	213	4338.29	2.12	.346

Data are presented as means ± SD. Distributions compared using the Kruskal-Wallis Test. Results considered significant if P<.05. Data collected from child-specific questionnaire completed by the mother

The same post hoc test with Bonferroni correction was applied to identify where the differences occurred between the groups. At 2.6y no significant differences were found between groups for social development however there was a significant difference between the moderate (Mean Rank = 4185.27) and mildly deficient (Mean Rank = 4401.92) groups (p = .004) at 3.2y with children of mildly deficient mothers scoring better than those who were moderately deficient. When considering children's fine motor score at 2.6y significant differences were observed again between the moderate (Mean Rank = 4118.37) and mildly deficient (Mean Rank = 4410.29) groups (p = <.001) with children of mildly deficient mothers scoring better, there were also significant differences between moderate deficiency and sufficient

(Mean Rank = 4772.01) groups (p = <.001) with those whose mothers were sufficient scoring best. However at 3.2y the only the moderate (Mean Rank = 4184.97) and mildly deficient (Mean Rank = 4435.95) groups remained significantly different (p = .001) with children of mildly deficient mothers scoring higher than those considered moderately deficient. Children's total development at 2.6y had significant differences again between the moderate (Mean Rank = 4112.38) and mildly deficient (Mean Rank = 4351.22) groups (p = .001) and the moderately deficient and sufficient (Mean Rank = 4722.63) groups (p = .001) with children of sufficient mothers achieving the highest scores.

Folate sufficient children obtained significantly higher scores in activity **(χ^2(2, *n* = 8408) = 8.41, *p* = .015, ε^2 = .02)** and prosocial behaviour **(χ^2(2, *n* = 8476) = 10.09, *p* = .006, ε^2 = .02)** at 3.2y. Significant differences in activity between the moderately (Mean Rank = 4237.69) and mildly deficient (Mean Rank = 4047.66) groups (p = .015) was observed with children of mildly deficient scoring lowest in comparison to the other two groups. Significant differences between the moderately (Mean Rank = 4197.65) and mildly deficient (Mean Rank = 4366.32) groups (p = .040) for prosocial behaviour with the sufficient group scoring the highest. Significant differences were also observed in language scores **(χ^2(2, *n* = 8168) = 21.47, *p* = <.001, ε^2 = .02)**, despite mean rank scores for the sufficient group and mildly deficient groups being similar (m=4221.03 and m=4238.46 respectively). Significant differences were observed between the moderately (Mean Rank = 4022.47) and mildly deficient (Mean Rank = 4238.46) groups (p = <.001). Significant differences were also observed in children's intelligibility **(χ^2(2, *n* = 8364) = 19.92, *p* = <.001, ε^2 = .02)**. The folate sufficient group scored lowest with

significant differences between the moderately (Mean Rank = 4155.40) and mildly deficient (Mean Rank = 4337.40) groups (p = .001) and mildly deficient and sufficient (Mean Rank = 3888.83) groups (p = .001). No significant differences were found for the remainder of the outcome variables tested (*Table 3.14*).

3.4 Discussion

This secondary data analysis assessed children's development at ~3y with the expectation that findings would provide a national context for folic acid research in a UK sample. The section begins by discussing the findings relating to each hypotheses and the differences observed between mothers who reported using folic acid at 32GW and those who did not with some possible explanations as to why they did not supplement. This is followed by a critical evaluation of ALSPAC data and a short conclusion.

3.4.1 Maternal folate status in late pregnancy: FA sufficient vs FA deficient at 32GW

Children of FA sufficient mothers scored slightly lower in all cognitive domains assessed by the WPPSI (perception IQ, verbal IQ and full-scale IQ) when compared to children whose mothers reported being FA deficient at 32GW. These were nonsignificant differences but nonetheless this was an unexpected finding. Evidence to date has generally reported a positive effect of maternal folic acid use on children's cognition (e.g. Ars *et al.*, 2019; Compan Gabucio *et al.*, 2021; Wang *et al.*, 2021; Caffery *et al.*, 2018; McNulty *et al.*, 2019; Villamor *et al.*, 2012; Veena *et al.*, 2010; Murphy *et al.*, 2007; Catena *et al.*, 2015; Bhate *et al.*, 2012; Chatzi *et al.*,

2012; Julvez *et al.*, 2009; del Rio Garcia *et al.*, 2009; Li *et al*, 2009; Gross *et al.*, 1974). Despite this, this finding is similar to those reported in a small number of studies included in the systematic review (*Chapter 2*) who also found no significant differences in children's cognitive development when mothers were FA sufficient or deficient (e.g. Tamura *et al.*, 2005; Campoy *et al.*, 2011; Li *et al.*, 2015) with detrimental effects only being found if mothers consumed very high doses (Valera-Gran *et al.*, 2014; Valera-Gran *et al.*, 2017). In each of these studies mothers continued supplementing past 12GW into the second and third trimesters. A possible explanation for this finding could be due to the small number of participants whose mothers were FA sufficient (n=18) in comparison to those who were deficient (n=858). Statistical tests were adjusted to account for the difference in group size. Cognitive data was collected during the CiF session and included a 10% sub-sample of ALSPAC participants. This sub-sample was selected randomly which would also help control these effects.

In addition to the cognitive data collected from children, ALSPAC mothers provided information on the child-specific questionnaires in relation to their children's social and motor development, temperament, language and communication, psychosocial adjustment and behaviour at ~3y. For these outcomes children whose mothers were FA sufficient scored significantly higher in social development, fine motor skills and total development at 2.6y and prosocial behaviour at 3.2y, and significantly lower in intelligibility at 3.2y. The significant differences observed in social development, fine motor skills and total development disappeared when measured again using the same scale at 3.2y. However, the proxy measures provided by mothers may not have

been reliable (Germain *et al.*, 2019). It is also possible that at 3y children were too young to accurately represent these particular areas of development.

3.4.2 Maternal folate level at 32GW: Sufficiency vs deficiency level (mild/moderate)

It was hypothesised that children whose mothers were folate sufficient at 32GW would achieve better developmentally that children whose mothers reported mild or moderate deficiency when children's cognitive, motor, social, emotional, behavioural and language development was assessed at ~3y. Additional analysis was used to determine if any FA intake at any level; even if classified as deficiency (<400µg/d) would be beneficial to children and at what level of FA would provide children with the most advantage.

Children's cognitive ability, specifically performance IQ and full-scale IQ, were significantly affected by FA level at 32GW with differences between moderate deficiency and sufficiency (performance IQ) and moderate and mild deficiency (performance and full-scale IQ) reported. ALSPAC findings showed that children performed best in the cognitive assessment when mothers were mildly deficient in FA at 32GW in comparison to those who were sufficient or moderately deficient at 32GW. This is surprising given that there is substantial evidence to suggest that sufficient use of maternal FA is advantageous for children's cognition (e.g. Caffery *et al.*, 2019; Veena *et al.*, 2010; Catena *et al.*, 2015). There is however some evidence to suggest that children's development might only be compromised if maternal nutrient deficiency during pregnancy is severe with the same negative effects not being seen with mild maternal deficiency (Prado & Dewey, 2014). This is due to a number of protective mechanisms which can buffer the negative effects of

deficiency to a degree including mothers pre-pregnancy nutritional status and homeostatic processes of the foetus (e.g. Tofail *et al.*, 2008; Prado *et al.*, 2012). Furthermore, one-carbon metabolism of which folate is a key component, is central to maintaining body homeostasis which is integral to body repair and human health of both mother and child (Suh *et al*., 2016).

When considering the other areas of development FA level was found to have a significant effect on children's social development (2.6y, 3.2y), fine motor skills (2.6y, 3.2y), total development (2.6y), activity (3.2y), language (3.2y), intelligibility (3.2y), and prosocial behaviour (3.2y). In the majority of cases ALSPAC children achieved the best scores when mothers were FA sufficient at 32GW. These children had better social, fine motor, gross motor and total development at 2.6y and social, and gross motor development, activity, sociability, communicative scores and prosocial behaviour. Children of FA sufficient mothers also achieved the lowest scores in emotionality and shyness. These findings are in line with the available evidence (e.g. Henry *et al.*, 2018; Steenweg-de Graff *et al.*, 2015). Current evidence and the studies contained in this thesis demonstrates that those who participate in research, but particularly in longitudinal studies tend to be motivated individuals practicing positive health behaviours including continued supplement use (Royal College of Paediatrics and Child Health, 2020). These positive behaviours have also been linked to children's physical and mental health and wellbeing, known to improve children's psychological and socioemotional development (Ars *et al.*, 2019; Compan Gabucio *et al.*, 2021; Caffery *et al.*, 2018; Henry *et al.*, 2018; Phelan, 2010). Motivated and health conscious mothers are also more likely to exhibit parental warmth and responsiveness, developing and maintaining strong bonds with

their children resulting in a secure attachment (Newland, 2015). This could help explain why children of folate sufficient mothers were achieving significantly better scores in these areas of development.

Although mean scores indicated that children of FA sufficient mothers were preforming best in motor, social, emotional, behavioural and language development, it wasn't always the case that there were significant differences between sufficient and deficient use at 32GW. When comparing children of sufficient mothers to those of deficient mothers, only three outcomes were affected by FA level; fine motor and total development at 2.6y (moderate deficiency/ sufficiency) and intelligibility (mild deficiency/ sufficiency). Significant differences were found between children whose mothers were mildly deficient and moderately deficient in fine motor and total development at 2.6y, social and fine motor development at 3.2y, activity, language, intelligibility and prosocial behaviour at 3.2y. However, it is possible that the cut-off levels between each category were too close to be meaningful. The notably small number of mothers who were folate sufficient at 32GW in comparison to those who reported being deficient could also have impacted on results. There are many reasons why only a small number of mothers continued supplementation in this sample. Early planning for ALSPAC began in the 1980's with data collection occurring between April 1991 and December 1992. Although evidence was accumulating in support of folic acid use in early pregnancy for the prevention of NTD affected pregnancies, clear guidance was only issued by MRC in July 1991. The lack of accessible folic acid knowledge in the general population would have had serious implications for the number of mothers choosing to supplement. Moreover, it would have taken some time for adjustment for medical professionals including GPs and

those providing antenatal care. Developing professional knowledge and incorporating new guidance into practice would understandably take time (Hughes, 2008). The research informing the MRC guidance in 1991 was focused on preventing NTD, therefore it was only recommended to supplement from preconception to 12GW. Mothers would not have known to continue beyond this time and medical professionals would not have recommended continuing supplementation without knowing the possible risks or benefits of doing so.

Despite relatively low numbers, the analysis investigating the impact of FA level on child development has offered some insight and supports the use of any amount of FA in late pregnancy. More significant results were found during this analysis possibly due to increased sensitivity by differentiating between the level of FA intake at 32GW. These results suggest that any use of FA use in late pregnancy could have a significant impact on children's development and that mothers don't necessarily need to maintain sufficient levels for children's development to experience a positive effect.

Analyses included a large number of ~3y children with relevant outcome data (n=8494) who were eligible for inclusion. Mothers were considered folate sufficient if they reported using folic acid in the 3 months prior to completing the ALSPAC survey at 32GW, if mothers reported not using the supplement at this late stage of pregnancy, they were considered folate deficient at 32GW. There was a large difference in participant numbers between the two groups with 241 mothers supplementing in comparison to 8540 who were not. Despite the unequal distribution, participants did not differ significantly in terms of background

characteristics. This comparison of folate sufficient and deficient ALSPAC mothers did however highlight some differences between the groups. Those who were FA deficient in late pregnancy were more likely to smoke and use alcohol during pregnancy and breastfeed for a shorter a shorter duration (m=10wks; SD=7.65)

Smoking and using alcohol while pregnant are risk behaviours which can significantly impact all children's development. ALSPAC mothers were recruited in 1991 when rates of maternal smoking and alcohol use during pregnancy were much higher than today (NHS, 2019; PHA, 2019). Furthermore, research has shown that age and SES are inversely correlated with these risk factors while location is another contributing factor (e.g. Royal College of Paediatrics and Child Health, 2020). There is some research to suggest that mothers who engage in these risk behaviours during pregnancy are more likely to adopt a poor parenting style (Chassin *et al*., 2005), which is closely linked to parent-child interaction and relationship quality and attachment (Wang *et al.*, 2016; Csala *et al.*, 2016). Additionally these risk factors are compounded by a lack of maternal awareness and difficulties faced by health professionals in communicating the importance of FA consumption during early pregnancy to the public, which decreases adherence to supplementation before and during pregnancy (Herter-Aeberli *et al*., 2020; Bitzer *et al*., 2013).

When these pregnancy risk factors are present, evidence suggests that mothers are less likely to partake in health promoting behaviours such as taking pregnancy supplements (Chassin *et al.*, 2005) or breastfeeding (Collins *et al*., 2011; Higgins *et al.*, 2010) and mediated by psychosocial factors such as maternal educational attainment, SES and income (Scott & Binns, 1999). There is growing evidence to suggest that breastfeeding improves child development. A cross-sectional study

(Deoni *et al.*, 2013) found that breastfeeding for 3 months increases white matter brain growth by 20-30% in 10-month to 4-year old children. At age 2y EBF children achieved more growth in the areas of the brain associated with language, emotional function and cognition compared to combination fed or formula fed infants. Moreover, children who were breastfed longer (>1y) had significantly enhanced brain growth in the areas of the brain dealing with motor function. A large UK study found that longer duration of breastfeeding, combination feeding or exclusively, was associated with less behavioural problems (Heikkila *et al.*, 2011), improved cognitive development, particularly in preterm children (Quigley *et al.*, 2012) and educational attainment (Heikkila *et al.*, 2014) at 5 years. These findings suggest that there is evidence of breastfeeding having a long-term effect on child development, behaviour and cognitive ability.

Less engagement in maternal risk behaviours and more in health promoting behaviours has been associated with an increased sense of family wellbeing (e.g. Wang *et al.*, 2016). Family wellbeing encourages effective connections and improves relationship quality which promotes the wellbeing of each individual in the family unit (Thomas *et al*., 2017). Newland's pathways to healthy adjustment model (2015) proposed that family wellbeing provides the foundations for positive parenting practices and subsequent child wellbeing and psychological, social and emotional development.

3.4.3 Critical evaluation of ALSPAC

The original design of the ALSPAC dataset was to investigate influences on health and development throughout the life span. A large amount of biological,

psychological and environmental data was therefore collected relating to pregnancy, delivery and later development and health of the children, mothers and family. The sample size, breadth and frequency of data collection, ongoing support and commitment from the study families is admirable and relatable to many as the Office of National Statistics (ONS, 2020) data shows that 85-88% of UK women within the age range of ALSPAC (age range 14-45y; m=23.27) will have at least one child (Boyd *et al*., 2013), therefore findings are relevant to the large majority of UK women and other high income countries in general.

In relation to this study, the dataset was subject to a number of limitations. In terms of the intervention, folic acid use was not quantified and was limited to only 2 retrospective self-report questions asking if they had taken folic acid/folate prior to 18GW and again prior to 32GW. Mothers were not asked to provide any additional information on the dosage consumed, the time of initiating supplementation or if the folic acid was contained in a multivitamin. This was an obvious limitation as research has shown that low doses (<400) and very high doses (>1000) can negatively impact developmental outcomes for children (e.g. Valera-Gran *et al.*, 2015; Valera-Gran *et al.*, 2017; Schlotz *et al.*, 2010). Research has also shown that the time of initiation is also critical (Prado & Dewey, 2014) with preconception use advised.

There is growing evidence detailing the important role of folate for neuron proliferation (Couperus & Nelson, 2006), DNA synthesis, repair and methylation (e.g. Allen *et al.,* 1993) and gene expression (e.g. Irwin *et al.*, 2018). Folate is a key component of one-carbon metabolism which plays an important role in children's

brain development and function (Emmerson *et al*., 2017) and has been shown to improve neuroplasticity (Jadavji *et al.,* 2017). Neuroplasticity has been defined as "the brains ability to modify, change and adapt both structure and function throughout life" (Voss *et al*., 2017, pg. 1657) and is closely linked to an individual's environment and experiences (Prado & Dewey, 2014) which would incorporate the child's social, emotional and behavioural experiences coupled with the home environment and parting style they are exposed to. ALSPAC mothers were not asked if folic acid was consumed as part of a multivitamin during pregnancy. If this was the case, it is impossible to attribute any positive or negative effects to one of many micronutrients. Research has demonstrated how each individual vitamin and mineral can have different and wide ranging positive and negative effects dependent on bioavailability and how each of these micronutrients interact. Folate status or maternal folic acid use was unable to be verified by maternal blood samples obtained during pregnancy or cord blood. Contact was made with ALSPAC researchers to inquire about the status and availability of biological data however, despite the extensive biobank and collection of serum folate from cord blood at birth the samples were deemed unreliable as they had been stored for 16 years before analysis. This was unfortunate as folate level obtained from biological samples could have been used not only to verify FA use but also to accurately differentiate mothers into groups by folate level; sufficient, mildly deficient and moderately deficient.

ALSPAC also experienced some issues with sampling due to the longitudinal nature of the study. This can be expected; however it could potentially have a significant impact on the results and interpretation. Attrition and decreasing response rates from mothers was problematic, particularly amongst mothers who had experienced

difficulties during pregnancy or following the birth. These difficulties were inclusive but not limited to, pregnancy complications or environmental problems such as inadequate housing or lack of social support. Children's response rates also decreased as they aged, this was evident when looking at the educational attainment of the ALSPAC enrolled children's sample. Children who had not participated or those lost to follow-up had a lower educational attainment than the national average and children who were enrolled performed better in terms of their education than the national sample (National Pupil Database). Furthermore, the difference in mean attainment increased with increasing completeness of participation in ALSPAC. This is a common occurrence in longitudinal research. Certain individuals are more likely participate in research in the first instance and continue participation through follow-up studies than others, associated typically with educational attainment, SES, internal motivation and the presence of positive health behaviours (Royal College of Paediatrics and Child Health, 2020).

Selection bias due to attrition could result in an underestimation of perinatal effects and later child developmental outcomes. The enrolled sample were more likely to be white and from an affluent background whereas those lost to follow-up were more likely to be male and have a lower household income. Level of income was assessed based on eligibility for free school meals. ALSPAC authors acknowledged (Boyd *et al.*, 2013) the over-representation of more affluent groups and under-representation of non-white groups when compared with the national population. These differences in ethnic composition between the enrolled and national samples could be attributed to regional differences and demographic changes within the UK and NI since the birth of the cohorts in 1991 and the FASSTT sample in 2005. Although a limitation

of ALSPAC, the under-representation of other ethnic groups could be advantageous for this current study as we were testing a homogenous ethnic group. Nevertheless, this must still be considered as the external validity of the study could be influenced.

3.4.4 Conclusion

Findings from this secondary data analysis generally aligned with the existing literature in relation to children's motor, social, emotional, behavioural and language development and to an extent cognition. In the ALSPAC sample, continued maternal folic acid use to 32GW significantly improved children's cognitive, social, motor, language and behavioural development. Differentiating between folate level at 32GW indicated that any folic use in late pregnancy could benefit children's development, even if consumption is inconsistent or considered insufficient. A number of limitations and the possible impact on these current findings were acknowledged.

Chapter 4

Children's psychological, behavioural, social and emotional development can benefit from continued maternal folic acid use throughout pregnancy: A follow-up of FASSTT children at 10 years old.

4.1 Introduction

This chapter describes and presents the findings from the final phase of this PhD which was a follow-up assessment of the Folic Acid Supplementation during the Second and Third Trimesters of pregnancy (FASSTT) trial now the child participants are 10 years old; FASSTT@10y. The chapter begins with a contextual description of the global FASSTT Trial followed by background information relevant to the FASSTT@10y study, the rationale for this most recent follow-up, followed by the study aim and objectives and the research hypotheses to be tested. The method section follows containing a detailed description of both global FASSTT and FASSTT@10y, including participant information, data collection procedure, ethical considerations, power calculation and statistical analysis conducted. The results section is presented next, detailing FASSTT@10y results, including a comprehensive analysis of the psychological effects observed, Hierarchical Multiple Regression Analysis, mediation analysis and comparison to normative data. The discussion section concludes this chapter and consists of a detailed discussion of the FASSTT@10y study with a comparison to earlier FASSTT findings (3y and 7y),

followed by the study strengths and limitations, implications and recommendations for future research and practice and a final conclusion.

4.1.1 Background to the study

The effects of maternal folic acid use during pregnancy on the development of the child have been extensively investigated for many years. The prevention of Neural Tube Defects (NTD) guided the development of NICE recommendations which state that women should supplement with 400µg/d of folic acid from preconception to the end of the first trimester of pregnancy (-4GW-12GW) (NICE, 2016). NTD's are a failure in the closing of the neural tube in the first few weeks of pregnancy (Stover, 2009), often before the mother even knows she is pregnant. This causes damage to the exposed underlying neural tissue which depending on severity and location of the lesion, can cause significant morbidity and mortality (Sutton *et al*., 2008).

The relationship between folate deficiency and NTD was hypothesised as early as 1965 (Hibbard *et al*., 1965). A number of early studies suggested that folic acid could reduce the risk of NTD (e.g. Smithells *et al.*, 1983; Mulinare *et al*., 1988; Bower & Stanley, 1989) and in 1991 an RCT was conducted by the British Medical Research Council to test the effectiveness of folic acid supplementation in the prevention of NTD recurrence (Medical Research Council, 1991). Results showed that women with a previous NTD- affected pregnancy could reduce their risk of recurrence by 72% by taking the now recommended 400µg/d of folic acid. Another RCT in Hungary found a 100% reduction in risk of a first occurrence of NTD in pregnancy if mothers supplemented with 800µg/d of folic acid (Czeizel & Dudas, 1992). Collective findings led the MRC to recommend pre-conceptual folic acid use

in all women, in addition to other public health measures to ensure adequate folate status.

Similarly, in America, the Centres for Disease Control and Prevention (1991) recommended that women who experienced a previous NTD-affected pregnancy should supplement with a high dose of folic acid (4000µg/d) from the time the begin to plan a pregnancy (CDC, 1991). The following year, the U.S. Public Health Service recommended that all women of childbearing age supplement with 400µg/d of folic acid (CDC, 1992) and was supported by the Institute of Medicine (1998). In 2009 the U.S. Preventative Services Task Force updated and published guidelines further reinforcing these recommendations. However, despite clear policy and guidelines NTD are still highly prevalent globally, but particularly in low income countries.

4.1.2 Global attitudes to folic acid food fortification

In order to reduce the number of first occurrence and recurrence NTD-affected pregnancies, mandatory folic acid fortification has been introduced in a total of 78 countries and is considered by some as one of the most successful public health initiatives in the recent past (Berry *et al*., 2010). Regulations were published by the World Health Organisation and the Food and Agricultural Organisation of the United Nations in 2006 to set target fortification levels, including maximum, minimum and legal minimum levels (WHO, 2006). In America, the U.S Food and Drug Administration initiated mandatory food fortification in 1998 (FDA, 1996) which has decreased the birth prevalence of NTD by approximately 35% or around 1300 babies per year (CDC, 2015). Other countries including Canada, Brazil, Argentina,

Chile, Costa Rica and South Africa have also reported declines in NTD's (19%-55%) since their initiation of folic acid food fortification (Chen & Rivera, 2004; Hertrampf & Cortes, 2004; Sayed *et al*., 2008; Ray *et al*., 2002, Lopez,-Camelo *et al*., 2010; Liu *et al*., 2004; de Wals *et al.*, 2007), however, the success rate is dependent upon a number of external factors.

4.1.3 Food fortification status in the UK

Recent data have shown the prevalence of NTD-affected pregnancies in the UK is 1.28 per 1000 total births equating to 19% live births and 81% of terminations (Morris *et al.*, 2016). Despite reports indicating the efficacy of folic acid fortification on NTD pregnancies and births, the introduction of such a programme has only recently been initiated (Haggarty, 2021). It is estimated that approximately 2000 NTD pregnancies could have been prevented since 1998 if the UK had participated in a fortification programme (Morris *et al.*, 2016). The Food Standards Agency (FSA) advised against mandatory fortification in 2002 due to concerns over the unintended effects such as the masking of vitamin B_{12} deficiency and an increased risk of colon cancer. In 2006 the Scientific Advisory Committee on Nutrition (SACN) were asked to consider the wider impact of fortification and provided preliminary recommendations to introducing mandatory fortification. In 2009 the SACN found no evidence that folic acid increased the risk of colon cancer however the recommendations remained unaffected. At this time, the FSA recommended mandatory fortification however no regulatory action was taken. Following an updated evidence review and risk assessment in 2016 the SACN recommendations remained unchanged (SACN, 2017)

The UK's decision not to initiate folic acid fortification earlier has been described as 'a missed opportunity' (Morris *et al.*, 2016) with authors stating that "failure to implement folic acid fortification… continues to cause avoidable terminations of pregnancy, stillbirths, neonatal deaths and permanent serious disability in surviving children" (pg. 604). However, fortification is not the only solution, education to raise awareness of the role of folic acid in pregnancy and the current recommendations could improve uptake in supplement use. A large population study in Europe (Bitzer *et al.*, 2013) found that only 17% of women were aware that folic acid supplementation could reduce the risk of NTD. This lack of knowledge could directly impact supplementation uptake and women's compliance. Bestwick *et al.* (2014) found UK uptake to be low in 2011-2012 with only 28% of women supplementing before pregnancy; a reduction from 40% in 1999-2001. The lowest rates were observed in Afro-Caribbean women (17%) possibly due to SES and associated health inequalities and women under 20 years old (6%) who are more likely to have an unplanned pregnancy and have less knowledge on the benefits of supplementing. Uptake levels have continued to reduce with Beverley *et al.*(2020) reporting that approximately only one-fifth of women consume folic acid before becoming pregnant which increases to three-fifths once pregnancy is confirmed. Additionally, the National Diet and Nutrition Survey (NDNS) (Beverley *et al.*, 2020) showed that 89% of women of childbearing age (16-49y) in the UK in 2019 had blood folate levels below the WHO cut-off for preventing NTD's (serum folate <7nmol/L) and has decreased over the past 11 years (Beverley *et al.*, 2020; Bates *et al.*, 2015). These results highlight folic acid uptake as an issue and offer support to the introduction of mandatory fortification in the UK whilst adhering to the stringent regulations detailed by the SACN and could benefit both mothers and children.

4.1.4 Developmental benefits of maternal folic acid supplementation

The benefits of periconceptional folic acid use, the development of the current guidelines and the implementation of food fortification programmes to reduce first occurrence and recurrence NTD pregnancies in the UK and beyond has been discussed in detail. Physical developmental benefits, *in utero* and after birth have also been reported. Other aspects of children's physical development have been shown to benefit from maternal folic acid use in pregnancy. Sufficient maternal folate levels have been shown to reduce instances of congenital heart defects (Botto *et al*., 2000; Czeizel, 1996, van Beynum, *et al*., 2010; Li *et al*., 2013) and other congenital malformations including oral clefts (Jahanbin *et al*., 2018; Wilcox *et al*., 2007), urinary tract abnormalities (Li *et al*., 1995) and limb deficiency (Shaw *et al*., 1995; Yang *et al*., 1997). Some studies have also shown adequate maternal folate to reduce preterm birth and low infant birth weight (Scholl & Johnson, 2000) and fetal death (Scholl *et al*., 1997). However, the benefits of supplementation are not limited to children's physical development, but rather extend to a number of other areas of development.

4.1.5 Early brain and neurodevelopment during pregnancy

Brain development begins at conception and develops rapidly during the first trimester. The neural plate grows and folds into the neural tube to form the beginnings of the spinal cord, nervous system and building the initial brain structure at approximately 22 days gestation (Couperus & Nelson, 2006). Once the neural tube closes the brain separates into three primal sections, the front brain, midbrain and hindbrain which grows and develops to form the various brain structures required to drive all bodily functions (Zou *et al*., 2021). The third trimester is when the cerebral

cortex supersedes the brain stem preparing the child for future cognitive, psychological, behavioural, social and emotional development (Baez-Mendoza & Schultz, 2013; Rubenstein, 2010). This indicates that supplementing into late pregnancy could be beneficial for children's brain development with 28GW to 42GW being a critical window for folic acid use.

Early cell division focuses on creating nerve cells which once in place develop axons and dendrites. These branching cell extensions connect with synapses which allow nerve signals to move between neurons and is depicted as child neurodevelopment (Prado & Dewey, 2014). Groups of neurons or pathways are refined continuously through overproduction of synapses and selective elimination based on the strength of connection and frequency of use. Through this process factors such as lived experience or external environment can influence neurodevelopment (Prado & Dewey, 2014) and lay the foundations for future cognitive development but also children's psychological, behavioural, social and emotional development which can have immediate and lasting effects; a process known as developmental programming (Almond & Currie, 2011).

4.1.6 Maternal folic acid use and children's neurological and cognitive development

The evidence to support the use of maternal folic acid in relation to children's cognitive and neurological development is well documented. Early observational research highlighted potential benefits for children's neurodevelopment (e.g. Bhate *et al.*, 2012; Chatzi *et al.*, 2012; Julvez *et al.*, 2009; del Rio Garcia *et al.*, 2009; Li *et al*, 2009; Gross *et al.*, 1974) and cognitive functioning (e.g. Villamor *et al.*, 2012; Veena *et al.*, 2010; Murphy *et al.*, 2007; Catena *et al.*, 2015), but was dependent on

sufficient use of the vitamin. Findings from the systematic review (*Chapter 2*) identified the restricted focus in terms of the duration of supplement use during pregnancy concentrating mainly on folic acid consumption during the early stages of pregnancy and not accounting for the potential benefits to be gained from continued use or supplementing in the later stages of pregnancy.

4.1.7 Contribution of FASSTT findings to the cognitive development literature

The FASSTT trial at Ulster University is a large, ongoing, multidisciplinary project spanning over 15 years. Initially designed and investigated by Biomedical Sciences, the School of Nutrition used the FASSTT RCT to measure homocysteine and folate levels during pregnancy on children's birth outcomes (McNulty *et al.*, 2013). The School of Psychology were early collaborative partners when Nutrition were interested in testing for and comparing the cognitive benefits associated with continued folic acid use during pregnancy (McNulty *et al.*, 2019). Collaboration continued with FASSTT@7y when Psychology tested for additional benefits in other areas of development (Henry *et al.*, 2018). The research has continued to grow with other disciplines providing their expertise to expand the knowledge relating to maternal folic acid use further. More recently, Epigenetics were able to test for genetic imprinting and gene expression, and this research continues using epigenetic investigating and analysis (Irwin *et al.*, 2019; Irwin *et al.*, 2018).

The relationship between extended maternal folic acid use (to 36GW) on children's brain function remains of great interest to Nutrition whose research is also ongoing in collaboration with the School of Computing (Caffery *et al.*, 2018; Caffery *et al.*, 2019). This area of research used the functional neuroimaging technique,

magnetoencephalography (MEG) to map brain activity by recording magnetic fields produced by the electrical currents in the brain (Singh, 2014). Findings indicated that children whose mothers continued supplementing were superior in the processing speed and verbal comprehension aspects of cognition in comparison to children whose mothers stopped supplementing at 12GW as recommended. Furthermore, neuronal responses during a language task were assessed and results suggested that these children also exhibited more efficient sematic language processing

4.1.8 Psychological development

The FASSTT research from a psychological perspective has focused on the other areas of child development which have received less attention in relation to maternal nutrition, and include children's psychological, behavioural, social and emotional development. Published findings to date tend to be positive suggesting that these developmental outcomes could indeed benefit from maternal folic acid use (e.g. Henry *et al.*, 2018). Sufficient and adequate folic acid use has been shown to improve children's mental and psychomotor development (Chatzi *et al.*, 2012; Villamor *et al.*, 2012, del Rio Garcia *et al.*, 2009; Bhate *et al.*, 2012) and social development (Julvez *et al.*, 2009; Bhate *et al.*, 2012, Chatzi *et al.*, 2012) while reducing reports of behaviour problems (Steenweg-de Graff *et al*, 2015; Roza *et al.*, 2010; Schlotz *et al.*, 2010), emotional problems (Chatzi *et al.*, 2012; Roza *et al.*, 2010; Steenweg-de Graff *et al.*, 2015) and motor delay (Roth *et al.*, 2011; Wehby & Murray, 2007). This research provides a restricted but promising insight into the potential benefits of supplementing during early pregnancy in terms of psychological, social and emotional development. The evidence needs to be

extended however to consider the effects of the dose and duration of supplement use required to provide optimum benefits.

4.1.9 Rationale

The evidence relating to wellbeing, its emotional and psychological components and how an individual's personality characteristics or traits can contribute to a positive state of wellbeing highlighted the need for a controlled assessment (*Chapter 1)*. The connection between personality and wellbeing (EWB and PWB) is established, however the process as to how children's psychological, social and emotional development influence certain personality traits is unclear. Investigations into how maternal folic acid can improve these areas of development to promote wellbeing is required considering the promising research into the positive effects of folic acid use on children's cognition. Thorough examination is warranted as all areas of children's development is intrinsically linked and typically occur together and the long-lasting effects positive wellbeing can provide.

Research suggests that Trait Emotional Intelligence (TEI) and Trait Resilience (TPR) could potentially be strong predictors of emotional, psychological and social development and subsequent wellbeing (e.g. Henry *et al.*, 2018). Behaviour can be an accurate indicator of wellbeing status and therefore must also be considered. However, to the best of the authors knowledge, the effect of maternal nutrition and folic acid use has on these particular traits and areas of development have not been studied outside of the FASSTT trial. FASSTT@10y provided a unique opportunity to examine emotional and psychological development of children whose mothers

adequately supplemented during pregnancy, and the design permitted a comparative analysis between recommended and continued use.

4.1.10 Trait Emotional Intelligence Theory

Emotions can impact attention, memory, learning, relationship development and our physical and mental health (Salovey & Mayer, 1990). Trait Emotional Intelligence (TEI) is defined as a constellation of capacities and self-perceived attitudes relating to emotion (Petrides & Furnham, 2001) with roots in personality psychology (e.g. Revelle & Scherer, 2009). The operational definition of TEI accounts for the characteristic subjectivity of emotional experience and therefore measures the construct using self-report rating scales more closely aligned with the subjective nature of emotion (Petrides, *et al.*, 2007). To support TEI theory research has shown that the same genes are implicated in both the development of individual differences in the Big Five personality traits and TEI domains (Vernon *et al*., 2008) connecting the construct of TEI to mainstream research (Petrides, 2010).

Being more emotionally intelligent, having emotional awareness and being able to effectively manage emotions of ourselves and others has been shown to have a significant impact on the social, psychological and physical development of both children and adults. Higher levels of TEI in childhood can help foster positive relationships and encourage empathy (e.g. Eggum *et al.*, 2011; Petrides *et al*., 2006) and has also been shown to improve engagement at school and increase attention (Raver *et al*., 2007) to achieve higher grades and control behaviour (Rivers *et al*., 2012).

Adults can also benefit from higher levels of TEI as it is linked to more positive social support networks (Mikolajczak *et al*., 2007) and improved marital relations in adulthood (Malouff *et al*., 2014). Research has shown that higher levels of TEI is associated with greater wellbeing (Austin *et al*., 2005), improved life satisfaction (Di Fabio & Saklofske, 2014) and a decrease in psychological disorders (Petrides *et al*., 2007). TEI has also been linked to better subjective and objective physical health (e.g. Mikolajczak *et al*., 2015; Martins *et al*., 2010), positive health related behaviours and less risk-taking behaviours (e.g. Fernandez-Abascal & Martin-Diaz, 2015; Saklofske *et al*., 2007).

4.1.11 Trait Psychological Resilience Theory

Resilience is a dynamic process that combines positive adaptation within the context of experienced or perceived adversity (Luthar *et al*., 2000) recognised as an important factor for positive health and wellbeing at all stages of life. However, as a construct it is difficult to operationally define (Windle *et al*., 2011). Block and Block (1980) first proposed the concept of Trait Psychological Resilience (TPR) where personality characteristics moderate the effects of stress to promote successful adaptation (Wagnild & Young, 1993). This proposal has been disputed by some (e.g. Masten, 2014) however a recent meta-analysis confirmed that personality and resilience were linked and TPR was negatively correlated with Neuroticism and positively correlated with Extraversion, Openness, Agreeableness, and Conscientiousness (Oshio *et al*., 2018).

Evidence appears to support the concept of resilience as “a constellation of psychological characteristics which cover the ability to resist, cope with and bounce

back from and succeed in the face of stressors" (Hartigh & Hill, 2022, p.g.2; Bryan *et al*., 2019). Oshio *et al*'s meta-analysis (2018) also identified a number of core components of TPR which include self-control, motivation for accomplishment, positive emotions, emotional stability and social engagement which correspond to a degree with the two higher order 'super-factors' of the Big Five (Carroll, 2002). Each component or dimension of TPR relates to a particular psychological function however these change depending on the measure used to assess due to inconsistencies in conceptualisation and operational definitions (Bryan *et al.*, 2019).

Children face many experiences throughout life which can directly impact their level of resilience and TPR. Risk factors include chronic poor health, poverty, family conflict, discrimination or negative life experiences (e.g. abuse, neglect, maltreatment) with negative outcomes including poor academic achievement or drop out, substance use, teenage pregnancy, crime, mental health disorders and emotional distress observed (e.g. Zolkoski & Bullock, 2012; Brooks, 2006; Resnick, 2000). However, evidence suggests that by supporting positive development including resilience, these negative outcomes could be avoided (e.g. Resnick, 2000).

Conversely, protective factors enable the child to change their response to an adverse event and potentially avoid the negative outcome. Zolkoski and Bullock (2012) reviewed the literature and reported that optimal resilience is achieved when protective factors are strengthened at the individual, family and community level simultaneously and many protective factors contribute to resilience at each of these levels. Children's temperament, autonomy, social skills, intelligence, gender and internal motivation can offer protection at the individual level (e.g. Alvord &

Grados, 2005; Benzies & Mychasiuk, 2009; Masten, 2001). Parenting style and responsive parenting, family structure, supportive interactions between parents and parents and children, social support and stable finances can promote protective factors at the family level (e.g. Benzies & Mychasiuk, 2009; Baumrind, 1991). At a community level, role models such as teachers, sports coaches, health workers and neighbours can help buffer resilience in effective environments and social structures (e.g. Alvord & Grados, 2005; Masten, 2001). Other fundamental protective factors identified in resilience research include self-regulation, self-concept and positive self-esteem (e.g. Benzies & Mychasiuk, 2009, Buckner *et al*., 2003). Werner and Smith (2001) report that resilient children positively used opportunities and resources available to them and considered hardships as learning experiences. This positive approach in life could be directly influenced by the personality traits of the child (Masten, 2014).

4.1.12 Peer Attachment Theory

Bowlby (1969) defined attachment relationships as a "lasting psychological connectedness between human beings" (p.g.194) forming in infancy initially between a baby and their primary caregiver, usually the child's mother. Attachment theory was developed to better understand this bond. According to Bowlby's theory the relationship quality, pattern and emotional availability of the mother generates the level of trust and security felt by the child. Together with individual experiences this can inform and determine the formation and quality of future relationships (Delgado *et al*., 2022). Attachment styles were later developed by Ainsworth *et al*. (1979) who distinguished between secure, ambivalent and avoidant attachment. Family structure shapes the first emotional bonds, habits, beliefs and values of a

person and these family experiences in combination with emotion determines the type of attachment style a person will develop in adolescence and as an adult (Bzostek & Berger, 2017).

From an early age the child will begin to link their experiences to their relational world and develop their 'internal working model' (Bowlby, 1977). This model assists them navigating and building relationships by enabling them to effectively evaluate the availability of their attachment figure and act accordingly (Duchesne & Larose, 2007). As the adolescent ages the model becomes integrated with their personality and helps guide their social development (Delgado *et al.*, 2022) while growing more independent from their parents. A meta-analysis by Weymouth *et al*. (2016) found the maintenance of close and positive bonds with parents throughout adolescence as vital for positive PWB and adjustment.

Moving out of childhood and into adolescence presents a new developmental phase and although cognitive, social and emotional changes are occurring, the attachment style formed throughout childhood remains relatively stable (Del Toro, 2012). With increasing autonomy adolescents become less reliant on parents but more so on their peers (Allen, 2008). These relationships can offer emotional and social support and security and become increasingly characterised as attachment bonds (Oldfield *et al*., 2016). This process of interacting with peers and making friendships is combined with less time spent with parents, changing the nature of the family relationships (Laursen & Hartl, 2013). Delgado *et al.*'s (2022) meta-analysis found that secure peer attachment predicted and promoted the creation of affective relationships based on communication, support, intimacy, trust and quality (Stern & Cassidy, 2018;

Nangle *et al*., 2003). Healthy and positive peer relationships are a powerful predictor of EWB and PWB in adolescence. Secure peer attachment was positively correlated with prosocial behaviour and negatively correlated with emotional difficulties and conduct problems (Schoeps *et al*., 2020).

4.1.13 The mediating effect of parenting style

Weymouth *et al.*'s (2016) acknowledgement towards the benefits of maintaining positive parent-child relationships into and throughout adolescence highlights the importance of an effective parenting style. Parents need to provide a safe and secure environment to enable their child to become autonomous and independent. The support they receive from their parents coupled with how available they perceive their parents to be to their needs determines the quality of their bond. Ideally the relationship should be characterised by warmth and the parents should remain important attachment figures into adulthood (Delgado, 2011). The different attachment styles developed during childhood can influence the child's later social development with peers and future romantic relationships. Delgado's (2011) study found that securely attached children were most able in gaining emotional autonomy from parents in comparison to those ambivalent and avoidant attachment styles and thus a better competence in relationship formation. Furthermore, the security of attachment has a strong influence on how well the children manage and regulate their emotions which is, in part, their emotional intelligence.

4.1.14 Theoretical connection to behavioural outcome

A recent study found that attachment style and quality with both parents and peers predicted both aggression and prosocial behaviour. Expectedly, the lowest levels of attachment quality produced the most aggression while the highest quality produced the most prosocial behaviour (Vagos & Carvalhais, 2020). Prosocial behaviour has been defined as voluntary social behaviours or intentions which aim to benefit someone else such as sharing, helping, caring, protecting or comforting (Silke *et al*., 2018) and is a necessary component for optimal social and emotional functioning (Shaffer & Kipp, 2010), associated with higher quality peer relationships, improved social competence and less aggressive and antisocial behaviours (e.g. Dekovic & Gerris, 1994; Saarni, 1990; Raskauskas *et al*., 2010; Barr & Higgins-D'Alessandro, 2007). The development of prosocial behaviour is essential for social connectedness and belonging (Wagaman, 2011) and engaging in these behaviours fosters social and cognitive adjustment particularly during childhood and adolescence (Lenzi *et al.*, 2013). This will help nurture relationships, interactions and understanding in the social context and encourage the defining positive behaviours and intentions towards others associated with prosocial behaviour (Eisenberg *et al*., 2018). A recent review of the literature found that prosocial behaviour was associated with an array of individual and environmental factors including but not limited to personality, emotional regulation, social skills, self-efficacy and relationship quality with family and peers, connected to both social development and individual differences (Silke *et al.*, 2018)

These are all important variables to consider and require thorough examination. If mothers are able to nurture these traits and qualities from pregnancy by

supplementing with folic acid, they are providing their children with a developmental advantage in terms of their TEI and TPR. These characteristics directly impact on children's wellbeing by protecting them from internalising and externalising behaviours and building their social identity. Theoretically these outcomes have been linked to parenting style and attachment therefore this research will examine if mediating effects exist between these factors and how they impact on children's psychological, behavioural, social and emotional development. FASSTT@10y study participants were approaching early adolescence and therefore their attachments were beginning to shift from family towards peer relationships. It was then important to investigate peer attachment as an outcome and possible mediator between folic acid and children's development including reported TEI, TPR and behaviour.

The FASSTT@7y investigation relied on parental proxy measures for the psychological assessment (Henry *et al.*, 2018). This was a valuable way to obtain information about the children's psychological development when their age could have prevented them from reliably self-reporting at the time. Germain *et al*., (2018) suggested that unlike observable measures, when it comes to subjective and interpretable concepts such as wellbeing, TEI and TPR the reliability of proxy measures is equivocal, therefore self-reporting is recommended and should be used whenever possible. FASSTT@10y exceeded prior investigations in that children were able to complete the measures themselves giving more accurate responses than previous parental ratings as they were 10 years old and able to reliably self-report.

As part of the FASSTT@7y investigation, children's cognitive scores were also compared to a nationally representative sample of British children of the same age (McNulty *et al.*, 2019). Children whose mothers continued supplementing scored significantly higher than the UK norm in verbal IQ, performance IQ, general language and full-scale IQ. This was tentative evidence of a beneficial effect on children's cognitive outcome due to the folic acid intervention. Differences between FASSTT@7y participants and UK children in terms of psychological development were not assessed at that time.

The FASSTT@10y study provided an opportunity to compare children's developmental outcomes, parenting style and attachment style with normative data, warranted by the findings when provisionally explored using FASSTT@7y data. The rationale for this analysis was to ensure the data and results were representative and to test if the FASSTT@10y sample differed significantly from the norm. There is an argument that people who voluntarily participate in research are somewhat different from those who do not. It is possible that participants were more motivated, health conscious with an active interest in improving their children's development and therefore willing to contribute to FASSTT@10y. These mothers were then more likely to adhere to supplementing and have a healthy and active lifestyle, factors both shown to improve children's development (Royal College of Paediatrics and Child Health, 2020). Furthermore, partaking in these positive health behaviours would suggest that all FASSTT@10y mothers (experimental and control) may be more likely to possess the qualities required for warm and responsive parenting and be more likely to have a secure attachment consisting of high quality interactions with their children (Cook, 2000).

To accurately assess if FASSTT@10y mother and child participants were above average in positive parenting and secure attachment a comparison to normative data is also recommended. Data relating to parenting style and attachment was collected by Winsler *et al*. (2005) and Finzi *et al*. (2000) respectively. If FASSTT@10y mothers and children differed significantly from these norms it would provide tentative evidence for the continued use of folic acid to improve children's psychological, social, emotional and behavioural development.

4.1.15 Other considerations

A perspective proposed and guided by the review was the possible predictive effect of children's physical growth on their psychological, socioemotional and behavioural development. Although not the purpose of FASSTT, it had the ability to test these potential relationships within an RCT design. Substantial research has found that children's developmental outcome improves with higher birth weights and longer gestations, peaking at 41-41GW (Hack *et al*., 1995; Gleason *et al.*, 2021). A child's birthweight is considered low if they weigh less than 2500g at birth a large proportion of low birth weight (LBW) children will be born preterm (<37GW) and have an increased risk of short and long-term consequences including mortality and motor and learning disabilities (Beck *et al.*, 2010; Glass *et al.*, 2015) and by school age at risk of deficits in other developmental domains (Bhutta *et al.*, 2002) including cognition, language, behaviour and school readiness (Upadhyay *et al.*, 2019; Beck *et al.*, 2010; Howard *et al.*, 2011; Dhamrait *et al*., 2021). LBW is primarily associated with poorer antenatal maternal health (Murphy *et al*., 2004), but negative outcomes can be mitigated by providing a positive and nurturing environment for children (Hack *et al.*, 1995).

Furthermore, the evidence detailing the relationship between head circumference and brain size and volume is relatively conclusive (e.g. Hack *et al*., 1989; Lipper at al., 1981) and an association with cognitive function has been proposed although dependent on various other factors including nutrition, environment and other anthropometric measures either directly or indirectly (Nicolaou *et al.*, 2020). In support of these early findings Yue *et al*. (2021) found that pre-term infants with delayed head growth were more susceptible to cognitive impairment by 2 years and at 10y higher birthweight and head circumference at birth were associated with improved cognitive ability (Veena *et al.*, 2010).

4.1.16 Aim of the study

The aim of this study was to use a RCT design to fully explore any potential relationships between maternal folic acid use and children's psychological, social, emotional and behavioural development including TEI, TPR and peer attachment at 10y. Moreover, an examination of continued supplementation through trimesters 2 and 3 was conducted to investigate if children attained any further developmental benefits above those gained from supplementation during trimester one only. It was also important to test for any potential mediating effects of peer attachment or parenting style on each of the significant developmental areas. However, it was also important to ensure that the FASSTT@10y sample did not differ significantly from UK normative data in terms of parenting style and peer attachment as this could impact the mediating effects, therefore this study also conducted a comparative analysis to UK data (Winsler *et al.*, 2005; Finzi *et al.*, 2000).

4.1.17 Hypotheses

1. Children belonging to the experimental group will score significantly higher in TEI, TPR and Prosocial Behaviour and significantly lower in internalising and externalising behaviours in comparison to the control group
2. Experimental group children will be more securely attached and display less anxious and avoidant attachment symptoms than children belonging to the control group.
3. Mothers of children in the experimental group will report more use of positive parenting practices than those in the control group.
4. Folate level at 36GW will significantly predict both global and individual dimensions of children's TEI, TPR, prosocial behaviour, secure attachment and mothers positive parenting.
5. Mother's parenting style and children's attachment style will mediate the relationship between maternal folate level at 36GW and children's TEI , TPR, behaviour difficulties and prosocial behaviour.
6. FASSTT@10y participants will not differ significantly in positive parenting and peer attachment when compared with normative data (Winsler *et al.*, 2005; Finzi *et al.*, 2000).

4.2 Methodology

4.2.1 Original FASSTT RCT Design

The original Randomised Control Trial (RCT), detailed elsewhere (McNulty *et al.*, 2013), was conducted in 2005/2006 and investigated the effect of Folic Acid Supplementation in the Second and Third Trimester and the health outcomes of the mother and newborn (FASSTT study; ISRCTN19917787). In summary this trial

involved 126 healthy pregnant women attending antenatal clinics at the Causeway Hospital, Coleraine, Northern Ireland. Participants were aged between 18 and 35 years, with singleton pregnancies and without pregnancy complications, who had taken folic acid supplementation during the first trimester of pregnancy and provided a non-fasting blood sample at 14GW and 36GW. Participants were excluded if they historically suffered from gastrointestinal, hepatic, renal or haematological disorders, vascular disease, epilepsy or had a previous NTD affected pregnancy, were the first degree relative of a woman who had an NTD affected pregnancy or suffered from NTD herself. Participants who were taking medication that interferes with B-vitamin metabolism and also those who had undergone *in vitro* fertilisation (IVF) treatment were also excluded.

The intervention took place over 26 weeks during the second and third trimesters of pregnancy. At the end of the first trimester (12GW) participants were randomly assigned to receive either 400µg/d of folic acid (n=59) or a placebo (n=60) until the end of pregnancy approximately 40GW. Mother participants mean age at the time of recruitment is shown in *Table 4.1*. Supplements were posted to mothers monthly in 7-day pillboxes and compliance was monitored by recording unused pills at collection. In those who completed the intervention an overall compliance rate of 93% was observed.

Table 4.1: Mother participant mean age and SD recorded at original FASSTT Trial recruitment in 2006.

Treatment	**M**	**SD**
Experimental Group (n= 59)	28.07	4.17
Control Group (n= 60)	29.43	3.43

4.2.2 Sampling and randomisation procedures

The intervention was a double-blinded, randomised placebo-controlled trial with an estimated sample size of 60 participants per group in order to power the study (80% at $\alpha = 0.05$). Recruitment for the original FASSTT study took place within the Northern Health and Social Care Trust (NHSCT) only due to ethical parameters, one of the six Health and Social Care Trusts in Northern Ireland. A total of 126 women completed the intervention in 2006. To qualify for inclusion participants must have supplemented 400 µg/d of folic acid for the recommended 12GW, pre-intervention. Participants were then randomly assigned to their treatment group at the beginning of trimester 2 after being stratified according to homocysteine concentrations present in blood samples collected pre-intervention *(Figure 4.1*). Mothers supplemented with either 400µg/d of folic acid or a placebo until the end of pregnancy and both supplements were identical in colour, size and shape. Random assignment was conducted by a staff member not involved in the study to ensure both researchers and participants remained blind to treatment group allocation.

4.2.3 FASSTT@ 10y recruitment

In 2016 the full sample of mother-child pairs (*n* =126) were approached to participate in the FASSTT@10y study (*Figure 4.2)*, 65 completed the psychological assessment (55%), 24 did not wish to participate (20%), 30 agreed to participate but failed to complete the assessment (25%) and 7 were uncontactable (6%). The sub-sample of mother-child pairs (*n*= 65) were recruited from October 2016 to December 2017 when children were approximately ten years old (M=10.76 SD= .18) (*Figure 4.2*). Participants and researchers were still blind to treatment group allocation at this follow-up assessment. Appointments for data collection were held in a quiet and

private room at Ulster University, local to the participants. Upon completion of the assessment children received a £20 All for One voucher as a gratuity.

Figure 4.1: Diagrammatic protocol of the FASSTT Offspring Trial

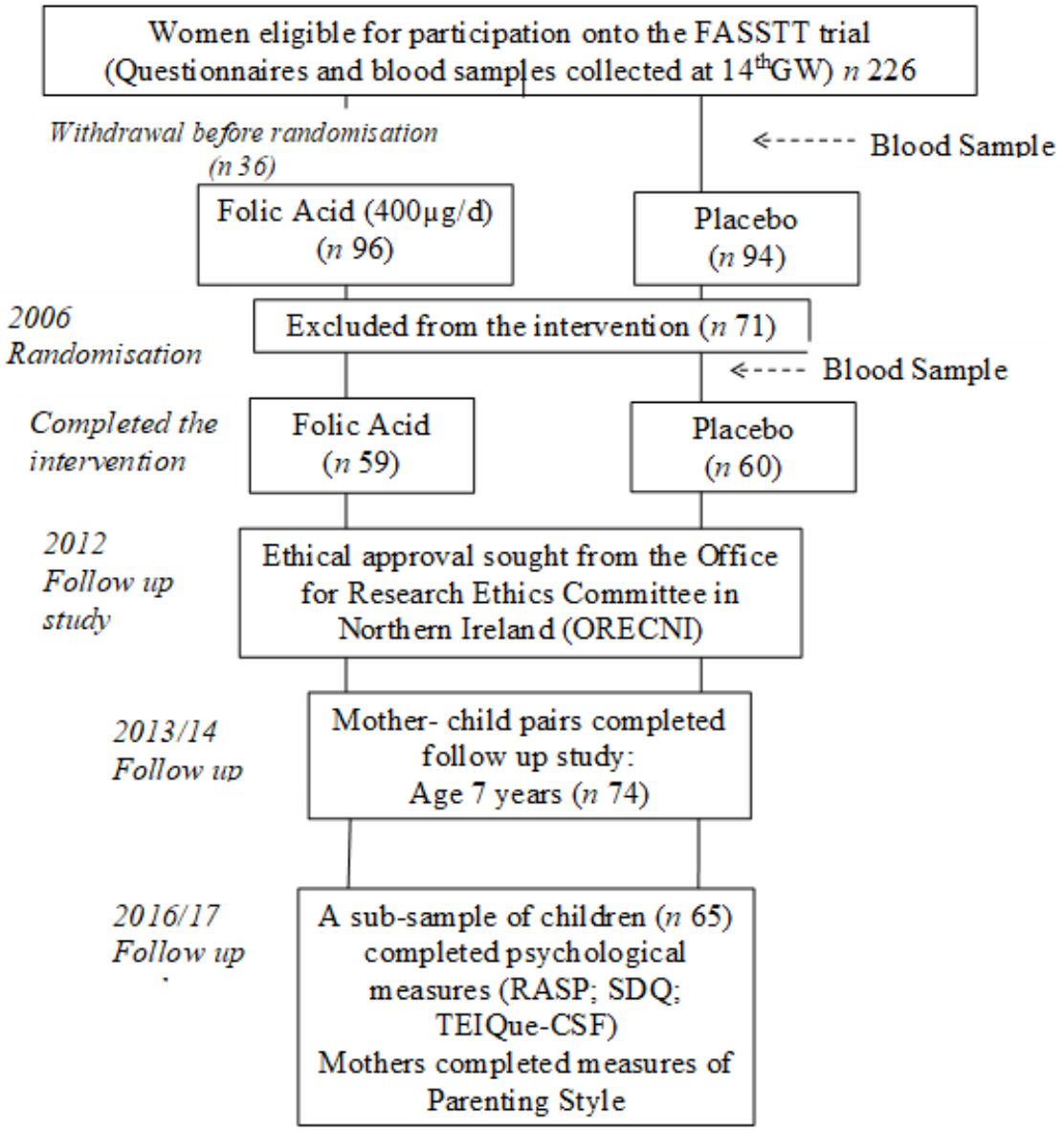
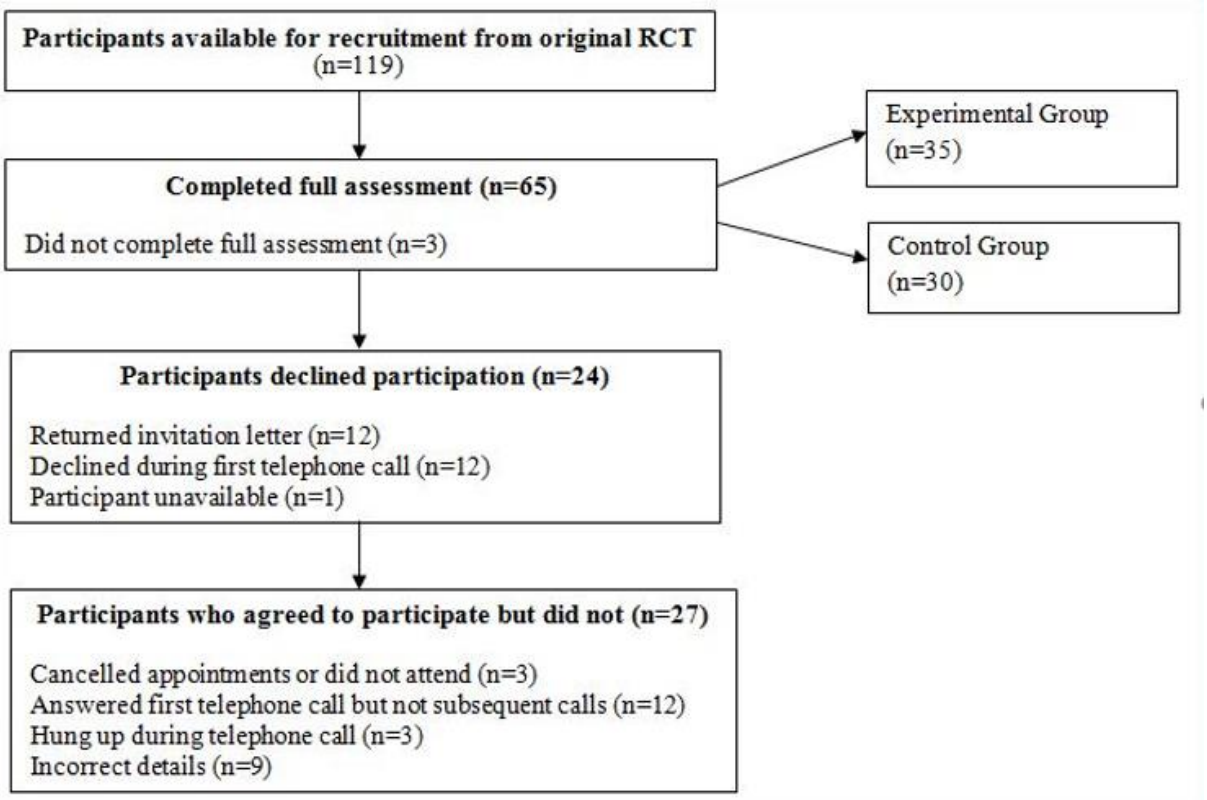


Figure 4.2: Participant flow of FASSTT@10y study population



4.2.4 FASSTT @ 10y: Child participant characteristics

A total of 65 children (31 males and 34 females) were assessed during the FASSTT@10y study. Of these included participants 35 had been originally assigned to the experimental group in 2006 and continued folic acid supplements throughout the pregnancy, and 30 had been assigned to the control group and received a placebo instead of a folic acid supplement. Participant information for each group at 10y are shown in *Table 4.2.* Any child whose mother had been recruited and included in the randomisation process in 2006 were eligible for inclusion. Participants were assigned a unique identification code to ensure confidentiality and anonymity.

Table 4.2: Child participant characteristics including distribution of sex, mean age and SD

Treatment	Males	Females	M	SD
Experimental Group (n= 35)	16	19	10.78	.12
Control Group (n= 30)	15	15	10.74	.22

4.2.5 Measures

A total of 5 self-report psychological measures were administered, 4 completed by the children and 1 completed by the parent. All scales were subject to a pilot trial before commencing data collection. *Table 4.3* outlines some characteristics of the measures used.

Table 4.3: Psychological measures and summary of characteristics that were administered to FASSTT@10y mother and child participants.

Measure	Type	Age range (y)	No of factors	No of items	Items reverse scored	Point scale used	Completion time (mins)
TEIQue-CSF	Child completed	8-12	Global	36	16	5	10-15
RASP	Child completed	12-19	7	40	0	6	10-15
SDQ	Child completed	11-17	5 or 3	25	5	3	5-10
ASCQ	Child completed	7-14	3	15	0	5	5
PSDQ	Parent completed	N/A	2/3 used.	20/32 used		5	5-10

Note. TEIQue-CSF – The Trait Emotional Intelligence Questionnaire Child Short Form; RASP – The Resiliency Attitudes and Skills Profile; SDQ – The Strengths and Difficulties Questionnaire; ASCQ – The Attachment Style Classification Questionnaire for Latency Age Children; PSDQ – The Parenting Styles and Dimensions Questionnaire

4.2.6 TEIQue-CSF

The Trait Emotional Intelligence Questionnaire Child Short Form (TEIQue-CSF) (Mavroveli *et al*., 2008; Mavroveli *et al*., 2009; Petrides, 2009) was specifically developed for children aged 8-12 years. The scale is comprised of 36 items relating

to 9 TEI dimensions and each has an illustrative example item listed in *Table 4.4*. Children responded on a 5-point Likert type scale (1 = disagree completely, 2= disagree, 3= neither agree nor disagree, 4=agree, 5= agree completely) with an average completion time of 10-15 minutes. A total of 16 items were reverse scored (items: 3, 6, 9, 12, 13, 15, 18, 21, 23, 24, 27, 29, 30, 33, 34, 35) before analysis.

Table 4.4: Emotional Intelligence dimensions and an illustrative example question, measured by TEIQue-CSF.

EI Dimension	Example Question
Adaptability	"I find it hard to get used to a new school year" (item 3)
Affective disposition	"I am a very happy kid" (item 22)
Emotion expression	"It's easy for me to talk about my feelings" (item 20)
Emotion perception	"It's easy for me to understand how I feel" (item 16)
Emotion regulation	"I'm not good at controlling the way I feel" (item 27)
Low impulsivity	"I do not like waiting to get what I want" (item 21)
Peer relationships	"The kids at school like playing with me" (item 10)
Self-esteem	"I feel great about myself" (item 4)
Self-motivation	"I try to do my homework as well as I really can" (item 19)

This instrument is available in over 20 languages and offers comprehensive personality profiles relating to emotion, providing a global TEI score. Global TEI scores range from 36 to 180 with higher scores reflecting higher TEI. Acceptable levels of internal consistency and temporal stability was found in an English sample (Cronbach $\alpha = .79$) (Mavroveli *et al.*, 2008), similar to that found in adult samples. Satisfactory levels of internal consistency were found in French and Greek samples ($\alpha = .80$) and maintained these high levels when tested separately by sex (Stassart *et al*., 2017; Babalis *et al*., 2013). Reliability within the FASSTT at 10y sample was $\alpha = .86$.

4.2.7 RASP

The Resiliency Attitudes and Skills Profile (RASP) (Hurtes & Allen, 2001) was developed to measure seven dimensions of TPR in 12 to 19-year old youths. The instrument is a 40-item scale on which children self-report how well they believe they cope with everyday challenges using a 6-point Likert type scale (1= strongly disagree, 2= moderately disagree, 3= slightly disagree, 4= slightly agree, 5= moderately agree, 6= strongly agree) with an estimated completion time of 10-15 minutes. *Table 4.5* lists each of the seven TPR dimensions and a corresponding item is provided.

Table 4.5: Resilience dimensions and an illustrative example question, measured by the RASP.

Resilience Dimension	Example Question
Insight	I can sense when someone is not telling the truth (item 21)
Independence	I say "no" to things I don't want to do (item 9)
Relationships	I choose my friends carefully (item 24)
Initiative	I try to figure out things I do not understand (item 30)
Creativity	I can imagine the consequences of my actions (item 6)
Humour	I look for the "lighter side" of tough situations (item 25)
Values Orientation	I stand up for what I believe is right (item 29)

This scale provides a score for each of the 7 dimensions and a global TPR score is the sum of all 7 dimensions, higher scores reflect strength in each domain and a higher global score corresponds to higher levels of TPR. Hurtes and Allen (2001) found strong internal consistency for global TPR ($\alpha = .91$) however Cronbach's Alpha coefficients were somewhat lower when calculated for each dimension

(relationships = .71, values orientation = .68, humour = .49, insight =.65, initiative = .53 and creativity = .68). High levels of internal consistency (α = 0.83) were also found for global TPR in an Italian sample (Sagone & Indiana, 2017). Reliability values in our sample for significant dimensions were Global TPR = .83 and creativity = .67. Windle *et al*. (2011) included the RASP in a systematic review assessing psychometric rigour and it was found to have satisfactory levels of content validity however, evidence for construct validity was limited and internal consistency was questionable as only one subscale out of seven was >.70. Despite the low reliability and validity it was the most appropriate self-report measure to use with this sample due to the small number of scales available to measure TPR in young, healthy participants.

4.2.8 SDQ

The Strengths and Difficulties Questionnaire (SDQ) (Goodman, 1997) is a brief emotional and behavioural screening questionnaire and is one of the most widely and internationally used brief questionnaires for assessing child problems (Goodman *et al*., 2010) in children aged 2 to 17 years. It is a brief multidimensional measure of psychosocial adjustment and is freely available in over 80 languages as both a self-report (11-17y) and a proxy report questionnaire (2-16y). The scale is comprised of 25 items with 5 subscales of 5 items each (*Table 4.6*) which measure emotional symptoms, conduct problems, hyperactivity/ inattention, peer relationship problems and prosocial behaviour. Each item is rated with a 3-point Likert type scale of 0 = Not True, 1 = Somewhat True and 2 = Certainly True. Five items were subject to reverse scoring before analysis (items: 7, 11, 14, 21, 25).

Table 4.6: *Dimensions measured by the SDQ and an illustrative example*

Difficulty Dimension	**Example Question**
Emotional Symptoms	"I worry a lot" (item 8)
Conduct Problems	"I fight a lot; I can make other people do what I want" (item 12)
Hyperactivity/ Inattention	"I finish the work that I'm doing, my attention is good" (item 25)
Peer Relationship Problems	"I have one good friend or more" (item 11)
Strength Dimension	**Example Question**
Prosocial Behaviour	"I try to be nice to other people, I care about their feelings" (item 1)

The total difficulties score is the summed score of each of the subscales, with the exception of the prosocial scale (Goodman, 1997). Higher scores on the prosocial behaviour subscale reflect strengths, whereas higher scores on the other four subscales reflect difficulties. A total difficulties score can also be calculated by summing the scores on the emotional symptoms, conduct problems, hyperactivity-inattention, and peer problems subscales (range = 0–40). It has an estimated completion time of 5-10 minutes and satisfactory levels of internal consistency (Yao 15-17y), test-retest reliability, concurrent validity and discriminant validity have been found (Yao *et al.*, 2009; Goodman, 2001; Muris *et al.*, 2003; Lundh *et al.*, 2008). Reliability within our sample was calculated for two models: a 5 factor model (Emotion $\alpha = .66$, Conduct $\alpha = .57$, Hyperactivity/ Inattention $\alpha = .58$, Peer Problems $\alpha = .39$, Prosocial Behaviour $\alpha = .62$, Total Difficulties $\alpha = .74$) and a 3 factor model (Internalising $\alpha = .66$, Externalising $\alpha = .70$, Prosocial $\alpha = .62$). Both models were tested as part of this research.

4.2.9 ASCQ

The Attachment Style Classification Questionnaire for Latency Age Children (Finzi-Dottan, 2012) is a self-report scale, developed to assess attachment style and externalising symptoms in 7-14-year olds. The scale is an adaptation of the adult attachment style scale (Mikulincer *et al*., 1990) and consists of 15 items with 5 items corresponding to a specific attachment style; secure, anxious or avoidant – a brief description and an example question of each attachment dimension is displayed in *Table 4.7.* Children responded on a 5-point Likert type scale (1= all wrong, 2= wrong, 3= a bit wrong/ a bit right, 3= right, 5=very right) with an average completion time of 5 minutes

Table 4.7: Dimensions measured by the ASCQ and an illustrative example

Attachment Dimension	**Example Question**
Secure Attachment	"I make friends with other children easily" (item 1)
Anxious Attachment	"Sometimes I'm afraid that other kids won't want to be with me" (item 5)
Avoidant Attachment	"I find it uncomfortable and get annoyed when someone tries to get too close to me" (item 12)

Based on their responses and highest score achieved on the scale, children are assigned to one of three attachment categories: Secure, anxious or avoidant. The measure is available in English and Hebrew. The scale has been found to be highly reliable for measuring attachment in school-aged children (Finzi *et al.* , 2000; Finzi *et al*., 2001) with satisfactory internal consistency, test-retest reliability, construct, discriminant and concurrent validity in both clinical and non-clinical samples (Al-Yagon & Mikulincer, 2004; Finzi *et al*., 1996). Reliability for our sample secure attachment $\alpha = .48$, Anxious attachment $\alpha = .66$ and avoidant attachment $\alpha = .61$

4.2.10 PSDQ

The Parenting Styles and Dimensions Questionnaire (Robinson *et al*., 1995) was developed to assess the parenting style of mothers and fathers of 4 to 12-year old children. The original instrument is a 32-item, self-report measure with each item being rated on a 5-point Likert-type scale (1= never, 2= once in a while, 3=about half of the time 4= very often, 5= always) with higher scores indicating more frequent use of the described behaviour. Estimated completion time is approximately 5-10 minutes. A brief description and an example question of each dimension is displayed in *Table 4.8* below.

Table 4.8: *Dimensions measured by the PSDQ and an illustrative example*

Positive Parenting	**Example Question**
Connection subfactor	"I encourage our child to talk about their troubles" (item 4)
Regulation/reasoning subfactor	"I explain to our child how we feel about their good and bad behavior" (item 3)
Autonomy granting subfactor	"I show respect for our child's opinions by encouraging our child to express them" (item 14)
Permissive Parenting	**Example Question**
Indulgent subfactor	"I threaten our child with punishment more often than actually giving it" (item 11)

Acceptable internal consistency and temporal stability were found in the original sample testing the 133-item scale (Robinson *et al.*, 1995). The short form containing 32 items also had adequate Cronbach's Alpha values for two of the parenting dimensions; authoritarian =.81, authoritative =.83, permissive parenting was slightly lower α =.65 (Robinson *et al*., 2001). Onder and Gulay (2009) adapted the scale to Turkish and maintained satisfactory reliability and validity for the authoritarian (α =.71) and authoritative dimensions (α= .84) but a low Cronbach's Alpha coefficient

for permissive parenting was noted (α = .38), high test-retest consistency also remained.

In the FASST @ 10y study the PSDQ was modified to include only 2 dimensions which aligns with the positive, resource focused approach. The authoritarian parenting dimension was removed, and the positive parenting (authoritative) and permissive parenting dimensions remained. The reliability in our sample for parenting style was positive parenting α = .72 and permissive parenting α = .77. Positive parenting contained a further 3 sub-factors as described in administration instructions: a) connection, warmth and support b) regulation/ reasoning induction and c) autonomy granting. Reliability was calculated for each dimension of positive parenting in our sample: parental connection, warmth and support α = .54, regulation/ reasoning induction α = .79, autonomy granting α = .63.

Excluding the Authoritarian dimension meant the scale was reduced from 32 to 20 items. In Canada, Biletchi *et al*. (2013) modified the PSDQ to include 21 items and identified three parenting styles. Exploratory Factor Analysis (EFA) showed that only 17 of 21 items loaded successfully. Internal consistency was lower than other populations with authoritative achieving α = .70 and permissive α = .59, below the recommendation but consistent with other cross-cultural research.

4.2.11 Procedure

At the time of the original FASSTT RCT, 52 mother-child pairs gave their consent to be re-contacted for future studies, and therefore were invited to take part in this follow-up by the researcher. Permission was obtained from the participants' consultant obstetrician to re-contact the remaining participants by letter inviting them

to take part in the follow-up study. Included in the letter were a Participant Information Sheet which participants were asked to read, and a stamped addressed envelope providing participants an opportunity to decline participation in the study. The researcher then got in contact with potential participants by telephone within one week; the study was explained to them again and a screening questionnaire was completed to ensure participant suitability. Children whose mothers verbally agreed to take part were invited to attend a one-off appointment lasting approximately two hours at Ulster University, Coleraine.

On arrival at the appointment the mother and child were met and accompanied to a quiet private room where the study was explained, and informed consent and assent was obtained from both the mother and child participants. The informed consent procedure was performed by the researcher who had full knowledge of the study, procedures and requirements. When obtaining consent and assent, the researcher ensured that the reason for undertaking the research, why they are being asked to participate and what they were being asked to do was fully understood by each participant. A full verbal explanation of the study and its requirements were given by the researcher and any questions/ queries were addressed. The benefits and risks of the study procedures and the rights of the participant were also explained. The child and researcher sat at a table where questionnaire responses were laid out as flash cards. For consistency, and to ensure understanding, the researcher read each questionnaire item to the child and the child responded by pointing to the flash card they felt was most appropriate. Once all four measures were complete the mother completed the final questionnaire. Upon completion of the full assessment children received their gratuity voucher.

4.2.12 Ethical Considerations

Ethical approval for the original RCT was granted in full, from the Office of Research Ethics Committees in Northern Ireland (ORECNI) on 25th August 2012 (05/Q2008/21), Approval from the Northern Health and Social Care Trust (NHSCT) was received on 28 December 2012. The mother participant was given both written and oral information regarding the study and adequate time to decide whether or not to participate and, if appropriate, then provide informed consent to the researcher. The study and its components were explained individually to each child participant who provided written assent in order to participate. To ensure confidentiality participants were assigned a unique study code for identification purposes, to ensure anonymity was protected. All participant data was kept on a password protected computer with access limited to those in the research team. Mothers and their children were reassured that their decision not to participate or withdraw from the study will not compromise any future care they may require in the Northern Health and Social Care Trust.

A substantial amendment was made to include the children at 10y to complete the measures and ethical approval was granted for the follow-up study on July 19, 2016 from ORECNI and on September 5, 2016 from NHSCT.

4.2.13 Sample size, power and precision

A priori analysis was calculated using G* Power (Faul *et al*.,, 2009) which estimated that a sample size of 21 participants per group was required to detect an observable difference in means of 10% between groups with a power of 80 % at $\alpha = 0.05$ using

independent t-tests. A total of 65 participants were recruited, 35 were experimental and 30 were control group participants. Effect size was calculated and reported post hoc using *Cohen's d* analysis.

4.2.14 Statistical Analysis

Data was entered into IBM SPSS Statistics (Version 24), cleaned and coded. Normality of participant background variables and psychological test scores was assessed using the Shapiro-Wilks test, and Levene's test was used to assess homogeneity of variance and confirmed the use of parametric tests. Chi-square was used to test for any significant differences in categorical variables and independent t-tests were used to find any significant differences in the remaining background characteristics including the serum folate and red blood cell folate levels at 12GW, 36GW and birth. The effects of folic acid vs placebo on each of the psychological measures were also examined using independent t-tests with Bonferroni correction with $p<.001$ considered statistically significant. As the assumptions of normality, homoscedasticity and multicollinearity and independence of observations were met, Hierarchical Multiple Regression Analysis (HMRA) was the most appropriate test to investigate if continued folic acid supplementation was a significant predictor of TEI, TPR, peer attachment and behavioural strengths and difficulties. In addition, further analysis was conducted to investigate if the relationship between maternal serum folate level at 36GW and children's TEI, TPR, behavioural difficulties or prosocial behaviour was mediated by positive parenting practices or children's attachment style. Finally, to compare FASSTT@10y parenting and peer attachment data to UK norms one-sample t-tests were conducted.

4.3 Results

This section details the results found during the FASSTT@10y study. Mothers folate status at 14GW (pre-intervention), 36GW (post-intervention) and birth (cord blood at delivery) is presented in the first instance, followed by the background characteristics of FASSTT@10y mother-child pairs. The section continues by listing each of the psychological effects tested in the FASSTT@10y sample and the level of significance found when the experimental and control groups were compared. An illustration and a detailed description of each significant finding is provided. Next, Hierarchical Multiple Regression Analysis (HMRA) was used to test if maternal folate level at 36GW significantly predicted children's developmental outcomes at 10y. The results section concludes with mediation analysis and a comparison to UK norms. This tests the mediating effect of parenting style and child peer attachment on the developmental outcomes significantly predicted by folate status at 36GW and if FASSTT@10y participants differed significantly to their UK counterparts. Significant mediators are illustrated using pathway diagrams.

4.3.1 Maternal folate status – pre and post intervention (FASSTT @ 10y)

The folate status of each treatment group was measured pre-intervention (~14GW), post-intervention (~36GW) and again at birth (cord blood). To ensure randomisation remained successful in the FASSTT@10y sub-sample independent t-tests were used to compare the groups folate levels at each of these time-points. No differences were observed between the groups pre-intervention as expected, however red blood cell (RBC) folate and serum folate levels were significantly higher in the experimental

group at 36th GW and following birth, again as expected (*Table 4.9*). This confirmed effective randomisation in the FASSTT@10y sample.

Table 4.9: Maternal folate status at 14th, 36th gestational weeks (GW) and birth for the 65 original FASSTT participants who participated in FASSTT@10y

	Experimental (*n*=35)	**Control (*n*=30)**	***p***	***95% CI***
RBC Folate levels 14th GW	1283.40 ± 648.33	1040.27 ±593.41	.122	-66.95, 553.21
RBC Folate levels 36th GW(a)	1829.82 ± 589.47	877.31 ± 328.18	<.001***	706.23, 1198.80
RBC Folate levels birth (b)	2202.82 ± 868.03	1511.66 ± 568.21	.003*	255.30, 1127.01
Serum Folate levels 14th GW	49.24 ± 21.69	50.78 ± 20.40	.771	-12.03, 8.96
Serum Folate levels 36th GW (a)	51.79 ± 19.26	21.84 ± 17.06	<.001***	20.61, 39.30
Serum Folate levels birth (b)	100.84 ± 37.03	64.07 ± 25.87	<.001***	19.54, 54.00

Note: Data are presented as means ± SD. Differences between groups were assessed using an independent samples t-test; P <.05 was considered significant. (a) Post-intervention following folic acid supplementation or placebo. (b) Cord blood collected at delivery. p<.05 **p<.01, ***p<.001*

4.3.2 Mother and child background characteristics

No significant differences between treatment groups were found when several maternal and child background characteristics were examined (*Table 3.10*). A combination of independent t-tests and chi-square tests were conducted comparing the treatment groups on a range of characteristics, listed in *Table 3.10.* This offers further support to the high quality of the RCT.

Table 4.10: General characteristics of FASSTT@10y participants

	Experimental (*n*=35)	**Control (*n*=30)**	***p***
Maternal Characteristics			

Age (y) [(a)]	29.43 ± 3.43	28.07 ± 4.17	.154
BMI (kg/m^2)	25.43 ± 5.62	25.04 ± 3.97	.746
Number of children	2.74 ± 0.83	2.93 ± 1.07	.416
Duration of folic acid use (weeks)	12.57 ± 7.21	16.23 ± 11.12	.115
Education attainment (y) [(b)]	19.52 ± 2.53	19.30 ± 2.48	.738
Parity (n)	0.86 ± 0.94	0.70 ± 0.79	.475
Smoking during pregnancy (%)	14.3	3.3	.128
Smoking at 10y follow-up (%)	14.7	6.9	.553
Alcohol use during pregnancy (%)	2.9	3.4	.912
Alcohol use at 10y follow-up (%)	85.3	79.3	.533
Married during pregnancy (%)	85.7	76.6	.339
Married at 10y follow-up (%)	91.2	82.8	.317
Homeowner at 10y follow-up (%)	85.3	79.3	.258
Child Characteristics			
Age at assessment (y)	10.74 ± 0.22	10.78 ± 0.12	.369
Born by caesarean (%)	28.6	30.0	.900
Sex (male) (%)	44.1	55.9	
Sex (female) (%)	51.6	48.4	.730
Exclusively breastfed from birth (%)	40.0	36.7	.804
Birth weight (g)	3481.37 ± 521.09	3473.00 ± 450.80	.945
Birth length (cm)	51.00 ± 2.44	50.93 ± 2.34	.909
Birth head circumference (cm)	34.59 ± 1.57	34.37 ± 1.28	.564
Weight at 10y (kg)	37.18 ± 7.18	38.84 ± 9.43	.945
Height at 10y (cm)	146.41 ± 7.90	146.69 ± 5.94	.874
Waist circumference at 10y (cm)	67.86 ± 7.77	70.37 ± 9.78	.254
Head circumference at 10y (cm)	54.54 ± 2.19	54.91 ± 2.42	.516

Note: Data are presented as mean ± SD or %. Differences between groups were assessed using independent t-tests (continuous variables) or chi-square test (categorical variables). $p<.05$ is considered significant. [(a)] Refers to the mothers age during the original FASSTT trial; [(b)] Years of formal education completed.

The following sections will focus on the developmental outcomes observed in FASSTT@10y participants and by comparing those in the experimental and control treatment groups. Children's TEI, TPR, behaviour, peer attachment and mothers parenting style are all considered to address research objectives 1-3 listed in the

introductory section of this chapter. All significant findings are displayed using histograms. Significance level and confidence intervals are also provided.

4.3.3 Psychological effects

The psychological outcomes of the experimental (n= 35) and control (n= 30) groups were compared using independent t-tests with Bonferroni correction (p<.001 considered statistically significant). Descriptive statistics for each scale administered including a total score, if available, and individual scores for each dimension are shown in *Table 4.11*. Global scores for TEI and TPR indicate a significant difference between experimental and control group conditions, moreover, significant differences were observed between groups regarding parenting style.

Children belonging to the experimental group scored significantly higher in both Global TEI **($t_{(63)}$ = 3.925, p = <.001, d = 0.99)** and total TPR scores **($t_{(63)}$ = 3.347, p = .001, d = 0.84)**. Experimental group participants scored somewhat lower in total behavioural difficulties however this was not a significant difference **($t_{(63)}$ = -2.236, p = .023, d = -0.56)**, *Figure 4.3*.

When considering the individual dimensions of the RASP, although not statistically significant, the experimental group scored somewhat higher in humour, relationships, independence, insight and values and slightly lower in initiative in comparison to the control group. Significant differences were observed between the groups with experimental group children scoring significantly higher in creativity **($t_{(63)}$ = 3.430, p = .001, d = 0.86)**, see *Figure 4.4.*

When measured using the SDQ, experimental group scores were approaching significance with a medium effect size, displaying less emotional problems, hyperactivity or inattention problems and less conduct problems **($t_{(63)}$ = -1.972, p = .053, d = -0.50)** than the control group. In addition, the experimental group reported less peer problems and more prosocial behaviour **($t_{(63)}$ = 2.149, p = .036, d = 0.54)** (*Figure 4.5*). Using the 3-factor model the experimental group scored somewhat lower in internalising and externalising behaviours, comparable to the 5-factor model (*Figure 4.6*).

Figure 4.3a: Significant differences in Global scores of TEI (TEI-Que) between experimental and control groups

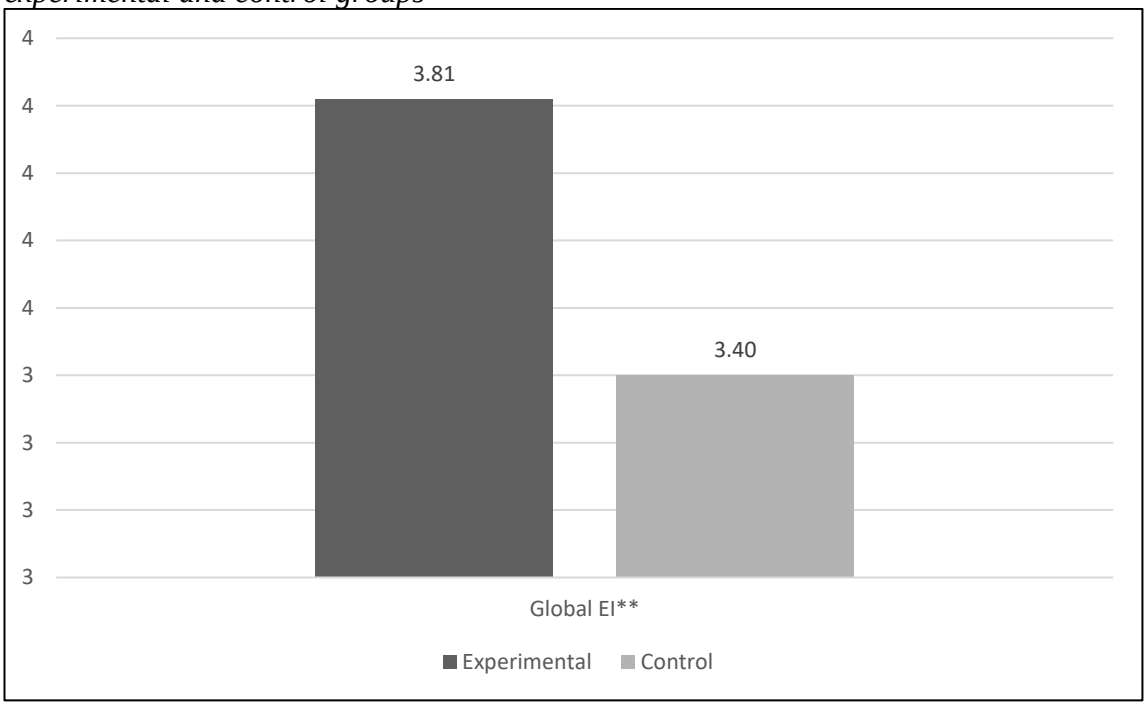


Figure 4.3b: Significant differences in Total TPR scores (RASP) between

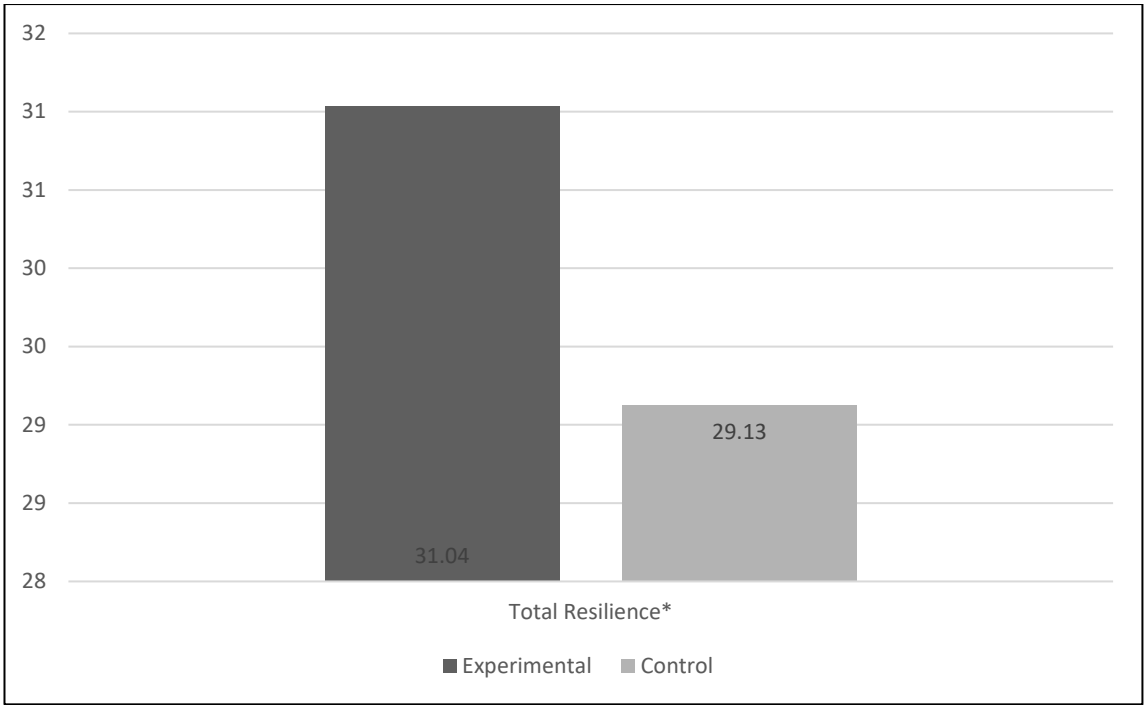


Figure 4.4: Individual dimensions of RASP measuring children's resilience

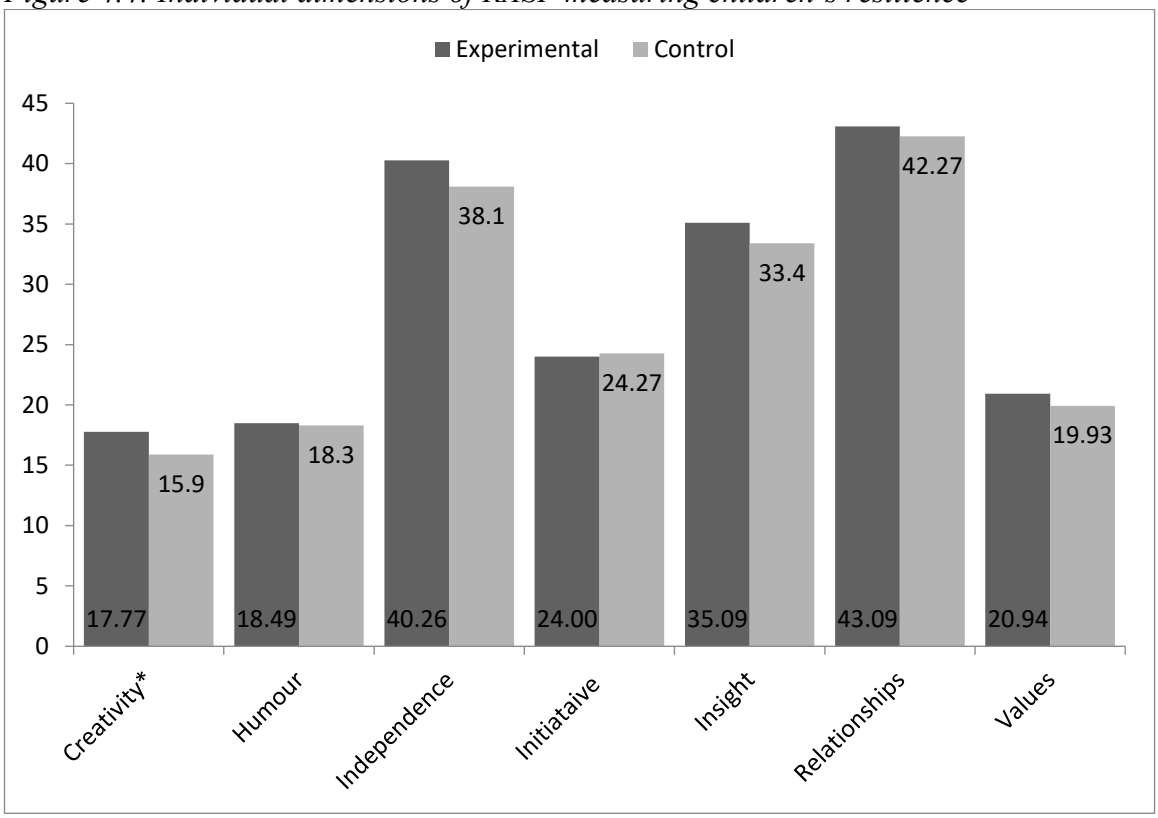


Table 4.11: Experimental versus control group descriptive statistics and main effects

	Experimental (*n*=35)	**Control (*n*=30)**	***t-value***	***p***	***95% CI Lower/Upper***		***Cohen's d***
TEIQue-CSF							
Global EI	3.81 ± .50	3.40 ± .31	3.925	<.001***	.20	.62	0.99
RASP							
Resilience Total	31.04 ± 2.28	29.13 ± 2.30	3.347	.001**	.77	3.04	0.83
Creativity	17.77 ± 1.94	15.90 ± 2.45	3.430	.001**	.78	2.96	0.85
Humour	18.49 ± 3.15	18.30 ± 2.73	.252	.802	-1.29	1.67	0.07
Independence	40.26 ± 3.74	38.10 ± 4.07	2.226	.030	.22	4.09	0.66
Initiative	24.00 ± 5.06	24.27 ± 2.60	-.461	.646	-1.42	.89	0.07
Insight	35.09 ± 3.18	33.40 ± 3.07	2.167	.034	.13	3.24	0.54
Relationships	43.09 ± 3.14	42.27 ± 4.14	.906	.369	-.99	2.62	0.22
Values orientation[(a)]	20.94 ± 1.98	19.93 ± 3.17	1.508	.138	-.34	2.36	0.38
SDQ							
Total Difficulties	8.91 ± 4.32	11.60 ± 4.83	-2.236	.023	-4.99	-.39	0.59

	Experimental (*n*=35)	**Control (*n*=30)**	***t-value***	***p***	***95% CI Lower/Upper***		***Cohen's d***
Emotional problems	2.21 ± 1.75	3.17 ± 2.12	-1.959	.055	-1.93	.02	0.49
Conduct Problems	1.67 ± 1.31	2.40 ± 1.63	-1.972	.053	-1.48	.01	0.49
Hyperactivity	3.76 ± 2.05	4.50 ± 2.26	-1.370	.176	-1.83	.34	0.34
Peer problems	1.27 ± 1.31	1.53 ± 1.36	-.776	.441	-.93	.41	0.19
Prosocial Behaviour	8.36 ± 1.62	7.53 ± 1.43	2.149	.036	.06	1.60	0.54
Internalising Problems	3.48 ± 2.54	4.70 ± 2.94	-1.761	.083	-2.59	.16	0.44
Externalising Problems	5.42 ± 2.89	6.90 ± 3.35	1.877	.065	-3.05	.09	0.47
ASCQ							
Secure Attachment	20.71 ± 2.47	19.73 ± 2.33	1.638	.106	-.22	2.18	0.41
Anxious Attachment	14.97 ± 2.06	16.27 ± 2.65	-2.212	.031	-2.47	-.12	0.55
Avoidant Attachment	12.29 ± 3.53	13.13 ± 3.28	-.998	.322	-2.54	.85	0.25
PDSQ							
Positive parenting	4.54 ± .30	4.32 ± .35	2.646	.010*	.05	.39	0.67
Warmth and Support	4.66 ± .44	4.55 ± .47	.966	.338	-.12	.34	0.24

	Experimental (*n*=35)	**Control (*n*=30)**	***t-value***	***p***	***95% CI Lower/Upper***		***Cohen's d***
Reasoning	4.50 ± .46	4.32 ± .58	1.369	.176	-.08	.43	0.34
Autonomy Granting	4.00 ± .62	3.82 ± .56	1.228	.224	-.11	.47	0.30
Permissive parenting	6.68 ± 2.11	6.63 ± 2.41	.076	.939	-1.09	1.17	0.02

Note. Experimental versus control group descriptive statistics and main effects
Bonferroni correction applied: p<.01 is considered significant
**p<.05 **p<.01 , ***p<.001 (a) t-value for unequal variances used*

Figure 4.5: Individual dimensions of SDQ measuring behaviour: five factor model

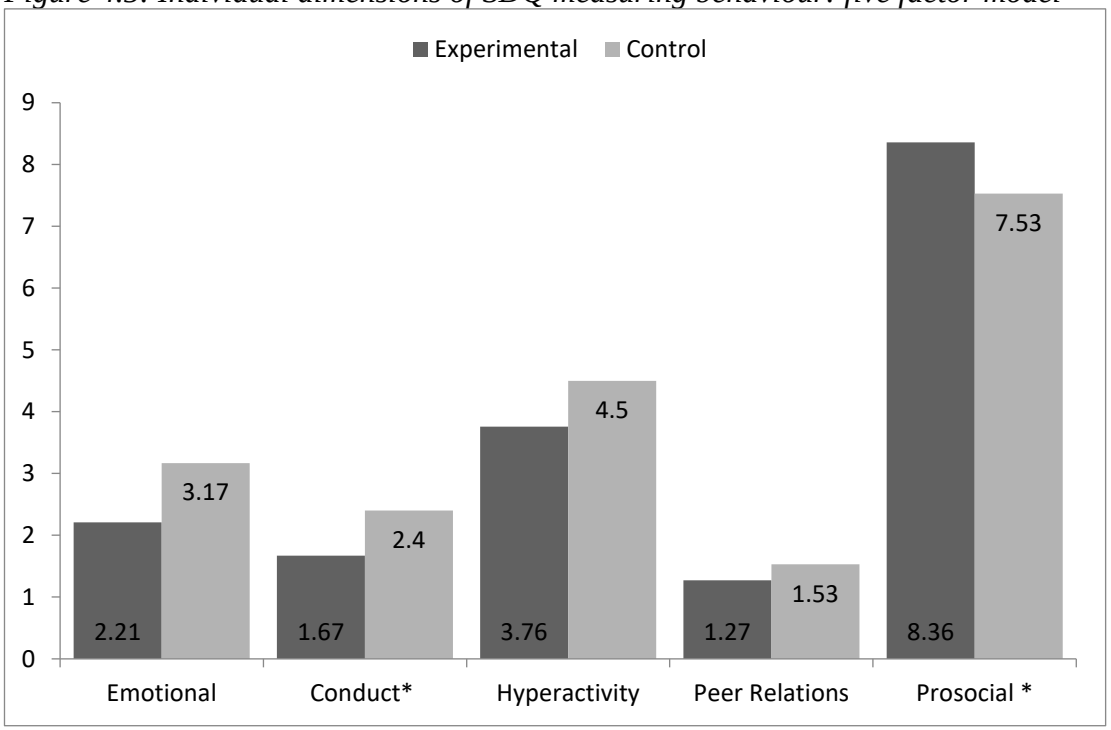
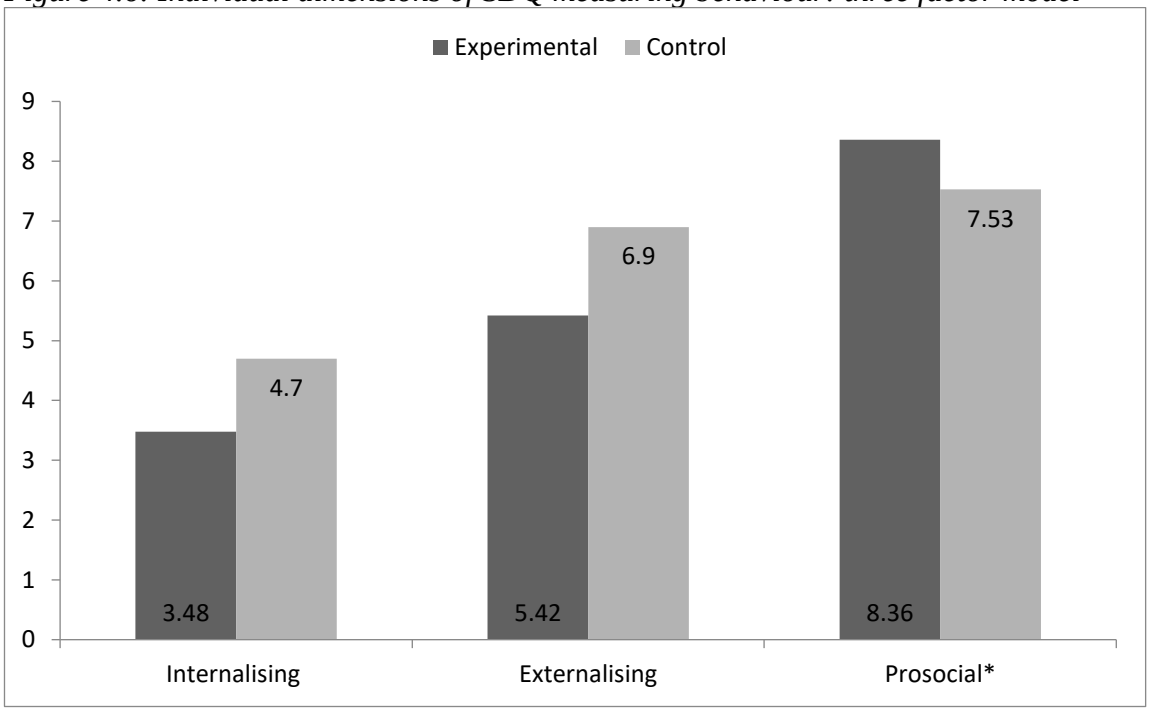


Figure 4.6: Individual dimensions of SDQ measuring behaviour: three factor model



According to ASCQ scores the experimental group displayed less avoidant and significantly less anxious attachment styles **($t_{(63)}$ = -2.212, p = .031, d = -0.56)** and were more likely to experience secure attachment *(Figure 4.7).*

Figure 4.7: Individual dimensions of ASCQ measuring peer attachment

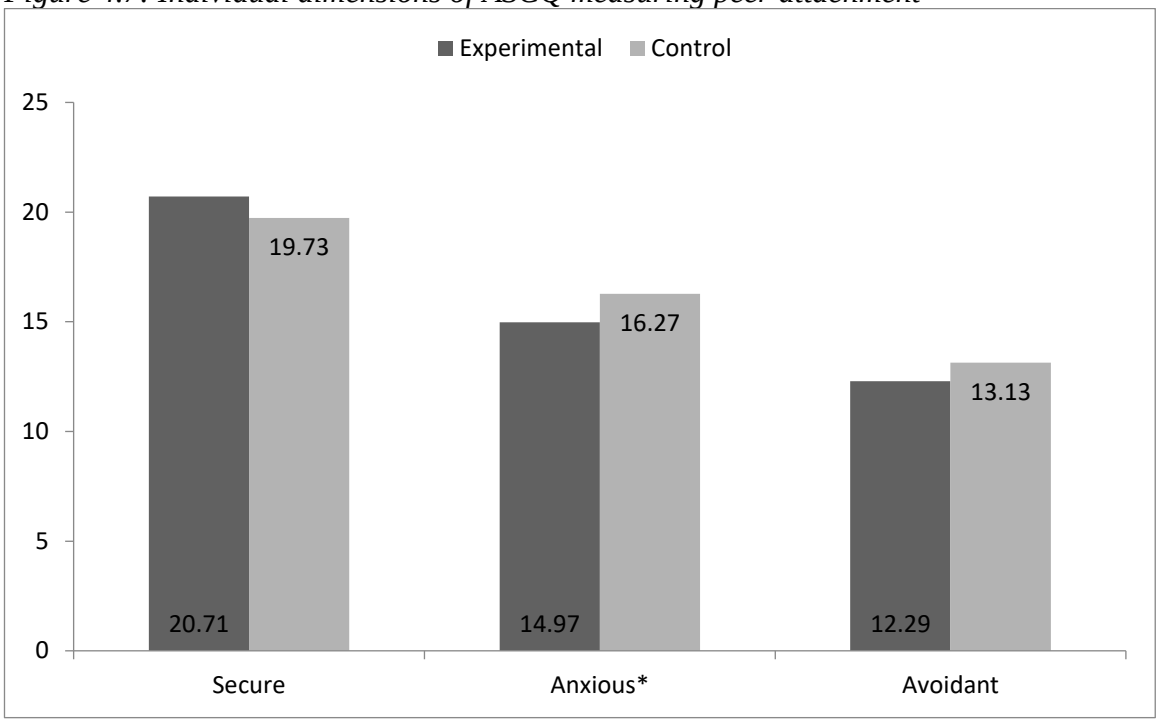
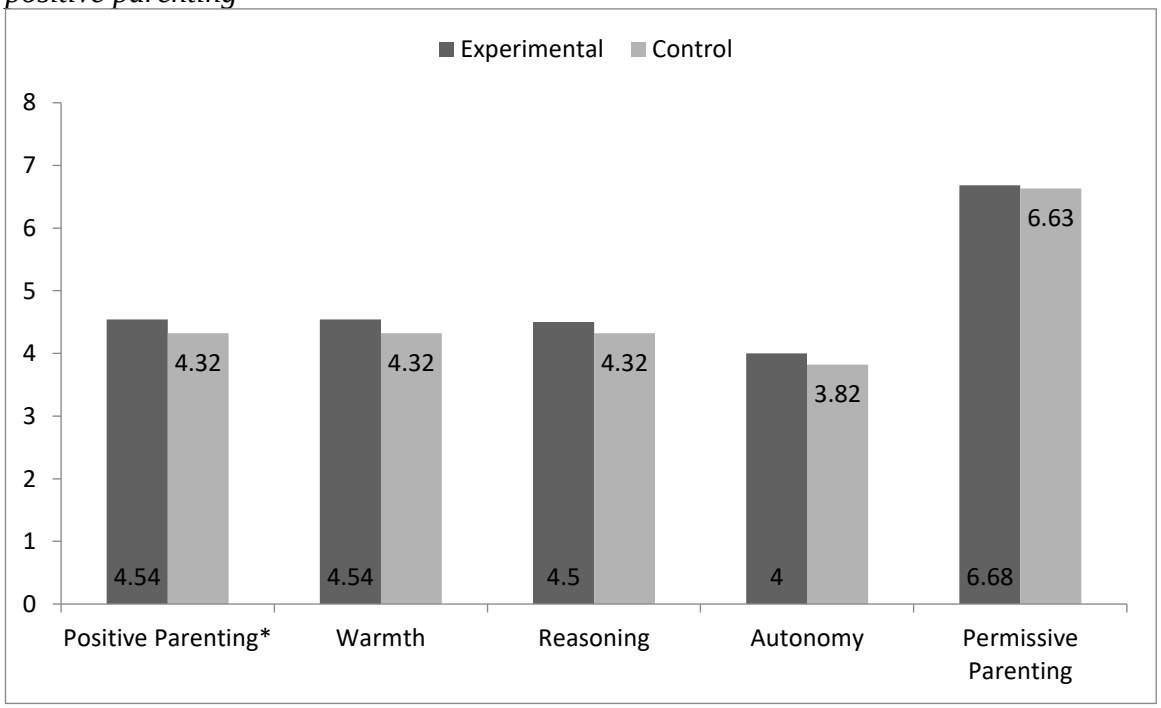


Figure 4.8: Individual dimensions of parenting style (PDSQ) and sub-dimensions of positive parenting



Parents provided ratings on their parenting style using the PSDQ. Approaching significance, mothers of the children in the experimental group reported higher levels of positive parenting **($t_{(63)}$ = 2.646, p = .010, d = 0.67)**, with higher levels of parental warmth, reasoning and autonomy granting dimensions in comparison to the control group. Surprisingly, the experimental group also reported slightly higher levels of permissive parenting. These differences were not significant (*Figure 4.8*).

4.3.4 Hierarchical Multiple Regression Analysis (HMRA)

Following the identification of significant differences between the groups and to address *Hypothesis 4*, HMRA was used to test the assumption that maternal folate status at 36GW would significantly predict children's developmental outcome, in particular, total TEI, total TPR, prosocial behaviour, secure attachment and positive parenting style. To investigate further, mediation analysis was conducted to test if mothers parenting style and children's peer attachment style mediated the relationship between maternal folate level at 36GW and children's developmental outcomes at aged 10y (*Hypothesis 5*).

4.3.5 TEI-Que: Global EI

The proposed relationships were analysed individually using HMRA. Global TEI was entered as the dependent variable with the child's anthropometric characteristics at birth and 10y entered as predictor variables in step 1 which accounted for a significant amount of variance (29%) (**$F_{(8, 45)}$ = 2.232; p = .035, R^2= .29**). Maternal characteristics including age and BMI were added for step 2 which only accounted for a further 2% of the variance (**$F_{(2, 43)}$ = 0.736; p = .485, R^2= .02**). The mother's

level of folate measured at 36GW was added to the model and accounted for a total of 39%, a significant amount of variance (**$F_{(1, 42)} = 5.173$; $p = .028$, $R^2 = .08$**). Serum folate level at 36GW and the child's head circumference at birth were significant predictors of children's TEI scores (*Table 4.12)*. Significant partial correlations were found between TEI and serum folate level at 36GW ($r_{(53)} = .433$, $p = .001$) including the child's head circumference at birth ($r_{(53)} = .319$, $p = .009$), weight at 10y ($r_{(53)} = .314$, $p = .010$), and mothers BMI ($r_{(53)} = -.242$, $p = .039$).

4.3.6 RASP: Total TPR and individual dimensions

The effect of these predictor variables on children's total TPR scores was then assessed. Step 1 (child's anthropometric measurements) accounted for 12% variance (**$F_{(8, 45)} = .769$; $p = .631$, $R^2 = .12$**) and Step 2 (maternal characteristics) yielded no additional support (**$F_{(2, 43)} = .003$; $p = .997$, $R^2 = .12$**). The addition of serum folate in Step 3 increased the variance by 7%, however this was nonsignificant (**$F_{(1, 42)} = 3.730$; $p = .060$, $R^2 = .19$**), no significant predictor variables were identified. A significant partial correlation was observed between resilience and serum folate at 36GW ($r_{(54)} = .303$, $p = .013$), no other significant values were found.

In terms of the individual dimensions of the instrument (Creativity, Humour Independence, Initiative, Insight, Relationships and Values Orientation), serum folate was identified as a significant predictor for Creativity (*Table 4.13*) and Values (*Table 4.14*). Serum folate at 36GW also explained a significant amount of variance, 26% (**$F_{(1, 42)} = 7.559$; $p = .008$, $R^2 = .26$)** and 22% respectively (**$F_{(1, 42)} = 5.358$; $p = .026$, $R^2 = .22$)** within the model. Furthermore, these were also the only RASP

outcomes to achieve partial correlations with serum folate ($r_{(54)}$ = .329, p = .008; $r_{(54)}$ = .290, p = .017). No significant predictors or partial correlations were found for the dimensions of Humour, Independence, and Insight. Initiative was partially correlated to child's sex ($r_{(54)}$ = -.258, p = .030), weight at 10y ($r_{(54)}$ = .279, p = .021), waist at 10y ($r_{(54)}$ = .303, p = .013) and maternal BMI ($r_{(54)}$ = .237, p = .042), however was not significantly predicted by any of the variables included in the model. Sex of the child was found to be a significant predictor in the Relationships dimension (β = -.427, $t_{(54)}$ = -3.178, p = .003), explaining a significant 20% of the variance in these scores (**$F_{(8, 45)}$ = 2.698; p = .016, R^2= .20**). This dimension was also partially correlated to sex ($r_{(54)}$ = -.437, p <.001), head circumference at birth ($r_{(54)}$ = -.222, p = .053) and height at 10y ($r_{(54)}$ = -.225, p = .051).

4.3.7 SDQ: Behavioural difficulties total score and individual dimensions

Another potential relationship examined was that between serum folate at 36GW and children's total difficulties. HMRA analysis identified that serum folate was not a significant predictor of total behaviour difficulties however this 3 step model did find a significant amount of variance at Step 1 (40%) (**$F_{(8, 44)}$ = 3.646; p = .002, R^2= .40**) and Step 2 (48%) (**$F_{(2, 42)}$ = 3.234; p = .049, R^2= .48**), a nonsignificant increase of 1% was observed following Step 3 (**$F_{(1, 41)}$ = 1.242; p = .271, R^2= .49**). These findings were attributed to anthropometric predictors including child's sex (β = .316, $t_{(53)}$ = 2.576, p = .019), birthweight (β = -.479, $t_{(53)}$ = -2.372, p = .022), length at birth (β = .396, $t_{(53)}$ = 2.483, p = .017) and maternal age (β = -.288, $t_{(53)}$ = -2.335, p = .025). A number of predictor variables were partially correlated to total difficulties including the child's birthweight ($r_{(53)}$ = -.247, p = .037), head circumference at birth

Table 4.12: HMRA with Total Emotional Intelligence score as the dependent variable

Variable	B	SE B	β	Sig.	95.0% Confidence Interval for B Lower	Upper
Reference group male=0						
Step 1: R2 = .29, f(8,45) = 2.332, p= .035						
Baby's Sex	-.193	.128	-.202	.139	-.452	.065
Baby weight (g)	.000	.000	-.266	.272	-.001	.000
Baby Length (cm)	-.011	.036	-.058	.762	-.084	.062
Baby head circumference (cm)	.141	.064	.436	.033*	.012	.270
Weight, kg (age10)	.001	.019	.018	.957	-.037	.039
Height, cm (age10	.028	.013	.378	.035	.002	.054
Waist Circumference 10y (cm)	-.018	.016	-.337	.247	-.050	.013
Head Circumference 10y (cm)	.012	.035	.054	.733	-.058	.082
Step 2: R2 Δ = .02, f(2,43) = 0.736, p= .485						
Baby's Sex	-.199	.129	-.208	.131	-.460	.062
Baby weight (g)	.000	.000	-.212	.397	-.001	.000
Baby Length (cm)	-.011	.037	-.059	.759	-.085	.063
Baby head circumference (cm)	.130	.068	.401	.061	-.006	.266
Weight, kg (age10)	.003	.019	.050	.881	-.035	.041
Height, cm (age10	.026	.013	.351	.055	-.001	.052

Variable	B	SE B	β	Sig.	95.0% Confidence Interval for B Lower	Upper
Waist Circumference 10y (cm)	-.017	.016	-.310	.300	-.049	.015
Head Circumference 10y (cm)	.013	.035	.061	.706	-.058	.084
BMI (Body Mass Index)	-.018	.015	-.167	.240	-.049	.012
Age (y)	-.001	.018	-.005	.973	-.038	.036
Step 3: R2 Δ = .08, f(1,42) = 5.173, p= .028						
Baby's Sex	-.160	.125	-.168	.205	-.412	.091
Baby weight (g)	.000	.000	-.276	.252	-.001	.000
Baby Length (cm)	-.008	.035	-.039	.830	-.078	.063
Baby head circumference (cm)	.136	.065	.420	.041*	.006	.266
Weight, kg (age10)	.002	.018	.043	.891	-.034	.039
Height, cm (age10	.021	.013	.284	.108	-.005	.047
Waist Circumference 10y (cm)	-.012	.015	-.218	.449	-.043	.019
Head Circumference 10y (cm)	.013	.034	.058	.707	-.055	.081
BMI (Body Mass Index)	-.012	.015	-.111	.419	-.042	.018
Age (y)	-.004	.018	-.032	.810	-.040	.031
Serum Folate C (nmol/l)	.004	.002	.304	.028*	.000	.007
Total R2 = .39						

*Values considered significant: *p<.05*

Table 4.13: HMRA with the Creativity dimension of the RASP score as the dependent variable and Serum folate at 36GW

Variable	B	SE B	β	Sig.	95.0% Confidence Interval for B Lower	Upper
Reference group male=0						
Step 1: R^2 = .09, f(8, 45) = .589, p= .781						
Baby's Sex	-1.099	.766	-.218	.158	-2.642	.443
Baby weight (g)	.001	.001	.108	.693	-.002	.003
Baby Length (cm)	-.042	.216	-.042	.847	-.478	.394
Baby head circumference (cm)	-.149	.383	-.088	.699	-.921	.623
Weight, kg (age10)	.018	.111	.062	.869	-.206	.243
Height, cm (age10	-.055	.077	-.143	.473	-.210	.099
Waist Circumference 10y (cm)	-.028	.093	-.098	.766	-.215	.159
Head Circumference 10y (cm)	-.119	.208	-.103	.569	-.538	.300
Step 2: R^2 Δ = .02, f(2,43) = .630, p= .537						
Baby's Sex	-1.071	.773	-.212	.173	-2.631	.489
Baby weight (g)	.000	.001	.081	.773	-.002	.003
Baby Length (cm)	-.064	.219	-.063	.772	-.505	.378
Baby head circumference (cm)	-.054	.404	-.032	.894	-.869	.761
Weight, kg (age10)	.021	.113	.071	.852	-.206	.248
Height, cm (age10	-.049	.078	-.126	.536	-.207	.109

Variable	B	SE B	β	Sig.	95.0% Confidence Interval for B Lower	Upper
Waist Circumference 10y (cm)	-.045	.096	-.156	.642	-.237	.148
Head Circumference 10y (cm)	-.101	.210	-.087	.634	-.525	.324
BMI (Body Mass Index)	-.008	.090	-.014	.929	-.190	.174
Age (y)	-.120	.110	-.172	.281	-.340	.101
Step 3: R^2 Δ = .13, f(1,42) = 7.559, p= .009						
Baby's Sex	-.798	.727	-.158	.279	-2.266	.670
Baby weight (g)	-2.227E-5	.001	-.005	.986	-.003	.003
Baby Length (cm)	-.037	.204	-.037	.856	-.449	.375
Baby head circumference (cm)	-.012	.377	-.007	.976	-.771	.748
Weight, kg (age10)	.019	.105	.062	.860	-.193	.230
Height, cm (age10	-.084	.074	-.216	.265	-.233	.066
Waist Circumference 10y (cm)	-.010	.090	-.033	.916	-.191	.172
Head Circumference 10y (cm)	-.105	.196	-.091	.594	-.501	.290
BMI (Body Mass Index)	.035	.086	.060	.689	-.138	.207
Age (y)	-.145	.102	-.209	.164	-.352	.062
Serum Folate C (nmol/l)	.028	.010	.406	.009**	.007	.048
Total R^2 = .26						

*Values considered significant: *p<.05 **p<.01*

Table 4.14: HMRA with the Values dimension of the RASP score as the dependent variable and Serum folate at 36GW

Variable	B	SE B	β	Sig.	95.0% Confidence Interval for B Lower	Upper
Reference male=0						
Step 1: R^2 = .10, f(8,45) = .620, p= .757						
Baby's Sex	-1.107	.782	-.214	.163	-2.682	.467
Baby weight (g)	.001	.001	.137	.614	-.002	.003
Baby Length (cm)	-.218	.221	-.211	.328	-.663	.226
Baby head circumference (cm)	-.082	.391	-.047	.835	-.870	.706
Weight, kg (age10)	-.096	.114	-.311	.404	-.324	.133
Height, cm (age10	.024	.078	.061	.758	-.133	.182
Waist Circumference 10y (cm)	.077	.095	.263	.422	-.114	.268
Head Circumference 10y (cm)	-.131	.212	-.110	.541	-.559	.297
Step 2: R^2 Δ = .02, f(2,43) = .392, p= .678						
Baby's Sex	-1.145	.794	-.222	.156	-2.745	.456
Baby weight (g)	.001	.001	.185	.515	-.002	.004
Baby Length (cm)	-.202	.225	-.196	.374	-.655	.251
Baby head circumference (cm)	-.187	.141	-.107	.654	-1.023	.649
Weight, kg (age10)	-.093	.116	-.302	.426	-.326	.140
Height, cm (age10	.014	.080	.034	.866	-.149	.176

Variable	B	SE B	β	Sig.	95.0% Confidence Interval for B Lower	Upper
Waist Circumference 10y (cm)	.094	.098	.322	.342	-.104	.292
Head Circumference 10y (cm)	-.142	.216	-.119	.516	-.577	.294
BMI (Body Mass Index)	-.042	.093	-.072	.651	-.229	.145
Age (y)	.092	.112	.130	.416	-.134	.319
Step 3: R^2 Δ = .10, f(1,42) = 5.358, p= .026						
Baby's Sex	-.903	.763	-.175	.244	-2.444	.638
Baby weight (g)	.001	.001	.111	.684	-.002	.003
Baby Length (cm)	-.178	.214	-.173	.410	-.611	.254
Baby head circumference (cm)	-.150	.395	-.086	.707	-.947	.648
Weight, kg (age10)	-.095	.110	-.309	.392	-.317	.127
Height, cm (age10	-.017	.078	-.043	.827	-.174	.140
Waist Circumference 10y (cm)	.125	.094	.428	.192	-.065	.316
Head Circumference 10y (cm)	-.145	.206	-.123	.484	-.561	.270
BMI (Body Mass Index)	-.005	.090	-.008	.960	-.186	.177
Age (y)	.070	.108	.098	.521	-.147	.287
Serum Folate C (nmol/l)	.024	.011	.351	.026*	.003	.046
Total R^2 = .22						

*Values considered significant: *p<.05*

($r_{(53)}$ = -.300, p = .015), waist circumference at 10y ($r_{(53)}$ = .288, p = .018), maternal age ($r_{(53)}$ = -.404, p = .001), and serum folate at 36GW ($r_{(53)}$ = -.324, p = .009).

The individual dimensions (Emotional Problems, Conduct Problems, Hyperactivity and Peer problems) were explored in further detail. Children's Hyperactivity and Inattention Symptom scores were significantly predicted by a number of variables (sex (β = .296, $t_{(53)}$ = 2.284, p = .028), birthweight (β = -.441, $t_{(53)}$ = -2.066, p = .045), length at birth (β = .337, $t_{(53)}$ = 1.994, p = .053), maternal age (β = -.279, $t_{(53)}$ = -2.141, p = .038) and maternal BMI (β = .326, $t_{(53)}$ = 2.432, p = .019)). Children's anthropometric measurements and maternal characteristics also accounted for a significant amount of variance in these scores, Step 1, 15% (**$F_{(8, 44)}$ = 2.157; p = .050, R^2= .15**) and Step 2, 29% (**$F_{(2, 42)}$ = 5.264; p = .009, R^2= .29**) cumulatively. Serum folate at 36GW (Step 3) was nonsignificant. Children's waist circumference at 10y was found to be a significant predictor Emotional Symptoms (β = .860, $t_{(53)}$ = 3.024, p = .004), explaining 23%, a significant amount of variance (**$F_{(8, 44)}$ = 2.956; p = .010, R^2= .23**). Children's head circumference at birth ($r_{(53)}$ = -.335, p = .007), waist circumference at 10y ($r_{(53)}$ = .369, p = .003) and maternal age ($r_{(53)}$ = -.370, p = .003) were partial correlates to this dimension of the SDQ. Conduct problems did not have a significant impact on the variance between scores however it was significantly predicted by sex of the child (β = .380, $t_{(53)}$ = 2.682, p = .011). Serum folate at 36GW ($r_{(53)}$ = -.332, p = .008), sex ($r_{(53)}$ = .320, p = .010) and maternal age ($r_{(53)}$ = -.242, p = .040) had significant partial correlations to Conduct Problems. No significant predictors or partial correlations were found in the dimension relating to Peer Problems.

4.3.8 SDQ: Prosocial behaviour

The same 3 step model and predictor variables was applied with prosocial behaviour as the dependent variable. Children's anthropometric measurements at birth and 10y accounted for a nonsignificant variance of 21% (**$F_{(8, 44)} = 1.460$; $p = .20$, $R^2 = .21$**). The addition of maternal age and BMI accounted for a further 1% of variance (**$F_{(2, 42)} = .287$; $p = .752$, $R^2 = .01$**). Serum folate at 36GW accounted for a total of 31%, a significant amount of variance, when added to the model (**$F_{(1, 41)} = .5.611$; $p = .023$, $R^2 = .08$**) and was the only significant predictor of prosocial behaviour (*Table 4.15*). Serum folate level at 36GW ($r_{(53)} = .278$, $p = .022$), child's sex ($r_{(53)} = -.241$, $p = .041$), birthweight ($r_{(53)} = -.306$, $p = .013$) and length at birth ($r_{(53)} = -.274$, $p = .023$) had significant partial correlations with prosocial behaviour.

4.3.9 ASCQ dimensions of attachment

In each of the three dimensions of attachment style none of the predictor variables explained the variance in the regression model, however, children's head circumference at 10y did significantly predict secure attachment ($\beta = .343$, $t_{(54)} = 2.095$, $p = .042$). No significant predictor variables were identified relating to anxious or avoidant attachment. Children's head circumference at birth ($r_{(54)} = .258$, $p = .030$) and 10y ($r_{(54)} = .309$, $p = .012$) was partially correlated to secure attachment, whereas waist measurement at 10y ($r_{(54)} = .250$, $p = .034$) was a partial correlation to anxious attachment. Serum folate ($r_{(54)} = -.295$, $p = .015$), maternal age ($r_{(54)} = -.338$, $p = .006$) and maternal BMI ($r_{(54)} = .260$, $p = .029$) were found to be partially correlated to avoidant attachment.

4.3.10 PSDQ: Parenting style total scores and individual dimensions

The same child anthropometric and maternal variables were applied to the regression model in relation to positive parenting styles . Serum folate at 36GW was identified as a significant predictor (β = .354, $t_{(54)}$ = 2.303, p = .026) and explained a significant proportion of variance (19%) (**$F_{(1, 42)}$ = 5.302; p = .026, R^2= .19**) and was partially correlated to positive parenting scores ($r_{(54)}$ = .270, p = .024), no other significant values were identified. This relationship was able to be explored further using the individual dimensions which comprise positive parenting style in the PSDQ. No significant values were found for parental warmth and support or regulation/ reasoning induction however autonomy granting was significantly predicted by serum folate at 36GW in step 3 of the model (*Table 4.16*) accounting for significant variance (36%) (**$F_{(1, 42)}$ = 7.831; p = .008, R^2= .36**). Head circumference at birth (β = .589, $t_{(54)}$ = 2.879, p = .006) and maternal BMI (β = .307, $t_{(54)}$ = 2.203, p = .033) significantly predicted autonomy granting. Serum folate ($r_{(54)}$ = .273, p = .023) and head circumference at birth were partially correlated to autonomy ($r_{(54)}$ = 326, p = .008). No significant predictors or partial correlates were found for permissive parenting style.

Table 4.15: HMRA with Prosocial Behaviour score as the dependent variable and Serum folate at 36GW

					95.0% Confidence Interval for B	
Variable	**B**	**SE B**	**β**	**Sig.**	**B**	**SE B**
Reference male=0						
	Step 1: R^2 = .21, f(8,44) = 1.460, p= .199					
Baby's Sex	-.505	.465	-.158	.284	-1.441	.432
Baby weight (g)	-.001	.001	-.237	.320	-.002	.001
Baby Length (cm)	-.060	.131	-.088	.648	-.324	.204
Baby head circumference (cm)	.106	.238	.088	.659	-.373	.585
Weight, kg (age10)	-.033	.066	-.173	.621	-.165	.100
Height, cm (age10)	-.008	.046	-.033	.860	-.100	.084
Waist Circumference 10y (cm)	-.014	.055	-.081	.793	-.125	.096
Head Circumference 10y (cm)	.233	.124	.312	.066	-.017	.482
	Step 2: R^2 Δ = .01, f(2,42) = 0.287, p= .752					
Baby's Sex	-.536	.474	-.168	.265	-1.494	.421
Baby weight (g)	-.001	.001	-.196	.429	-.002	.001
Baby Length (cm)	-.053	.134	-.078	.691	-.323	.216
Baby head circumference (cm)	.073	.252	.061	.773	-.435	.581
Weight, kg (age10)	-.029	.067	-.154	.666	-.164	.106
Height, cm (age10	-.015	.047	-.060	.758	-.110	.081

Variable	B	SE B	β	Sig.	95.0% Confidence Interval for B	
					B	SE B
Waist Circumference 10y (cm)	-.008	.057	-.044	.890	-.123	.107
Head Circumference 10y (cm)	.235	.126	.315	.070	-.020	.490
BMI (Body Mass Index)	-.040	.055	-.112	.467	-.151	.070
Age (y)	.018	.065	.043	.779	-.114	.150
Step 3: R^2 Δ = .08, f(1,41) = 5.611, p= .023						
Baby's Sex	-.396	.454	-.124	.389	-1.313	.522
Baby weight (g)	-.001	.001	-.262	.271	-.002	.001
Baby Length (cm)	-.039	.127	-.057	.760	-.295	.217
Baby head circumference (cm)	.096	.239	.080	.689	-.387	.579
Weight, kg (age10)	-.030	.063	-.161	.634	-.159	.098
Height, cm (age10)	-.033	.045	-.135	.473	-.125	.059
Waist Circumference 10y (cm)	.010	.054	.058	.849	-.100	.120
Head Circumference 10y (cm)	.233	.120	.312	.059	-.009	.475
BMI (Body Mass Index)	-.018	.053	-.051	.733	-.125	.088
Age (y)	.005	.062	.011	.937	-.121	.131
Serum Folate C (nmol/l)	.014	.006	.339	.023*	.002	.027
Total R^2 = .31						

*Values considered significant: *p<.05*

Table 4.16: HMRA with the Autonomy dimension of the PSDQ score as the dependent variable and Serum folate at 36GW

Variable	B	SE B	β	Sig.	95.0% Confidence Interval for B Lower	Upper
Reference male=0						
Step 1: R^2 = .17, f(8,45) = 1.122, p= .367						
Baby's Sex	.151	.171	.128	.383	-.194	.495
Baby weight (g)	.000	.000	-.115	.661	-.001	.000
Baby Length (cm)	-.028	.048	-.119	.565	-.125	.069
Baby head circumference (cm)	.175	.086	.440	.047*	.002	.347
Weight, kg (age10)	-.009	.025	-.128	.720	-.059	.041
Height, cm (age10)	.000	.017	-.005	.979	-.035	.034
Waist Circumference 10y (cm)	.007	.021	.103	.743	-.035	.049
Head Circumference 10y (cm)	.043	.046	.158	.363	-.051	.136
Step 2: R^2 Δ = .07, f(2,43) = 2.060, p= .140						
Baby's Sex	.170	.168	.145	.316	-.168	.508
Baby weight (g)	.000	.000	-.232	.378	-.001	.000
Baby Length (cm)	-.034	.047	-.145	.475	-.130	.061
Baby head circumference (cm)	.225	.087	.566	.014*	.048	.401
Weight, kg (age10)	-.012	.024	-.165	.638	-.061	.038
Height, cm (age10	.005	.017	.059	.755	-.029	.040

Variable	B	SE B	β	Sig.	95.0% Confidence Interval for B Lower	Upper
Waist Circumference 10y (cm)	-.001	.021	-.016	.959	-.043	.041
Head Circumference 10y (cm)	.046	.046	.169	.322	-.046	.138
BMI (Body Mass Index)	.031	.020	.236	.117	-.008	.071
Age (y)	-.035	.024	-.217	.146	-.083	.013
Step 3: R^2 Δ = .12, f(1,42) = 7.831, p= .008						
Baby's Sex	.230	.157	.196	.150	-.087	.547
Baby weight (g)	.000	.000	-.314	.206	-.001	.000
Baby Length (cm)	-.028	.044	-.120	.525	-.117	.061
Baby head circumference (cm)	.234	.081	.589	.006**	.070	.398
Weight, kg (age10)	-.012	.023	-.173	.596	-.058	.034
Height, cm (age10)	-.002	.016	-.026	.886	-.035	.030
Waist Circumference 10y (cm)	.007	.019	.100	.733	-.033	.046
Head Circumference 10y (cm)	.045	.042	.166	.297	-.041	.130
BMI (Body Mass Index)	.041	.018	.307	.033	.003	.078
Age (y)	-.041	.022	-.252	.073	-.085	.004
Serum Folate C (nmol/l)	.006	.002	.384	.008**	.002	.010
Total R^2 = .36						

*Values considered significant: *p<.05 **p<.01*

4.3.11 Mediation Analysis

Serum folate level at 36GW predicted children's outcomes of Global TEI, prosocial behaviour and mother's positive parenting including the dimension of autonomy and the creativity and values dimensions of TPR. It was therefore important to consider if these were causal findings or mediated by parenting style or children's attachment style. To investigate further, mediation analysis was conducted to test if the relationship between maternal serum folate level at 36GW and children's TEI was mediated by parenting style or children's attachment. Secure attachment was found to be a significant mediator between serum folate at 36GW and children's TEI (*Figure 4.9*). Children of mothers with higher levels of folate in late pregnancy achieved significantly higher scores in the secure attachment dimension of the ASCQ (p = .001, *CI*, .032, .115) resulting in significantly better scores in TEI (p = , *CI*, .000, .049). Additionally, Avoidant attachment significantly mediated serum folate at 36GW and children's TEI (*Figure 4.10*). Children of mothers with a lower serum folate level at 36GW were significantly more likely to report higher levels of avoidant attachment (p = .052, *CI*, -.070, .000) which in turn, significantly reduced children's TEI scores (p = .013, *CI*, -.069, -.009). No mediating effects of anxious attachment were observed. Positive parenting behaviour nor its individual dimensions did not predict children's TEI, TPR, total difficulties, prosocial behaviour or any of the individual dimensions therefore the mediating effects could therefore not be fully tested.

Figure 4.9: Pathway diagram illustrating the significant mediating effect of secure attachment between serum folate level at 36GW and children's Emotional Intelligence.

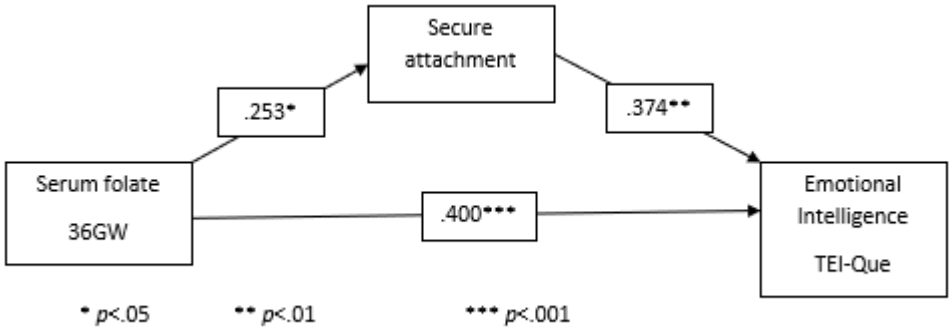
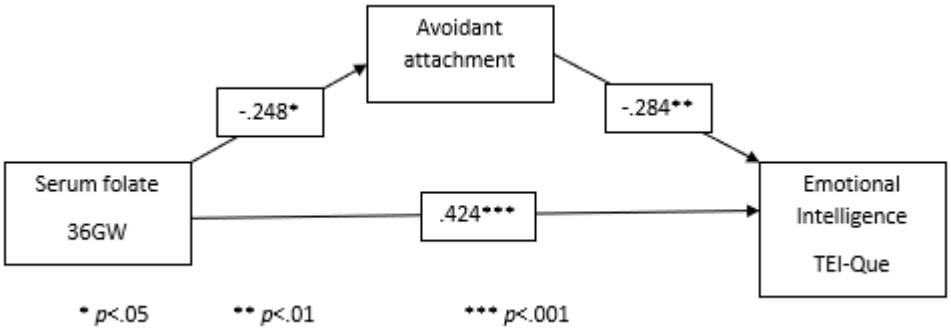


Figure 4.10: Pathway diagram illustrating the significant mediating effect of avoidant attachment style between serum folate level at 36GW and children's Emotional Intelligence.



4.3.12 Comparative analysis of FASSTT@10y and UK normative data

One sample t-tests were conducted to compare FASSTT participants to normative data in relation to parenting style (Winsler *et al.*, 2005) and attachment (Finzi *et al.*, 2000), with the expectation that all FASSTT participants would not be significantly different from the UK norm. Mean normative scores for positive parenting style as reported by mothers was $M = 4.05$ ($SD = .32$) and FASSTT experimental and control group members reported a mean score of 4.54 ($p = <.001$, *CI:* .384, .599) and 4.32 respectively ($p = <.001$, *CI:* .140, .405), both significantly higher than the norm.

Mean normative scores were also obtained for each of the three attachment styles: secure (*M* = 3.77, *SD* = .55), avoidant (*M* = 2.59, *SD* = .52) and anxious attachment (*M* = 2.79, *SD* = .83). The scores for secure attachment were significantly higher for both treatment groups (Experimental *M* = 4.14, *SD* = .49, *p* = <.001; Control *M* = 3.95, *SD* = .47, *p* = .047). However, anxious attachment was also reported as higher for both treatment groups (Experimental *M* = 2.99, *SD* = .41, *p* =.006; Control *M* = 3.25, *SD* = .53, *p* = <.001). Significant differences were not observed regarding avoidant attachment (Experimental *M* = 2.46, *SD* = .71, *p* = .273; Control *M* = 2.63, *SD* = .66, *p* = .761). During this analysis both treatment groups scored significantly higher in comparison to the norm in positive parenting, secure and anxious attachment. However, it is worth noting that the experimental group did score better than their control group counterparts.

4.4 Discussion

The aim of this study was to assess the development of FASSTT child participants at 10 years old and to examine if children whose mothers received the supplement full-term had developmental advantages in the areas of TEI, TPR, behaviour and peer attachment, compared to children whose mothers stopped supplementing at the recommended 12GW. Furthermore, an investigation into the potential mediating effects of parenting style and attachment and how these children compared to the norm was also of interest.

Findings from the FASSTT@10y study demonstrated that children belonging to the experimental group rated significantly higher on Global TEI and total TPR and the

creativity dimension of the RASP. Parents of these children were also significantly more likely to adopt a positive parenting style than those in the control group. The control group scored higher in total difficulties and anxious attachment and lower in prosocial behaviour, however these were not significant differences. HMRA identified serum folate at 36GW as a significant predictor of children's Global TEI, prosocial behaviour and the dimensions of creativity and values in addition to positive parenting, and its dimension of autonomy granting. Secure and anxious peer attachment were found to mediate the relationship between maternal folate at 36GW and children's TEI.

Supplementary HMRA also found that in some instances children's anthropometric measurements or maternal characteristics were significant predictors of children's developmental outcomes. Head circumference at birth was a significant predictor of TEI and the autonomy granting dimension of the PSDQ. Maternal BMI was also found to be a significant predictor of autonomy granting. These findings offered support to evidence included in the systematic review (*Chapter 2*), linking child growth to developmental outcomes. Moreover, the parenting and attachment styles of FASSTT participants were compared to normative data and found to be significantly better for both treatment groups.

4.4.1 Adding to existing FASSTT findings

Results at 3y and 7y indicated a potential cognitive benefit for FASSTT trial participants (McNulty *et al.*, 2019). A pilot study conducted on a sub-sample of FASSTT participants during the 7y assessment indicated that continued folic acid use throughout pregnancy could promote children's Global TEI and total TPR. The

treatment groups differed significantly in all individual dimensions of the RASP but not in total behaviour difficulties or any of the SDQ individual dimensions at 7 years old (Henry *et al.*, 2018). FASSTT @10y offers further support to earlier findings in this sample with Global TEI and TPR scores remaining significantly higher for those in the experimental group. Individual dimensions of scales however did not differ between groups with the exception of creativity (RASP) with the experimental group scoring better than controls.

Exploratory HMRA analysis at 7 years found serum folate at 36GW to be a predictor of EI and resilience (Henry *et al.*, 2018) however the latter did not remain significant at 10y with only the individual RASP dimensions of creativity and values appearing to remain receptive to folate. Serum folate at 36GW was not found to predict prosocial behaviour at 7 years however it was at 10 years. Additional analysis at 10y found that serum folate at 36GW predicted the autonomy granting dimension of positive parenting as measured by the PSDQ, an area not previously explored. Although some differences were observed there was general consensus in the findings between the studies at 7 and 10 years.

The discrepancies uncovered could be attributed to a number of factors. One possible explanation could be that these assessments at 10 years were completed by the children which we would expect would give a more accurate representation of their development in comparison to the parental ratings used at 7 years (Germain *et al.*, 2019). Missing data is another complication which can impact analysis and results if not managed correctly (Coffman *et al.*, 2016). During this study instruments were administered by the researcher to the participant, therefore missing data was

infrequent, any instances were recorded in the database and analysis was conducted using either listwise or pairwise deletion of cases dependent upon the statistical test being applied.

The FASSTT@7y study (Henry *et al.*, 2018) concluded that the explanation for significant findings was likely to be complex with possible mediating factors. To understand the social and emotional development of children key factors to investigate were parenting style, the relationship between mother and child and the subsequent style of peer attachment which children develop from childhood. FASSTT@10y was therefore designed to further examine the relationship between maternal folic acid use and children's development in more depth and explore the possible mediating effects of parenting style and children's peer attachment.

4.4.2 Adding to the folic acid literature

It is difficult to draw comparisons between FASSTT@10y and the existing literature due to the differences in design, exposure conditions and outcomes measured, however, in general this study supports the evidence that is available. When considering the effect of supplementing past 12GW, a small number of studies which included women who had adequate folate levels (through supplementation or diet) from preconception until the end of trimester 2 (approximately 28GW) found positive associations between folate and children's development including mental and social development at 2y (Bhate *et al.*, 2012), neurodevelopment at 3y (Villamor *et al.*, 2012) and cognition at 10y (Veena *et al.*, 2010). These findings are similar to the cognitive improvements reported in FASSTT@3y, 7y and 10y (McNulty *et al.*, 2019; Caffrey *et al.*, 2019).

There has been some investigation into the effects of maternal folic acid use in late pregnancy (>20GW to delivery) using an RCT design. In these intervention studies women supplemented as recommended during trimester one and continued supplementation from 20GW. Campoy *et al.* (2011) found no association between continued maternal folic acid use and children's cognition at 6.5y, whereas Catena *et al.* (2015) found a positive association between folic acid use and executive function at 8.5y in the same sample as measured as part of a clinical assessment. Although conflicting, this difference in results can be explained, at least in part by the outcomes being measured. Cognition and executive function are independent constructs with cognitive development referring to how an individual's perceives, thinks and understands incorporating memory, problem solving and decision making processes (VandenBos, 2015). Executive function on the other hand refer to a family of top-down mental processes or higher level cognitive skills used to control and coordinate cognition and behaviour (Diamond, 2013). Defining these constructs clarifies how significant differences were observed in executive function but not in general cognitive development.

Although these studies were similar to the design and intervention of FASSTT they cannot be directly compared as the purpose of these studies was to examine the effects of fish oil on children's cognitive development; folic acid was used as a comparator only, but significant findings do still offer some support. An earlier study by Tamura *et al* (2005) however found no associations between maternal folic acid use in late pregnancy (>19GW to delivery) and children's mental and psychomotor development at 5y. These findings should be considered with caution due to the

differences in study design and various limitations; this was a small observational study with no information provided on the time of initiation or duration of use and also the sample was not homogeneous with FASSTT@10y participants.

The systematic review (*Chapter 2*) found that the available research tended to focus on children's cognitive development, with some research exploring the potential associations between maternal folate consumption and language development and concluding that adequate use in early pregnancy improved children's language and communication skills at 18 months (Chatzi *et al.*, 2012) and reduced the risk of severe language delay at 3y (Roth *et al.*, 2011). Limited evidence examining the effect of maternal folic acid use on children's psychological, emotional and behavioural development was found during the search.

When considering studies based on outcomes similar to those measured in FASSTT@10y, findings are limited and tend to have a negative focus. Low folate levels during the first trimester was found to increase the risk of behaviour problems (Roza *et al.*, 2010) and emotional problems (Steenweg-de Graff *et al.*, 2015). This posed the question as to whether sufficient or continued folic acid use could reduce the risk of behaviour problems in children and increase their prosocial behaviour. FASSTT@10y reported that although these differences occurred, they were not significant.

Wehby and Murray's (2007) study found folic acid to result in a marginally significant poorer performance in the personal/ social domain. When compared to other available evidence this is a relatively unexpected finding, however no

information was provided on the dose used or the time of initiation possibly due to the use of older data (1988-1991). As the evidence into social, emotional and behavioural development is limited these findings offer some comparison for FASSTT@10y until the evidence develops further. These findings provide a context and offer some support to the FASSTT@10y study through observational data. Henry *et al*'s (2018) study however was the first to test the effects of folic acid supplementation as an intervention on children's psychological, emotional, social and behavioural development using an RCT design. It was the findings from this study (FASSTT@7y) that justified further investigation at 10y.

It is important to be mindful of the potential positive or negative effects of physical growth and development. A possible link was uncovered between a number of anthropometric measurements including birthweight an later BMI, head circumference and children's development which concurred with some instances in the literature (e.g.Veena *et al.*, 2010, Julvez *et al.*, 2009; Campoy *et al.*, 2011, Bhate *et al.*, 2012). FASSTT@10y found that TEI and the autonomy dimension of positive parenting was positively predicted by both head circumference at birth and maternal folate at 36GW. This indicates that continued supplementation could increase head circumference at birth and contribute to improved TEI possibly through improved cognition. The parents of children who display greater TEI are in turn more likely to encourage autonomy through parenting practices as hypothetically children would be more socially, psychologically and behaviorally adept.

The supplementary comparative analysis to UK norms provided further insight into those who participated in FASSTT@10y. As randomisation balances observed and

unobserved participant characteristics (Misra, 2012) it was hypothesized that FASSTT@10y participants would not be significantly different to the general UK population. However, as previously discussed some would argue that voluntary participants are somewhat different to non-participants and those lost to follow-up. It was particularly important to consider any possible differences in relation to the sample for the potential mediating factors. Results showed that both experimental and control group participants differed significantly from the UK norm. This suggests that perhaps retained participants were more likely to adopt a positive parenting style and children present as more securely attached with those in the experimental group scoring better than the control. This provides tentative evidence for the continued use of folic acid to improve children's psychological, social, emotional and behavioural development.

4.4.3 Strengths and limitations of the FASSTT @ 10y Trial Study design

This trial has many strengths, the first is the study design. Rigorous and well-conducted RCT's provide the best estimates of the impact of an intervention (Karanicolas *et al*., 2010) and can identify and examine causal relationships if well designed (Hariton & Locascio, 2018). The randomised double-blind placebo control design applied to FASSTT is considered the 'gold standard' of intervention studies (Bhide *et al*., 2018) due to the randomisation of treatments, blinding of participants and researchers, relatively low attrition, objective measures of children's development and thorough consideration of potential confounders. These factors reduced the risk of bias throughout the duration of the study.

Randomisation was used to reduce the effects of selection bias by balancing both observed and unobserved characteristics of the participants between the treatment groups (Misra, 2012). For FASSTT@10y, this helps attribute any differences found in developmental outcome to the folic acid intervention. However, the possibility of selection bias at the time of recruitment could not be eliminated completely and may have been introduced inadvertently through voluntary participation, evident from the normative data and analysis. As the study relied on volunteers it is possible that those who participated in the first instance but even more so at the later follow-up investigations were more likely to be motivated and conscientious parents. Parents with qualities such as these are more likely to engage well with their children, therefore more likely to adopt a more positive parenting style and form close attachments with their children than those who declined participation or who dropped out in the early stages of the trial. These attributes would then understandably have a positive effect on children's developmental outcomes. Randomisation also removes most confounding by all known and unknown factors by reducing the possibility that an observed outcome is due to an extraneous factor. However, selection bias could also have been introduced due to the use of a placebo. This could negatively impact recruitment with some declining the opportunity to participate due to the possibility of being randomly assigned to the control group.

This was a double-blind study where both the participants and researchers were blind to treatment conditions (folic acid or placebo) at the time of intervention and at each follow-up assessment. Blinding is important feature of RCT design in order to reduce the risk of performance and detection bias. Original FASSTT participants were randomly assigned to their treatment group and stratified according to their

homocysteine concentration by a member of staff independent to the study. Additionally, all participants were administered the same outcome measures whilst still under the blinded conditions of the trial. This double-blind procedure reduced the possibility of unconscious information or personal bias occurring.

Clinical trials are complex with many strengths but not without limitations. RCT's are prone to attrition and loss to follow-up and FASSTT was no exception. The sample size recruited for FASSTT@10y could be considered relatively small with a total of 65 participants completing the study (35 experimental participants and 30 control participants). However, FASSTT@10y recruitment was limited to those who had completed the intervention in 2006 (n=126). The loss to follow up was 45% and considered high according to Dumville *et al*. (2006). Despite this the study remained adequately powered to detect observable differences with confidence and the effect size for significant findings was high in most cases despite the reduced number of participants. A reasonable explanation for the small sample was due to ethical considerations; original FASSTT participants were recruited from one hospital in one of six HSCT's in Northern Ireland. This single site approach kept the study manageable for the original researcher in 2006 however, if both hospitals with maternity units (Causeway and Antrim) contained in the Northern Trust had been included it could have increased participant numbers considerably. As protocols differ between Trusts it would not have been possible to recruit outside of this Trust without individual ethical approval.

In order to be clinically useful, the validity of the study was an important factor to consider when conducting this RCT. Although inherently high in internal validity it

was important this was not at the expense of the external validity due to the importance of this research for public health. Highly selective eligibility is often a concern with RCT design (Rothwell, 2006), however with FASSTT the aim was to be as inclusive as possible. A wide range of women (18-35y) with a singleton pregnancy and without complications were eligible if they had supplemented with 400µg/d of folic acid during the first 12GW as recommended. As all newly pregnant women are referred to an antenatal clinic by their GP therefore a wide reach to all pregnant women in the catchment area was expected to offer as many women as possible the opportunity to participate.

Women were only excluded if they had a previous NTD pregnancy or had a first degree relative with NTD due to the increased dose of folic acid taken in early pregnancy to aid the neural tube closure. Additionally, women with certain chronic disorders often requiring medication were excluded due to the possible interference with B-vitamin metabolism. The restricted approach to recruitment was a limitation and could impact the external validity and generalisability of the findings when applied to other populations outside of NI such as the UK and Europe. Using normative data to compare FASSTT@10y parenting style and attachment data was a valuable and important addition to FASSTT@10y analysis in order to assess how FASSTT@10y participants compared to those outside of the NI context. Restricted recruitment is unlikely to impact generalisability to NI residents in other HSC Trusts due to the small geographical and population size of the country, although not impossible. The population of N.I has also changed considerably in the last decade, the FASSTT sample could therefore be less representative of the NI population now than it was in 2006. There is also a chance that participants who volunteered and met

the eligibility criteria may not have been entirely representative of the population from which they were derived e.g. women who volunteered were generally more likely to adopt a positive parenting approach. Although every action was taken throughout the study to minimize the threat to external validity these are factors could still have had a negative impact.

The rigid and clinical conditions associated with RCT can increase the risk of other biases such as non-compliance and attrition or loss to follow-up (Misra, 2012). During the FASSTT intervention compliance was measured and found to be high at 93% with folic acid use checked weekly by a FASSTT researcher. An inherent strength of the intervention was the levels of folate were obtained from maternal blood samples at 14GW and 36GW. Mothers reported their folic acid use during the first 12GW which was confirmed by blood folate levels. Two measures of folate were collected: RBC folate and serum folate. Further blood samples were collected from cord blood following the birth of the child. These objective measures provide accurate folate levels at each time point rather than relying on the self-report alone as used in other research which is an obvious limitation.

Developmental outcomes

Adding to the study's strength, children's developmental outcomes and mothers parenting style were measured with various instruments, carefully selected to be the best fit for use in the FASSTT sample. These were piloted before data collection commenced to ensure children understood the questionnaire items and how to respond using the flash cards. All instruments achieved high internal consistency

within the FASSTT sample for each of the global scores (TEI, TPR, total difficulties and positive parenting). Instrument developers of the RASP (Hurtes & Allen, 2001) reported low internal consistency in some dimensions (<.60), and a systematic review (Windle *et al*., 2011) found questionable internal consistency in 6 of the 7 dimensions however, this was irrelevant in this instance as no significant differences were observed in any individual dimensions aside from creativity which achieved a value of α=.67; marginally below the accepted value of .70.

The other instruments used were less susceptible to problems with internal consistency. An exception to this was the permissive parenting dimension of the PDSQ which struggled to meet satisfactory internal consistency reported populations (Robinson *et al.*, 2001; Onder & Gulay, 2009). This was attributed to an issue with factor loadings when the short form was developed, however this was not found in FASSTT@10y where acceptable levels were recorded. An explanation for this could be due to the removal of the authoritarian parenting dimension. In keeping with the rationale of this study, the focus was on a positive approach to data collection. The removal of this dimension added strength to FASSTT@10y as the instrument was more likely to elicit accurate responses from parents and reduce likelihood of missing data when it focused on the positive rather than the negative aspects of parenting. However, as the PSDQ was adapted in this way direct comparisons cannot be made to other study populations. This measure was useful for parents to reflect and evaluate the perception of their own parenting. As parenting is a two-way interaction process (Maccoby & Martin, 1983), an additional measure to assess the children's perception of their parents parenting style would be a useful addition to increase validity.

Navaneetham *et al*. (2019) conducted a scoping review to identify scales which measure children's perception of parenting style. An extension of the PSDQ was identified (G1 version), developed by Robinson *et al*. (2001). This child completed scale measures Adolescents' Perceptions of Parenting Style during Childhood in the context of the three main parenting styles measured by the PSDQ: authoritative, authoritarian, and permissive parenting. This scale was developed for use with adolescents aged 13-18y and could be an important consideration for future FASSTT follow-up investigations. Alternatively, the Perceptions of Parents Scale (POPS) assesses parental involvement and level of autonomy support from the perspective of the child (Grolnick *et al*., 1991) and is suitable for use in children from 8y and measures children's perception of parents autonomy support, involvement and warmth, linking directly to the positive parenting dimension of the PSDQ.

Although appropriate instruments were used and administered to the child by a trained researcher, the timing of the tests could potentially have been an issue. Data collection took approximately 2 hours. During this time children completed a cognitive assessment lasting 1 hour 30 mins followed by a short break before completing the psychological assessments. Regardless of the break it was a long session for children and when the time came for the psychological assessment children could have been experiencing fatigue or boredom resulting in less accurate responses. There is no evidence to suggest this is the case, but it is worth noting. To counteract the possibility of this occurring, and to ensure the instrument items were understood the researcher asked the question verbally and the participant responded using flash cards illustrating the responses on the Likert scale.

Analysis technique

Statistical analysis was carefully designed and executed with diligent testing of test assumptions to confirm appropriate use (e.g. the use of parametric tests including chi square, independent t-tests, HMRA, mediation and one-sample t-tests). Independent t-tests compared the mean differences between all developmental outcomes including the individual dimensions reported by experimental and control groups The application of Bonferroni correction reduced the risk of a type I error and returning false positive results. A considerable number of t-tests were completed therefore the p value was corrected from $p<.05$ to $p<.01$. The mediation analysis conducted followed the Baron and Kenny (1986) method which is and remains the most widely used method in health and social science research (Pardo & Roman, 2013). Researchers have recognised some limitations associated with this technique (e.g. Pardo & Roman, 2013; Krause *et al*., 2011), however a systematic search of the literature was conducted by MacKinnon *et al*. (2007) to ascertain the frequency of mediation analysis in psychology research. Results showed that the Baron and Kenny method was the most frequently cited method primarily included in APA journals including a mix of study designs and analyses in many fields of psychology including quantitative psychology (methods), social, clinical, health, developmental, cognitive and educational psychology. This would indicate that it remains an accepted method to test for mediation.

Comparative evidence

Some intervention studies have been conducted since the implementation of FASSTT in 2006 (Catena *et al.*, 2015; Campoy *et al.*, 2011; Li *et al.*, 2009; Li *et al.*, 2015), however these trials were designed to investigate the role of micronutrients

during the second and third trimesters on children's cognitive function with folic acid as a comparator and not the intervention. Some beneficial effects of supplementing with folic acid only were observed in relation to cognition during the child's first year of life (Li *et al.*, 2009) and at 8.5y (Catena *et al.*, 2015). Areas of development beyond cognition have received little recognition in the literature particularly using an RCT design therefore controlled research into potentially beneficial effects on children's psychological and socioemotional development was imperative. A number of observational studies support FASSTT@10y findings reporting improved social, emotional and behavioural development when mothers start supplementing early in pregnancy and take the recommended dose (e.g. Roza *et al.*, 2010; Steenweg-de Graff *et al.*, 2015).

4.4.4 Implications for research and practice

FASSTT@10y findings generally support the existing literature and extend the evidence firstly due to the lack of available RCT's examining the impact of folic acid on children's development and secondly by investigating the effect of supplementation beyond the first trimester. Although consisting of a series of trials, FASSTT was the first RCT to be conducted which investigated the causal relationship between maternal folic acid use in pregnancy and children's psychological, behavioural and socioemotional developmental outcomes. These are areas that have only been explored in a limited capacity using observational or non-randomised studies. The internal validity of this RCT and the external validity of previous research enables the findings to be understood and translated into the realm of public health practice, a key aim of this research.

A significant finding in the FASSTT@10y study was that children in the experimental group scored significantly higher in the creativity dimension of the RASP. This indicates that language development could be a potential mechanism as children's creativity has been linked to language development (Howe *et al*., 2014; Holmes *et al.*, 2019). Language has been successfully linked as an important factor to each of the outcomes measured in FASSTT@10y: TEI, TPR, behaviour and attachment (e.g. Levickis *et al.*, 20118; Daugaard *et al*., 2017; Mayer & Salovey, 1993; Alvord & Grados, 2005; Benzies & Mychasiuk, 2009; St Clair *et al*., 2018; Safdar & Zahrah, 2016) and evidence illustrates how the different areas of children's development are intrinsically linked rather than being separate, isolated traits or skills. We can then assume that as children's language skills improve as they age, they become more able to label and discuss their feelings which helps regulate their emotions. As they become more effective at communicating, they become more self-aware and aware of the thoughts and feelings of others, then their social skills improve which boosts their ability to form close attachments to their peers (Schoeps *et al.*, 2020).

This research could also have important practical implications for public health. The Developmental Origins of Health and Disease (DOHaD) emphasizes the role of prenatal exposures to various environmental factors in determining the health or risk of disease throughout the life course (Sharp *et al.*, 2018). Similarly, development that occurs during childhood can have significant consequences in later adulthood in terms of health and wellbeing (Children's Research Network, 2017). Accurately identifying any factors such as good health and folic acid supplement use during pregnancy could potentially benefit an individual's short and long-term health and

development. This is an important issue and therefore every effort should be made to address it.

4.4.5 Conclusion

This stage of the PhD study aimed to examine if children's psychological, behavioural, social or emotional development could benefit from continued maternal folic acid use for the duration of the pregnancy. Results indicate that children's TEI, TPR, behaviour and peer attachment could indeed benefit from continued maternal folic acid supplementation. The potential benefits were uncovered initially during the FASSTT@7y study and further supported by this current research when the children were 10 years of age.

Chapter 5

Integrative discussion and future directions

5.1 Chapter overview

The overarching aim of this PhD thesis was to examine the role of maternal folate status during pregnancy on children's psychological, behavioural, social and emotional development using a resource-focused approach to investigate any beneficial effects experienced by children in their development. This concluding chapter will summarize and integrate each of the studies contained in this thesis. The implications and future directions are then discussed followed by strengths and limitations of the PhD and recommendations for research, policy and practice, concluding with some final thoughts.

5.2 Summary of the main research findings

The empirical studies and research contained in this thesis achieved the overarching aim of this PhD by examining the role and positive impact of maternal folate status during pregnancy on children's psychological, behavioural, social and emotional development using a resource-focused model based on positive psychology theory. Each individual study added significantly to the prenatal nutrition and child development literature.

5.2.1 Systematic review

The systematic review conducted as part of this thesis collected and comprehensively evaluated all existing evidence examining the effect of maternal

folate status during pregnancy on children's development in the first 12 years of life. A narrative review was considered most appropriate due to the lack of homogeneity in the available research. Historically evidence has implemented a deficit model focusing on the negative effects of folate deficiency during early pregnancy and using folic acid in early pregnancy as a preventive measure against NTD, with little research into the impact on other aspects of children's development beyond their physical development. The majority of research that was available was observational with few intervention studies.

Distinctively, this review added to existing evidence by applying a resource-focused approach to explore the developmental benefits experienced by children whose mothers supplemented with folate at any time during their pregnancy. The aim was to evaluate the positive impact of folate on seven key areas of children's development that have received less recognition in the literature (cognitive, motor, psychomotor, social, emotional, behavioural and language development). Main findings from the review suggested that adequate (daily use) and sufficient folate intake (~400µg) during pregnancy could improve children's development with the majority of studies reporting a beneficial effect (e.g. Chatzi *et al.*, 2012; Roza *et al.*, 2010; Steenweg-de Graff *et al.*, 2015; Villamor *et al.*, 2012; Scholtz *et al.*, 2010; Julvez *et al.*, 2009; Wehby & Murray., 2007; Bhate *et al.*, 2012; Veena *et al.*, 2010; Roth *et al.*, 2011; Catena *et al.*, 2015; Li *et al.*, 2009). Some studies found children to be at higher risk of behaviour, emotional and peer problems with more instances of hyperactivity and mental health disorders if the mother was folate deficient (Roza *et al.*, 2010; Steenweg-de Graff *et al.*, 2015; Scholtz *et al.*, 2010) while a small number of studies found no effect on cognitive or neurodevelopment (Tamura *et al.*,

2005; Campoy *et al.*, 2011; Li *et al.*, 2015). The remaining studies reported a negative effect but with atypical folic acid use (Handel *et al.*, 2016; Valera-Gran *et al.*, 2014; Valera-Gran *et al.*, 2017). This review highlighted the lack of consensus regarding the ideal folate conditions required to achieve the best developmental outcomes for the mother and child.

The systematic review surmised that clear information around when supplementation should begin, for how long and at what dosage is required and needs to be available to mothers, health professionals, policymakers and academics in order to support informed decision-making. There has been a growing interest in the application of positive psychology in research, therefore a systematic review implementing a resource-focused rather than a deficit model was imperative. To the authors knowledge this is the first review considering the beneficial impact of maternal folate status during pregnancy on children's development and is an initial step to help balance the risks and benefits associated with folic acid use. Moving forward it is critical that research clarifies the optimal dose, duration of use and time of initiation in order to give children the best developmental advantage possible in their first 12 years.

The review process highlighted an abundance of research in the realm of folate deficiency, particularly in early pregnancy and the negative impact on children's physical development, and to a lesser degree children's neurological and cognitive development. This trend emphasised a need for research to take a different perspective. A gradual shift towards investigating the effect of folate deficiency during early pregnancy on children's language, social, emotional and behavioural

development followed, and later branched out into looking at the potential benefits of folate sufficiency on children's development. The required time of initiation and duration of folate use remained focused on early pregnancy (<12GW) due to the recommendations advising health professionals. At the time the review was conducted, some research into continued folate use beyond the 12GW recommendations had been conducted however the information provided was limited and inconsistent (e.g. Tamura *et al.*, 2005; Catena *et al.*, 2015; Campoy *et al.*, 2016; Villamor ett al., 2012; Schlotz *et al.*, 2010; Veena *et al.*, 2010).

5.2.2 ALSPAC

The ALSPAC study comprised a secondary data analysis of the Avon Longitudinal Study of Parents and Children (ALSPAC) data (Golding, 1989). The original aim of this study was to compare and validate FASSTT@10y findings in a large, independent UK sample. However due to methodological and data limitations this study was redesigned and a stand-alone secondary data analysis of ALSPAC was conducted.

Data from an ALSPAC sub-sample (*Phase I* recruits) were analysed to investigate if children whose mothers were supplementing with folic acid at 32GW had a developmental advantage at 3 years old compared to those who had reported they were not supplementing at 32GW. Two analyses were conducted in an attempt to examine the queries surrounding optimal folic acid dose, it was hypothesised that children of mothers who reported being folate sufficient at 32GW would achieve higher scores in IQ, resilience, social, motor and language development when compared to those who reported being folate deficient at 32GW. A second analysis

was conducted to investigate the effect of folic acid dose and less than adequate maternal folate levels at 32GW on children's development. It was hypothesised that that children whose mothers were folate sufficient at 32GW would achieve better scores in IQ, social development, motor development, resilience and language and have less behavioural problems than children whose mothers reported mild or moderate deficiency. It was also expected that children whose mothers reported being mildly deficient would score better in these developmental areas than children whose mothers were moderately deficient.

Unexpectedly, results showed that the folate sufficient group scored lower although not significantly in all domains of the WPPSI at 4y. However, these children did score significantly higher in social development, fine motor and total development at 2.6y and prosocial behaviour at 3.2y but significantly lower in intelligibility than those whose mothers reported being folate deficient at 32GW. Results also found that sufficient folate had a weak but significant negative effect on children's performance IQ and Full-Scale IQ. Folate level had a significant positive effect on various aspects of children's development at ~3years including children's social development, fine motor and total development at 2.6y, and for social and fine motor scores at 3.2y. These findings could be attributed to the use of parental proxy measures which research suggests could be less reliable than self-report for subjective measures such as these (Germain *et al.,* 2019). Another explanation could be that the mothers and children who continued as research participants through a number of follow-up investigations tend to be more motivated. These individuals also tend to practice more positive health behaviours including folate uptake and adherence (e.g. Royal College of Paediatrics and Child Health, 2020; Ars *et al.*,

2019; Compan Gabucio *et al.*, 2021), and tend to score more highly on positive parenting and develop secure attachments with their children (Cook, 2000). Understandably, the children whose mothers continued supplementing would score better in these areas of development measured during ALSPAC.

ALSPAC analysis produced some results that corresponded with FASSTT findings at 3y, 7y and 10y. Similar constructs were measured in ALSPAC children, albeit at a younger age (~3y) and included social, emotional and behavioural development. In accordance with FASSTT findings (e.g. Henry *et al.*, 2018; McNulty *et al.*, 2019; Caffery *et al.*, 2018; Irwin *et al.*, 2019; Irwin *et al.*, 2018), social, emotional and behavioural outcomes of ALSPAC children appeared to benefit when mothers reported supplementing with folic acid at 32GW in comparison to those who had not supplemented. These ALSPAC findings are generally in line with existing evidence which considered the developmental benefits for children whose mothers supplemented in pregnancy (e.g. Chatzi *et al.*, 2012; Roza *et al.*, 2010; Steenweg-de Graff *et al.*, 2015; Villamor *et al.*, 2012; Schlotz *et al.*, 2010; Julvez *et al.*, 2009; Wehby & Murray, 2007; del Rio Garcia *et al.*, 2009; Bhate *et al.*, 2012; Veena *et al.*, 2010; Roth *et al.*, 2011; Catena *et al.*, 2015; Li *et al.*, 2009), however research into maternal folic acid use in late pregnancy is limited and conflicting (e.g. Caffrey *et al.*, 2019; Henry *et al.*, 2018; Tamura *et al.*, 2005; Catena *et al.*, 2015; Campoy *et al.*, 2016; Villamor ett al., 2012; Schlotz *et al.*, 2010; Veena *et al.*, 2010).

FASSTT@7y participants completed the same cognitive assessment (WPPSI) as ALSPAC CiF participants at 4y but were older. Although no significant differences were found between folic acid sufficient and deficient groups in ALSPAC,

significant differences were found in FASSTT@7y and 10y (McNulty *et al.*, 2019; Caffery *et al.*, 2018). The age difference between study participants could offer some explanation as to why the FASSTT@7y experimental group children had a cognitive advantage and the ALSPAC folic acid sufficient children did not. Piaget's theory of cognitive development (Piaget, 1964) suggests that children's intelligence changes as they grow, passing through a sequence of stages which reflect the increasing sophistication of children's thought, determined by a combination of interacting biological and environmental factors (Black & Hoeft, 2016; Voss *et al.*, 2017). These stages include sensorimotor (birth-18/24 months), preoperational (2-7y), concrete operational (7-11y) and formal operational (adolescence to adulthood). Each developmental stage involves a different type of thinking and therefore a different type of intelligence (Piaget, 1964). According to Piaget, ALSPAC participants were at their mid preoperational stage of development were children use symbolic rather than logical thought and are still learning to express how they think through language. FASSTT@7y children however were transitioning into the concrete operational stage characterised by logical thought, developing language and visio-spatial skills, domains which are tested by WPPSI. This highlights the potentially pivotal role language plays in optimal cognitive, psychological, social and emotional development in children.

Secondary data analysis using ALSPAC data was bound by the limitations of ALSPAC in terms of design, methodology and risk of bias and posed a number of difficulties when exploring it in such a focused area. These included limited information on folic acid use, unsupported by biological samples and issues with developmental measures. Although the same constructs were assessed, different

standardised tests were completed, and at a different age to FASSTT@7y and FASSTT@10y participants and extraneous variables were difficult to control. These differences were problematic and prohibited the use of these data for validation of FASSTT findings. Loss to follow-up and attrition requires careful consideration during analysis. Comparing non-participants to those who remained engaged revealed a difference between the groups. This supported the assumption that those who continue participation tend to be different to those that don't, supporting the rationale for conducting the normative tests in FASSTT@10y.

Despite these difficulties, ALSPAC results did justify further investigation using an RCT design and did offer some support to FASSTT@3y findings as children were reported to achieve better in prosocial behaviour, social, motor and language development at 3y when mothers were folate sufficient at 32GW. The findings of FASSTT@3y and ALSPAC combined allow us to speculate that a relationship exists between folic acid use in later pregnancy and children's development. However, further research is required to fully investigate this relationship thoroughly. This study also offers some tentative support to FASSTT@7y and 10y investigations. The challenges of external validity faced by FASSTT were addressed and a national context for findings was provided. In conjunction these results indicate that children's cognitive, social, emotional and behavioural development could benefit from folic acid use in late pregnancy and that FASSTT@7y and 10y could be generalizable outside of NI.

5.2.3 FASSTT@10y

The FASSTT@10y study was a continuation of the ongoing **F**olic **A**cid **S**upplementation during the **S**econd and **T**hird **T**rimesters of pregnancy (FASSTT) trial at Ulster University (Henry *et al.*, 2018; H. McNulty *et al.*, 2019; B. McNulty *et al.*, 2013). Child participants were now 10 years old, and the study aim was to use an RCT design to test if continued maternal folic acid use throughout pregnancy provided children with any additional developmental benefits in comparison to those who ceased supplementation at the recommended 12GW. The mediation effects of parenting style and children's attachment were also assessed due to the proposed theoretical interactions between the variables (e.g. Sanvictores & Mendez, 2022). Additional analysis using normative data was informed by similar analysis conducted during FASSTT@7. Experimental and control participants were compared to a UK norm and experimental group children achieved significantly higher scores in verbal IQ and full-scale IQ scores. No significant differences were found between the FASSTT control group and the UK mean scores (McNulty *et al.*, 2019). These findings provided tentative support that maternal folic acid use can be advantageous for children's cognitive development and warranted further investigation.

FASSTT@10y tested a number of hypotheses that children belonging to the experimental group would score significantly higher Trait Emotional Intelligence (TEI) and Trait Psychological Resilience (TPR) and prosocial behaviour and significantly lower in behavioural difficulties (internalising and externalising) than those in the control group, with folate level at 36GW a significant predictor of the outcomes. Those children in the experimental group should also be more securely

attached and less anxious with their mothers adopting a more positive parenting approach than their control group counterparts. Furthermore, it was expected that FASSTT@10y participants would not be significantly different from UK normative data in positive parenting and attachment, and that mother's parenting style and children's attachment would mediate maternal folate level in late pregnancy and children's developmental outcome at 10y.

FASSTT@10y findings supported some of the research hypotheses with experimental group participants reporting significantly higher levels of global TEI, TPR and creativity as measured by the RASP. No significant differences were found in children's prosocial behaviour, total behavioural difficulties or its individual dimensions or attachment style. HMRA analysis identified folate at 36GW as a predictor of TEI, creativity, values orientation and the autonomy granting dimension of positive parenting. Additionally, mediation analysis only found folic acid at 36GW and TEI to be mediated by secure and anxious attachment but not positive parenting. No other developmental outcome was mediated by positive parenting or attachment. Results relating to the normative data also found that all FASSTT@10y participants (experimental and control) were significantly different from the UK norm. These results indicate that continued folic acid use throughout pregnancy could enhance children's psychological, behavioural, social and emotional development in the specific areas of EI, resilience, prosocial behaviour and peer attachment at 10 years old and we can speculate that there is some relationship between folate level at 36GW and attachment.

FASSTT@10y tested for a causal relationship between maternal folic acid use on specific areas of children's psychological, social, emotional and behavioural development, namely, TEI, TPR, prosocial behaviour, peer attachment and mothers parenting style. The stringent requirements of the RCT ensured the time of initiation, dose and duration of folic acid use were all controlled in order to effectively test if using folic acid for a longer duration during pregnancy would provide children with a developmental advantage. The novel measures of development (TEI and TPR) in relation to maternal folic acid intake during pregnancy are important factors to consider due to the significant impact they have on children's global development, health and wellbeing (Henry *et al.*, 2018; H. McNulty *et al.*, 2019; B. McNulty *et al.*, 2013; Caffery *et al.*, 2018; Irwin *et al.*, 2019; Irwin *et al.*, 2018; Bhate *et al.*, 2012; Chatzi *et al.*, 2012; Julvez *et al.*, 2009; del Rio Garcia *et al.*, 2009; Li *et al*, 2009; Gross *et al.*, 1974; Villamor *et al.*, 2012; Veena *et al.*, 2010; Murphy *et al.*, 2007; Catena *et al.*, 2015), and the wider impact this can have on family wellbeing (Newland, 2015). Theoretically the outcomes measured have been linked to parenting style and attachment (e.g. Bowlby, 1969; Elicker *et al.*, 1992; Alhusen *et al.*, 2013; Bost *et al.*, 1998). This research therefore investigated the potential mediating effects of these factors and found that children's attachment style mediated the relationship between maternal folate level at 36GW and children's TEI. Additionally, folate level at 36GW significantly predicted children's TEI, prosocial behaviour the creativity and values dimensions of resilience and the autonomy dimension of positive parenting style. However, it is important to consider other factors which could influence these areas of development such as temperament or personality, play opportunities and style and family structure due to their impact on wellbeing (EWB, PWB and SWB).

5.3 Implications and future directions for research

Individually and in combination this collection of evidence provides a unique and valuable insight into the benefits of maternal folic acid use during pregnancy, particularly relating to continued use until delivery (FASSTT@10y) and highlights important issues relating to research. The available observational evidence clearly shows the benefits of early initiation and consuming the recommended dose for children's physical, cognitive and to a lesser degree psychological development and although limited at present the literature is growing. ALSPAC analysis further supports the use of adequate and sufficient folic acid use for children's early psychological, social, emotional and behavioural development. However, further experimental studies in the area are required which would enable additional high-quality systematic reviews and meta-analyses to be conducted in order to develop, update and implement policy and inform health practitioners in order to educate mothers using a positive perspective. This would have important implications for preconception care offered to others and would help address any issues associated with uptake and adherence of folic acid before and during pregnancy. By raising awareness through education health care providers could work towards helping mothers make informed choices about supplementing and the benefits of continued folic acid use during pregnancy.

5.3.1 Biopsychosocial model of child development

Research is also needed to gain a deeper understanding of the biological, psychological and environmental factors involved in maternal folic acid use and subsequent child development. Research into brain function and neurological processes clarifies that the children's brains subject to substantial growth and

development during pregnancy and early childhood, and as a result are highly responsive to external factors and internal activity (Black & Hoeft, 2016; Voss *et al.*, 2017). This neuroplasticity means that children's brains are more vulnerable to stressors but equally more capable of resilience if supported effectively (Shonkoff, 2011). The child's environment has been shown to have a significant impact on their biological development filtering through to their psychosocial development and behaviour (Black & Hoeft, 2016).

Children live in ecological systems which can risk or promote healthy development. The biopsychosocial model of development uses an interdisciplinary approach to examine how these three domains interact during a child's early years of development. Positive biopsychosocial development using a resource focused approach with roots primarily in social work was proposed by (Saleebey, 2008). Exploratory research has identified sensitive periods when the brain is most plastic and susceptible to external influences; when synaptic connections are being refined (Prado & Dewey, 2014). Applying a resource focused biopsychosocial model in research and practice is advantageous for all children as it ultimately serves to nurture and support, but it may be even more beneficial for children who experience adversity.

Disadvantaged children from low SES appear to have an increased risk of poor health outcomes. In 2021 approximately 17% of NI residents lived in relative poverty while 12% lived in absolute poverty (Department of Communities, 2015). These children are more vulnerable to environmental stress, more likely to face increased family transitions, unresponsive caregiving, community violence and

antisocial behaviour and a lack of social support (Evans, 2004). Policies and guidance relating to maternal folic acid during pregnancy could be considered a resource focused biopsychosocial intervention for optimal child development. This research suggests it can significantly improve children's cognitive, psychological, social, emotional and behavioural development by encouraging positive aspects of preconception and antenatal care through the uptake and adherence of folic acid supplementation and educating women on the benefits of supplementing.

5.3.2 Biological implications and future research

FASSTT@10y identified causal relationships between the variables measured and findings from this study suggest that continued supplementation could give children a developmental advantage at 10 years old. A possible explanation for this could be that sufficient folate is available during a critical window of fetal brain development in later pregnancy (>28GW). Research has shown that brain structures and functioning begin to develop in sequence during the early prenatal period, throughout pregnancy and postpartum (Courperus & Nelson, 2006). Structurally the cerebellum begins developing first. This area of the brain is responsible for motor control and spans from conception to the postnatal period (Zou *et al.*, 2021) whereas the striatum, which is responsible for coordinating several aspects of cognition develops rapidly during late gestation. In particular, this area of the brain is activated by rewards in social situations (Baez-Mendoza & Schultz, 2013). Animal research has found that social contact and parenting conditions can impact striatal anatomy and physiology and have long-lasting effects on behaviour, therefore this area of the brain plays a critical role in attachment and maintaining the bond (Baez-Mendoza &

Schultz, 2013). The striatum receives much of its information from the cerebral cortex which is responsible for the child's cognitive, psychological, behavioural, social and emotional development (Rubenstein, 2010). The third trimester marks the beginning of the rapid growth and development of this brain structure as the other areas of the brain and spinal cord which control bodily functions are now fully developed (Zou *et al.*, 2021).

Myelination and neuronal connectivity occurs largely postnatally but begins early in the third trimester (Prado & Dewey, 2014) and has a great influence on cognition and IQ (Almond & Currie, 2011). Sensory pathways for basic functions such as vision and hearing develop first in the first postnatal year, followed by language and cognition which develop into adulthood (Roth *et al.*, 2011). Folate is required for all fetal growth and development including that of the brain (Tamura *et al.*, 2005). It is therefore possible that children's brain growth, development and functional ability could be determined and manipulated by maternal folic acid use. This research shows that although brain development is a life-long process which occurs from conception, there may be a window of opportunity for folate to provide optimal benefit to children's brain development and structures of the brain directly responsible for cognitive, psychological, social, emotional and behavioural development. This suggests that the time of initiation and duration of folic acid use during pregnancy could be an important factor with continued use providing additional benefits for children.

In 2000, Seligman acknowledged how an understanding into the heritability of negative states such as aggression and depression was growing but the knowledge

into the genetic contribution of gene-environment interactions was limited. FASSTT research began to explore these connections identified by Seligman (2000) in relation to folic acid, cognition (e.g. Caffery *et al.*, 2018; McNulty *et al.*, 2019) and epigenetics. Epigenetic effects on children's psychosocial development in a randomised trial of folic acid supplementation in the second and third trimester (EpiFASSTT), used a biopsychosocial approach to go further than previous FASSTT trials. These trials investigated not only the effects of continued maternal folic acid use on children's cognitive, social and emotional development, but also explored any correlations with epigenetic changes at birth. This study was designed to test if the genome changes persist in the longer-term by examining the intergenerational and transgenerational potential of environmental factors on DNA methylation (e.g. Irwin *et al.*, 2019; Irwin *et al.*, 2018; Caffery *et al.*, 2018). This research aligns closely with the DOHaD framework which considers the effects of environmental stressors in the prenatal period on later health and disease therefore contributing significantly to public health.

The EpiFASSTT project continues to actively investigate the epigenetic effects of maternal folic acid supplementation on children's development within the FASSTT sample (e.g. Irwin *et al.*, 2019; Irwin *et al.*, 2018; Caffery *et al.*, 2018). Previous research has shown that genetic factors could determine the fundamental developmental potential of a child, but their environment has crucial influences of the developmental ability achieved. Further exploration into the gene-environment interaction in terms of psychological, social and emotional development is warranted following the promising results found in the FASSTT@10y sample. Guided by positive psychology this thesis suggests that positive experiences during childhood

could enhance a number of developmental areas but particularly in social and language development.

5.3.3 Environmental implications and future research

Boosting children's psychological, social and emotional development can improve physical and mental health throughout life which can promote a more positive state of wellbeing (Lamers *et al.*, 2012; Howell *et al.*, 2007; Chida & Steptoe, 2008; Public Health England, 2015; Smedegaard *et al.*, 2016. Research has shown that a sense of wellbeing can be fostered, maintained or changed through social interactions. Bronfenbrenner's (1977) ecological systems theory considers how child development is determined by an intricate system of relationships ranging from a child's immediate environment to broad societal and cultural values which interact and influence each other. According to Bronfenbrenner (1977) the child's environment can be categorised into 5 systems (the microsystem, the mesosystem, the exosystem, the macrosystem, and the chronosystem) with the microsystem containing the child's most immediate environment including family, peers and school being the most influential. Relationships within this system are bi-directional therefore the child can influence and be influenced by those in their microsystem, viewing a child's implicit and explicit environment as a crucial mechanism in their development. This provides some explanation as to why parenting style and peer attachment are important influences in development and subsequently how they affect the wellbeing of the child and the family as a whole.

Aside from parenting style and attachment, other factors which enrich a child's environment can have a significant impact on all aspects of their development

include their family structure, number of siblings, play opportunities and the types of play they engage in. The literature indicates that children living with two married biological parents are at least risk of adverse developmental outcomes, most notably in the socioemotional domain (Bzostek & Berger, 2018). Family structure is complex but known to be impacted by factors including SES which contributes to a higher number of unmarried births which in turn is associated with family instability, more frequent transitions and poorer socioemotional development (e.g. McLanahan, 2004). These factors can increase familial stress and conflict, influence the environment children are living and learning in and contributes to the level and quality of parenting and resources available (Amato, 2005). Central to family structure and dynamics are siblings. Sibling relationships and how they are embedded within families directly affect development and are key to individual and family wellbeing (EWB, PWB and SWB). These relationships consist of both positive and negative attributes and are linked to marital and parental systems and are subject to frequent and at times emotionally intense interactions (McHale *et al*., 2014).

Play is another key factor to be considered and is inherently linked to family structure and siblings but also to peers. Play is often described as ‘the work of a child’ (Lillard, 2013), and research has identified its crucial role in children’s cognitive and neurodevelopment (Romero-Ayuso *et al.*, 2021), psychological, social, emotional and behavioural development (Bundy *et al*., 2016), particularly during early childhood. The first 1,000 days of life (<2y) is a unique period of vulnerability and opportunity due to sensitive periods of brain development (Cusick & Georgieff, 2016). The activities and play children engage in builds synaptic connectivity

influencing all aspects of development including motor skills, language, socialization, creativity, learning, problem-solving, mastery and emotional intelligence (Romero-Ayuso *et al.*, 2021). Play that connects cognitive, socioemotional and motor development provides optimal conditions for developing a complex and integrated brain structure (National Research Council, 2000). Play can be classified into two broad categories; free play and guided play both of which provide valuable learning experiences (Lillard, 2013). Playful learning is child-centered and hands-on, constructivist and affectively positive spanning both free play and guided play (Lillard, 2013). Ideally the child uses their imagination and experiences to develop their play content, however the parent should strive to provide a conducive play environment. Regardless of the type of play, positive parenting and its individual dimensions of parental warmth, connection, regulation and reasoning and autonomy granting links closely with play furthering children's development (Kerns & Barth, 1995).

5.3.4 Cognitive and psychological implications and future research

Rothbart stated that "understanding temperament is central to our understanding of development, linked to individual differences in personality and neuronal function" (2007, pg 207). Personality originates from temperament and is shaped by experiences which incorporate a set of cognitions about the self and others, the physical and social world, values and attitudes to name a few (Rothbart, 2007). Temperament is evident from birth and can have significant impacts on early parental bonds, parenting style and attachment (e.g. Putnum *et al.*, 2002; Wachs *et al.*, 2005), subsequently helping to sculpt personality. Broad personality traits such as the Big Five (Extraversion, Neuroticism, Conscientiousness, Agreeableness and

Openness) have been shown to account for differences in TEI and TPR (e.g. Vernon *et al.*, 2008; Carroll, 2002). However, narrow traits such as optimism, hope, self-efficacy and motivation also contribute significantly to global wellbeing (e.g. Diener & Lucas 1999). Based in positive psychology theory these internal processes promote focus, confidence and a positive outlook, buffering against any adversity (Folkman & Moskowitz, 2000). This would suggest that these narrow traits are important areas to explore further.

FASSTT (7y and 10y) investigations have shown that traits such as TEI and TPR are significantly impacted by continued maternal folic acid use. FASSTT children were also subject to a developmental advantage in other areas of development including cognition. It appears an intricate relationship exists between attachment, emotional development, including EI and resilience and language ability. A significant difference was observed in creativity, measured by the RASP during FASSTT@10y. This suggests that language development and language ability could be a potential mechanism of action and also highlights the important role of play. There is some research indicating that children's language can benefit from adequate and sufficient folic acid use, particularly in early pregnancy (e.g., Roth *et al.*, 2011; Chatzi *et al.*, 2012). FASSTT@10y warrants speculation on the contribution of language, it was not measured within FASSTT@10y sample, but it is an interesting line for future research.

Research suggests that children's receptive and expressive language can benefit from maternal folic acid use, particularly in early pregnancy (e.g. McNulty *et al.*, 2019; Caffery *et al.*, 2018) which has been linked to children's psychological,

socioemotional and behavioural development (e.g. Henry *et al.*, 2018; Roza *et al.*, 2010; Steenweg-de Graff *et al.*, 2015; Schlotz *et al.*, 2010; Bhate *et al.*, 2012; Handel *et al.*, 2016; Roth *et al.*, 2012). Further research is therefore required to explore language as an underlying mechanism. Although not measured in FASSTT@10y, language development was able to be explored at a basic level using ALSPAC data when children were three years old. Applying a whole body or holistic approach to understand how children develop would assume that language development would occur in synchrony with other areas of development and could be a possible mechanism of action and a potential mediator.

Receptive and expressive language are interlinked but distinct constructs, both central to children's social development and supporting social relationships (Dockrell & Marshall, 2015). Language learning begins before birth during the last trimester as the foetus can hear its mother's voice (Moon *et al*., 2013). These prenatal interactions and the child's awareness of language from the womb have been shown to affect the child's later linguistic preferences (Karmiloff *et al*., 2001). In a study by Mampe *et al*. (2009) it was found that the cries of new-born babies mimicked language they heard before birth in tone and rhythm. This reflexive behaviour is initially non-intentional but quickly babies learn that crying can make the mother respond. This is communication and is characterised as a transmission of information through noises, eye contact and facial expression (Piazza *et al*., 2020), language on the other hand emerges as children begin to organize and understand their world, scaffolded by a rich set of predetermined cognitive skills to support language learning (Rose *et al*., 2009). These include perceptual, memory attention and reasoning skills, and the ability to draw analogies and to create and influence

representations of objects, actions and the minds of others (Moll & Tomasello, 2010). These cognitive abilities help children to establish the rules of language, formulate new words and understand their meaning and subsequently facilitate the demands of social experiences and relationships (Piazza *et al.*, 2020). Thus, the development of language is not dependent on cognition alone but also the child's EI and social interactions which are directly influenced by a number of environmental factors including the number of siblings, play and interaction with other children and motivation and reinforcement in the family environment. There has also been some research into the pace children develop language and how explicit comparison to their peers can have a long-term impact on children if they perceive their ability to be less (e.g. Young *et al.*, 2002). As children get older and master language, they become more able to communicate with others effectively and efficiently (Dockrell & Marshall, 2015) further expanding their social skills and subsequent emotional and psychological development such as EI and resilience.

5.4 Thesis contributions to policy and practice

Policy makers and analysts are informed and guided by the evidence when developing or updating the guidance. Thus, it is imperative that the research is extensive and of high technical quality for them to consider the problem, adjust their thinking, propose different actions while orienting their professional stance to enhance the policies under development or review (Vivek & Nanthagonan, 2021). The multi-method design applied to this thesis has the ability to meet these requirements, due to the mix of methodologies utilised to produce evidence high in scientific rigor.

The introductory chapter of this thesis (*Chapter 1*) detailed the components of wellbeing including but not limited to social and emotional development. Although many areas of child development exist, NICE (2012) has recognised the vital roles social and emotional development have on children's global development, health and wellbeing and developed policy to encourage optimal development in these areas with specific targeting to vulnerable groups based on their level of risk. Research has shown that positive social and emotional development is crucial for all children impacting in all areas of their lives throughout childhood and into adulthood (Antonucci *et al.*, 2004; Allen, 2008). However, a number of other elements are equally integral for a state of positive wellbeing and include physical, mental and psychological health and development. Wellbeing has been categorised as hedonic (emotional) or eudemonic (psychological) with accumulating evidence to support links to personality and character strengths (e.g. Diener *et al.,* 2003; Kokko *et al.*, 2013; Diener & Lucas, 1999; Chen *et al.*, 2012; Diener & Oishi, 2005). Intricately linked is social wellbeing with positive social experiences bolstering the other areas of wellbeing to promote a positive state (Cicognani *et al*, 2014). Optimal wellbeing reduces the risk of internalising and externalising behaviour problems while promoting prosocial behaviours (Erikson *et al.*, 2011), this also feeds into an individual's sense of wellbeing through social, emotional and psychological experiences.

The positive psychology movement and the shift in model from deficit to resource focused has meant that the three pieces of research conducted for this thesis aligned to the policies and the associated research gaps identified by NICE (2012). In relation to the PH11 guideline and the Programme Development Group (PDG)

suggestions, the systematic review addressed the effectiveness of interventions to improve the nutrition of mothers and children under 5 years. The included studies captured a range of populations including ethnic minorities, low income countries and low SES groups (PH11a). The review also collated all good quality studies which utilized folic acid as a public health intervention to improve nutrition in the UK (PH11c). The quality and risk of bias assessment examined and scored the measurement and validation of folic acid before and following an intervention. Although self-reporting was used in some observational studies, validation through blood samples was present in most cases (PH11e). When considering PH40 guideline detailed by the Public Health Interventions Advisory Committee (PHIAC), the review addressed a number of identified research gaps. The effectiveness of folic acid as a health intervention to improve children's social and emotional development and wellbeing (PH40a), and the operational definitions and associated measures for social and emotional development from each study were collated (PH40b). However, a thorough examination of construct validity followed by an assessment of scale suitability would be required to fully meet this research limitation and is beyond the scope of the review contained in this thesis. To a certain extent the differential impact of early intervention on the development of different samples was achieved (PH40d) as study populations differed and were compared as part of the analysis process.

The FASSTT@10y study also addressed a number of research gaps identified in relation to PH11 and PH40. Currently there is a lack of evidence about the effectiveness of interventions to improve the nutrition of mothers and their children under 5 years (PH11b) and the children's social and emotional development and

wellbeing (PH40a). It is also a well-designed RCT to improve the nutrition of women before and during pregnancy (PH11c) through adequate, sufficient and sustained folic acid use during pregnancy. Unlike many interventions, folic acid was not a self-report measure but controlled, accurately measured and biologically validated before and after the intervention, thus fulfilling PH11d. This study used evidence based operational definitions for TEI, TPR, peer attachment and parenting style which informed the scales administered during the data collection process which met the requirements of PH40b. As noted in the discussion section of the FASSTT chapter (*Chapter 4*), operationally defining these constructs remains problematic however the inclusion of TEI and TPR was justified.

The ALSPAC dataset was also useful to consider some of the requirements listed by the PDG and PHIAC that remained unmet by the review and RCT. Due the extensive sample incorporating all ages, ethnicities, income levels and SES groups, the ALSPAC study when applied as a folic acid intervention study was beneficial for investigating the nutrition of mothers and children under 5 years (PH11a), comparing these groups was within in the scope of ALSPAC, however conducting this analysis was beyond the scope of this PhD. ALSPAC also provided an opportunity to query the effective components of an intervention found in FASSTT on a wider scale (PH11e) but more importantly this study had the ability to consider the numerous operational definitions of social and emotional development and the associated parent and child completed measures which could be evaluated in an attempt to combat any discrepancies and contribute to the advancement of a theoretically sound and universal operational definition for constructs notoriously difficult to define and measure including emotional intelligence but particularly resilience.

5.5 Strengths and limitations of the research

Individually and in combination these studies provide robust and reliable evidence, useful for informing policy on folic acid use during pregnancy as well as UK fortification programmes, adding substantially to the psychological and biomedical literature. In order to counteract the methodological strengths and limitations of each individual study a multi-method approach was applied (Vivek & Nanthagopan, 2021). Multi-method research has been defined as a combination of distinct approaches or methods used in parallel or in sequence which stand-alone until inferences are being made (Johnson *et al*., 2007). The aim of using multiple methods and the reason for utilizing this approach in this thesis was to move beyond a descriptive analysis to contribute to a better understanding and interpretation of phenomena through the application of various data sources as recommended by Anguera *et al*. (2018). This design has the ability not only to investigate the area of interest more thoroughly, but it can be useful to strengthen the rigor of a discipline and help it progress.

The application of the multi-method design was an inherent strength and although demanding in terms of time and ability, conducting each study showed researcher competency for all of the required methodologies. Each study was carefully designed to address the research questions posed and ultimately meet the aims and objectives of the thesis. This design was the most appropriate to meet the needs of the thesis as it enabled the subsequent methods to significantly contribute to the learning outcomes of the other studies through the use of complementary methods to maximize research strengths. However, the limited evidence available in the area meant triangulation of data and findings was challenging. It was difficult to value

each study equally when some were more vulnerable to the effects of limitations than others. Each of the studies were novel in some capacity therefore it was difficult to draw conclusions, triangulate and compare with a limited evidence base. It is however important to note that due to the limited evidence available each of the studies were carefully designed to answer the research questions, subject to high quality methods and analyses to ensure any supportive evidence found was robust.

This thesis was developed with each study running concurrently and sequentially which could be described as an embedded multi-method design (Vivek & Nanthagopan, 2021). This enabled each study to work synchronously to minimize any weaknesses and develop the strengths. Conducting the systematic review was a critical component of the research. Searching the literature confirmed the shortcomings in available evidence and subsequently identified a gap where this thesis could focus and surmised that clarity on the optimal dose, time of initiation and duration of use was required. FASSTT@10y provided a unique opportunity to use a longitudinal RCT design where folic acid dose and time of initiation were controlled while the duration of use was compared to test for any potential benefits for children's psychological, behavioural, social or emotional development. ALSPAC held promise as an effective validation study for FASSTT@7y and @10y, being a large, independent UK study and theoretically ideal for combatting the limitations of the FASSTT RCT. Despite being unable to compare with the findings of FASSTT@7y and FASSTT@10y, ALSPAC did provide some support for FASSTT@3y in terms of cognitive, psychological, social and emotional

development allowing us to speculate that a relationship did exist between maternal folic acid use and children's development.

A strong relationship exists between folic acid use, medical practice and biomedical research, characterised typically by a deficit and its use as a treatment for the prevention of illness in mother and child. A review of international wellbeing indicators found that four dominant international models, including the WHO and UNESCO, consult based on deficit approaches rather than strengths-based approaches with limited use of subjective data to capture a child's will and desire. This has meant that guidelines are focused on the rights and needs of the child and fail to consider what the children themselves want to increase their wellbeing. Measurements of child wellbeing are therefore most commonly measured and understood in negative terms (Marjanen *et al*., 2016). This review indicated that this is an international issue, embedded deeply into current research culture.

As evidence continues to advance, new theoretical perspectives are expanding knowledge and developing innovative treatments. One such perspective is positive psychology which recommends a resource focused model which focuses on an individual's positive experiences, attributes and skills, shifting the focus from illness to wellness (Seligman, 2000). Positive psychology has gained momentum in recent years with resource models being successfully applied in numerous areas including health, cognitive and clinical psychology and other disciplines (Hobfoll, 2002). The application of a positive, resource-focused model in the context of this thesis will ultimately raise awareness of the importance of preconception care and the potential benefits of maternal folic acid use. There is a growing body of evidence surrounding

knowledge and awareness of preconception health and care due to the important implications they can have on fertility, pregnancy outcome and immediate and long-term health and development of the child (Stephenson *et al.*, 2018; Fleming *et al.*, 2018; Barker *et al.*, 2018). According to evidence, women appear to be aware of preconception care including folic acid use (Olowokere *et al*, 2015; Temel *et al*., 2015). Despite this, research indicates that the majority of participants do not request or implement preconception care before becoming pregnant (Olowokere *et al.*, 2015). Similar results were found in India (Sunila et al., 2019) and in Sweden with less than half of mothers reportedly supplementing before pregnancy, even when the pregnancy was planned (Stern *et al.*, 2015). Research in the Netherlands found low levels of knowledge on the benefits of supplementing with folic acid during pregnancy and the insufficient intention to seek out preconception care (Temel *et al.*, 2015). This lack of awareness is a global issue, and a combination of education and structural interventions have been recommended to improve and maintain awareness of preconception care and in particular, maternal folic acid use (e.g. Olowokere *et al.*, 2015; Temel *et al.*, 2015; Akinajo *et al*., 2019).

If health practitioners, policy makers, pregnant mothers and the general population are more aware of the benefits supplementation could have on their child's development, and there is an understanding as to how these benefits translate into positive health and wellbeing in adulthood perhaps early uptake, adherence and continued use would become commonplace. Poor uptake and adherence to folic acid undermines public health effectiveness and some would argue that despite knowledge and intention, it does not always translate into action (e.g. Herter-Aeberli *et al.*, 2020; Bitzer *et al.*, 2013; Olowokere *et al.*, 2015; Temel *et al.*, 2015).

However, there is also substantial evidence to suggest that folic acid adherence can be improved by increasing knowledge for both pregnant mothers and those providing healthcare (e.g. Felipe-Dimog *et al*., 2021; Martin *et al*., 2017), through interventions such as community-based health education (e.g. Kamau *et al*., 2019), available and effective antenatal care (Tarekegn *et al*., 2019), counselling techniques to encourage consumption (e.g. Siekmans *et al*., 2017; Felipe-Dimog *et al.*, 2021). Although maternal adherence to folic acid alone or in combination with other vitamins is generally low across all countries, much of the literature is reflective of uptake and adherence during early pregnancy or in low and middle-income countries (e.g. Felipe-Dimog *et al.*, 2021; Siekmans *et al.*, 2017).

In the UK, McDougall *et al*. (2021) found corresponding results to those outlined above but specifically to preconceptional folic acid use. Poor adherence was reported (31.5%), despite the majority of mothers actively planning their pregnancies (64.8%) with a focus on periconceptional health recommended by authors for effective public health. European evidence reported that although a high majority of women were aware of folic acid (82.8%) only 45.5% supplemented, consumption did not differ between those who planned their pregnancy and those who did not (Fulford *et al*., 2014). Supplement use was highest in those who practiced other positive health behaviours such as eating a healthy diet, having an active lifestyle and being non-smokers (McDougall *et al.*, 2021). Supplement adherence improved when those providing antenatal care were challenged to elicit and correct the pregnancy beliefs of women who believed they were less susceptible to the consequences of non-adherence.

As with all research, this thesis and each of the individual studies were not immune to limitations. However, every effort was made to reduce any negative effects and to incorporate and develop research strengths where appropriate. The omission of language development as an outcome measure is a notable limitation. The systematic review identified that maternal folic acid use could have a significant impact on children's language acquisition and development. Closely related to language is social development where children begin to understand and learn the rules of language in a context individual to them. A key aspect in fostering children's social development and subsequent psychological, emotional, behavioural and language development is play. Play is critical to all areas of children's development and despite its necessity, it is a notoriously difficult concept to define. There is agreement however that play involves the child being actively engaged in an activity which serves as a learning opportunity to problem solve, make decisions, follow rules and use self-control (Romero-Ayuso *et al.*, 2021). Engaging in play activates the brain, building and strengthening neuronal connections to enhance language, social competence, motor abilities whilst nurturing creativity and imagination (Bundy *et al.*, 2016).

Play was not measured or considered in any of the three studies contained in this thesis and FASSTT@10y did not provide the opportunity to measure language on this occasion. However, ALSPAC to a degree, did investigate the potential impact of maternal folic acid use on aspects of children's language at 3 years old, which would be a time of rapid development in terms of language and communication. As children begin school and form friendships the rules of language change and further development will occur. Language development at an older age was unable to be

measured due to data limitations. The findings from these studies would indicate that those with better social, emotional and psychological attributes and abilities would be more effective communicators both verbally and non-verbally.

A specific limitation in relation to the systematic review is the period of time that lapsed between the updated systematic search which took place in February 2018 and thesis completion. Research has advanced in this area which could mean other pieces of research could be eligible for inclusion. Unfortunately, time constraints did not allow a more recent search of the literature. In preparation for publication these searches will be updated, and any eligible studies will be assessed for quality and risk of bias and included in the systematic review.

5.6 Recommendations

This is an exciting area of research with the intricacies of child development and its relationship with maternal folic acid use during pregnancy needing further exploration. The research included in this thesis suggests that language and play are key to the psychological, social, emotional and behavioural development of a child, therefore exploring these constructs as potential mechanisms of action is an important step for future research. Mascha *et al*. (2013) recognised that focused research designed to understand the mechanisms behind the observed effects between an intervention and outcome is often under pursued in experimental research.

This thesis demonstrated how certain aspects of social development such as attachment can significantly mediate psychological, behaviour, and emotional

development. A recent advancement of a meta-theoretical paradigm suggested that a construct which initially mediates could evolve into a construct that moderates (Karazsia & Berlin, 2018). This is an area that requires further exploration as the integration of conceptual mediation and moderation can offer a more thorough understanding of the complexity between the dependent nature of these independent constructs (Karazsia *et al*., 2014). Time of measurement was recognised as an important factor, therefore the longitudinal nature of FASSTT could provide the conditions to explore if the mediators could also be moderators. Distinguishing between mediation and moderation could provide an opportunity to delve deeper into potential mechanisms while exploring the nature of causality. Baron and Kenny (1986) suggested that in some instances were mediation is questionable certain variables can be treated as moderators to resolve the issue. In relation to this thesis, parenting style was not found to be a significant mediator between maternal folic acid use and child developmental outcome however, testing for moderating effects of parenting style could yield some important and significant results relating to parenting style not detected in these studies.

Emotional intelligence, resilience, attachment and behaviour were useful measures of development to consider due to the limited evidence available in relation to these areas of development and maternal folic acid use. In recent years these developmental outcomes have received increasing attention due to the positive psychology movement and the focus on wellbeing, nationally and internationally which has helped shape and operationally define these concepts to an acceptable standard. However, including other psychological constructs such as hope, optimism and self-efficacy would be a useful addition to the evidence.

Resource-focused models have been described by a large body of literature and have been more or less widely used in practice for many years (Seligman & Csikszentmihalyi, 2000). Resource orientation focuses on an individual's strengths to maintain and improve health and contrasts with the approach of deficit. These approaches are usually treated separately in the literature, rarely considering the possibility or application of a shared-resource orientation model. Research in this respect is lagging behind interventions used in practice. Moving forward, research needs to use an integrated approach, providing sound evidence, based on the risks and benefits associated with an intervention. Conducting research in this way will provide balanced and complete findings, valuable for advancing research, practice and policy. Taking this approach is critical for health promotion and encouraging positive health behaviours. Keeping individuals informed of the benefits of an intervention is equally as important as clarifying any risks involved and focusing on strengths and benefits will undoubtably enhance all areas of development and foster an improved sense of wellbeing.

5.7 Conclusion and final thoughts

Each of the included studies indicate that maternal folic acid use during pregnancy benefits children's development beyond physical neurological and cognitive factors with positive effects being observed in children's psychological, behavioural, social and emotional development. FASSTT@10y findings uncovered that children's emotional intelligence and resilience are two character strengths which could benefit from continued supplementation into late pregnancy with advantages also being observed in peer attachment and prosocial behaviour. Despite the methodological and analytical difficulties associated with the ALSPAC dataset, exploratory findings

did offer some support to the folic acid evidence base, including the FASSTT@10y study.

The findings from these studies suggest that the guidance informing preconception and antenatal care requires updating. Going forward, new research needs to adopt the positive, resource focused approach concentrating on the beneficial effects of supplementation to promote children's health, wellbeing and development. In time this will help balance the literature looking at the positives and negatives of supplementing and account for the effects of dose, time of initiation and duration of use. This will ensure researchers, practitioners and public health officials are able to make informed decisions based on literature with the ability to weigh up both the risks and benefits for both mothers and children.

Based on the findings provided by these pieces of research we would recommend that all women consume adequate and sufficient folic acid for the duration of their pregnancy and into lactation. This ensures that optimal levels are maintained at all stages of children's brain development providing the best foundation for the other key areas of development (psychological, behavioural, social and emotional) to build on. Being more mindful of the benefits of supplementing during pregnancy for children's development, coupled with the understanding of why it is important for both mothers and children we expect would only promote uptake and use. It could also help foster more secure attachments beginning at birth which could contribute to a more positive parenting style increasing the wellbeing of the child but also of the parents, siblings, wider family and society.

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Appendices

Appendix 2.1

Systematic Review Protocol

Title

Can maternal folic acid supplementation during pregnancy impact on the children's psychosocial development?

Researchers

Ms Lesley-Anne Henry, Prof Tony Cassidy & Dr Marian McLaughlin

Background

Folate is a naturally occurring B-vitamin, essential to the body to synthesize, repair and methylate DNA. There is an increased demand for folate during pregnancy to aid rapid cell division and growth; vital for fetal, placental and maternal development (1) Folic acid is a synthetic form of folate, and a dosage of 400ug/d is recommended to all women from preconception to the end of the first trimester of pregnancy (2). The physical benefits of folic acid supplementation are well documented in the literature; the most notable is protection against first occurrence and reoccurrence of neural tube defects (3); affecting over 900 pregnancies per year in the UK (4). Folic acid supplements have also been shown to reduce the risk of congenital heart defects (5), cleft palette (6). Premature delivery (7) and low infant birth weight (7).

Another popular research perspective investigates the impact of maternal folic acid on children's neurocognitive development. Recent findings suggest that a deficiency in maternal folate levels negatively impacts on the child's cognitive performance (8), whereas optimal levels positively impact on language and motor development at age four (9). Supplemental folic acid during early pregnancy has also been shown to reduce the risk of severe language delay (10) and autistic spectrum disorders (ASD) in children (11).

The evidence available exploring the impact of folic acid supplementation on children's social and emotional development is limited; one study found that higher maternal folate status lowered the risk of children Internalising and externalising

problems (12). However, children's development tends to occur simultaneously and it is important to establish a link between maternal nutrition and subsequent child development. It is therefore important to review the available literature regarding folic acid intake during the prenatal and perinatal stages of pregnancy and explore the potential effect on the children's psychosocial development.

Research question and aims:

The overall objective of this review is to collect and evaluate the literature about whether mothers taking supplemental folic acid during pregnancy is associated with an improvement in the children's psychosocial development; physical, psychological, social, emotional and cognitive development from birth to age 11.

Methods:

Search strategy

- Biomedical database: CINAHL, EMBASE, MEDLINE (Ovid), PsycINFO, AMED, Nutrition and Food Sciences (including Nutrition Abstracts and Reviews), ProQuest Complete, Web of Science (including SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI, BIOSIS-PREVIEWS, KCI-KOREAN, RSCI & SciELO-CI).
- Social science database: Applied Social Sciences Index and Abstracts (ASSIA), International Bibliography of the Social Sciences (IBSS) AND Sociological Abstracts,
- Internet Search engines: Google & Google scholar
- Grey literature search: Science Direct, Sage Psychology, conference proceedings or other studies published in abstract form only, grant awards and theses or dissertations
- Hand searching the reference lists of all included studies
- Hand searching the reference lists of relevant reviews, commentaries, or other non-research articles identified during the initial scoping search. Commentaries or letters on specific studies are also reviewed to see if they contain content that should be noted during data extraction or risk of bias assessment of the original report.

Inclusion

- No publication year or language limits will be imposed (to prevent inherent bias)

Exclusion

- Articles with no original data e.g. editorials, reviews
- Retracted articles

Screening and selecting

Types of study to be included: RCT's, prospective cohort, retrospective cohort, cross-sectional or case-control study design

Condition or domain being studied: folate is a water-soluble B-complex vitamin required for cell growth and division, and adequate folate intake is necessary to prevent a wide variety of health conditions for both the mother and child. Some published studies suggest that taking folic acid, a synthetic form of folate during pregnancy encourages better cognitive development in the child and as children tend to develop physically, cognitively and psychologically or emotionally in concurrence. It makes sense that taking the supplementation would also be beneficial to the children's psychological development.

Participants/ population:

Inclusion:

All mothers who had supplemented with folic acid during pregnancy

All male and female offspring from birth to 11 years

Exclusion:

Children 12+ years

Children with any diagnosed developmental disorder e.g. autism

Non-human animals including laboratory animal studies or pets

In silico studies or in vitro models utilizing organs, tissues, cell-lines or cellular components.

Intervention(s), exposure (s):

Inclusion:

Exposure to folic acid via supplement prior to and/ or during pregnancy

Mothers exposed to normal levels of folate during the second and third trimesters

Exclusion:

Exposure to multivitamin containing folic acid prior to and/ or during pregnancy

Comparator(s)/ Control:

Inclusion:

No exposure to folic acid

Mothers exposed to low levels (exposure below detection levels) of folic acid

Mothers exposed to high levels of folic acid

Exposure to normal levels of folate and ceased at 12 weeks' gestation

Exposure to multivitamins containing folic acid

Exclusion:

No exclusion

Outcome(s):

Primary:

Psychological measures of health, wellbeing and development

Secondary:

Intelligence and psychological, cognitive, emotional, social and language development

Quality assessment

CASP checklist for inclusion and exclusion

NICE risk assessment checklist

Data extraction and analysis

Title/Abstract Review:

Two members of the team will independently screen the titles and abstracts from each search to determine whether a reference meets the inclusion criteria; studies that are not excluded based on title and abstract will be screened through full text review. Screeners will be trained and have knowledge of the project and an initial pilot phase will occur to improve the clarity of the inclusion and exclusion instructions and to improve accuracy and consistency amongst screeners.

Studies are not considered further when the title or abstract clearly indicate that the study does not meet the inclusion criteria. In the case of screening conflicts, screeners will independently review their screening results to confirm the inclusion/exclusion decision and, if needed discuss discrepancies with the other screener. Any articles with unresolved screening conflicts at the title and abstract phase will be included in the full text review.

Full-text Review:

After completion of the title/abstract screen, full text articles are retrieved for those studies that either clearly met the inclusion criteria or where eligibility to meet the inclusion criteria is unclear. Full-text review will be conducted by one member of

the review team with a second member of the team confirming any exclusion determination of the first reviewer. True disagreements will be resolved by discussion involving other members of the team.

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Appendix 2.2

Table 1: Critical Appraisal Skills Programme (CASP) Checklist for Cohort Studies used to assess study quality.

		Yes	Can't tell	No
A	**Are the results of the study valid?**			
	1. Did the study address a clearly focused issue?			
	HINT: A question can be 'focused' In terms of a) The population studied b) The risk factors studied c) The outcomes considered d) Is it clear whether the study tried to detect a beneficial or harmful effect?			
	2. Was the cohort recruited in an acceptable way?			
	HINT: Look for selection bias which might compromise the generalisability of the findings: a) Was the cohort representative of a defined population? b) Was there something special about the cohort? c) Was everybody included who should have been included?			
	Detailed questions			
	3. Was the exposure accurately measured to minimize bias?			
	HINT: Look for measurement or classification bias: a) Did they use subjective or objective measurements? b) Do the measurements truly reflect what you want them to (have they been validated)? c) Were all the subjects classified into exposure groups using the same procedure			
	4. Was the outcome accurately measured to minimize bias?			

	HINT: Look for measurement or classification bias: a) Did they use subjective or objective measurements? b) Do the measures truly reflect what you want them to (have they been validated)? c) Has a reliable system been established for detecting all the cases (for measuring disease occurrence)? d) Were the measurement methods similar in the different groups? e) Were the subjects and/or the outcome assessor blinded to exposure (does this matter)?			
	5a. Have the authors identified all important confounding factors			
	List the ones you think might be important, that the author missed.			
	5b. Have they taken account of the confounding factors in the design analysis?			
	HINT: Look for restriction in design, and techniques e.g. modelling, stratified-, regression-, or sensitivity analysis to correct, control or adjust for confounding factors			
	6a. Was the follow up of subjects complete enough?			
	6b. Was the follow up of the subjects long enough?			
	HINT: Consider a) The good or bad effects should have had long enough to reveal themselves b) The persons that are lost to follow-up may have different outcomes than those available for assessment c) In an open or dynamic cohort, was there anything special about the outcome of the people leaving, or the exposure of the people entering the cohort?			
B	**What are the results?**			
	7. What are the results of the study			
	HINT: Consider a) What are the bottom line results? b) Have they reported the rate or the proportion between the exposed/unexposed, the ratio/the rate difference? c) How strong is the association between exposure and outcome (RR,)? d) What is the absolute risk reduction (ARR)?			

	8. Are the results precise?			
	HINT: Look for the range of the confidence intervals, if given.			
	9. Do you believe the results?			
	HINT: Consider a) Big effect is hard to ignore! b) Can it be due to bias, chance or confounding? c) Are the design and methods of this study sufficiently flawed to make the results unreliable? d) Bradford Hills criteria (e.g. time sequence, dose-response gradient, biological plausibility, consistency)			
C	**Will the results help locally?**			
	10. Can the results be applied to a local population?			
	HINT: Consider whether a) A cohort study was the appropriate method to answer this question b) The subjects covered in this study could be sufficiently different from your population to cause concern c) Your local setting is likely to differ much from that of the study d) You can quantify the local benefits and harms			
	11. Do the results of this study fit with other available evidence?			
	12. What are the implications of this study for practice?			
	HINT: Consider a) One observational study rarely provides sufficiently robust evidence to recommend changes to clinical practice or within health policy decision making b) For certain questions observational studies provide the only evidence c) Recommendations from observational studies are always stronger when supported by other evidence			

Table 2: Critical Appraisal Skills Programme (CASP) Checklist for RCT's used to assess study quality.

		Yes	Can't tell	No
A	**Are the results of the study valid?**			
	1. Did the study address a clearly focused issue?			
	HINT: A question can be 'focused' In terms of a) The population studied b) The intervention given c) The comparator given d) The outcomes considered			
	2. Was the assignment of patients to treatments randomised?			
	Consider: a) How was this carried out, some methods may produce broken allocation concealment b) Was the allocation concealed from researchers and patients?			
	3) Were all of the patients who entered the trial properly accounted for at its conclusion?			
	Consider: a) Was the trial stopped early? b) Were patients analysed in the groups to which they were randomised?			
	Detailed questions			
	4. Were patients, health workers and study personnel 'blind' to treatment?			
	HINT: Think about a) Patients? b) Health workers? c) Study personnel?			
	5) Were the groups similar at the start of the trial?			
	Consider: Look at a) Other factors that might affect the outcome such as age, sex, social class, these may be called baseline characteristics			

	6) Aside from the experimental intervention, were the groups treated equally?			
B	**What are the results?**			
	7. How large was the treatment effect?			
	Consider: a) What outcomes were measured? b) Is the primary outcome clearly specified? c) What results were found for each outcome? d) Is there evidence of selective reporting of outcomes?			
	8. Was the estimate of the treatment effect precise?			
	Consider: a) What are the confidence limits? b) Were they statistically significant?			
C	**Will the results help locally?**			
	9) Can the results be applied in your context? (Or to the local population?)			
	HINT: Consider whether a) Do you think that the patients covered by the trial are similar enough to the patients to whom you will apply this? if not how to they differ?			
	10. Were all clinically important outcomes considered?			
	Consider: a) Is there other information you would like to have seen? b) If not does this affect the decision? c) Was the need for this trial clearly described?			
	11. Are the benefits worth the harms and costs?			

	Consider: a) Even if this is not addressed by the trial, what do you think?			

Appendix 2.3

Table 1: The PRISMA checklist was completed as part of the systematic review process.

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Does maternal folic acid supplementation during pregnancy impact on children's development in the first 12 years of life? A systematic review.	12
ABSTRACT			
Structured summary	2	Background: Research has confirmed that folic acid use (preconception to 12GW) prevents NTD's but its impact on other areas of child development is less consistent. Objectives: To comprehensively evaluate evidence on the impact of folic acid on 7 key areas of children's development (cognitive, motor, psychomotor, social, emotional, behavioural and language development) Data sources: An online search was conducted on 13 relevant biomedical and social sciences databases and grey literature. Eligibility criteria: For inclusion, studies needed to be experimental (randomised or non-randomised), Mothers must have supplemented with folic acid (FA) or dietary folate (DF) during pregnancy at detectable levels at the first assessment. A comparative group of either no FA use, low/ high levels or MV (without FA) use. Children under 12y must have provided at least one measure of development (cognitive, motor, psychological, social, emotional, behavioural or language), multiple measures were acceptable. Study appraisal: All articles meeting the inclusion criteria were subject to a title and abstract and full-	13-14

		text review. Key data was extracted from 19 eligible studies by 3 independent reviewers followed by a quality (CASP) and risk of bias (NICE) assessment. Data synthesis: Meta-analysis was not possible due to heterogeneity amongst the studies in terms of design, intervention and outcome. Results: 13 studies found a beneficial effect of folic acid supplementation on children's cognitive, motor, psychomotor, social, emotional, behavioural and language development, 3 studies found no significant effect, 4 studies found children were at higher risk of developmental disorders if the mother was folate deficient during pregnancy and 3 reported a negative effect however this was atypical use. Limitations: A number of limitations and improvements are acknowledged. Implications of key findings: To our knowledge this is the first review exploring the impact of folic acid on children's development. Further research is required in this area to allow for robust reviews and meta-analyses to be conducted. This will be a useful resource for both policy makers and researchers. Conclusions: This review suggests that folate intake during pregnancy could benefit or improve key areas of children's development	
INTRODUCTION			
Rationale	3	The physical benefits of folic acid (FA) supplementation are clear, and the neurocognitive benefits are also well documented. However, the evidence available exploring the impact of maternal folate status on other key areas of children's development including social, emotional, behavioural, motor and language development is limited. The current evidence focuses on non-use, insufficient or over-use of folic acid during pregnancy and the possible detrimental effect to child development. Driven by this deficit model the focus has been on preventing developmental problems with sufficient folic acid use. Generally, this was a useful approach to guide research and inform policy on using folic acid to prevent negative aspects of development such as NTD's and child developmental disorders. However, in order for policy makers to make informed decisions the risks should be balanced with the potential benefits associated with supplementing. The recent shift to a more positive approach	17-18

		exploring the potential beneficial effect of sufficient maternal folic acid use on children's development has highlighted conflicting evidence and an absence of systematic reviews. Consequently, it is imperative a high quality systematic review is conducted to investigate the potential benefits associated with maternal folic acid use during pregnancy on children's development.	
Objectives	4	Using experimental research this review explores the role of maternal folic acid supplementation during pregnancy on children's development, particularly, their psychosocial development from birth to 12 years. This is achieved by comparing sufficient vs insufficient use, high folate vs. normal folate levels, early vs. late use and folic acid vs MV (without FA).	19
METHODS			
Protocol and registration	5	A protocol was developed to guide the research team during the review process. This was not published but can be viewed in Appendix 1.	
Eligibility criteria	6	For inclusion studies must be experimental by design (randomised or non-randomised), Mothers must have supplemented with folic acid (FA) or dietary folate (DF) during pregnancy at detectable levels at the first assessment. A comparative group of either no FA use, low/ high levels or MV (without FA) use. Children under 12y must have provided at least one measure of development (cognitive, motor, psychological, social, emotional, behavioural or language), multiple measures were acceptable. Studies were excluded if the mother supplemented with MV containing FA during pregnancy or within 4 weeks preconception, or the mother was undernourished or HIV infected, children were over 12 years old or presented with any developmental disorder (e.g. ASD) and all non-human studies were excluded.	23-24
Information sources	7	An online search was conducted encompassing 13 biomedical and social science electronic databases and relevant grey literature (May-July 2016). CINHAL, Ovid (PsychINFO, Medline, AHMED, Embase), Nutrition and Food Sciences, ProQuest Complete, Web of Science, Scopus, TRIP, Applied Social Sciences Index and Abstracts (ASSIA), International Bibliography of the Social Sciences	22-23

		(IBSS) AND Sociological Abstracts. An internet search and grey literature search was also completed to find anything that may have been missed. This included Google Scholar, Science Direct, Sage Psychology, conference proceedings or other studies published in abstract form only, grant awards and theses or dissertations (Ethos). There was no publication year or language limits imposed however only articles with original data was included i.e. no reviews, editorials or retracted articles. In addition, reference lists of included articles and relevant reviews were also hand-searched to ensure all important studies had been included. An updated search was conducted in February 2018.	
Search	8	(OVID search including Medline, PsychINFO, Embase and AMED) "folic acid*" or folate* or "vitamin* B_9" or "Vitamin* B_{12}" AND "perinatal nutrit*" or "pregnan* nutrit*" or "preconception* nutrit*" or "maternal nutrit*" or "periconception* nutrit*" AND "emotion* intelligen*" or EI or "emotion* quotient*" or EQ or "emotion* develop*" or "intelligen* quotient*" or IQ or "cogni* abilit*" or "psycholog* develop*" or "psychosocial* develop*" or "language develop*" or "verbal* IQ" or "Verbal* intelligen* quotient*" or "emotion* adjust*" or "emotion* matur*" or "social* develop*" or cogniti* or wellbeing or "well being" or well-being or resilien* or coping Search was conducted by one reviewer with assistance from an Ulster University Research Librarian	21-22
Study selection	9	Articles identified during the systematic search were subject to a title and abstract review by 3 independent reviewers. Those meeting the inclusion criteria were included for full-text review. In the case of screening conflicts, reviewers independently screened the article again to confirm the inclusion/exclusion decision and discrepancies were discussed amongst the team. Any articles with unresolved screening conflicts were included for full text review.	24-25
Data collection process	10	A data extraction form was developed specifically for this review by LH and agreed by the team. The form was piloted on 3 RCT and 3 cohort papers and adjusted as necessary to ensure all relevant key data was being recorded.	25
Data items	11	Key data was extracted by one reviewer and included publication information, study and participant characteristics and exposure and outcome details including; authors, publication year, journal of	

		publication, study design, study location, mother participant age, number recruited, folate dosage, time spent supplementing, blood sample availability, length of follow up, number at follow up, outcomes observed, and measure used.	
Risk of bias in individual studies	12	Study quality was assessed using the Critical Skills Appraisal Programme (CASP) by 3 independent reviewers. Risk of bias was measured using the National Institute for Health and Clinical Excellence (NICE) tool by 1 reviewer as inter-rater reliability had already been confirmed. Reporting quality meant bias could not be accurately assessed as in some cases the relevant information was missing. Selection, performance, attrition and detection bias was possible in all studies with some at more risk than others.	27-30
Summary measures	13	A combination of confidence intervals, p values, risk ratios, beta and SE of beta.	
Synthesis of results	14	Heterogeneity amongst the studies in terms of design, intervention, and outcome measurements no attempt to summarise the effect by meta-analysis was made.	

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Possible publication and reporting bias.	
Additional analyses	16	None	
RESULTS			
Study selection	17	A total of 1237 papers were identified at the initial search. Following the title and abstract review 56 articles were identified as meeting the inclusion criteria, after full-text review 38 were excluded and the remaining 18 progressed for data extraction. An updated search identified an additional 17 studies, 10 were excluded based on information presented in the abstract and 7 progressed for a full-test review, 1 study met the inclusion criteria and was included in	

		the review. The main reasons for exclusion were that mothers supplemented with MV including FA and therefore the outcome could not be attributed to FA alone or that folic acid was not used or measured during pregnancy.	
Study characteristics	18	The 19 eligible studies included 4 RCT's and 15 prospective cohort designs. The main outcomes were children's cognitive, motor, psychomotor, social, emotional, behavioural or language development. The children ranged from 1 month to 11 years and mother/child background characteristics are detailed in Tables 4-6. Many of these studies were follow up investigations to established cohorts (12/19).	
Risk of bias within studies	19	19 articles were assessed for risk of bias. Analysis provided a preliminary conclusion.	
Results of individual studies	20	A total of 19 articles investigated the effect of FA or DF or a combination of both during pregnancy on different key areas of children's development.	
Synthesis of results	21	13 studies found a beneficial effect of FA supplementation on children's cognitive, motor, psychomotor, social, emotional, behavioural and language development, 3 studies found no significant effect, 4 studies found children were at higher risk of developmental disorders if the mother was folate deficient during pregnancy and 3 reported a negative effect however this was atypical use. A beneficial effect on cognition was reported in 8 articles and a non-significant effect in 3 articles. 1 article reported a detrimental effect of FA on cognition however this was in relation to high doses. 2 articles reported a beneficial effect on motor development. 1 reported a non-significant effect on children's psychomotor development and 1 reported a detrimental effect of high doses of FA. 2 studies found a beneficial effect and 1 found a marginal negative effect of FA on social development. 3 articles reported a beneficial effect on emotional and behavioural development and 1 reported a beneficial effect on language and 1 reported a detrimental effect however this was in conjunction with SSRI use. 1.Chatzi (2012) measured the neurodevelopment of 553 18m old children in Greece using BSID-III. Compared to non-users, daily intake of 5mg FA was associated with a 5-unit increase on the scale of receptive communication and a 3.5 increase on the scale of expressive communication.	

		2. Roza *et al* (2010) examined the association between FA use before 12GW and behavioural and emotional problems in 4,214 18m old children and found that FA protected against internalising (OR of no supplement use 1.65; 95% CI 1.24, 2.19) and externalising problems (OR 1.45; 95% CI 1.17, 1.80) after adjustment. BW and head circ did not mediate the association. 3. Steenweg-de Graff (2012) found 3y old children (3209) were at higher risk of emotional problems when mothers did not supplement with FA or started supplementing in late pregnancy (OR 1.45; 95% CI 1.14, 1.84) compared to children whose mothers supplemented from early pregnancy. 4. Villamor (2012) found that for each 600µg/d increment in total folate intake (FA+DF) before 12GW 3y old children (n=1210) scored 1.6 points higher (95% CI 0.1-3.1; p=.04) in receptive language, a test that predicts overall intelligence. 5. Scholtz (2010) found that low folate levels (FA+DF) in early pregnancy were associated with increased hyperactivity (RCF; beta=-.24; p=.013; TFI; beta -.24; p=.022) and peer problems (RCF; beta -.28; p=.004) TFI beta=-.28; p=.009) in 8.75y old children (n=100). 6. Julvez (2009) investigated the association between early FA use and 4y old children's neurodevelopment (n=420). Verbal (b=3.98, SE=1.69) motor (b=4.54, SE=1.66), verbal-executive function (b=3.97, SE=1.68) scores, social competence (b=3.97, SE=1.61) and a lower rate of inattention symptoms (OR=0.46; 95% CI 0.22, 0.95) were associated with reported FA use after adjustment. 7. Wehby (2007) early FA use was associated with improved gross-motor development (OR 1.78; 95% CI 0.94, 3.38) and a borderline poorer performance in the personal social domain (OR 0.51; 95% CI 0.28, 0.93) in a sample of 6774 children. 8. Del-Rio Garcia (2009) evaluated the mental and psychomotor development of 253 children during their first year in relation to maternal DF intake and found that folate deficiency (<400µg/d) reduced the mental development of genetically susceptible children if their mother carried the TT genotype (beta=1.8; 95% CI = -3.6, -0.04; p for interaction =0.07).	

		9. Bhate (2012) investigated the relationship between DF measured at 28GW and 34GW on 3 areas of children's development at 2y (n=123). Adequate folate was associated with higher motor development scores (28GW p= .018; 34GW p= .05), mental development scores at 28GW (std beta= 0.178; p= .045) and 34GW (std beta= 0.199; p= .027) and social development scores at 28GW (std beta= 0.194; p= .028) and 34GW (std beta= 0.223; p= .013) 10. Veena (2010) examined if FA during pregnancy was associated with children's neurodevelopment in children aged 9-10y (n=536). Maternal folate had an independent positive association with learning ability and long-term storage and retrieval (beta=0.10 95% CI 0.01-0.19), visuospatial ability (beta= 0.10; 95% CI 0.01, 0.19) and attention and concentration (beta= 0.10; 95% CI 0.02, 0.18) after extensive adjustment. 11. Handel (2016) detected a significant association between long-term SSRI use and delayed language competence in 3y old children (n=51747) but only when mothers supplemented with FA simultaneously. After 4-8 weeks of simultaneous use the risk ratio reached 4.5 (95% CI 2.5, 8.0) for intermediate delay and 5.7 (95% CI 2.5, 13.0) for most delay using the best language category for reference. 12. Valera-Gran (2014) examined the association between very high doses of FA+DF (>5000µg/d) and children's neuropsychological development after 1y old (n=2213) and found very high doses significantly lowered children's psychomotor scores (-4.35 points; 95% CI -8.34, 0.36) and increased their risk of delayed psychomotor development (OR= 1.59 ; 95% CI 0.82, 3.08), this was not statistically significant. 13. Roth (2011) assessed the severe language delay (defined as 1 word or unintelligible utterances) in 3y old children (n=38954) and found that adjusted ORs for 3 patterns of exposure to maternal dietary supplements (no supplement as the reference) were 1.04 (95% CI, 0.62–1.74) for other supplements but no FA; 0.55 (95% CI, 0.35–0.86) for FA only; and 0.55 (95% CI, 0.39–0.78) for FA in combination with other supplements, demonstrating a clear protective effect of folic acid supplementation during pregnancy 14. Tamura (2005) explored the association between FA in late pregnancy and children's neurodevelopment at 5y old (n=355) and found no significant differences in development scores between	

		the low and normal folate groups. 15. Valera-Gran (2017) further examined the association between very high doses of FA (above the tolerable upper intake level, >1000µg/d) and children's neuropsychological development at 4-5 y old (n=1682) and found a negative association in global verbal (beta= -2.49; 95% CI -4.71, -0.27) verbal memory (beta= -3.59; 95% CI, -6.95, -0.23) cognitive function of posterior cortex (beta= -2.31; 95% CI -4.45, -0.18) and cognitive function of left posterior cortex (beta= -3.26; 95% CI -5.51, -1.01). 16. Catena (2016) conducted an RCT exploring the long-term effects of 5-MTHF (FA alone) on children's attention networks at 8.5y (n=136) and found a beneficial effect on children's executive function; interference p= .01 and alertness p= .03) in comparison to the other intervention groups (FO and FO+5-MTHF) 17. Campoy (2011) implemented an RCT to assess the long-term effects of 5-MTHF on children's cognitive development at 6.5y (n=154) but found no significant effects on cognition 18. Li (2009) investigated the benefits of maternal MV use on children's mental and psychomotor development using FA as a comparator (n=995)using an RCT. MV was associated with mean increases in mental development raw scores for 1y old children of 1.00 and 1.22 points when compared to FA only and FA+FE respectively, no significant differences was observed in psychomotor development raw scores. 19. Li (2015) conducted an RCT to examine the effect of MV on children's intellectual development at 7-10y using FA only as a comparator (n=1744) and found no evidence to suggest a different effect on intellectual development between MV, FA only and FA+FE.	
Risk of bias across studies	22	A preliminary conclusion is provided after analysing the eligible studies	
Additional analysis	23	None	
DISCUSSION			

Summary of evidence	24	Nineteen articles from various locations and populations were reviewed and the majority (13/19) reported a beneficial effect of adequate folate (folic acid or dietary folate) on child development. Others (4/19) studied the impact of folate deficiency and found children were at higher risk of experiencing developmental problems or difficulties, a small number of studies reported no significant effect on development (3/19) and 3 reported a detrimental effect (11, 12, 15) however, 2 of these examined the adverse effects associated with high doses of folate and in the other study mothers were long-term Selective Serotonin Reuptake Inhibitors (SSRI) users simultaneously supplementing with folic acid during pregnancy.	
Limitations	25	This review has some limitations. • The review protocol was not registered however this did not affect the integrity of the study and was used diligently by the research team to guide the review. • The evidence investigating the impact of FA on children's social, emotional, behavioural and language development was scarce which makes synthesising and drawing conclusions difficult. In addition, the observed effect in the included articles was quite small in magnitude when compared to the protective effect of folic acid on NTD's. A potential explanation could be that unlike NTD, development is a subtle and gradual process requiring a long observation period therefore attrition and measurement errors could counteract the potential positive effect of supplementing. • Most (15/19) of the included studies were non-randomised but the evidence from 2 RCT's supported the positive results of the cohort studies. • Achieving high reporting quality in longitudinal research is difficult. All included studies were longitudinal therefore it is possible the quality of reporting was low. This can lead to misinterpretation of the results. Furthermore, transparency was an issue highlighted during the risk of bias assessment. Many details were not reported or reported elsewhere but this was attributed to the follow-up design of large established datasets. • The gold standard GRADE system was unable to be applied to accurately assess the risk of bias. • There was major heterogeneity in the studies in terms of design, intervention, and outcome	

		measurements and a meta-analysis was not possible. There is a possibility of publication bias if some studies finding negative results were not published or available in the grey literature. A formal assessment through meta-analysis was not possible.	
Conclusions	26	This review suggests that folate intake during pregnancy could benefit or improve key areas of children's development (cognitive, motor, psychomotor, social, emotional, behavioural or language)	
FUNDING			
Funding	27	This review was completed as a chapter of a doctoral thesis. The PhD studentship was funded by the Biology and Biological Sciences Research Council (BBSRC) and the Economic and Social Research Council (ESRC) and from the Northern Ireland Department for Economy (Grant Ref: ES/N000323/1 'EpiFASSTT'). This work was also supported by funding from HSC Research and Development Division of the Public Health Agency, Northern Ireland (Enabling Research Award STL/5043/14).	

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

Appendix 3.1

Table 1: Complete child based questions variable list

Filename	Variable code	Variable label	Timepoint	Questionnaire/ Assessment
KF	kf512b	DV: Social achievement score	2y 6m	My little study daughter/son
KF	kf528b	DV: Fine motor score	2y 6m	My little study daughter/son
KF	kf540	DV: Gross motor score	2y 6m	My little study daughter/son
KF	kf545	Total development score	2y 6m	My little study daughter/son
KG	kg620b	DV: EAS emotionality (prorated)	3y 2m	My 3 year old boy/girl
KG	kg621b	DV: EAS activity score (prorated)	3y 2m	My 3 year old boy/girl
KG	kg622b	DV: EAS shyness score (prorated)	3y 2m	My 3 year old boy/girl
KG	kg623b	DV: EAS sociability score (prorated)	3y 2m	My 3 year old boy/girl
KG	kg870	DV: Language score	3y 2m	My 3 year old boy/girl
KG	kg898	DV: Intelligibility score	3y 2m	My 3 year old boy/girl
KG	kg899	DV: Communicative score	3y 2m	My 3 year old boy/girl
KJ	kj493	Social Development Score	3y 6m	My son's/ daughter's health and behaviour
KJ	kj519	Fine Motor Score	3y 6m	My son's/ daughter's health and behaviour
KJ	kj535	Gross Motor Score	3y 6m	My son's/ daughter's health and behaviour
KJ	kj643	Emotional difficulties score (RR)	3y 6m	My son's/ daughter's health and behaviour
KJ	kj644	Conduct difficulties score (RR)	3y 6m	My son's/ daughter's health and behaviour
KJ	kj645	Hyperactivity score (RR)	3y 6m	My son's/ daughter's health and behaviour
KJ	kj646	Prosocial score (RR)	3y 6m	My son's/ daughter's health and behaviour
KJ	kj647	Total behavioural difficulties score (RR)	3y 6m	My son's/ daughter's health and behaviour
CIF	cf811	Performance IQ Wppsi	49m	Children in Focus
CIF	cf812	Verbal IQ Wppsi	49m	Children in Focus
CIF	cf813	Fullscale IQ Wppsi	49m	Children in Focus
CIF	cf948	Control Parenting	12m	Children in Focus
CIF	cf949	Warmth Parenting	12m	Children in Focus

Table 2: Complete background variables list

Filename	Variable	Variable label	Timepoint	Questionnaire

	code			
	cidB2894	Unique pregnancy identifier	N/A	
KZ	kz021	Sex	Birth	Child baseline sample
KZ	kz030b	Birthweight from obst data	Birth	Child baseline sample
KZ	kz031b	Head circ from obst data	Birth	Child baseline sample
KA	ka035	Any breastfeeding	4w	My young baby boy/girl
KA	ka036	Latest report of breast feeding	4w	My young baby boy/girl
KB	kb275	Ever breast-fed	6m	My daughter/son
KB	kb276	No. times a day currently breast feeding	6m	My daughter/son
KB	kb279	Age in weeks when breast feeding stopped	6m	My daughter/son
KB	kb280	Duration of breast feeding	6m	My daughter/son
A	a525	PRES marital status	8GW	Your environment
A	a600	Mums opinion of neighbourhood	8GW	Your environment
A	a636	Neighbourhood quality index	8GW	Your environment
B	b003	NO of previous PREGS	18GW	Having a baby
B	b143	Taking folic acid during this PREG	18GW	Having a baby
B	b665	Tobacco smoked in 1ST 3MTHS of PREG	18GW	Having a baby
C	c113	Taken folic acid in last 3MTHS	32GW	Your pregnancy
C	c645a	Mums highest educational qualification	32GW	Your pregnancy
C	c755	Social Class - Maternal	32GW	Your pregnancy
C	c804	Child ethnic background	32GW	Your pregnancy
D	dw002	Pre-pregnancy weight (Kg)	12GW	About yourself
D	dw021	height (cm)	12GW	About yourself
E	e041	Had caesarean section	8w	Me and my baby
E	e695	Age of mother at birth	8w	Me and my baby
F	f304	Home ownership status	8m	Looking after the baby
F	f805	Financial difficulties score	8m	Looking after the baby
G	g502	No. of children in household now	21m	Caring for a toddler

Appendix 3.2

Antenatal Questionnaire Administration

Table 1: 'Your Environment' (A) questionnaire completed by mothers who enrolled before 14GW

Questionnaire	Section	Contents
Your Environment	A	Your home environment
A	B	Chemicals and Medicines in your environment
A	C	Electrical equipment
A	D	Things you do
A	E	Your household
A	F	Your social environment

Table 2:'Having a baby' (B) questionnaire completed by mothers at 18GW

Questionnaire	Section	Contents
Having a Baby	A	Your previous pregnancies
B	B	Your health
B	C	Your reactions to becoming a parent
B	D	Your feelings
B	E	Occupation
B	F	Recent events
B	G	Activities and lifestyle
B	H	About yourself

Table 3: 'Your Pregnancy' (C) questionnaire completed by mothers at 32GW

Questionnaire	Section	Contents
Your Pregnancy	A	Plans and expectations
C	B	Your present health
C	C	Your diet
C	D	Your own childhood
C	E	Your environment and lifestyle
C	F	Your feelings
C	G	Infant feeding
C	H	Education and occupation
C	I	Being a parent
C	K	Your early sexual experiences

Table 4: 'About Yourself' (D) questionnaire documented details on the mother's history.

Questionnaire	Section	Contents
About Yourself	A	Your medical history
D	B	Your partner
D	C	You and your parents
D	D	Your family and friends
D	E	Your outlook on life

Table 5: 'Your Home and Lifestyle' questionnaire was completed by mothers who enrolled after 18GW and combined 'Your Environment' and 'Having a Baby' questionnaires.

Questionnaire	Section	Contents
Your Home and Lifestyle	A	Your home environment
	B	Chemicals and medicines
	C	Things you do
	D	Your household
	E	Your lifestyle
	F	Your social environment

Postnatal Questionnaire Administration

Table 6: 'Me and My Baby' (E) questionnaire completed 8 weeks after delivery

Questionnaire	Section	Contents
Me and My Baby	A	Labour and delivery
E	B	Your health and lifestyle in pregnancy
E	C	Your health now
E	D	Your feelings
E	E	Life events
E	F	Looking after your baby
E	G	Support and help

Table 7: 'Looking after the Baby' (F) questionnaire was completed by mothers approximately 8 months after birth

Questionnaire	Section	Contents	
Looking After the Baby	A	Your Health	
F	B	Looking after a baby	
F	C	Your feelings	CCEI and EPDS questions
F	D	Recent events	
F	E	Your home	
F	F	Your household	
F	G	Your partner	
F	H	Your occupation and lifestyle	
F	I	Being a parent	
F	J	Your social environment	
F	K	Chemicals in your environment	

Table 8: 'Caring for your Toddler' (G) questionnaire was completed by mothers approximately 21months after birth

Questionnaire	Section	Contents
Caring for a	A	Your health

Toddler			
G	B	Being a parent	
G	C	Your family and friends	
G	D	Your feelings	Crown-Crisp Experiential Index and Edinburgh Postnatal Depression Scale
G	E	Recent events	
G	F	Your home	
G	G	Your household	
G	H	Your partner	
G	I	Your occupation and lifestyle	
G	J	Your neighbourhood	
G	K	Equipment for babies and toddlers	
G	L	Chemicals in the environment	
G	M	Health services	

Child Specific Questionnaire Administration

Table 9: 'My Young Baby Boy/ Girl' (KA) questionnaire completed by mother approximately 4 weeks after delivery

Questionnaire	Section	Contents	Questionnaire battery if applicable
'My Young Baby Boy/ My Young Baby Girl'	A	You and your baby	
KA	B	Feeding	
KA	C	Sleeping	
KA	D	Crying	
KA	E	Vomiting and stools	
KA	F	Your baby's health	
KA	G	Looking after your baby	
KA	H	About your baby	Denver Developmental Screening Test

Table 10: 'My Son/ My Daughter' (KB) questionnaire was completed by mothers approximately 6 months after birth

Questionnaire	Section	Contents	Questionnaire battery if applicable
My Son/ My Daughter	A	Your baby	
KB	B	Accidents and injuries	

KB	C	Feeding	
KB	D	Sleeping and crying	
KB	E	You and your baby	HOME inventory
KB	F	Brothers and sisters	
KB	G	Problems and treatment	
KB	H	Temperament	Carey Infant Temperament Scale
KB	I	Milestones	Denver Developmental Screening Test
KB	J	Growth	

Table 11: 'My Study Son/ Daughter' (KF) questionnaire was completed by mothers approximately 30 months (2.5y) after birth

Questionnaire	Section	Contents	Questionnaire battery if applicable
My Study Son/ Daughter	A	Your child's health	
KF	B	Sleeping and crying	
KF	C	You and your child	
KF	D	Upsetting events	
KF	E	Milestones	

Table 12: 'My Three Year Old Girl/ Boy (KG) questionnaire was completed by mothers approximately 38months (3y) after birth

Questionnaire	Section	Contents	Questionnaire battery if applicable
My Three Year Old Girl/ Boy	A	Problems and treatment	
KG	B	Accidents and injuries	
KG	C	Your child's environment	
KG	D	Child care	
KG	E	Feeding	
KG	F	Temperament	EAS Temperament Scale
KG	G	Growth	
KG	H	Understanding and talking	MacArthur Toddler Communication Questionnaire
KG	I	More about talking and listening	

Table 13: 'My Son/ Daughter's Health and Behaviour' (KJ) questionnaire was completed by mothers approximately 42 months (3.5y) after birth.

Questionnaire	Section	Contents	Questionnaire battery if applicable
My Son/ Daughter's	A	Your Child's	

Health and Behaviour		Health	
KJ	B	Sleeping and crying	
KJ	C	You and Your child	
KJ	D	Upsetting events	
KJ	E	Milestones	
KJ	F	Strengths and difficulties	Revised Rutter Parent Scales for Preschool Children
KJ	G	Handedness	

DDVT

Milestones	**Item included in questionnaire**	
Items relating to fine motor development	2.6y	3.6y
Can bang together two similar objects that he is holding	✓	×
Grabs objects using the whole hand	✓	✓
Can pick up a small object using forefinger and thumb only	✓	✓
Will use a pencil and scribble	✓	✓
Can build a tower putting on object on top of another	✓	✓
Can build a tower of 4 bricks	✓	✓
Can build a tower f 8 bricks	✓	✓
Can copy a vertical line with a pencil	✓	✓
Will turn the pages of a book	✓	✓
Can wiggle his/ her thumb	✓	✓
Can copy a circle and draw it more or less	✓	×
Can draw a circle	×	✓
Can build a tower of 6 bricks	✓	✓
Can fit shapes in a board	✓	✓
Can thread beads on a string	✓	✓
Can use his/ her right hand to draw	✓	✓
Can use his/ her left hand to draw	✓	✓
Can copy a plus sign and draw it more or less	×	✓
Can copy a square and draw it more or less	×	✓
Can undo big buttons	×	✓
Can fasten big buttons	×	✓

Milestones	**Item included in questionnaire**	
Items relating to gross motor development	2.6y	3.6y
From a standing position can stoop and return to standing	✓	✓
Can kick a ball	✓	✓
Can throw a ball	✓	✓
Can balance on one foot for at least 1 second	✓	×
Can balance on one foot for at least 4 seconds	×	✓
Can jump up and down	✓	✓

Can walk	✓	✓
Can walk backwards 5 steps	✓	✓
Runs	✓	✓
Can walk up steps	✓	×
Can walk up steps – one foot on each step	×	✓
Can hop	✓	×
Can hop at least twice on one foot	×	✓
Can walk on tiptoe	✓	×
Can walk on tiptoe for at least 9 feet	×	✓
Can stop from a full run within 2 steps	×	✓
Can walk down steps like a adult – one foot on each step	×	✓
Can jump over an obstacle (e.g. toys on floor)	×	✓

Milestones	**Item included in questionnaire**	
Items relating to social skills	2.6y	3.6y
Is able to drink from a cup	✓	×
Is able to drink from a cup without spilling it	×	✓
Indicates what he/ she wants without crying for it	✓	×
Asks for what he/ she wants without crying for it	×	✓
Copies me doing the housework	✓	✓
Helps in the house with simple tasks	✓	✓
Can take clothes off with help	✓	✓
Can out shoes on without fastening them	✓	✓
Can wash and dry hands	✓	✓
Eats with and spoon and/ or fork	✓	✓
Can put t-shirt on by him/ herself	✓	✓
Can get dressed without help	✓	✓
Can brush teeth with help	✓	✓
Can get dressed without help	✓	✓
Plays card games or board games	✓	✓
Prepares breakfast cereal to eat	✓	✓

Milestones	**Item included in questionnaire**	
Items relating to communication skills	2.6y	3.6y
Can bang together two similar objects that he is holding	✓	×
Grabs objects using the whole hand	✓	✓
Can pick up a small object using forefinger and thumb only	✓	✓
Will use a pencil and scribble	✓	✓
Can build a tower putting on object on top of another	✓	✓
Can build a tower of 4 bricks	✓	✓
Can build a tower f 8 bricks	✓	✓
Can copy a vertical line with a pencil	✓	✓
Will turn the pages of a book	✓	✓
Can wiggle his/ her thumb	✓	✓
Can copy a circle and draw it more or less	✓	×
Can draw a circle	×	✓

Can build a tower of 6 bricks	✓	✓
Can fit shapes in a board	✓	✓
Can thread beads on a string	✓	✓
Can use his/ her right hand to draw	✓	✓
Can use his/ her left hand to draw	✓	✓
Can copy a plus sign and draw it more or less	×	✓
Can copy a square and draw it more or less	×	✓
Can undo big buttons	×	✓
Can fasten big buttons	×	✓

Revised rutter scale

Nowadays my child	**Certainly True**	**Sometimes True**	**Not True**
Tries to be fair in games			
Is restless, runs about or jumps up and down, does not keep still			
Is considerate of other people's feelings			
Is squirmy, fidgety			
Destroys own or others belongings			
Is spontaneously affectionate to family members			
Fights with other children			
Is not much liked by other children			
Volunteers to help around the house or garden			
Us worried, worries about many things			
Tends to do things on his own, rather solitary			
Is irritable, quick to fly off the handle			
Will try to help someone who has been hurt			
Appears miserable, unhappy, tearful or distressed			
Has twitches, mannerisms or tics of the face and body			
Bites nails or fingers			
Is disobedient			
Is kind to younger children			
Has poor concentration, or short attention span,			
Tends to be afraid of new things or new situations			
Helps other children who are feeling ill			
Is fussy, or over-particular			
Tells lies			
Has wet or soiled himself in the past 12 months			
Comforts a child who is upset			
Has a stutter or a stammer			
Has other speech difficulty			
Plays imaginatively, enjoys 'pretend' games			
Bullies other children			
Is attentive			

Nowadays my child	Certainly True	Sometimes True	Not True
Gets n well with other children			
Doesn't share toys			
Cries easily			
Is a forceful, determined child			
Blames others for things			
Shares out treats with friends			
Gives up easily			
Is inconsiderate of others			
Is an independent, confident child			
Kicks, bites other children			
Is kind to animals			
Stares into space (stares blankly)			
Tries to stop quarrels or fights			

Appendix 4.1

Reflexivity

Researcher Reflection

'It is not sufficient to have an experience in order to learn. Without reflecting on this experience it may quickly be forgotten, or its learning potential lost' (Gibbs, 1988, p. 9, as cited in McCarthy, 2011).

Although uncommon in experimental studies, reflexivity could be a useful endeavour to improve transparency and potentially reduce bias by providing a context for the researchers methodological, analytical and interpretive choices. Reflexivity requires the researcher to examine their research practice and critically reflect on, and define their role, consider the complex interactions between the author, other members of the research team and participants and remain mindful of our own background and motivations for conducting the research. These factors could influence any findings or interpretations, therefore by exploring their impact on the research process. Reflection, I believe could only strengthen the credibility of experimental research. This enables researchers to share lessons learnt, changes they would make or suggestions on how to improve the research process in the future.

I began researching in this area early in my career for a number of reasons. I was a motivated researcher and enthusiastic student in the final year of my undergraduate degree. This was a project that was gathering momentum within the department following an interdisciplinary collaboration with Biomedical Sciences and when the opportunity arose, I was keen to get involved both for the research experience and

for my dissertation topic. I entered into the project with confidence and with more experience than most following a successful research placement and a number of assistant positions.

It was an area I had much personal interest in. My mother was born with NTD which meant I was considered high risk during my own pregnancy in 2006, like participants in the study I continued supplementing with folic acid throughout my pregnancy albeit at a higher dose of 500µg/d. My son was born in 2007 and therefore a similar age to those who participated in FASSTT and I felt I could relate to those who had taken part. These similarities undoubtably kept me motivated, engaged and excited about the findings, highlighting how my actions during pregnancy could give my child a boost in areas of his development that I hadn't considered.

Despite my own background and personal motivations I strived at all times to be an objective researcher and conducting the research contained in this PhD and writing the thesis resulted in a great deal of personal and professional growth and development. I always felt confident in my research abilities however each study and chapter posed its own unique difficulties which I needed to overcome and resolve in order to produce high quality research. My experience with systematic review processes was limited prior to the PhD and a methodology I found particularly challenging despite learning a new and valuable set of research skills. I completed various training opportunities to assist with this piece of research including Cochrane training and followed advice from those who were experienced in this method such as research librarians based at Ulster. Each skill was developed and consolidated

through the practical experience of conducting the review. I found the available literature was problematic due to the lack of research in the area, and as folic acid or folate generally is consumed in combination with other vitamins, usually with iron or as part of a multivitamin, the intervention of interest was already incredibly narrow. Due to the focus on nutrition and biomedical science, child developmental outcomes beyond those considered physical were also limited. Therefore, widening my search strategy to incorporate folic acid or folate and child cognitive, psychological, social, emotional, behavioural and language development resulted in an adequate number of suitable papers but contained heterogeneous samples. This determined the use of a narrative review and vetoed the possibility of a meta-analysis which was disheartening.

The lack of new research into the area between my first and second literature search was surprising. This suggests that research is on the cusp of transitioning and incorporating resource-focused models. The systematic review process has improved in recent years, there are now management tools and aids to assist the process which were not available to me at the time. These would be incredibly useful to improve the credibility of results. In retrospect using referencing software would also have helped manage each stage of the review, particularly when working independently as a team through the development of inclusion and exclusion criteria and the title, abstract and full text review stages.

The FASSTT@10y trial was the study in which I felt most comfortable, and although familiar due to my heavy involvement with the FASSTT@7y study (Henry

at al., 2018) it was still an extensive learning experience. Through this study I was able to work 'on the ground', meeting with participants to collect data, which in itself was rewarding. As I was in control of every aspect of this study, I was confident that it was rigorous and the challenges presented by FASSTT@10y were minimal in comparison to the other research pieces in the PhD. As part of this study I had the opportunity to work closely with the wider EpiFASSTT team. This gave me the opportunity to learn about folic acid from the perspective of each discipline but also share what I was finding from a psychological standpoint, enabling me to get a broader understanding of the research and incorporate this information into the thesis.

In contrast, ALSPAC presented numerous difficulties. When identifying potential datasets to use for a comparative analysis in the early stages ALSPAC appeared ideal. The volume of data initially was difficult to digest and manage however, once all relevant variables were selected and delivered it was much less daunting. The complications with the data did not become apparent until analysis was well underway with inconsistencies and discrepancies peppered throughout the results. At this stage all data from birth to 12y was included in analysis and this is when it became clear that all additional children recruited in Phases II and III had not contributed to the pregnancy questionnaires and therefore had no folic acid information. These reasons coupled with a lack of control over the dataset, I did not have the same confidence in scientific rigor which I had felt during the FASSTT@10y study. The data was difficult to decipher and following it was clear that ALSPAC, as a comparative or validation study for FASSTT@10y was not possible. Despite this, I was able to consider the FASSTT@3y findings as all

ALSPAC children up to 4 years were Phase I recruits. Although not directly comparable any significant findings would provide support for FASSTT and other evidence available in the area. Working with this dataset was arduous and an extensive learning experience however it provided unvaluable lessons on data management, handling and analysis that can only be gained through population based studies.

I also found writing the ALSPAC chapter problematic. The literature which used the data reported inconsistent methodologies, including participant numbers and recruitment details for example. This made me query if others encountered similar problems, and if so, what steps they took to overcome them. It was disappointing not being able to use the data as it was initially intended however it still provided supporting evidence to what we had learnt previously and added knowledge about the other areas of development that we had not investigated at the earlier age of 3y during FASSTT. I believe I learnt the most about research through this study. Although full of potential, I felt the data was chaotic, inconsistent and difficult to manage. Each day seemed to present a new obstacle to overcome and for these experiences I am grateful. It did however make me reflect and showed me that I can still learn something new each day should it be a skill, how I react to, think about and manage a problem but also how I would do things differently.

Looking back I should have asked ALSPAC researchers and data handlers more questions, such as asking for help navigating the catalogue of variables and assistance to narrow down my variable list at the application process. Many of the

problems I encountered only became apparent during analysis but perhaps those working with the data on a daily basis could have provided me with some insight. Overall however, I feel like I got the best from the data but perhaps raising awareness of data problems such as inconsistent inputting and labelling in the ALSPAC database could be useful for future researchers.

On a personal level, I found it difficult returning to the PhD following a period of maternity which was followed by uncertainty around COVID lockdown and restrictions and a second period of maternity. Thankfully all data had been collected prior to COVID and therefore did not have any negative impact in that respect. This lapse in time however from 2019 to 2022 brought about many changes in a university setting, not all conducive to conducting and writing research. Throughout the PhD I had underestimated the time involved for the stages involved in each individual study and this was also true with the time involved in writing. I am proud to have had the PhD experience and have gained so much knowledge in an area of great personal and public interest. But also that I have learnt so much about myself, my limitations, boundaries and flaws but also my goals, aspirations, motivation and my ability to persevere when I felt like I couldn’t.