UNIVERSITY OF SOUTHAMPTON

FACULTY OF MEDICINE, HUMAN DEVELOPMENT AND HEALTH

School of Medicine

COGNITIVE PERFORMANCE DURING CHILDHOOD AND EARLY ADOLESCENCE IN INDIA: RELATIONSHIPS TO BIRTH SIZE, MATERNAL NUTRITION DURING PREGNANCY AND POSTNATAL GROWTH

By

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ABSTRACT

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COGNITIVE PERFORMANCE DURING CHILDHOOD AND EARLY ADOLESCENCE IN INDIA: RELATIONSHIPS TO BIRTH SIZE, MATERNAL NUTRITION DURING PREGNANCY AND POSTNATAL GROWTH

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Previous research, mainly from developed populations, has shown that a variety of early life factors (prenatal and postnatal), and socio-demographic factors that influence early life growth and development, are important determinants of childhood cognitive function. The main objective of the current study was to examine maternal nutritional indices, body size at birth, postnatal growth and infant feeding practices as predictors of childhood cognitive function independently of socio-demographic factors, in an Indian population.

The Mysore Parthenon Cohort comprises 674 babies born in the Holdsworth Memorial Hospital, Mysore, India, in 1997-1998. Maternal anthropometry and serum vitamin D, vitamin B12, folate and homocysteine concentrations were measured at 30±2 weeks of gestation. Detailed newborn anthropometry was performed. Data on breast-feeding was collected during the first, second and third year follow-up visits. The children had repeat anthropometry every 6-12 months until adolescence. Their cognitive function was assessed at age 9.5 years (n=542) and 13.5 years (n=545) using three core tests from the Kaufman Assessment Battery for Children and additional tests measuring learning, long-term retrieval/storage, short-term memory, reasoning, verbal fluency, visuo-spatial ability, and attention and concentration. Data on the parents' socio-economic status and education, maternal intelligence and home environment were recorded.

For each SD increase in birthweight, length, head (HC) and mid-upper-arm (MUAC) circumferences, and sum of skinfolds (SS) at birth there was a 0.10-0.14 SD increase in cognitive scores, independent of socio-demographic confounders ($P<0.05$). The strongest associations were with learning and visuo-spatial ability. Effects of similar size were found for gain in length, HC and MUAC during infancy and early childhood, and gain in SS during late childhood ($p<0.05$). HC at birth and head growth during early childhood were the strongest predictors, independent of other body measurements. Offspring cognitive scores increased by 0.12-0.16 SD per each SD increase in maternal serum folate concentrations during pregnancy ($p<0.05$). Maternal vitamin B12 concentrations were inversely related to some cognitive domains in the child ($p<0.05$), apparent mainly in Hindus. Breast-feeding duration was unrelated to children's cognitive function. There were positive and independent associations of maternal education and intelligence with children's cognitive function ($p<0.001$). The variance in cognitive function explained by maternal folate status (1-3%), newborn HC and childhood head growth (4-9%) and current socio-demographic factors (6-17%) appeared to be additive (total variance explained 16-25%).

In conclusion, maternal folate status in pregnancy, HC at birth, head growth during infancy and early childhood, and current socio-demographic factors independently predict childhood cognitive function in this cohort. The findings suggest a need for investment in integrated interventions, targeting both the mother and the child, to improve prenatal and postnatal nutrition and maternal education, in order to promote optimal cognitive development among Indian children.

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Declaration of Authorship

I, **SARGOOR RAJAGOPALAN VEENA** declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

COGNITIVE PERFORMANCE DURING CHILDHOOD AND EARLY ADOLESCENCE IN INDIA: RELATIONSHIPS TO BIRTH SIZE, MATERNAL NUTRITION DURING PREGNANCY AND POSTNATAL GROWTH

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
3. Where I have consulted the published work of others, this is always clearly attributed;
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
5. I have acknowledged all main sources of help;
6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
7. Either none of this work has been published before submission, or parts of this work have been published as: [please list references below]:

Signed: ……

Date: …….

Publications are listed overleaf

Publications

1. Veena SR, Krishnaveni GV, Wills AK, Kurpad AV, Muthayya S, Hill JC, Karat SC, Kiran KN, Fall CHD, Srinivasan K. Association of birthweight and head circumference at birth to cognitive performance in 9- to 10-year-old children in South India: prospective birth cohort study. *Pediatr Res* 2010; 67: 424-429
2. Veena SR, Krishnaveni GV, Srinivasan K, Wills AK, Hill JC, Kurpad AV, Muthayya S, Karat SC, Nalinakshi M, Fall CHD. Infant feeding practice and childhood cognitive performance in South India. *Arch Dis Child* 2010; 95: 347-354
3. Veena SR, Krishnaveni GV, Srinivasan K, Wills AK, Muthayya S, Hill JC, Kurpad AV, Yajnik CS, Fall CHD. Higher maternal plasma folate but not vitamin-B12 concentrations during pregnancy are associated with better cognitive function scores in 9-10 year old children in South-India. *J Nutr* 2010; 140: 1014-1022
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5. Veena SR, Hegde BG, Ramachandraiah S, Krishnaveni GV, Fall CH, Srinivasan K. Relationship between adiposity and cognitive performance in 9-10 year old children in south India. *Arch Dis Child* 2014; 99: 126-134

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Author's Contribution

The Mysore Parthenon Cohort was set up by Caroline Fall, Jacqueline Hill and GV Krishanveni in 1997-1998. I have played a major role in following-up the cohort in Mysore since 1999, working along with Dr. Krishnaveni. Together we headed a research team and managed overall research activities, recruited research assistants, psychologists and computing staff. We were trained in conducting the stress test, under the guidance of Professor Dirk Hellhammer, University of Trier, Germany. I underwent training in cognitive function assessment, along with three psychologists, under the guidance of Professor Krishnamachari Srinivasan, St. John's National Academy of Health Sciences, who is my supervisor in India. I trained the research assistants in anthropometry, blood pressure and bioimpedance measurements and conducted inter- and intra-observer variation studies. I was involved in blood collection, and supervised the cognitive function assessment, which was administered by the psychologists. I and Dr Krishnaveni conducted the stress tests, including salivary sample collection and its processing.

I underwent training in conducting a Systematic Review, with guidance from Hayley Denison. I carried out the database search for a Systematic Review, retrieved relevant articles and extracted the data. I, along with Sarah Kehoe carried out the quality assessment.

I prepared the data analysis file with the help of Vanessa Cox and Patsy Coakley. I analysed the data myself with guidance from Professor Clive Osmond, Ella Marley-Zagar and Dr. Sarah Crozier. I interpreted the results with guidance from my supervisor Professor Caroline Fall and co-supervisors Dr. Catharine Gale and Professor Krishnamachari Srinivasan. I have typed this thesis myself. The 9.5 year cognitive function data were collected before I registered for this PhD and some results have already been published (page xv). The 13.5 year data were collected during my PhD registration period.

List of Abbreviations

CNS	Central nervous system
SES	Socio-economic status
LBW	Low birthweight
IUGR	Intrauterine growth restriction
HC	Head circumference
HPA	Hypothalamic-pituitary-adrenal axis
CRH	Corticotrophin releasing hormone
GDM	Gestational diabetes mellitus
IQ	Intelligence quotient
BMI	Body mass index
DHA	Docosahexaenoic acid
IGF	Insulin-like growth factor
BDNF	Brain-derived neurotrophic factor
SGA	Small for gestational age
Hb	Haemoglobin
PUFA	Polyunsaturated fatty acid
SD	Standard deviation
BSID	Bayley Scale of Infant Development
KABC	Kaufman Assessment Battery for Children
WISC	Wechsler Intelligence Scales for Children
WHO	World Health Organization
HMH	Holdsworth Memorial Hospital
MRCLEU	Medical Research Council Lifecourse Epidemiology Unit
OGTT	Oral glucose tolerance test
MUAC	Mid-upper-arm-circumference
MTHFR	Methylenetetrahydrofolate reductase
IOV	Inter observer variation
SLI	Standard of living index
MIQ	Maternal intelligence
IQR	Inter quartile range
SS	Sum of skinfolds
CRD	Centre for Reviews and Dissemination
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
MeSH	Medical subject headings
FFQ	Food frequency questionnaire
MDI	Mental development index
PDI	Psychomotor development index
ICDS	Integrated child development service
UNICEF	United Nations International Children's Emergency Fund

CHAPTER 1 INTRODUCTION

Good cognitive development and function is important for achieving optimal social and health outcomes throughout life. Recent evidence links poor fetal growth to lower 'human capital' outcomes such as educational attainment, adult income or assets[1]. A variety of inter-linked factors influence prenatal and postnatal growth and development and play an important role in determining cognitive performance, independently or mediated partly/completely by one another. This thesis describes a study carried out as part of a programme of research into the developmental origins of health and disease in India. The work presented here has been conducted on ~600 healthy children from a birth cohort for whom information has been collected at various time points starting from the prenatal period to early adolescence. The main objective of the study was to explore possible pathways linking early life growth and development and its related factors to cognitive ability during childhood and early adolescence.

1.1 Background

1.1.1 Definition of cognitive function: The terms cognitive function, cognitive ability, cognitive performance are used throughout this thesis. In brief, all these terms refer to general mental ability/intelligence. Cognitive function is complex, includes multiple mental processes such as memory, attention, perception, action and problem solving. These processes correlate well within each other but vary between individuals[2,3] and are capable of undergoing adaptation to environmental challenges.

1.1.2 Implications of cognitive skills: Both in developed as well as developing countries early life cognitive skills make a significant contribution to academic achievement (qualitative as well as quantitative), and thus to better employment opportunities, income and spending choices and assets[4-6]. These skills are also associated with health and quality of life as age advances. Evidence from many studies indicates that better cognitive skills in early life predict reduced morbidity (coronary heart disease, hypertension, stroke, lung cancer and dementia) and all cause mortality in later life[7,8]. Although higher intelligence is linked with reduced risk of a range of adverse health outcomes, evidence suggests it is not associated with risk of most types of cancer[9]. Reduced cognitive function is likely to be associated with poorer lifestyle (smoking, alcohol consumption, physical activity, dietary intake), and in turn contributes to increased morbidity and mortality in later life[10-12]. There

are reports linking higher childhood cognitive ability with consumption of a healthier diet (more fruits, vegetables, fish, whole meal bread and less consumption of chips, biscuits etc), increased likelihood of being vegetarian, increased physical activity, reduced prevalence of overweight/obesity, hypertension, reduction or cessation of smoking and higher alcohol intake in adulthood[13-17]. Thus better cognitive skills contribute to 'health and wealth' not only at an individual level but also the society at large.

In developing countries the rising burden of communicable and non-communicable diseases is an important public health concern. Consequently increasing financial investment towards treatment, loss of working hours, premature retirement and reduced life expectancy can affect economic conditions at an individual and national level. In developing countries many people cannot afford even basic treatment for many diseases. Additionally widespread poverty, malnutrition and illiteracy have led to general lack of awareness about the factors influencing health and disease outcomes. Better cognitive ability during early life (childhood, adolescence and early adulthood) not only contributes to academic growth but also helps in understanding the role of various modifiable factors (diet, nutrition, physical fitness, smoking, etc) related to health and disease in late adulthood and old age. This helps in increased awareness, promoting healthier lifestyle and thus a better quality of life as age advances. Therefore identifying factors influencing cognitive skills during childhood and adolescence and appropriate initiatives to improve these factors are important to promote health, economic growth and human capital outcomes.

1.2 Cognitive development

Cognitive development refers to changes observed in mental ability over a period of time. It depends on neurodevelopment and physical development. Neurodevelopment and physical development originate in early gestation and continue throughout infancy, childhood, adolescence and adulthood before levelling off, followed by decline in old age[18-20].

The process of human central nervous system (CNS) development starts prenatally from the day of conception and continues throughout the nine months of gestation. During this period the CNS develops as a thin layer of unspecified tissue (neural tube) and differentiates into the brain and spinal cord. During this stage brain development includes

the processes of neurogenesis, proliferation, migration, axonal outgrowth, synaptogenesis, differentiation, and apoptosis that results in a system that can process sensory input and organize actions. Further, CNS development continues in the postnatal period, mainly by reorganization of the brain structure. Brain development takes place rapidly during the last trimester of pregnancy and the first two years of life and continues throughout childhood, adolescence and adulthood. This period includes the processes of axonal and dendritic development, synaptogenesis, neuronal and synaptic pruning, and myelination, with increasing brain maturity and changes in neurotransmitter sensitivity. Brain volume increases fourfold from birth to 10 years, and head circumference, which correlates with brain volume, rises from birth and peaks at 5 to 10 years of age[18-20]. The rate of development and maturity of different areas of the brain varies (Figure 1.1)[21]. In the first two years of life areas (the pre- and post-central gyrus, temporal lobe, and occipital lobe) related to the most basic functions i.e. sensory (taste, smell, touch, hearing, vision) and motor skills (walking, moving) develop rapidly. In early childhood, development of the areas related to language development (left hemisphere) and spatial orientation (right hemisphere) gain prominence. In late childhood and adolescence the areas (frontal lobes including hippocampus and cingulated gyri) which are involved mainly in the execution of more complex functions (like planning, organization, problem solving, and memory) attain their maturity[20,21]. The mental abilities developed during early life remain stable as age advances. There are studies reporting considerable stability in mental abilities over time[22]. It has also been reported that low linguistic complexity in early adulthood is associated with lower mental ability score and higher incidence of dementia in old age[23].

Physical development, which reflects nutrition, health, motor function, exploratory behaviour and positive reinforcement from caregivers, also contributes to cognitive development possibly through the factors that influence both physical and CNS development. Physical development, similar to neurodevelopment, starts prenatally from the day of conception and continues postnatally through the second decade of life and possibly early adulthood[9]. Physical growth in general is a regular process. However the velocity or rate of growth, reflecting physiological events, varies at different periods of development. It occurs rapidly during the later part (third trimester) of gestation and the first 2 years of postnatal life. The rate of growth decreases during childhood. During adolescence growth spurts dramatically until complete maturity with marked changes in body composition measures, such as bone, muscle, fat and reproductive organs[20].

Figure 1.1 Development of the human brain

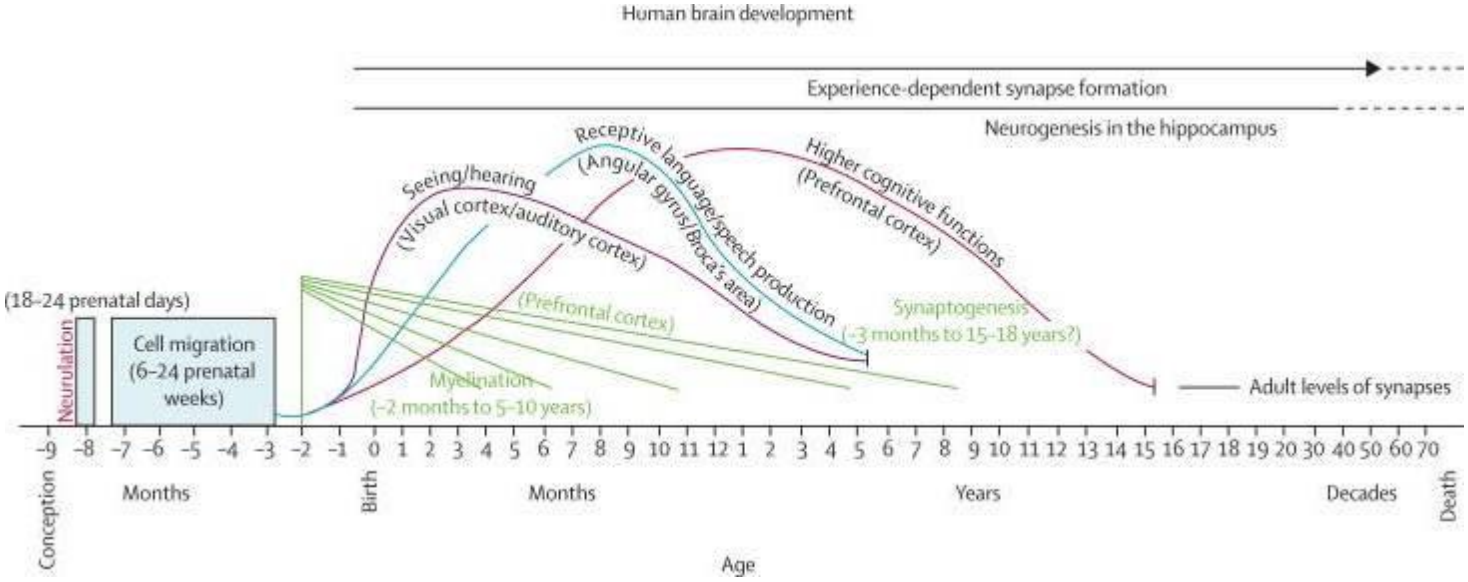


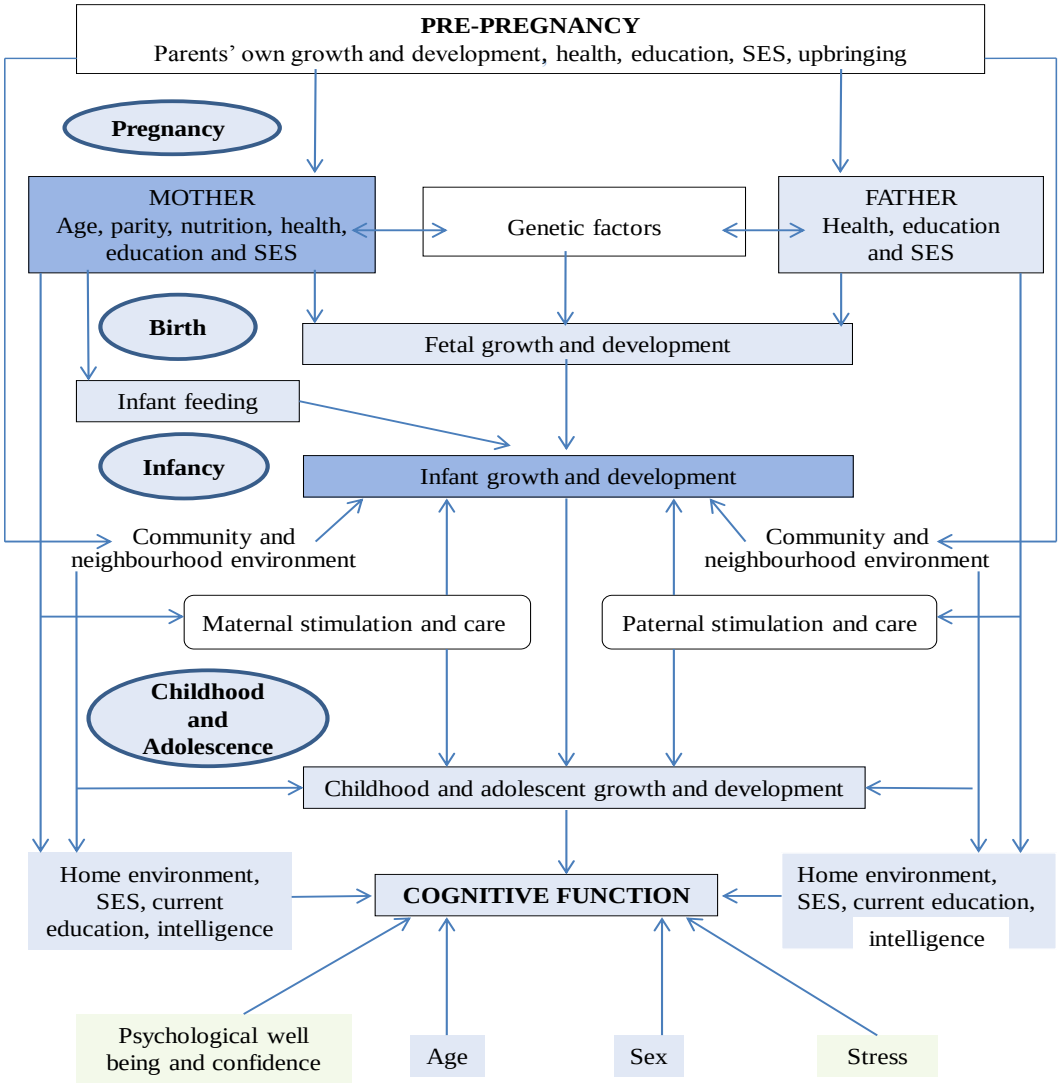
Figure reproduced with permission copyright © American Psychological Association (Thompson RA, Nelson CA. Developmental science and the media: early brain development. *Am Psychol* 2001; 56: 5-15) [21]

Mechanisms involved with human growth and development are complex. They include numerous molecular interactions and anatomical transformations which are vulnerable to a variety of factors operating at varying periods of development starting from the prenatal period to the second decade of life by which time growth (physical and neural) will be completed. Thus the occurrence of differences in tempo in growth may have profound implications in determining cognitive function and behavioural development during early life and in turn their relationship to health and human capital outcomes as age advances. Therefore it is important to examine cognitive ability during childhood and adolescence, and its determinants to explore the possible pathways linking them before recommending necessary interventions where appropriate.

1.3 Factors influencing cognitive function development

Optimal cognitive development depends on a variety of factors influencing intrauterine life, postnatal life and childhood/adolescence. These factors can be i) genetic factors ii) prenatal factors (pre-pregnancy and pregnancy), for instance maternal nutrition, parity, parents' health, education and socio-economic status (SES) and iii) postnatal factors such as infant feeding, parental stimulation and care, SES, education and intelligence, home, neighbourhood and community environment and current nutrition. In the context of existing literature, the links between these factors and the likely pathways through which they might determine cognitive function are illustrated in Figure 1.2. Maternal and paternal factors such as maternal nutrition and parity, education and SES, together with genes influence fetal growth and development including nutrient stores, musculoskeletal and neurodevelopment. Maternal education and SES also influence infant feeding practice. Similarly, parental health, education and SES influence parenting practice (stimulation and care) and home environment. These factors, which are linked to each other, together with birth size, and the neighbourhood and community environment, contribute to nutrition, and physical and neurodevelopment during infancy, childhood and adolescence. All these factors together with the child's sex, age, psychological wellbeing and confidence are likely to influence cognitive performance, either directly or indirectly.

Figure 1.2 Flow diagram illustrating links between possible factors and likely pathways in predicting cognitive function



SES Socio-economic status

Blue shade indicates availability of data in my study

No shade indicates non-availability of data in my study

Green shade indicates availability of data in my study but plays no part in this thesis

1.3.1 Genetic factors and cognitive ability: Cognitive function is multidimensional. It has been proposed that 'general cognitive ability' (general intelligence), often termed the '*g*' factor accounts for individual differences in cognitive performance[24,25]. The '*g*' factor is a variable reflecting ability across various tests of cognitive function such as reasoning, spatial, verbal, perceptual organization and memory abilities. Since its proposal, several studies (twins, families and adoption) have widely acknowledged that '*g*' accounts for 50% of variance in individual differences in cognitive function. General cognitive ability is highly heritable. Several studies including genome wide linkage and association scans,

have identified many candidate genes and polymorphisms associated with '*g*' and other specific tests[26-29]. However their individual effects are small and are not well replicated. Further, genetic influence on memory tends to be smaller, although other dimensions such as verbal and perceptual organization abilities show similar amounts of genetic influence.

1.3.2 Environmental factors and cognitive ability: Although general cognitive ability has been attributed partially (~50%) to genetic factors, a variety of environmental factors predict cognitive performance. There is evidence from a large body of research linking cognitive function during childhood and adolescence with the family's SES[5,30-33]. The family's SES has an important influence on nutrition, parenting practices, and accessibility to learning environments and materials. Parental education and intelligence also influences offspring cognitive performance[34-37]. Cognitive ability is also determined by the home and neighbourhood environment (exposure to violence, toxins, smoking and alcohol consumption, parental care and access to a cognitively stimulating environment)[5,30,38-41].

These factors which are linked to one another can also influence fetal growth and postnatal nutrition, growth and development[42-47] and are therefore possible potential confounders of any association between prenatal and postnatal factors with cognitive function. The socio-demographic factors may confound any association found between, for example, prenatal and postnatal growth and offspring cognitive function, but some effects may also be mediated by acting through fetal development or through postnatal nutrition, parental care, stimulation, growth and development.

1.3.3 Prenatal factors and cognitive function

1.3.3.1 Intrauterine and developmental origins of cognitive performance: In the last couple of decades a large body of research linking poor fetal growth with health and disease in later life has shed light in understanding the factors influencing health outcomes in later life. Inadequate nutrient supply to the growing fetus in utero during critical periods of organ development and resulting in low birthweight (LBW) can lead to adverse health outcomes (cardiovascular disease, insulin resistance, type 2 diabetes and hypertension) in later life by altering (fetal programming) the structure and function of developing organs[48].

The concept of 'fetal programming' represents the prenatal factors influencing the conditions in postnatal life and is based on the concept of fetal adaptation and later non-

adaptation. During intrauterine life there is sequential growth and development of the organs occurring mainly during the period of rapid cell division. This 'critical period' of organ growth and development is vulnerable to nutritional insults. The growing fetus adapts to inadequate nutrient supply by prioritizing the growth of vital organs like the brain by redistribution of blood flow and compromising abdominal visceral and musculo-skeletal growth[48]. Thus programming prepares the growing fetus to withstand the 'new environment' and survive in the new environment with the available nutrition. Although the brain is spared, the effect of programming could interfere with optimal neurodevelopment and function. A variety of insults (maternal nutrition, smoking and alcohol, psychosocial stress, blood pressure) during the critical period of development can alter the utero-placental transmission of nutrients, oxygen supply, growth factors etc. Consequently the fetus is exposed to macronutrients and micronutrients deficiencies, imbalance in growth factors and hypoxia. This in turn leads to alterations in overall fetal growth including neurotransmitter as well as neuroendocrine systems and structural brain development[49,50]. It is also possible that these prenatal factors can influence the maternal brain and behaviour which in turn influence the postnatal neurodevelopment and function[51,52]. It has been reported that the changes in maternal brain and behaviour could possibly interfere with child care leading to impairment in the postnatal neurodevelopment and function[53]. These pathways are not mutually exclusive and play an important role in determining cognitive function and behaviour in the offspring later in life.

1.3.3.2 Birth size and cognitive function: Size at birth is a product of a fetus' trajectory of growth. It is used as a marker of the fetal intrauterine environment to study the consequences of reduced fetal growth with later health outcomes. In both developed and developing countries there is evidence from many studies, including a recent systematic review[54], demonstrating associations between low birthweight or smaller head circumference at birth and poorer cognitive function in later life (childhood and adulthood)[41,55-69]. The evidence has been reported from 'high-risk' individuals (born LBW, premature, or with intrauterine growth restriction (IUGR))[55,56,67-69], as well as from those who were born at full-term, and across the normal range of birthweight and/or head circumference (HC)[41,57-66,]. Some studies have reported linear associations between birth length and childhood cognitive ability[41,64,66]. A summary of the findings from the above studies linking birth size and cognitive performance in children is presented in Table 1.1.

Table 1.1 Summary of the findings from studies linking birth size with childhood cognitive function

Author, Year of publication, Sample size, Age, Country, Term or pre-term births	Birth measurements	Results (after adjustment for potential confounders)	Test instrument or tests used for cognitive function assessment
[41]Malacova et al; 2009; N=55,533 and N=55,240 Age 8.2 Y Australia Term born	Birthweight Birth length Head circumference	Higher percentage of optimal birthweight, length and head circumference was positively related to reading and writing scores Birth weight-Reading : 0.24 score; writing : 0.24 score Birth length-Reading : 0.35 score; writing : 0.51 score Birth head-Reading : 0.46 score; writing : 0.37 score Effect of birthweight was modified by educational status of the residing area	The reading test - multiple choice, short- and open-response questions The writing test - writing a short story, fable or an anecdote.
[57]Brennan et al; 1985 N=14,541 vs N=282 Age 8 months, 4 Y and 7 Y USA Term born	Appropriate head circumference vs small head circumference	No significant differences in the scores for mental and motor development scales and intelligence quotient (IQ) scores between children having small head circumference at birth compared to those with appropriate head circumference No information about confounders	At 8 months- Bayley Mental and Motor Development Scales At 4 years-Stanford-Binet scale IQ 7 years-Wechsler Intelligence Scale IQ
[58]Richards et al; 2001 N=3,900 Age 8, 11 and 15 Y UK	Birthweight	A positive linear association existed between birthweight category and cognitive score at age 8 years, with an estimated standard deviation score of 0.44 between the lowest and highest birthweight groups. The findings were similar at age 11 and 15 years No gestational age data	At age 8 reading comprehension, word pronunciation, vocabulary, and non-verbal reasoning At age 11 verbal and non-verbal intelligence, arithmetic, word pronunciation, and vocabulary At age 15 verbal and non-verbal intelligence, reading comprehension, and mathematics
[59]Gale et al; 2006 N=633 Age 4 and 8 Y UK; Term born	Head circumference	Head circumference was positively associated with full scale, verbal and performance IQ at 4 years but not at 8 years. Full scale, verbal and performance IQ increased by 2.41, 1.65, 2.52 points for 1SD increase in head circumference at birth	Wechsler Preschool and Primary Scale of Intelligence at 4 years Wechsler Intelligence Scale for Children at 8 years
[60]Heinonen et al; 2008 N=1,056 Age 4 ½ Y Finland Term born	Birthweight; Body mass index (BMI), Length; Head circumference	Lower Birthweight and BMI were associated with lower scores of general reasoning and/visual-motor integration; general reasoning and/or visual-motor integration decreased by >1.20 points per1 SD decrease in weight or BMI at birth. For 1 SD decrease in length or head circumference at birth, abilities across all of the cognitive domains decreased by >1.31 points	Columbia Mental Maturity Scale Beery scale Kiese and Kozielski Verbal Competence Test Wettstein Language Comprehension Test

[61]Silva et al; 2006 N=11,244 Age 10 y UK Preterm and term born	Birthweight	Birthweight was positively associated with verbal and non-verbal tests, and reading, language and maths tests Cognitive function increased by 0.046 SD units for 1 SD unit increase in birthweight The effect of SES was greater than the effect of birthweight	The British Ability Scale The Shortened Edinburgh Reading Test The Friendly Maths Test The Pictorial Language Comprehension Test
[62]Tong et al; 2006 N=723 Age 2, 4, 7 and 11-13 Y Australia Preterm and term born	Birthweight Birth length Head circumference	Lower Birthweight, shorter length and smaller head was associated lower mental development index at 2 years, general cognitive index at 4 years and full scale IQ at 7 and 11-13 years. At age 2 years, cognitive performance decreased by 0.97 points per 100 g decrease in birthweight. But at age 7 and at 11-13 years the association attenuated with increasing age and not significant. The association of birthweight was greater in boys than girls at age 2 and 4 years. Effect of length and head after adjustment not reported	Bayley Scales of Infant Development at age 2 years McCarthy Scales of Children's Abilities at age 4 years The Wechsler Intelligence Scale for Children (revised) at ages 7 and 11–13 years.
[63]Broekman et al; 2009 N=1,645 Age 8-12 Y Singapore Preterm and term born	Birthweight Birth length Head circumference	Positive association of birthweight, length and head circumference with non verbal reasoning ability. For every 1 kg increment in birthweight, 1cm in birth length, and 1 cm in head circumference, there was a corresponding increase in IQ of 2.19, 0.49, and 0 .62 points respectively The association remained similar even after excluding preterm and those with extreme birth measurements	Raven's Standard Progressive Matrices test
[64]Pongcharoen et al; 2012 N=560 Age 9 Y Thailand Preterm or term not known	Birthweight Birth length	Positive association of birthweight with performance IQ, 0.09 SD increase in performance IQ/SD increase in birthweight Positive association of birth length with full scale (0.10 SD) and performance IQ (0.14 SD)/SD increase in birth length	Wechsler Intelligence Scale for Children–III edition Raven's Colored Progressive Matrices (non verbal)
[65]Rahu et al; 2010 N=1,822 Age 7-13 Y Estonia; Term born	Birthweight	Positive association of birthweight with IQ score IQ score increased by 0.7 point per 500 g increase in birthweight	Raven's Standard Progressive Matrices test
[66]Yang et al; 2011 N=11,899 Age 6.5 Y Belarus Term born	Birthweight Birth length	Positive association of birthweight and birth length with full scale, verbal and performance IQ full scale, verbal and performance IQ increased by 0.82, 0.81, 0.65 points/1SD increase birthweight respectively full scale, verbal and performance IQ increased by 0.62, 0.53, 0.59 points/1SD increase birth length respectively	Wechsler Abbreviated Scales of Intelligence

[67]Mcgregor et al; 1998 N=131 + N=131 Age 6 and 12 months Country-Brazil Term born	Appropriate birthweight vs low birthweight-term	Low birthweight-term infants had poorer motor (4.2 points at age 6 months and 7 points at age 12 months) and psychomotor development (7.3 points at 6 months and 9.9 points at age 12 months) than appropriate birthweight infants. The difference increased between 6 and 12 months. Low birthweight infants were affected by home environment and maternal illiteracy	6 and 12 months -Bayley Scale for Mental and Psychomotor development Index
[68]Villar et al; 1984 N=205 Age 2 years and 3 years Guatemala Term born	Normal birthweight IUGR low ponderal index (LPI) IUGR-adequate ponderal index(API)	IUGR-API at 2 years scored lowest in overall cognitive composite score (mean score 38) compared to IUGR-LPI (mean score 48) and normal birthweight (mean score 83)	At 2 years Composite Infant Scale At 3 years Tests for reasoning, verbal processes, memory, learning, and perceptual analytical scales
[69]Chaudhari et al; 2004 N=180 (low birthweight) and N=90 (controls) Age 12 Y India Preterm small for gestation and very low birthweight (birthweight <2kg)	Birthweight Low vs normal	Intelligence and academic performance of low birthweight children were lower than that of controls. Low birthweight children also had poor visuo-motor perception, motor incompetence, reading and mathematics learning disability IQ score was poorer in low birthweight children (mean IQ score 89.5) compared to controls (97.2). Pre term small for gestational age children had the lowest mean (85.4) IQ score No information about confounders	Wechsler Intelligence Scale, Bender Gestalt test for visuo-motor perception, Wide Range Achievement Test for specific learning disability, Draw-a-person screening test for emotional problems and Movement Assessment Battery for motor competence

1.3.3.3 Maternal factors during pregnancy and cognitive function

1.3.3.3.1 Maternal smoking and cognition: Maternal smoking during pregnancy (nicotine toxicity) interferes with placental transfer, uterine blood flow and nutrient supply. Thereby the fetus is exposed to reduced nutrient, blood and oxygen supply resulting in fetal growth restriction. Nicotine also acts as a neuroteratogen interfering with the structure and neurotransmitter activity of the developing brain[70,71]. Evidence linking maternal smoking and cognitive function is not consistent. Some studies have reported reduced intelligence and poor academic achievement during childhood and adolescence in relation to maternal smoking[70,72-74]. However, recent studies reported that such effects are confounded by maternal factors such as maternal intelligence and education[75,76]. While a study reported a dose response effect of smoking on cognitive ability[73] another did not find any association[77].

1.3.3.3.2 Maternal alcohol consumption and cognition: Evidence from neuroimaging studies in humans suggest that prenatal alcohol exposure leads to reduced fetal brain volume and may permanently alter cognitive function[70]. Recent meta-analyses reported poor mental development index during infancy[70,78] and deficits in learning, intelligence and memory during childhood in relation to maternal alcohol drinking during pregnancy[70,79,80].

1.3.3.3.3 Maternal psychosocial stress and cognition: The activity of two important stress responsive systems, the hypothalamic-pituitary-adrenal (HPA) axis and the placenta, possibly accounts for an association between prenatal stress and poor cognitive abilities in later life[49,50,81]. During pregnancy corticotrophin releasing hormone (CRH) is released by the hypothalamus and placenta. Release of hypothalamic CRH induces adrenocorticotrophin production which in turn stimulates the synthesis and release of glucocorticoids (cortisol) into systemic circulation. At the same time elevated maternal glucocorticoids regulate the HPA axis activity via negative feedback by interacting with glucocorticoid receptors which are present in the CNS. In contrast to the role of cortisol in negative feedback regulation of the HPA axis, cortisol increases placental CRH production resulting in a positive feedback allowing simultaneous increase in CRH, adrenocorticotrophin and cortisol in maternal and fetal compartments. Fetal exposure to excessive cortisol is regulated by a placental enzyme (11 β -HSD2) required to convert cortisol into inactive form. The level of this enzyme increases as pregnancy advances protecting the fetus during critical period of development and drops towards the end of

pregnancy promoting transplacental transfer of cortisol in sufficient quantity required for organ maturation including CNS in the fetus. Maternal stress during pregnancy can affect the activity of the HPA axis (reduced negative feedback) and the placenta (reduced activity of placental enzyme) resulting in excessive materno-fetal transfer of cortisol. Exposure of the growing fetus to abnormal levels of cortisol can interfere in fetal maturation, growth and differentiation of CNS during early life and in turn interfere with cognitive performance and behaviour later in life[49,50,81,82]. There is growing evidence linking prenatal stress with poor mental development index and language impairment during infancy[83-85], poor working memory, inhibitory control, intelligence, attention and academic achievement during childhood[86-89] and lower intelligence score during adolescence[90].

1.3.3.3.4 Maternal gestational hypertension and cognitive function: The term 'gestational hypertension' refers to the development of high blood pressure (systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg) in a previously normotensive pregnant woman after 20 weeks of gestation. It includes mutually exclusive categories: a) gestational hypertension (new-onset hypertension without proteinuria) and b) pre-eclampsia (new-onset hypertension with proteinuria). Gestational hypertension can result in IUGR and interfere in neurodevelopment possibly by reducing blood flow and oxygen supply. As a result offspring cognitive function may be impaired in later life. Studies examining the link between gestational hypertensive disorders and offspring cognitive ability have reported inconsistent findings. While two studies reported no association between maternal gestational hypertension and cognitive function during early childhood and adolescnce[91,92], other studies reported lower verbal ability and lower intelligence score in children[93] and adult men[94] and poor pro-social behaviour during childhood and adolescence[95] born to mothers with gestational hypertensive disorders. Another study in children found better cognitive ability among those who were born to mothers with preeclampsia compared to those born to mothers with hypertension alone[96]. An inflammatory state with accompanying oxidative stress interfering in fetal growth, including neurodevelopment, could be a possible pathway linking gestational hypertension and cognitive function[97].

1.3.3.3.5 Maternal gestational diabetes mellitus and cognitive function: Glucose is an important nutrient necessary for fetal growth including neurodevelopment. Therefore one would expect that higher maternal glucose concentrations may enhance brain development and consequently better cognitive function later in life. However, studies examining

cognitive outcome in the offspring of mothers with gestational diabetes mellitus (GDM) are inconsistent. While a case-control study reported a higher risk of language impairment in offspring born to GDM mothers compared to controls[98], another recent review has reported no difference in cognitive ability; however, children born to mothers with GDM performed less well in tests of fine and gross motor functions[99]. Further, there are studies reporting inverse associations of offspring cognitive outcomes with the severity of GDM assessed by glycosylated haemoglobin level and ketonuria, suggesting that offspring cognitive performance could be within normal limits in well-controlled GDM[99]. A recent study, using Mendelian randomisation, demonstrated that maternal and offspring genetic variants which are associated with glucose levels were not related to intelligence quotient (IQ) in 8 year old children. However maternal, but not offspring, genetic variants related to type 2 diabetes were associated with an increase in the child's IQ[100]. The authors concluded that a causal pathway linking in utero exposure to glucose and child's IQ is unlikely.

1.3.3.4 Maternal nutrition and cognitive function

Nutritional requirements during pregnancy increase to meet the physiologic changes in gestation and fetal demands for growth and development. Maternal nutrition has a pivotal role in regulation of fetal growth, including neurodevelopment, and affects the lifelong health and productivity in the offspring. It is well known that maternal undernutrition results in IUGR with the resultant reduction of birth size in the offspring[101].

1.3.3.4.1 Maternal nutrition and offspring birth size: Maternal nutrition is the primary source of nutrition for the developing fetus. Both macronutrients and micronutrients are required for optimal fetal development. Observational studies have demonstrated that prenatal body mass index (BMI) and weight gain, markers of maternal prenatal nutritional status, predict offspring birth size[102-106]. Evidence from observational, experimental and famine studies have shown the influence of maternal protein-energy intake on offspring birthweight[107-110]. Observational and experimental studies have found that children of mothers who consumed micronutrient-rich foods more often, or received micronutrient supplementation during pregnancy had higher birthweight[111-113].

1.3.3.4.2 Maternal nutrition and offspring neurodevelopment: Maternal body composition, macronutrients (carbohydrate, protein and fat) and micronutrients such as B

vitamins (B6, B12 and folate), vitamin D, fatty acids (Docosahexaenoic acid (DHA)) and minerals (for example iron, iodine and zinc)) are likely contributors to offspring neurodevelopment[114]. While macronutrients serve as building blocks in overall CNS development, micronutrients enable neurogenesis, axonal and dendritic growth, myelination, synaptogenesis, neurotransmitter production and transmission[114]. As neurodevelopment occurs rapidly during pregnancy any impairment in the nutritional supply to the fetus may interfere with development of the brain, neurotransmitter synthesis and their transmission. Further, the degree of alteration in the structure and function of the nervous system could depend on the timing, duration, type and severity of prenatal nutritional deprivation. Thus cognitive function later in life depends on the timing, degree and extent of the alterations and the area of the brain affected[114,115]. Recently, animal studies have reported that prenatal protein malnutrition in rats resulted in alterations in hippocampus formation, an important area in the brain involved in learning and memory[115,116]. Another study in baboons reported that 30% reduction in the dietary intake during pregnancy resulted in impaired cell proliferation and maturation in the cerebral cortex. These changes were accompanied by decreased cerebral insulin like growth factor (IGF-I), brain derived neurotrophic factor (BDNF), and glial neurotrophic factor S-100β leading to altered cerebral development and metabolism[117].

1.3.3.4.3 Maternal nutrition and offspring cognitive function: As neurodevelopment depends on both prenatal macronutrients and micronutrients, one would expect maternal nutritional status during pregnancy to be a potential predictor of offspring cognitive ability in later life. In general, studies in both developed or developing countries, investigating the associations of maternal nutritional status during pregnancy (anthropometric indices, macronutrients and micronutrients) with offspring cognitive function are limited. There are some observational studies investigating the associations of maternal folate[118-123], vitamin B12[118,120,122,123] and vitamin D[124,125] status with offspring cognitive function during infancy and childhood. While some found an association, others did not. Although iron is important for neurodevelopment[114], studies reporting association between maternal iron status and offspring cognitive function are scarce. Apart from observational studies, experimental studies examining the effect of supplementation of micronutrients like folic acid, vitamins B12, vitamin D and iron, and studies examining the relationship of maternal body composition with offspring cognitive function are limited. I am carrying out a systematic review of childhood and adolescent cognitive function in relation to maternal nutritional status during pregnancy and the details are described in chapter 4.

1.3.4 Postnatal factors and cognitive function

As mentioned in section 1.2, cognitive function depends not only on prenatal neurodevelopment but also on postnatal neurodevelopment which takes place rapidly in infancy and continues into childhood and adolescence. It is therefore possible that nutrition and body composition during infancy and childhood contributes to neurodevelopment and influences later cognitive function.

1.3.4.1 Infant feeding and cognitive function: Nutrition during infancy is important for growth and neurodevelopment which take place rapidly in the first 2 years of life. Breast milk, rich in long-chain polyunsaturated fatty acids, is an important source of nutrition for early life neurodevelopment[126]. Further, breast-feeding may promote neurocognitive development by means of better mother-baby bonding and sensory stimulation[127,128]. There is evidence from observational studies, studies among high-risk individuals (born small-for gestational age (SGA) or very low birthweight)[36,127-134] and from a randomized trial[135] reporting a beneficial effect of breast-feeding on cognitive performance. Several studies, mainly from developed countries, report that children who were breast-fed rather than formula-fed in infancy scored better in cognitive function tests. But the effect, though significant, was small (ranging from 2-8 developmental quotient points)[126, 136,137]. However, some observational studies[138-144], and another randomized controlled trial among preterm infants[145], found no association between breast-feeding and later cognitive ability**.** The role of confounding factors could be a possible reason for inconsistent findings. Parents' SES and intelligence/education are strongly related to childhood cognitive performance (details are reported in section 1.3.2). These factors are also related to initiation and duration of breast-feeding and may confound the association of breast-feeding with cognitive function through postnatal nutrition, stimulation, growth and development. In developed countries, mothers of higher SES and education are more likely to initiate breast-feeding soon after birth and feed their babies for a longer duration[146]. In contrast, in developing countries breast-feeding may be unrelated to, or inversely related to, maternal SES and education. In India, a recent survey reported that early termination of breast-feeding was associated with higher maternal SES and education[147]. Therefore data from developing countries may be helpful in addressing the confounding effects. But data from developing countries are very few[131,132]. Apart from breast-feeding, age at introduction of complementary foods in infancy, and the quality of those foods, may also

influence later cognitive ability. But the literature is limited. One study reported that meat consumption from 4-12 and 4-16 months was positively associated with children's psychomotor but not mental developmental indices up to 24 months of age[148]. Another study reported that 4 year old children who consumed more fruits, vegetables and home-prepared foods in infancy (6 and 12 months) had higher full-scale and verbal intelligence scores, independent of confounding variables[149]. A recent study examining the association of children's dietary patterns at 6, 15 and 24 months of age with their cognitive function at 8 years reported that after adjustment for potential confounders, higher scores on a discretionary dietary pattern (biscuits, chocolate, sweets, soda and crisps) at all ages were associated with 1–2 point lower IQ scores[150]. They also found that breast-feeding pattern at 6 months and homemade contemporary dietary pattern (herbs, legumes, cheese, raw fruit and vegetables) at 15 and 24 months were associated with higher IQ (1-2 point) scores; positive association at 6 months and no association at 15 or 24 months between home-made traditional diet pattern (meat, cooked vegetables, desserts) and IQ scores; while ready-prepared baby foods pattern at 6 and 15 months was negatively associated, the ready-to-eat foods pattern at 24 months was positively related to IQ scores.

1.3.4.2 Childhood nutrition and cognitive function: Neurodevelopment continues through childhood and may be influenced by childhood diet. A balanced diet comprising protein, energy, fat and micronutrients is important for optimal neurodevelopment. These nutrients contribute to cognitive development directly through effects on brain development and the synthesis and functioning of neurotransmitters. Additionally, better nutrition improves health status. Therefore children can be active and will have better interactions within family, school and neighbourhood which enable the children to acquire better cognitive skills. Nutritional deprivation is largely of concern in developing countries, attributed mainly to poverty and poor living conditions. Observational, longitudinal and supplementation studies report that poor nutrition (protein-energy) is related to deficits in childhood cognitive test scores (IQ and school grades) and suggested that these associations are likely to be confounded by socio-economic factors[6,151]. Further, these studies, though not conclusive, reported deficits in specific functions like reasoning, perceptual-spatial abilities and fine motor skills in relation to poor nutrition. Prenatal supplementation with continued supplementation during infancy (first two years) rather than during childhood had a beneficial effect on childhood cognitive abilities[151]. There is evidence that not only macronutrients, but also micronutrients influence cognitive performance[152]. An observational study reported that adolescents with vitamin B12

deficiency scored poorly in tests of fluid intelligence, spatial ability and working memory[153]. A recent cross sectional study reported an inverse association for vitamin B12 concentrations and a linear association for haemoglobin (Hb) concentrations with mental processing index among school aged children[154]. Evidence from randomised controlled trials using iron supplements have found improved cognitive test scores in the intervention group compared to controls[155,156]. A recent randomized control trial using high and low concentrations of a combination of micronutrients and n-3 (omega-3) fatty acids reported that, except for some small differential effects, lower micronutrient concentrations were as effective as higher concentrations on cognitive function[157].

Apart from macronutrients and micronutrients there is evidence from some studies, mainly from developed countries, that childhood dietary patterns also contribute to cognitive skills. A longitudinal study among New Zealand children (at age 3.5 and 7 years) found that total IQ scores at 3.5 years was 3.96 points higher in those children who ate breads or cereals four or more times per day compared to those who did not consume them at the same level[158]. This study also found an inverse association between IQ score (with a decrease of 3-4 points) and daily margarine consumption among all study children and in SGA children. At 7 years, full scale IQ score was 3.6 points higher in children consuming fish weekly compared to those who did not[158]. In a cross sectional study among 6-16 year old US children while higher intake of polyunsaturated fatty acids (PUFAs) was associated with an increase (2.15 score) in short-term memory, higher intake of cholesterol was associated with a decrease (2.67 score) in the same ability[159]. However they did not find any association of total and saturated fat intakes with cognitive function. Among Finnish children aged 5 years higher intakes of PUFAs were found to be associated with better visual motor skills, and higher intakes of protein were associated with better speech and language skills[160]. Another longitudinal study in the UK, reported that a 1 standard deviation (SD) increase in the 'processed' (high fat and sugar content) pattern of diet at 3 years of age was associated with a 1.67 point decrease in IQ at 8.5 years[161]. They also found that 1 SD increase in the 'health-conscious' (salad, rice, pasta, fish, fruit) pattern at the time of cognitive function assessment (8.5 years) was associated with a 1.20 point increase in IQ. However they did not find associations of dietary patterns at 4 and 7 years with childhood cognitive function. Evidence from some studies reported that, not only dietary pattern, but also meal times (breakfast and mid morning snack in particular) were found to be associated with cognitive performance[152].

1.3.4.3 Growth in infancy and childhood and cognitive function: Some studies have examined the effects of weight and/or length/height or HC during infancy and early childhood with cognitive function in childhood and early adolescence. However the results are not consistent. While some studies found an increase in cognitive test scores with weight gain during infancy and early childhood[60,66,162], others did not find any association[62,163]. Another study reported small negative effects on cognitive test scores in relation to weight gain from birth to 10 years[61]. Similarly some studies both from developed and developing countries reported a positive association between cognitive test scores and length/height[60,61,64,66,164,165] and HC[59-61] during infancy and/or childhood or adolescence. It has also been reported mainly from low- and middle-income countries that stunting during infancy and early childhood, attributed mainly to poor nutrition and poor living conditions, was associated with poor cognitive ability during childhood and adolescence[6,151,166]. There are some studies from developed countries reporting associations of childhood cognitive function in relation to adiposity (BMI) at the time of assessment. The findings were inconsistent. A cross sectional study among 8-16 year old children reported that higher BMI was associated with poorer performance in tests of visuo-spatial ability and working memory independent of potential confounders[167]. Another study among children aged 7-9 years found that higher BMI and fat mass (total and central adiposity) was associated with lower response in performance of a task that requires greater amounts of cognitive control (NoGo task) but not in performance of a task that requires lesser amounts of cognitive control (Go task)[168]. This study also found that higher BMI was associated with lower school achievement (spelling and arithmetic). While higher total fat mass was associated with lower scores for reading and spelling, higher regional (central adiposity) fat mass was associated with lower scores for reading, spelling and arithmetic. In contrast another cross sectional study did not find any association between BMI and cognitive skills (attention, memory and language) among children aged 6–19 years[169]. However they found association between lower BMI and poor memory performance in girls.

Apart from postnatal nutrition and body composition, a variety of environmental factors such as SES, parental education, intelligence and home and neighbourhood environment (already described in section 1.3.2), which influence prenatal growth and neurodevelopment also contribute to postnatal growth, including neurodevelopment. There is evidence mainly from low- and middle-income countries that these factors are likely to interact with each other in predicting cognitive performance. It has been reported that

LBW/SGA term born children scored poorly in mental and psychomotor development during infancy and verbal ability during early childhood compared to normal birthweight children. However, LBW children performed well in these tests if their parents were more educated and were exposed to better stimulation and if they were from better socio-economic background[170].

1.4 Cognitive function; Dimensions and assessment

As mentioned in section 1.2 and depicted in figure 1.1, sensory-motor abilities can be assessed during early postnatal life from infancy to 3 years of age. Usually these skills are assessed by the Bayley Scale of Infant Development (BSID). Beyond 3 years higher cognitive function includes multiple cognitive dimensions (domains). Neuropsychological test batteries such as the Kaufman Assessment Battery for Children (KABC)[171] and Wechsler Intelligence Scales for Children (WISC)[172] are commonly used to assess various cognitive domains. The tests that are used in my study are based on some of the eight broad dimensions described in the Carroll model[173]. The model describes the three-stratum structure of cognitive abilities (Figure 1.3). It is based on the factor analysis of variables from measures of various psychological tests (more than 460 datasets) covering about six decades of intelligence testing in various populations of different age, gender and culture. Initial factor analysis of an individual dataset was carried out to identify the factors representing basic cognitive functions. It includes ~70 narrow functions like reasoning, writing, reading, spelling, memory span, visualization, sound discrimination etc. that can be measured by individual tests. Subsequent factor analyses were carried out to identify broad domains and a general intelligence factor. The outcome of the factor analysis suggests three layers or strata of cognitive abilities, with each stratum representing the variation in factor loadings at the next lower level. The 3rd stratum, general intelligence is a composite measure of eight broad domains of cognitive ability (2nd stratum) which correlates with general intelligence and of 70 basic narrow functions (1st stratum) which are underlying the eight broad domains. The eight broad domains and their description are illustrated in Table 1.2.

Figure 1.3 The three stratum theory of cognitive abilities – Carroll model[173]

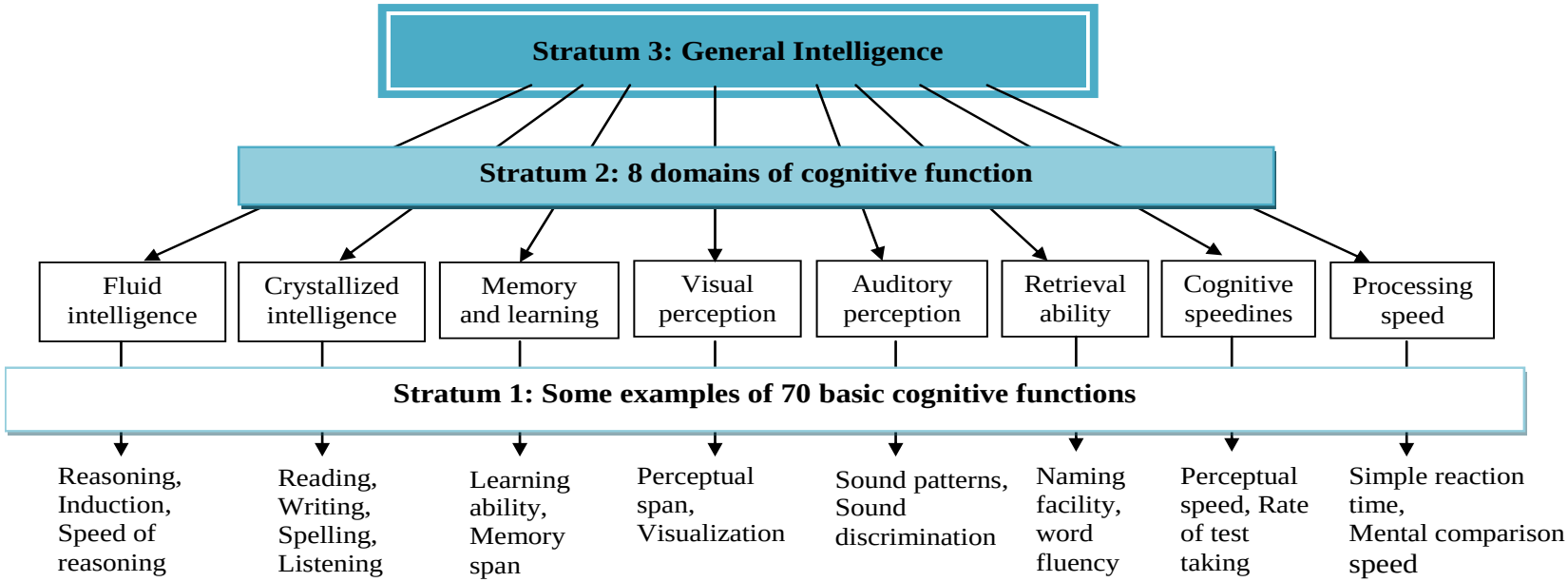


Table 1.2 Eight broad domains of cognitive function and their description

Cognitive domain	Description
Fluid intelligence	Basic reasoning and problem solving ability based on unfamiliar information and procedures that requires minimum learning
Crystallized intelligence	Reasoning and problem solving ability based on previous learning, acquired knowledge, procedures and experience
Memory and learning	Ability to learn and store information in the immediate awareness and use the information within a couple of minutes (short-term memory)
Visual perception	Ability to process, analyze, think in response to visual stimuli or patterns including the ability to store and recall visual patterns
Auditory perception	Ability to process, analyze, and think in response to auditory stimuli, including the ability to process and discriminate sound patterns which are presented in difficult conditions
Retrieval ability	Ability to learn and store the information for a long time and to recall the stored information fluently and efficiently (long-term memory)
Cognitive speediness	Ability to process tasks rapidly; it involves focused attention
Processing speed	Time taken to respond or react to stimuli or a task (reaction time; usually within fractions of seconds).

1.4.1 Using western instruments in developing countries: Due to differences in culture, language and social conditions the psychological instruments developed for western countries are difficult to use in developing countries. Hence they require suitable adaptations, translation and back translation, and validity testing before using them. Validity is the extent to which the test measures the underlying cognitive ability, for example short-term memory[174]. It can be tested by correlating the score of one test with that of another test measuring the same cognitive ability. Reliability indicates the extent to which the test yields the same score when the individuals are assessed using the same test again and again[174]. It can be assessed by a) testing the level to which an individual gets same score whenever the same test is administered; b) the extent to which an individual obtains the same score when a similar test with different, but equivalent, items is administered; and c) by checking the internal consistency i.e. the extent to which the scores of the different items are consistent with the scores within a scale.

1.5 Studying cognitive function in the Indian population; the rationale

India, a developing country, is undergoing rapid socio-demographic and economic transition. In-spite of this, the economic growth and overall development is lagging behind and cannot be compared to that of developed countries. To add to this, the burden of infectious and non-infectious disease and morbidity and mortality related to them is a public health concern[175]. This is largely due to a rising population, widespread poverty, poor living conditions, poor nutrition (multiple nutritional deficiencies), illiteracy, general lack of awareness about the factors influencing health and disease outcomes, and inadequate access to health care.

1.5.1 Maternal and child nutrition in India[176]: Maternal undernutrition is still widely prevalent. About 36% of women aged 15-49 years are undernourished and 12% are stunted and about 58% of pregnant women are anaemic. Micronutrient deficiencies (like vitamin B12, folate and vitamin D) are common. Although there are national programmes like the integrated child development service, mid-day meal programme to promote better childhood nutrition, childhood undernutrition is widely prevalent. About 20% of the children below 5 years of age suffer from wasting and about 43-48% are underweight and stunted due to chronic undernutrition.

1.5.2 Birthweight in Indian children: About 30% of Indian children weigh <2.5 kg at birth[177]. Mean birthweight in India is around 2.7 kg, almost 800 g lower than among white Caucasian babies in high-income countries[178,179]. Low birthweight is mostly due to IUGR rather than prematurity[180], probably related to maternal chronic macronutrient and micronutrient deficiencies[112].

1.5.3 Infant feeding in Indian children: Despite the World Health Organization (WHO) guidelines[181] on infant feeding practices (initiating breast-feeding within an hour of birth, exclusive breast-feeding for 6 months, introduction of nutritious complementary foods from 6 months, and continued breast-feeding up to 2 years), breast-feeding is initiated within an hour of birth in only 23% of children, about 46% of children under 6 months of age are exclusively breastfed and about 56% of children aged 6-9 months are provided with the recommended semi-solid complementary foods and breast milk[176].

1.5.4 Status of education, employment and socio-economic status in India: According to the 2005-2006 National Family Health Survey-3 report[176], the literacy rate has substantially progressed in the last 50 years**.** Nearly 78% of men and 55% of women aged 15-49 years are literate. A higher proportion of those in urban areas are literate, compared with their rural counterparts. Among men 88% from urban and 72% from rural area of residence are literate. Corresponding figures for women are 75% and 46% and the urban-rural difference is much higher for women than for men (29 % vs.16 %). Education level varies in different states. In general, women completing 12 or more years of education ranges from 5% to 37%; corresponding figures for men are 12 to 38%. Unemployment, particularly in women, is another concern. In total about 87% (84% urban and 89% rural) of men and 43% (30% urban and 49% rural) of women are employed. The majority of women (59%) are agriculture workers. The nature of jobs among men varies. Only a small (7%) percentage of men and women are in higher grade jobs. According to the National Family Health Survey-3[176], wealth index, an index of SES, was defined by combining information on 33 items which includes household assets and characteristics such as ownership of consumer items (mattress, pressure cooker, chair, cot/bed, table, electric fan, radio/transistor, black and white television, colour television, sewing machine, telephone, computer, refrigerator, watch or clock, bicycle, motorcycle or scooter, animal-drawn cart, car, water pump, thresher and tractor), household electrification; drinking water source; type of toilet facility; type of flooring; material of exterior walls; type of roofing; cooking fuel; house ownership; number of household members per room. Based on the wealth index, about 48% of urban and 7% of rural households were found to be in the highest socio-economic strata.

Among children, with the introduction of National programmes like compulsory primary education through Sarva Shiksha Abhiyan (Education for All Movement), school enrolment has considerably improved. Nearly 90% of children aged 6-11 years both in urban and rural areas are enrolled in primary schools. However, dropout rates are causing great concern. About 72% (74% urban and 71% rural) of primary school aged children and 51% (60% urban and 47% rural) of secondary school aged children attend primary and secondary school respectively. Nationally more than 1 in four children aged 6-17 years are not in school at all. The school attendance rate for boys and girls are the same in urban areas. In rural areas, however, the school attendance rate for girls is 12% lower than that of boys. Although gender inequality is not apparent at the primary school level, at age 15-17 years, 49% of boys and 34% of girls were attending secondary school. About 50% of

schools lack basic infrastructure. With increasing competition for getting entry into higher education, rising academic pressure from childhood is another important concern. Although there is no evidence to link academic pressure and childhood cognitive function it is possible that increasing academic stress interferes with behaviour, physical activity and social interaction and thus influences cognitive function and academic achievement.

Children born and reared under the above described socio-economic conditions are more likely to be deprived of good nutrition, parental care and a stimulating environment than children born and brought up in a population with higher socio-economic background. It is possible that these circumstances, together or independently, can have a significant impact on the child's growth and development including cognitive development. Most of these circumstances are modifiable. It is therefore important to study cognitive function in early life and identify the factors influencing it. In India, studies investigating the impact of environmental, prenatal and postnatal factors on cognitive performance during childhood and adolescence are scarce. Some studies reported poor childhood cognitive performance in relation to maternal vitamin B12 deficiency during pregnancy[122], LBW[69,182], poor SES[183], childhood body composition[154,166,183] and the children's micronutrient (for example zinc, fatty acid) status[154,184]. Also, studies comparing the cognitive abilities between Indian children and children from developed countries are scarce. A recent cross sectional study among socially, nutritionally and educationally deprived Indian tribal children reported that the growth curve for median IQ scores of the tribal children was placed just above the 5th percentile value of British children at younger age (5 and 6 years). However as age advanced the growth curve was placed just below the 5th percentile value of British children[183]. Evidence from developing countries like India linking small size at birth and postnatal life, poor maternal and child nutrition with poor cognitive performance would strengthen advocacy for policies to invest in maternal and child health. Further, better cognitive ability will enhance awareness about healthy lifestyle, and contribute to better academic growth and employment. Studying cognitive function in developing countries will also contribute to worldwide understanding about the differences in the role of confounding factors mediating or modifying the effect of maternal nutrition, birth size, breast-feeding and postnatal nutrition, growth and development in predicting cognitive function. Therefore identifying the factors predicting deficits in cognitive ability and initiating appropriate interventions to improve them will potentially improve the health, economic growth, human capital outcomes and overall national development.

1.6 The Mysore Parthenon study

The Mysore Parthenon Study is a prospective observational study initiated during 1997-98 with the objective of examining the incidence of GDM and its determinants in an Indian population[185].

1.6.1 Study setting and methods: Between June 1997 and August 1998, pregnant women attending the antenatal clinic of the Holdsworth Memorial Hospital (HMH), Mysore were recruited. At 30±2 weeks gestation, these women underwent a 100g, 3-hour, oral glucose tolerance test. Of the 830 women who participated in the study 674 of the women delivered at HMH. Anthropometric measurements of the newborn babies were carried out. The methodology is described in detail in chapter 2.

1.6.2 Results: The prevalence of GDM was 6.2% (n=49). Shorter and fatter women had higher concentrations of glucose and measures of insulin resistance and higher prevalence of GDM. The babies born to mothers with GDM were bigger in all measurements, especially body fat (skinfold measurements), as well as skeletal size (length), and muscle mass (MUAC), compared to neonates born to non-diabetic mothers. Even in the offspring of non-diabetic mothers, macrosomic changes were seen across the range of maternal fasting glucose concentrations. Irrespective of maternal diabetes, the babies were smaller compared to their western counterparts.

1.6.3 Follow-up: The Mysore Parthenon Study created a cohort of children who were followed-up subsequently. The main aim of the follow-up of these children was to study the short- and long-term effects of maternal gestational glucose/insulin status on their growth, development and health outcomes[186,187]. Details of the Mysore Parthenon Study are described in chapter 2. This became the foundation of my thesis.

The children were measured, and health and developmental monitoring was done during annual visits until the age of 5 and every 6 months thereafter. Infant feeding data was collected. At 5, 9.5 and 13.5 years, in addition to the routine anthropometry, blood and biochemical parameters (Hb, plasma glucose and insulin concentrations, lipids and micronutrients (vitamin B12 and folate) concentrations were measured. Additionally at 9.5 years children's dietary data were collected and grip strength was also measured. Pubertal growth was assessed every 6 months from year 9 onwards. Cognitive performance was

assessed at 9.5 years and again at 13.5 years. At 13.5 years in addition to cognitive performance children's behaviour and stress response were assessed. Details regarding follow-up are described in chapter 2.

1.6.4 Strengths of the Mysore Parthenon Cohort: The strengths of this cohort are a) detailed anthropometric measurements, glucose tolerance, micronutrient (serum folate, vitamin B12, homocysteine and vitamin D) concentrations and blood pressure of all the women in pregnancy, and their husband's weight and height at that time; b) detailed neonatal anthropometry including weight, length, head and arm circumferences and skinfold thickness, placental morphology and gestational age; c) detailed anthropometry of children from age 1 to 13.5 years including adiposity measures, infant feeding data, children's cardiovascular risk markers (glucose/insulin, insulin resistance, lipid profile and blood pressure), Hb and micronutrient (vitamin B12 and folate) concentrations measured at 5, 9.5 and 13.5 years, pubertal assessment from age 9 onwards, dietary and grip strength data at 9.5 years, and behaviour and stress response at 13.5 years; d) parental anthropometry and glucose/insulin status at 5 and 9.5 years following the index pregnancy and e) parental education, occupation, income, SES, home environment and maternal intelligence data.

Using these data I will be able to examine cognitive function during childhood and adolescence in relation to socio-demographic factors (SES, parental education, occupation, income, maternal intelligence and home environment), prenatal factors (maternal nutrition, neonatal body size and gestational diabetes) and postnatal factors (breast-feeding, postnatal growth including adiposity and current body composition). I will be able to investigate whether socio-demographic factors mediate or modify the effects of prenatal and postnatal size in predicting childhood and adolescent cognitive performance.

None of the women in my cohort has ever smoked or consumed alcohol. Less than 1% of the mothers had hypertensive disorders during pregnancy. Information about the severity and details of the clinical management of GDM, maternal stress during pregnancy, placental vascularity, exclusive breast-feeding and type of complimentary foods, parental stimulation and care and neighbourhood environment was not collected. Thus I will not be able to examine the contribution of these factors in determining cognitive function in this cohort.

The objective of my study is to test the following hypotheses:

1.6.5 The hypotheses:

1.6.5.1 Primary hypotheses

1. Smaller size at birth (birthweight, length, head, mid-upper-arm circumferences and sum of skinfolds) is associated with poorer cognitive ability.
2. Greater gain in body size (head, mid-upper-arm circumferences, height and sum of skinfolds) from birth to childhood is associated with better cognitive ability, strongest for the gain in head circumference during early childhood (from birth to five years).
3. Maternal overweight/obesity and/or underweight, and shorter height during pregnancy are associated with poorer cognitive ability in the offspring.
4. Lower maternal vitamin D, vitamin B12 and folate concentrations during pregnancy are associated with poorer cognitive ability in the offspring.

1.6.5.2 Secondary hypotheses

1. Gestational diabetes mellitus in mothers is associated with poorer cognitive ability in their offspring.
2. Longer duration of breast-feeding is associated with better cognitive ability.

I am describing the detailed methodology involved, and the results obtained, in the course of the study in the subsequent chapters. My PhD was carried out during the data collection for the 13.5 year follow-up of the Parthenon children. Prior to my PhD, I published some of the cognitive function results from the 9.5 year follow-up (references 188-191); during the time of PhD, I also analysed some cognitive function and other data from the 9.5 year follow-up. In this thesis I will report combined results from the 9.5 and 13.5 year studies, which also include new analyses of the data from 9.5 years (including associations with childhood growth, and a comparison of the strength of associations of early life factors (prenatal, infant and early childhood nutrition and growth) and current socio-demographic factors with cognitive function.

CHAPTER 2 METHODOLOGY

Figure 2.1 Mysore, Karnataka, India

2.1 Setting

2.1.1 Mysore-CSI Holdsworth Memorial Hospital (HMH): Mysore, a city with a population of more than a million is located in the southern Indian state of Karnataka (Figure 2.1). It has a large government hospital providing services to the population living in Mysore and surrounding villages. It also has a few government primary health centres, 10 large, and several small private hospitals. HMH a century old mission hospital built in 1905 and governed by the Church of South India is situated in a relatively poor and crowded area at the centre of the city (Figure 2.2). Initially for the first half of the century it provided mainly maternal health services to people in Mysore and later on extended its services to all the other medical specialties. Currently it is a charitable 350-bedded multispecialty hospital providing services to a large section of the population from all socio-economic categories in Mysore as well to around 25 % of the population from neighbouring villages.

Figure 2.2 CSI Holdsworth Memorial Hospital

2.1.2 HMH deliveries, birth records and building a research unit: Around 2000-2500 women visit HMH every year for delivery (around 10-15% of all hospital deliveries in Mysore). Birth records of these deliveries containing the babies' birthweight, length and head circumference (HC) have been preserved routinely since 1934. This unique collection of birth records led to the setting up of a study in collaboration with the Medical Research Council, Lifecourse Epidemiology Unit (MRC LEU), Southampton, UK, which contacted several long established hospitals in India before finding the availability of birth records at HMH. As part of the collaboration, initially people born in HMH were identified through details from birth records by a house-to-house survey and studied for the prevalence of coronary heart disease and type 2 diabetes and their relationship to birth size. Later on many other studies were carried out based on the birth records.

Findings from one of these studies[192] suggesting that maternal hyperglycaemia could increase the risk of type 2 diabetes in the offspring resulted in setting up of the Mysore Parthenon Study. In the year 2001 a research block (Figure 2.3) was built within the hospital premises to conduct research studies exclusively in the field of developmental origins of health and disease.

Figure 2.3 Research block

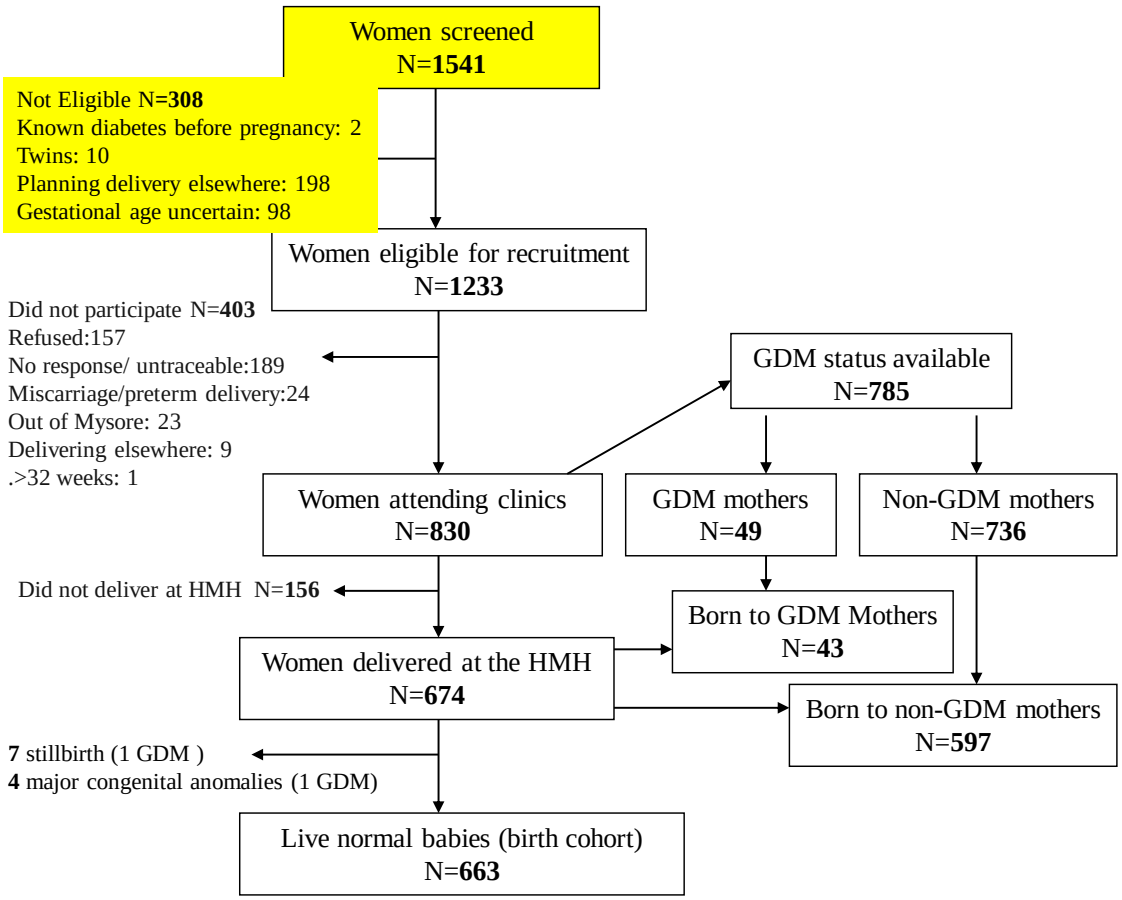
2.1.3 The Mysore Parthenon Study[185-187]: This hospital based prospective birth cohort study was designed in the year 1997-98. The main objective of this study was to investigate the incidence and determinants of GDM in an Indian population and its short- and long-term effects on the growth, development, health and disease outcomes in their offspring.

2.2 Study population

2.2.1 Study on glucose intolerance in pregnancy: Between June1997 and August 1998, 1541 women booking consecutively into the antenatal clinic of HMH were screened. Of them 1233 (80%) women satisfying the eligibility criteria (not known to have diabetes before pregnancy, had a singleton pregnancy of less than 32 weeks gestation (determined by their last menstrual period) and planned to deliver at HMH) were enrolled into the study. 830 (67%) of them participated in the study. This study was part of the PhD programme of Dr Jacqueline Hill a research fellow from Southampton, UK.

2.2.2 Deliveries and birth cohort: Due to the traditional practice of the women visiting their maternal home for delivery as well as accessibility to a number of maternity units closer to their area of residence, many women attending the antenatal clinic at HMH finally visit another hospital for delivery. Of the 830 women studied, 674 (81%) delivered at HMH. 11 children (7 still born (1 born to mother with GDM) and 4 with major congenital anomalies (1 born to mother with GDM)) were excluded. The remaining 663 (41 GDM) babies constitute this birth cohort. Figure 2.4 depicts the study cohort during pregnancy and at birth. For the past 13 years I and Dr Krishnaveni, who started this birth cohort, have been supervising the subsequent follow-ups. Children from this cohort are the participants of my study.

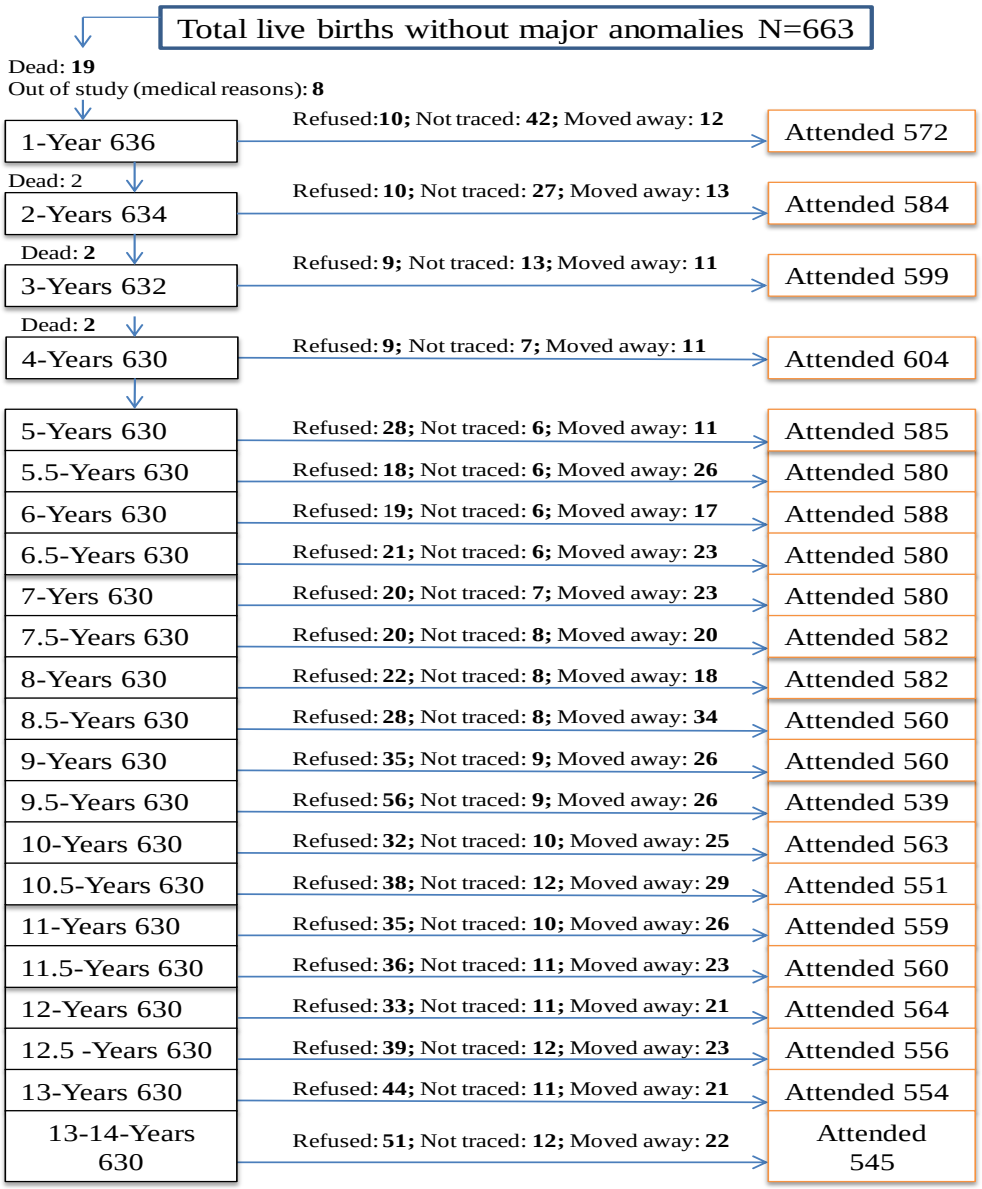
Figure 2.4 Flow chart describing study population during pregnancy and at birth



2.2.3 Follow-up from 1 to 13 years: All the children were invited for follow-up to track their growth and development. 8 children found to have medical problems, which could have affected their normal process of growth and development, were excluded from the study. 25 children died between age 1 and 4 years. Of the remaining 630, all available and willing children were followed up annually on the child's birthday (± 4 wks) till the age of

5 and thereafter every 6 months after the birthday (± 4 wks). An overview of the available study cohort and participants in each follow-up and the reasons for non-participation are presented in Figure 2.5.

Figure 2.5 Flow diagram depicting available study cohort, number of participants and reasons for non-participation at each follow-up



2.3 Clinical investigations and data collection

2.3.1 Pregnancy: In this section I describe the data collected at the time of recruitment and the methodology related to maternal anthropometry and blood testing carried out at 30±2 weeks of gestation.

2.3.1.1 Data collection at the time of recruitment: Details of maternal age, parity,

religion, area of residence (classified as urban or rural based on their address), SES, education, occupation and history of smoking and alcohol consumption were recorded. None of the mothers had ever smoked or consumed alcohol. Women taking medications and vitamin supplements were recorded. Body composition measures (weight and height), education and occupation of their husbands were also recorded.

2.3.1.2 Maternal anthropometry: Detailed anthropometry of the mothers including height, weight, circumferences (mid-upper-arm and head), skinfold thicknesses (biceps, triceps, subscapular and suprailiac) and external pelvimetry (intercristal, interspinous and external conjugate diameters) was carried out using standardized methods.

2.3.1.3 Blood test: All the willing women had a 100g, 3-hour oral glucose tolerance test (OGTT). After collecting fasting blood sample, a 100g oral glucose load was administered and further blood samples were collected 30, 60, 120 and 180 minutes later for plasma glucose and insulin assay. The 100g OGTT was used rather than the usual WHO 75g OGTT, because it was the established test used by clinicians in HMH. Complete OGTT data were available for 785 women. Glucose was measured using a standard hexokinase method. Between batch coefficients of variations were <1.5%. Gestational diabetes was defined using Carpenter-Coustan criteria[193] (presence of two or more plasma glucose concentrations ≥ 5.3 mmol/L (fasting), 10.0 mmol/L (60 minutes), 8.7 mmol/L (120 minutes) and 7.8 mmol/L (180 minutes). GDM was diagnosed in 49 (6.2%) women. Women who were found to be diabetic were referred to one of several consultant obstetricians in HMH, who managed their subsequent clinical care. We did not collect information about subsequent severity and management of GDM because different consultants had widely differing management protocols, and we did not think this information would be meaningful in a research context.

Apart from glucose/insulin assay, maternal serum vitamin D, vitamin B12, folate and homocysteine concentrations were also measured using stored serum samples (frozen at -80^{o}C for ~8 years). Serum vitamin D concentrations were measured by radioimmunoassay[194]. Intra- and inter-assay coefficients of variations were 8.8% and 10.8% respectively. Serum vitamin B12, folate and homocysteine concentrations were analysed using microbiological assays[195-197]. Intra- and inter-assay coefficients of variations were <8% for the vitamin B12 and folate assays and for the homocysteine assay, between-day coefficients of variations were <3%.

2.3.2 At birth: Detailed anthropometric measurements (Figure 2.6, 2.7) including weight, lengths (crown-heel and crown-rump), circumferences (head, mid-upper-arm (MUAC), abdomen and chest), skinfold thicknesses (triceps and subscapular) were carried out within 72 hours of birth using standardized procedures. Gestational age, based on the last menstrual period, was available for all babies. Placental morphological features (weight, length, breadth and number of cotyledons) and Apgar score were also recorded.

Figure 2.6 Birthweight

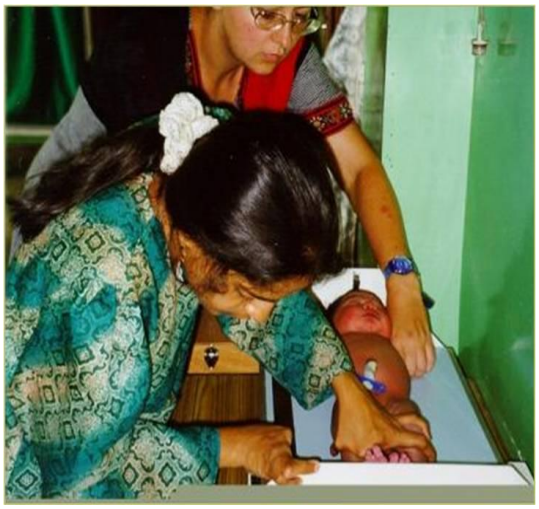


Figure 2.7 Birth length

2.3.3 At follow-up from 1 to 13 years: At each follow-up, detailed body composition measures were recorded using standardized methods. Details of measurements at birth and subsequent follow-ups and their protocol are described in Table 2.1 and appendix 1 respectively. From age 5 onwards, in addition to anthropometry, body fat percentage was measured using bioimpedance technique. Until the age of 5 their developmental milestones were assessed using standard tests described in standard paediatric text books[198], and information on the child's health and vaccination were collected.

At 5 and 9.5 years of age, apart from routine body composition measurements, detailed investigations were conducted. Children underwent an OGTT, and their haemoglobin, plasma glucose/insulin, vitamin B12, folate and homocysteine (only at age 5 years) concentrations and lipid profile were measured. The methodology for vitamin B12, folate and homocysteine assays was similar to that explained in section 2.3.1.3. From the age of 9 years data on the date of menarche and sexual maturity (by physical examination) using Tanner scale were recorded[199]. Additionally, at 9.5 years hand grip strength, plasma cortisol and corticosteroid binding globulin concentrations were measured and dietary data (food frequency questionnaire and 24 hour recall) were also collected. At both time points,

the parents' glucose/insulin concentrations and body composition measures were measured and their SES, education and occupation were recorded. In addition, methylenetetra-hydrofolate reductase (MTHFR677) genotype in the parents and children was assessed.

Table 2.1 List of anthropometric measurements carried out at birth and at follow-up

Weight (kg)	Measured naked using an electronic weighing scale to the nearest 5 g at birth (Seca, Germany), and thereafter in minimal clothing, to the nearest 100 g (Seca, Germany or Salter, UK).
Height/length (cm) (crown-heel and crown-buttock or sitting height)	Measured using Harpenden infant stadiometer during first two years and Microtoise wall-mounted stadiometer (CMS Instruments, UK) thereafter, to the nearest 1mm. An assistant helped to maintain the child's posture for the first 2 years. From four years onwards, sitting height was measured using a stool of known height
Head circumference (cm)*	Measured at the level of maximum occipito-frontal diameter (farthest point of the occipital protuberance in the back and just above the eyebrows in front) to nearest 1 mm using a fibreglass anthropometric tape.
MUAC (cm)*	Measured at the mid-point between acromion and olecranon processes to the nearest 1mm using a fibreglass anthropometric tape.
Abdominal circumference (cm)*	Measured at the level of umbilicus to the nearest 1mm at the end of expiration using a fibreglass anthropometric tape. Measured up to the age of 6 years.
Chest circumference (cm)*	Measured at the level of xiphisternum to the nearest 1mm at the end of expiration using a fibreglass anthropometric tape. Measured up to the age of 9.5 years.
Waist circumference (cm)	Measured at the mid-point between lower border of 12^{th} rib and highest point of the ileac crest at the level of mid-axillary line to the nearest 1 mm at the end of expiration using a fibreglass anthropometric tape. Measured from age 5 years onwards.
Hip circumference (cm)	Measured at the level of greater trochanter to the nearest 1 mm using a fibreglass anthropometric tape. Measured from age 11 years onwards.
Subscapular skinfold (mm)	Measured just below the inferior angle of the scapula along the natural direction of the skin cleavage using Harpenden skinfold calipers (CMS Instruments, UK). Readings taken at the end of 5 seconds.
Triceps skinfold (mm)	Measured at the intersection of horizontal mid arm line and the vertical line at the most posterior point of the triceps using Harpenden calipers. Readings taken at the end of 5 seconds.

*At birth these measurements were carried out using a blank tape, marked and measured against a fixed ruler.

At the age of 9-10 years detailed cognitive performance was assessed in these children and the tests used are described in detail in section 2.7.1 and Table 2.2a and 2.2b.

2.3.4 Infant feeding data: At one, two and three years of follow-up, information on infant feeding was obtained by asking mothers the same set of questions: How was the baby fed from birth (breast, bottle, breast+bottle or other)?; If breast-fed, was the baby still being breast-fed?; If no longer breast-fed, what was the age (months) at which breast-feeding stopped? In addition, at one year follow-up, mothers were asked the age (months) at which their baby started taking solid foods regularly.

2.4 Relationship with the families of study cohort

Continuous follow-up of these children helped me to get to know each child and his/her family at a personal level that resulted in creating a trusting environment and building excellent rapport between the families and the research team. Frequent interactions with the families during follow-up and their continued co-operation helped in achieving a high participation rate (85+ %) and enabled us to plan new studies and investigations which in turn helped the parents to know their children's health and wellbeing.

2.5 Current study at 13-14 years (March 2011-May 2012)

2.5.1 Team (Figure 2.8): The team comprised four research assistants, a data manager and two psychologists. I was involved in training the team in different areas of data collection (anthropometry, administering questionnaire and cognitive function assessment with the help of a senior psychologist from St John's Research Institute, Bangalore, India) and conducted inter-observer variation studies before the commencement of the study. My data manager, with the help of the computing department of the MRC LEU, Southampton, was instrumental in establishing a computer database using Microsoft access software package. I ran day-to-day clinics and was involved in supervising the appointments, field visits, processing and storing of blood and saliva samples, data entry and was responsible for the quality of data collection. Every day data from the data sheets were double entered. Regular meetings with the team were conducted to assess the progress of the study and to identify problems. Recognition and appreciation of the team for their good work followed by discussions and exchange of ideas helped to resolve the difficulties encountered during the study and kept the team in an excellent form for the smooth conduct of the study.

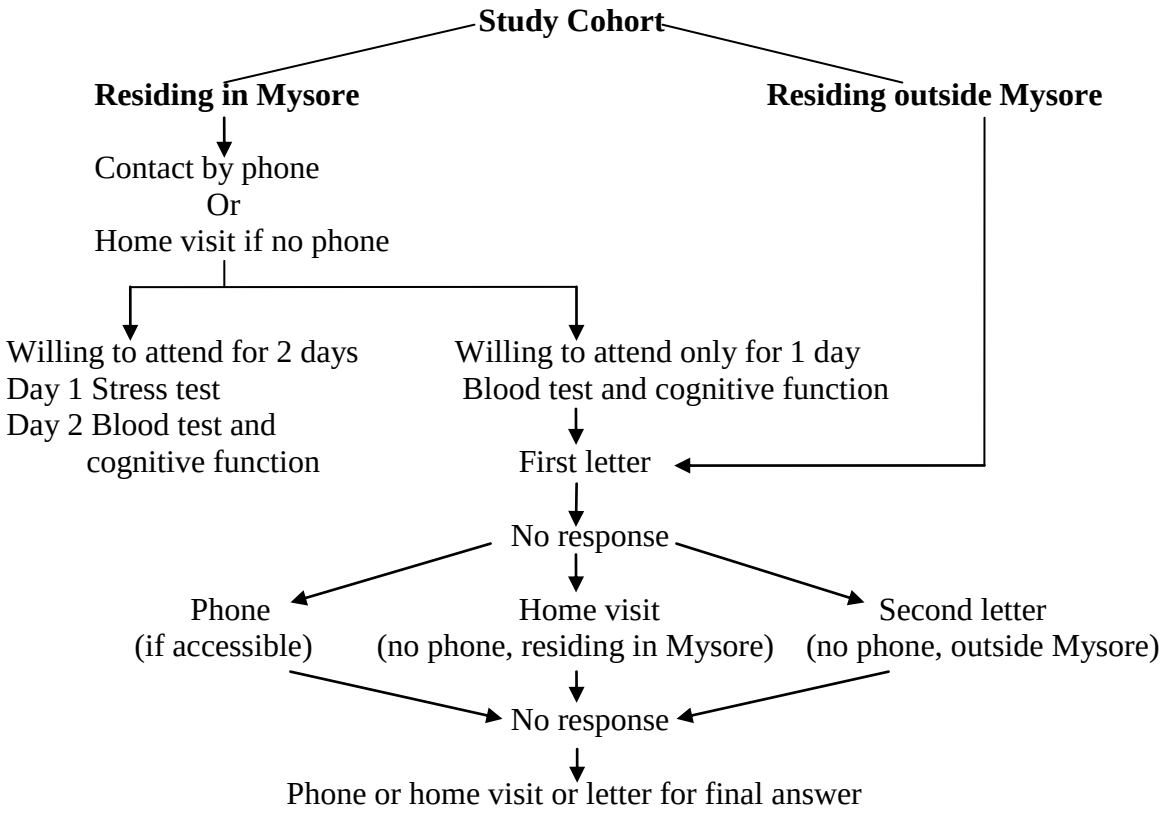
Figure 2.8 Research team

2.5.2 Planning and training: More detailed investigations were planned for the 13.5 years follow-up. In addition to routine anthropometry, the study was designed to investigate biochemical parameters, micronutrients, blood pressure measurement and cognitive function and behavioural assessment in the entire cohort and to examine stress responses in a sub sample of this cohort. It was also planned to assess maternal intelligence, temperament and home environment. To select a suitable battery/questionnaire for cognitive function assessment, maternal intelligence and home environment, I visited St. Johns' Research Institute, Bangalore, India, and discussed suitable tests applicable to our children with a senior psychiatrist and a senior psychologist who had experience in conducting cognitive function assessment in children. A senior psychologist from the same institute provided training to masters' level psychologists regarding administration and scoring of the tests/questionnaires used before the commencement of the study. To learn the procedures involved in stress test in children, I visited Trier and obtained training in the conduct of this test under the guidance of Prof. Dirk Hellhammer, who devised this test. Ethical approval for the study was obtained from the HMH Research Ethical Committee**.**

2.5.3 Appointments: Appointments to participate in the study were made 6 months after the child's 13th birth day (± 3months). Fixing a date for this follow-up was based on whether the child was willing to attend the clinic for two consecutive days (stress test on the first day and blood test and cognitive function assessment on the next day) or was willing to attend only for the blood test and cognitive function assessment. As it was

planned to examine stress responses in a sub sample only, it was decided to book the children residing in Mysore for the stress test (easy accessibility and strict monitoring of the protocol involved in stress test). Therefore the study cohort was divided into two groups: a) residents of Mysore and b) residents outside Mysore. For the residents of Mysore, the child's family was contacted by phone or by visit if phone was not available and details of the study were explained and were asked to attend the clinic. If they were willing, a date to attend the clinic for stress test (day 1) and blood test and cognitive function assessment (day 2) were assigned and an information sheet describing the study details printed in local language as well as in English were provided (appendix 2). For the residents outside Mysore and also for those residing in Mysore but willing to attend the clinic only for blood test and cognitive function assessment, a letter, along with the information sheet, was sent to the parents a week prior to the clinic date asking them to bring the child to the research clinic. If they did not attend on the given date, they were contacted by phone to give another date. If they were not accessible by phone, another letter was sent asking them to bring the child at a later date if they were living outside Mysore, or a field staff visited their home if they were living in Mysore to assign another date. Finally all the non-participants were contacted by phone or letter or home visit to get a final answer. Details about assigning appointments are illustrated in Figure 2.9.

Figure 2.9 Mode of assigning appointment date for attending the research clinic



2.5.4 Instructions on the day before the clinic

2.5.4.1 Stress test participants: A research assistant visited the house and explained the details of the study. Instructions to be followed before visiting the research clinic on day 1 (stress test) were provided. Upon completing the stress test (day1), parents were asked to visit the research clinic next morning (day 2) with the child fasted for blood test.

2.5.4.2 Remaining participants: A research assistant contacted the parents by phone if available or visited their house if residing in Mysore and reminded them about the tests and to visit the research clinic next morning with the child fasted.

For all the participants parents were also informed that the child's cognitive function assessment would be carried out after the blood test and after providing breakfast.

2.6 Clinic - Child

In this section I am describing the methodology related to anthropometry, blood testing and cognitive function assessment.

2.6.1 Consent (Figure 2.10a and b): Upon arrival at the research clinic, the child and his/her mother/or the relative accompanying the child were questioned to ensure that the child had fasted. Details of the investigations were explained again to confirm that they had understood the nature of the tests. Written consent from the mother or the accompanying person and assent from the child were obtained (appendix 2).

Figure 2.10a Consent

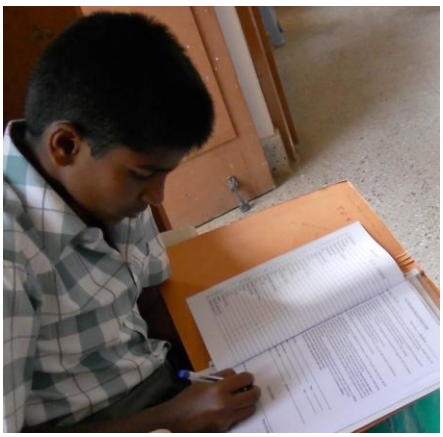


Figure 2.10b Assent

2.6.2 Venesection and blood test (Figure 2.11a and b): A local anaesthetic cream (EMLA^{TM} 5% contains Lidocaine (2.5%) and Prilocaine (2.5%) in a cream base) was applied over the venesection site (preferably ante-cubital fossa) for 30-45 minutes to de-sensitise the skin. A fasting blood sample was taken for haemoglobin, plasma glucose, insulin, micronutrients (vitamin B12, folate and homocysteine), lipid profile assays and for DNA analysis. Of the 545 children who attended the clinic, blood samples were collected from 533 children. Blood sampling was not done in 12 children (4 children- difficult venesection and 8 children refused venesection).

Figure 2.11a EMLA application

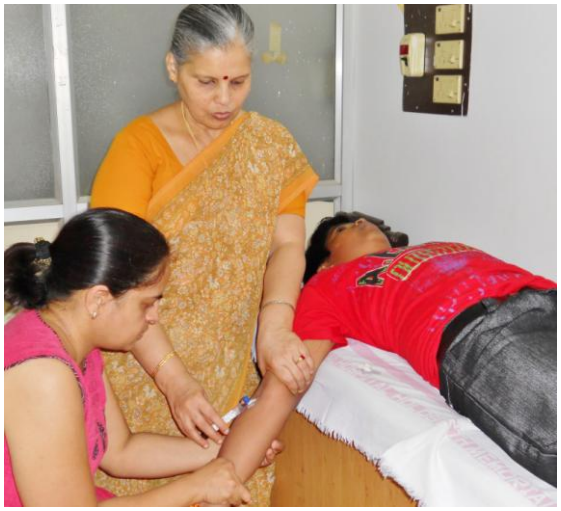


Figure 2.11b Blood sampling

2.6.3 Questionnaire: The questionnaire was designed to collect the details about the child's health problems in the previous year and current medication history (appendix 2)

2.6.4 Anthropometry (Figure 2.12a-f): Team members were trained for the measurements and the procedures were standardized by an inter observer variation (IOV) study before the commencement of the study (appendix 3). The anthropometric measurements and protocol were the same as for the previous years (Table 2.1 and appendix 1 respectively).

2.6.5 Body fat measurement (**Figure 2.13**)**:** Fat mass was measured using a bioimpedance analyser (Bodystat, Quadscan 4000, Isle of Mann, UK), with the child rested for 5 minutes beforehand in supine position.

In addition to the above investigations, blood pressure was measured and data on date of menarche and sexual maturity (by physical examination) were also recorded.

Figure 2.12a Height

Figure 2.12b Weight

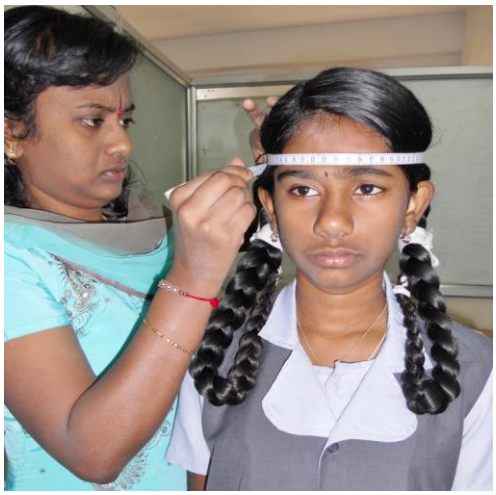


Figure 2.12c Head circumference

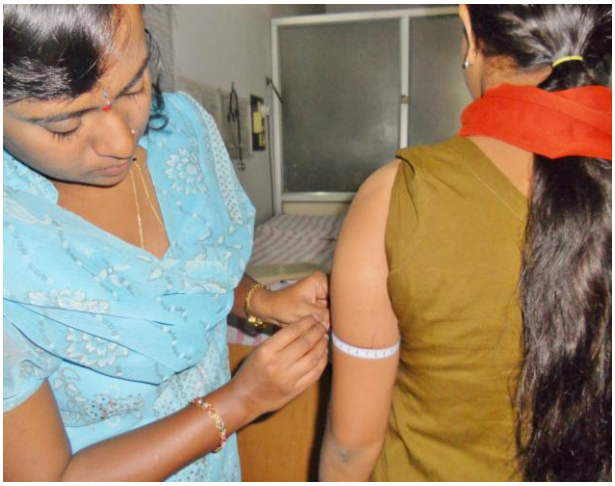


Figure 2.12d Mid-upper-arm circumference

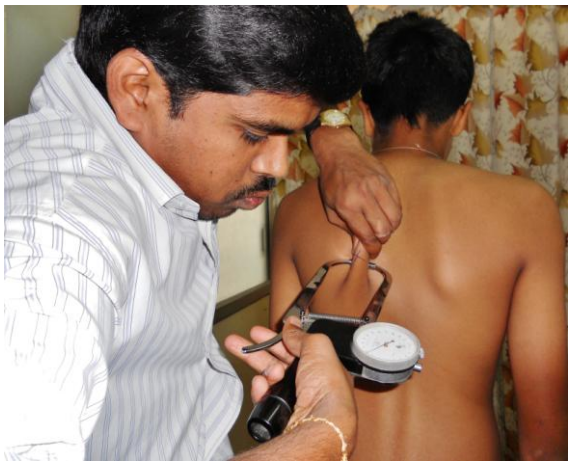


Figure 2.12e Subscapular skinfold

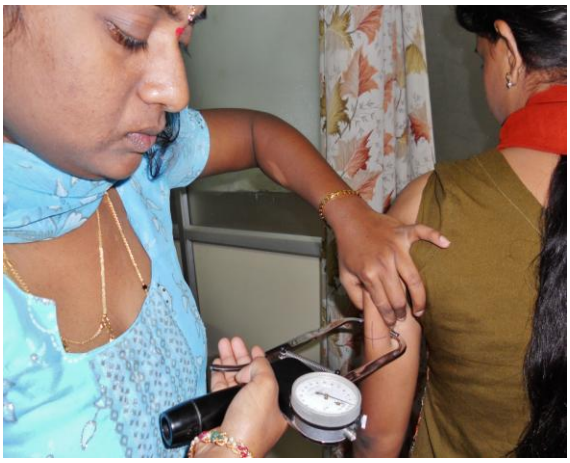


Figure 2.12f Triceps skinfold

Figure 2.13 Bioimpedance

2.7 Cognitive function assessment

2.7.1 Cognitive tests (Figure 2.14a-f): The cognitive measures that I used in my study consisted of a series of neuropsychological tests applicable for use in school aged children related to specific cognitive domains. The cognitive battery included 3 core tests from the KABC-II edition[171] (Atlantis, word order and pattern reasoning) and additional tests, 1 each from WISC-III (coding)[172], Koh's block-design [200,201] and neuropsychological assessment tool[202]. These tests covered the domains of learning, long-term memory and

retrieval ability (Atlantis), short-term memory (Word order), reasoning (Pattern reasoning), language production (Verbal fluency), visuo-spatial ability (Koh's block design), and attention and concentration (WISC-III edition), which are consistent with the Carroll model[173]. I used these tests as they are typical tests relevant to everyday life. These tests assess the day-to-day problem solving abilities which are more likely to be associated with academic performance and behavioural outcome of an individual. The description, protocol and the instructions including scoring of these cognitive tests are described in Table 2.2a and b, appendix 1 and 2 respectively.

2.7.2 Adaptation, validity and reliability of cognitive tests in my study: The process of adaptation and validation for these tests was already done by a team from St Johns' Research Institute, Bangalore, India in collaboration with faculties from the Tilburg University, Netherlands. The language and culture of the population studied for validation is similar to our population. Validation included extensive adaptation process of the tests to ensure their applicability in the local cultural context. The adaptation initially included judgemental (qualitative) procedure consisting of iterations of translating, piloting and modifying the instrument (instructions, examples and items) based on the construct, language, culture, theory and familiarity applicable to the local cultural context[203]. This was followed by a statistical (quantitative) procedure evaluating the adequacy of the adapted version using structural equation modeling, correlation tests and MANOVA to test the performance of the adapted version against gender and age. Subtests showed relatively high loadings on the general cognitive factor largely replicating the Cattell-Horn-Carroll model underlying the original KABC-II and external relations with demographic characteristics such as children's age, gender and scholastic achievement, were as expected[204].

2.7.3 Assessment: Cognitive function was assessed during childhood (9-10 years) and early adolescence (13-14 years). Before the commencement of the study the psychologists who administered cognitive function tests were trained and the procedures were standardized by IOV study (appendix 3). After ensuring that the child had breakfast, all tests were administered in the morning hours to each child in a single session of 60 to 90 minutes in separate rooms free from distraction, in the local Kannada language.

Table 2.2a Description of the cognitive tests (Tests from KABC-II[171])

Atlantis Initially nonsense names for pictures of fish, plants and shells were taught to the child by the examiner. Then the child was asked to point to the correct picture among an array of pictures when the nonsense name of that picture was read out. The test measures learning ability/long-term storage and retrieval, associative memory.	 **Figure 2.14a Atlantis**
Word order The examiner reads out a series of names of common objects. The child was then asked to touch the series of silhouettes of common objects in the same order as read out by the examiner. An interference task (colour naming) was added between the stimulus and the response for the more difficult items. The test measures memory span, short-term memory, working memory.	 **Figure 2.14b Word order**
Pattern Reasoning The examiner shows the child a series of stimulus (images) that form a logical linear pattern with one missing stimulus. The child was then asked to complete the pattern by selecting the missing stimulus from a set of 4 to 6 options shown. Most stimuli are abstract, geometric shapes. The difficulty of the task increases as the test progresses. The test measures reasoning abilities such as induction and deduction and fluid reasoning.	 **Figure 2.14c Pattern reasoning**

Table 2.2b Description of the cognitive tests (Additional Tests[172,200-202])

Verbal fluency[202] A neuropsychological test of language production in which the child was asked to name as many first names as possible in 1 minute. The test measures broad (long-term) retrieval ability; speed and flexibility of verbal thought process.	 **Figure 2.14d Verbal fluency**
Koh's block design[200,201] A psychometric test of intelligence in which the child was asked to reproduce a series of picture designs presented on test cards using groups of 4, 9, or 16 multi-coloured blocks. The test measures visuo-spatial problem solving ability, visual perception and organization	 **Figure 2.14e Koh's block design**
Coding-WISC-III[172] Initially examiner taught the children about substituting specific symbols for numbers presented in the boxes. Then the child was asked to substitute specific symbols corresponding to the numbers presented in the boxes, and to complete as many items as possible in 2 minutes. The test measures visual-motor processing speed and coordination, short-term memory, visual perception, visual scanning, cognitive flexibility and attention	 **Figure 2.14f Coding-WISC-III**

2.8 Maternal intelligence

Maternal intelligence was assessed using the Revised Bhatia's Short battery of Performance Tests of Intelligence for Adults[205]. It is a non-verbal performance intelligence test and provides a quick assessment of the performance intelligence and has been found to give a good approximation of the full scale IQ. It consists of Koh's Block Design (Figure 2.15a) and Passalong (Figure 2.15b) subtests. Details of the tests, instructions for administering the tests including scoring are described in appendix 2.

Figure 2.15a Koh's Block Design Test

Figure 2.15b Passalong Test

2.9 Questionnaire

2.9.1 Home environment/inventory: The Home Observation for Measurement of the Environment Inventory- Early Adolescent version[206] is designed to measure the quality and quantity of stimulation and support available to a child in the home environment. The focus is on the child in the environment and the child as a recipient of inputs from objects, events and transactions occurring in connection with the family surroundings. It consists of 60 items clustered into 7 subscales measuring physical environment, learning materials, modelling, fostering self sufficiency, regulatory activities, family companionship and acceptance. Description of these subscales, are presented in Table 2.3. The list of items used and the scoring are described in appendix 2. The home environment was assessed by a trained research assistant at the adolescent's home through a combination of direct observation and interviewing the mother in the presence of the adolescent.

Table 2.3 Description of home environment scales

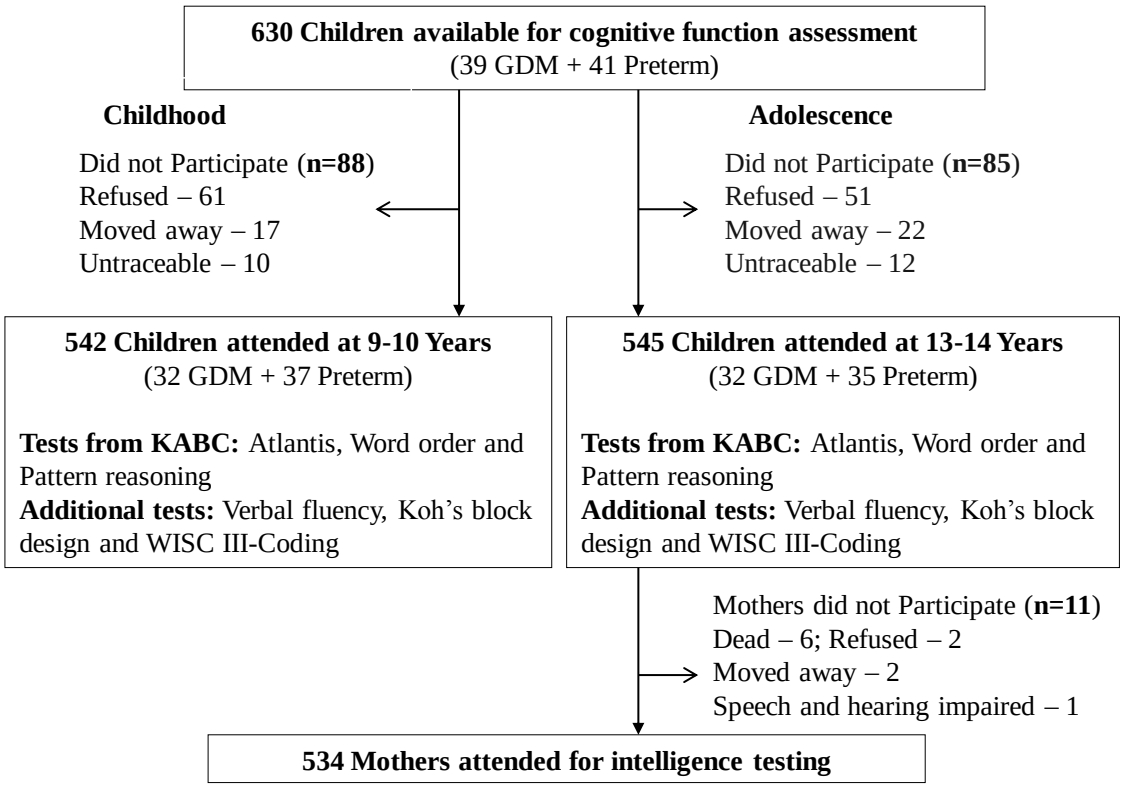
Scales	Description
1.Physical environment (7 items)	It indicates whether the environment where an adolescent resides is safe, clean, pleasing and less noisy with sufficient space and light.
2.Learning materials (10 items)	It indicates parental support for learning and development through the provision of objects/equipments/books needed to encourage skill development and problem solving ability.
3.Modeling (10 items)	It identifies mature and socially responsible behaviour of the parent making themselves as role model to the adolescent. Involves teaching via practice, not via preaching.
4.Fostering self-sufficiency (6 items)	It indicates parent's efforts in providing/training useful skills related to health, studies, lifestyle etc. thus enabling the adolescent to acquire skills to become self-sufficient.
5.Regulatory activities (10 items)	It identifies whether parent has set rules and regulations in daily routine/lifestyle to maintain an ordered household.
6.Family companionship (8 items)	It indicates the role of the family in daily routines, social outings as a group and to identify the adolescent as part of an active family.
7.Acceptance (9 items)	It identifies a positive parent-child relationship.

2.9.2 Socio-economic status: Current SES was assessed using the Standard of Living Index (SLI) designed by National Family Health Survey-2[207] which derives a score based on type and size of the house, household sanitary facilities, source of water and power supply, cooking fuel used, ownership of house/property, land, livestock and household assets (appendix 2). Current education, occupation and income of the parents' were also recorded (appendix 2).

2.10 Participation rate

Of the 630 available children, a total of 542 (86%) children underwent cognitive function assessment at the age of 9-10 years. In the current study (at age 13-14 years) 545 (86.5%) children and 534 (98%) mothers of these children took part in the study. Figure 2.16 illustrates study participants, reasons for non-participation and cognitive tests administered at both ages.

Figure 2.16 Flow diagram illustrating study participants, reasons for non-participation and cognitive tests administered during childhood and adolescence



2.11 Summary

An overview of the data collected from the children and their parents at various time points are presented in Table 2.4.

Table 2.4 Summary Table

Age (Year)	Children	Mothers	Fathers
Birth	Anthropometry, Placental morphology Gestational age, Mode of delivery Apgar score	Anthropometry Oral glucose tolerance test, Vitamin D, B12, folate and homocysteine concentrations Age, parity, religion, area of residence and education	Height and weight Education SES
1-4	Anthropometry, Infant feeding Developmental mile stones and health	None	None
5	Anthropometry, Bioimpedance, Health, Blood pressure Oral glucose tolerance test, lipid profile, haemoglobin, Vitamin B12, folate and homocysteine concentrations Methylenetetrahydrofolate reductase (MTHFR677) genotype	Anthropometry Oral glucose tolerance test, Education MTHFR677 genotype	Anthropometry Fasting glucose and insulin concentrations Education, SES MTHFR677 genotype
6-9	Anthropometry, Bioimpedance	None	None
9-10	Anthropometry, Bioimpedance, Health, Blood pressure Hand grip strength, Sexual maturity Oral glucose tolerance test, lipid profile, haemoglobin, Vitamin B12, folate, and cortisol concentrations, diet Cognitive function assessment	Anthropometry Oral glucose tolerance test, Education	Anthropometry Oral glucose tolerance test Education, SES
10-13	Anthropometry, Bioimpedance, Sexual maturity	None	None
13-14	Anthropometry, Bioimpedance, Health, Blood pressure Sexual maturity, Stress response Haemoglobin, fasting glucose, insulin, vitamin B12, folate, and salivary cortisol concentrations, lipid profile Cognitive function and behavioural assessment	Maternal intelligence Maternal temperament Home environment Education	Education SES

CHAPTER 3 COGNITIVE ABILITY DURING CHILDHOOD AND ADOLESCENCE

3.1 Introduction

As described in the introductory chapter a variety of environmental factors (socio-demographic, prenatal and postnatal) influencing neurodevelopment are determinants of childhood and adolescent cognitive function.

In this chapter I describe cognitive performance during childhood and early adolescence in the Mysore Parthenon cohort. I examine associations of socio-demographic factors, neonatal anthropometry, gestational diabetes, breast-feeding duration, childhood and adolescent body composition and postnatal growth with these outcomes. In the course of the analysis I test the following hypotheses:

- Smaller size at birth (birthweight, length, head and mid-upper-arm circumferences and sum of skinfolds) is associated with poorer cognitive ability.
- Larger body size during childhood and adolescence (head and mid-upper-arm circumferences, height and sum of skinfolds) is associated with better cognitive ability in the corresponding period.
- Greater gain in body size (head and mid-upper-arm circumferences, height and sum of skinfolds) from birth to childhood is associated with better cognitive ability, strongest for the gain in head circumference from birth to 5 years.
- Longer duration of breast-feeding is associated with better cognitive ability.
- Gestational diabetes mellitus in mothers is associated with poorer cognitive ability in their offspring.

3.2 Data preparation

3.2.1 Data cleaning: Before analysis, I checked the data for systematic errors. Missing values were identified and coded appropriately. Range checks were done to ensure that values were within the expected range.

3.2.2 Statistical methods: All analyses were carried out using Stata (version10.0). For all measurements, histograms were plotted to determine whether they were normally

distributed. The most appropriate transformations were used for variables with skewed distributions. The child's skinfolds thicknesses were log transformed (Figure 3.1a and b). For pattern reasoning score square root transformation was the most appropriate (Figure 3.2a and b). Fisher-Yates transformation (details in appendix 4) provided the best approximation of a normal distribution for Koh's block design score (Figure 3.3a and b).

Figure 3.1a Distribution of neonatal subscapular skinfold

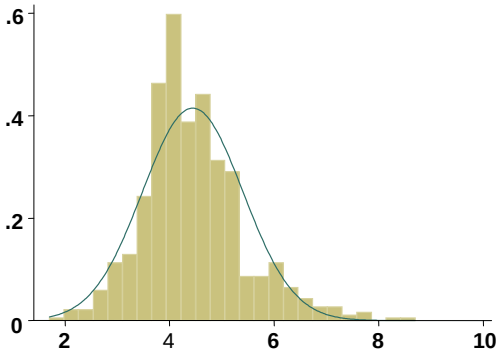


Figure 3.1b Log transformed subscapular skinfold

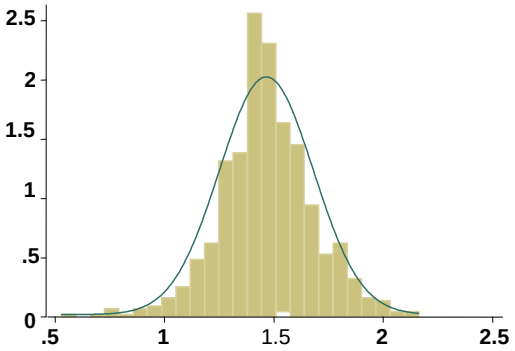


Figure 3.2a Distribution of childhood Pattern reasoning

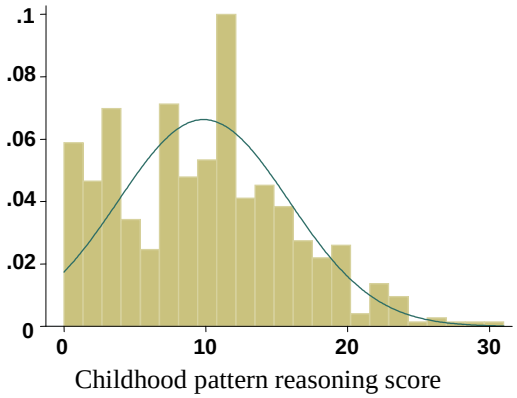


Figure 3.2b Square root transformed Pattern reasoning

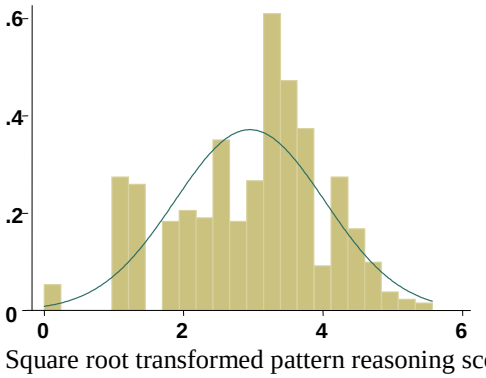


Figure 3.3a Distribution of childhood Koh's block design

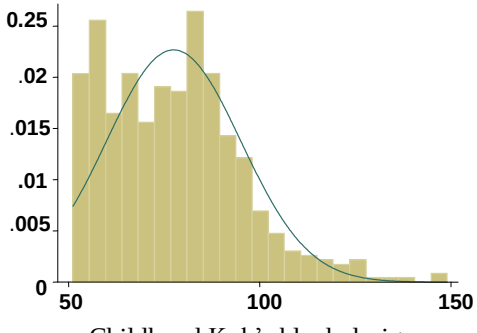
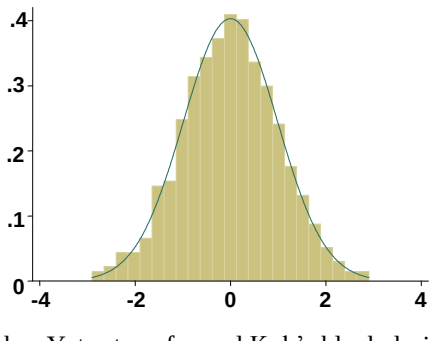


Figure 3.3b Fisher-Yates transformed Koh's block design



Differences in the means of exposure or outcome variables between two groups, such as between boys and girls and offspring of mothers with and without GDM, were compared using t-tests. Chi-Squared tests were used to test differences in proportions between groups. Correlations between continuous variables (for example cognitive test scores during childhood and adolescence) were examined using Pearson's correlation. Spearman's correlation was used to examine correlations between categorical variables (for example parity, income and occupation). Linear regression analyses were used to determine predictors of cognitive performance during childhood and adolescence, adjusting for potential confounders. In order to facilitate interpretation, internally standardized *Z*-scores ((subject's value–population mean)/standard deviation of population) of all the cognitive test scores and exposure variables were used in the regression model where appropriate and are expressed in units of standard deviations (SD).

I chose categories for duration of breast-feeding based on their meaningfulness from a public health perspective, (eg. having a bin for <3 months) and also from a statistical perspective (i.e. having enough subjects in each bin), and to reduce the effect of outliers and maintain ordering. Breast-feeding duration was split into 6 categories (<3, 3-5, 6-8, 9-11, 12-17 and 18+ months). For tabulations, mean values for cognitive scores are presented for each category. For regression models, I used the categorised duration as a continuous variable. Likelihood ratio tests were performed to examine departure from linearity of breast-feeding duration categories and cognitive function. I also examined the association between breast-feeding duration and cognitive function using breast-feeding duration in months (non-categorised) as a continuous variable. A quadratic term was used to examine a non-linear or threshold effect of breast-feeding duration.

Interaction terms were used to test for differences in the associations between various early life exposures and sex, and to examine interactions between small for gestational age (SGA)/low birthweight (LBW) and breast-feeding duration, in relation to cognitive scores. After ensuring that there was no interaction between exposure and sex in predicting cognitive ability, sexes were pooled in all analyses with adjustment for sex. The presence of confounding in the association between exposure (for example birth size, postnatal growth) and outcome was evaluated by observing the change in the effect size before and after adjustment for potential confounding factors in multiple regression analyses. Generalised estimating equation models were used to compare the associations of birth size with childhood and adolescent cognitive function.

3.2.3 Missing data: Birth size, current body composition and outcome data were available for all the children who participated at both time points. Of the early life factors, breast-feeding duration, GDM status and postnatal conditional growth data were not available for 5%, 5% and 13% of the children respectively. Since these variables are main exposures of interest and also there were no differences in birth size, socio-demographic characteristics and the cognitive test scores among those who had these data compared to those who did not, I did not consider imputing missing data, and analyses were carried out using all the available data. Of the socio-demographic factors, data for maternal intelligence (MIQ) and home environment were missing for ~7% and ~35% of the children respectively. Maternal educational attainment was greater and occupation of the main bread winner was higher, and standard of living index (SLI), sum of skinfolds at birth, reasoning and visuo-spatial ability scores during childhood were lower in those who had MIQ data compared to those who did not (appendix 5). Maternal education, and occupation and income of the main bread winner, and the scores for learning during childhood, and all the cognitive tests scores during adolescence were higher in those who had home environment data compared to those who did not (appendix 5). Furthermore in multiple regression analyses while examining the associations between early life factors and cognitive function adjusting for these factors would have reduced the sample size considerably leading to over or under estimation of the confounding effect of these factors. Therefore in order to maintain the sample size and to reduce bias I imputed home environment and MIQ data. Each of these variables was imputed by replacing the original variable with two newly constructed variables: a) a binary variable which took the value 0 if the original variable had a known value and 1 if it was missing; b) the mean value of the original variable when it was missing. In order to assess the risk of bias in my study due to missing data, I also tested for differences in the slope of predictor variables (maternal, prenatal and postnatal factors) with cognitive function in those with or without MIQ and home environment data by using regression models which included the binary 'missingness' variable and an interaction term (binary variable*predictor variable). A significant interaction signified a difference in slopes. In 2256 analyses, spanning all predictors and outcomes, and the full range of models, only 48 (2%) showed statistically significant interactions, fewer than the 5% expected by chance. I did not therefore take this further (eg. stratified analysis), and concluded that missing data were unlikely to bias my findings.

3.3 Study population

Of the 630 available children, cognitive function was assessed for 542 (261 boys) and 545 (259 boys) children during childhood (age 9-10 years) and early adolescence (age 13-14 years) respectively. Study participants in childhood were not different from non-participants in gestational age, birth measurements, sex ratio and maternal parity. Compared to parents of participants, parents of non-participants had higher SLI and better educational attainment, occupation and income ($p<0.05$ for all; data presented in appendix 6). These findings were similar during adolescence except that non-participants had larger skinfolds at birth compared to participants ($p<0.05$ for all).

3.4 Description of cognitive function

There are no standard norms for cognitive function tests scores and they were treated as continuous scores. Cognitive function test scores during childhood and adolescence are presented in Table 3.1. The mean test scores of my study children at age 9-10 years were similar to those from another Indian population of the same age (personal communication with Dr. Srinivasan, St John's Research Institute, Bangalore).

Table 3.1 Description of cognitive function

Tests of cognitive function (score)	N	Mean (SD)	Range
Childhood			
Learning, long-term retrieval/storage (Atlantis)	**542**	67.7 (17.2)	18 to 107
Short-term memory (Word order)	**542**	16.4 (2.5)	9 to 28
Reasoning ability (Pattern reasoning)*	**542**	10.0 (5.0,14.0)	0 to 31
Verbal fluency (Verbal fluency)	**542**	16.1 (4.9)	5 to 40
Visuo-spatial ability (Koh's block design)*	**542**	76.8 (63.1,88.3)	51.2 to 149.1
Attention and concentration (Coding-WISC-III)	**542**	32.7 (8.1)	8 to 61
Adolescence			
Learning, long-term retrieval/storage (Atlantis)	**545**	79.9 (14.4)	24 to 108
Short-term memory (Word order)	**545**	18.9 (3.8)	10 to 29
Reasoning ability (Pattern reasoning)	**545**	15.5 (6.6)	1 to 33
Verbal fluency (Verbal fluency)	**545**	21.3 (5.6)	9 to 42
Visuo-spatial ability (Koh's block design)	**545**	83.0 (25.9)	38.4 to 145.7
Attention and concentration (Coding-WISC-III)	**545**	47.7 (11.0)	20 to 93

*Skewed variable; values are medians (inter quartile range (IQR))

3.4.1 Correlation between childhood and adolescent cognitive function

There were positive correlations among the cognitive test scores both during childhood and adolescence (Table 3.2a), greatest for learning and reasoning, reasoning and visuo-spatial ability. There were also positive correlations between test scores during childhood and the corresponding test scores during adolescence (Table 3.2b), especially between learning, reasoning and visuo-spatial ability.

Table 3.2a Correlation coefficients (r) between cognitive function test scores during childhood and adolescence

Childhood	**Learning**	**Short-term memory**	**Reasoning**	**Verbal fluency**	**Visuo-spatial ability**	**Attention**
Learning	**1.0000**					
Short-term memory	0.34***	**1.0000**				
Reasoning	**0.45*** **	0.35***	**1.0000**			
Verbal fluency	0.23***	0.27***	0.25***	**1.0000**		
Visuo-spatial ability	0.33***	0.29***	**0.46*** **	0.15***	**1.0000**	
Attention	0.22***	0.25***	0.36***	0.32***	0.21***	**1.0000**
Adolescence	**Learning**	**Short-term memory**	**Reasoning**	**Verbal fluency**	**Visuo-spatial ability**	**Attention**
Learning	**1.0000**					
Short-term memory	0.33***	**1.0000**				
Reasoning	**0.39*** **	0.35***	**1.0000**			
Verbal fluency	0.18***	0.23***	0.28***	**1.0000**		
Visuo-spatial ability	0.26***	0.33***	**0.50*** **	0.10*	**1.0000**	
Attention	0.20***	0.21***	0.37***	0.40***	0.31***	**1.0000**

Values in red indicate greatest correlation; *p<0.05; ***p<0.001

Table 3.2b Correlation coefficients (r) between childhood and adolescence cognitive function test scores

	Childhood cognitive function					
Adolescent cognitive function	**Learning**	**Short-term memory**	**Reasoning**	**Verbal fluency**	**Visuo-spatial ability**	**Attention**
Learning	**0.61*** **	0.33***	0.40***	0.19***	0.29***	0.23***
Short-term memory	0.33***	**0.52*** **	0.33***	0.21***	0.25***	0.25***
Reasoning	0.38***	0.35***	**0.61*** **	0.33***	0.46***	0.36***
Verbal fluency	0.18***	0.22***	0.18***	**0.44*** **	0.14***	0.30***
Visuo-spatial ability	0.26***	0.29***	0.45***	0.11***	**0.62*** **	0.24***
Attention	0.20***	0.24***	0.33***	0.30***	0.26***	**0.56*** **

Values in red indicate greatest correlation; ***p<0.001

3.5 Relationship to age

The age range of the study participants during childhood was 8.9 to 10.6 years (Mean (SD) 9.7 (0.3) years). Corresponding figures during adolescence were 13.2 to 13.9; 13.5 (0.1). Age at the time of testing was positively associated with attention and concentration during childhood (β=6.0 (score) per year; p<0.001) and adolescence (β=6.8; p=0.045). There were no significant effects of age on other cognitive test scores.

3.6 Relationship to sex

Girls scored better than boys in short-term memory, reasoning, verbal fluency and attention and concentration during childhood and adolescence (Table 3.3).

Table 3.3 Cognitive performance during childhood and adolescence in boys and girls

Tests of cognitive function (score)	**Boys (n=261)**	**Girls (n=281)**	
	Mean (SD)	**Mean (SD)**	**P**
Childhood			
Learning, long-term retrieval	67.6 (17.7)	67.8 (16.7)	0.9
Short-term memory	16.1(2.5)	16.7 (2.5)	0.02
Reasoning ability*	9.0 (4.0, 13.0)	11.0 (6.0, 14.0)	0.004
Verbal fluency	14.7 (4.1)	17.4 (5.2)	<0.0001
Visuo-spatial ability*	76.9 (63.7, 87.8)	76.2 (63.1, 88.4)	0.7
Attention and concentration	30.2 (7.5)	35.1 (7.9)	<0.0001
Adolescence	**Boys (n=259)**	**Girls (n=286)**	**P**
Learning, long-term retrieval	80.1 (14.0)	79.7 (14.8)	0.8
Short-term memory	18.5 (3.6)	19.3 (4.0)	0.02
Reasoning ability	14.7 (6.4)	16.2 (6.7)	0.01
Verbal fluency	19.8 (4.6)	22.7 (6.1)	<0.0001
Visuo-spatial ability	84.7 (26.1)	81.4 (25.6)	0.1
Attention and concentration	44.3 (9.8)	50.7 (11.2)	<0.0001

*Skewed variable; values are medians (IQR); P value is for the difference between boys and girls

3.7 Relationship to socio-demographic factors

In this section I describe socio-demographic factors (parity, parents' SLI, education, occupation and income of the main bread winner, rural or urban residence, MIQ and home environment) and their relationship to cognitive function. Of the 542 and 545 children who participated during childhood and adolescence, MIQ data was available for 508 and 534 mothers respectively. Home environment data was available for 359 children, as we could not collect data for adolescents living outside Mysore. Since there were strong positive correlations of current SLI, parental education, occupation and income and with the corresponding data collected during pregnancy and childhood (ρ=0.4 to 0.9; $p<0.0001$), I used these variables, collected during childhood and adolescence, to examine their relationship with cognitive function in the corresponding period.

3.7.1 Description: The socio-demographic characteristics of the study participants during childhood and adolescence were similar. Therefore I have presented only the adolescent data (Table 3.4). About half the children were first born. Among mothers, a third had received <10 years of education, 10 years and >10 years of education. Corresponding figures for fathers were 29%, 24% and 44%. Occupation and income were divided into 5 categories. The majority of participants were urban residents. While a large proportion of mothers (26%) scored low (<70), only a small proportion (<2%) scored high (>130) in the intelligence tests.

3.7.2 Correlation between socio-demographic factors (Table 3.5): Parity was negatively correlated with maternal education and home environment. SLI, parental education, occupation, income, MIQ and home environment were positively correlated with one another.

3.7.3 Socio-demographic factors and cognitive function: Lower maternal parity, higher SLI, parental education, occupation and income, urban residence, higher MIQ and higher home environment score were associated with better cognitive function in the children (Table 3.6; Figure 3.4a and b). The strength of these associations was similar at 9.5 and 13.5 years. Strongest associations were consistently with learning, reasoning and visuo-spatial ability.

Table 3.4 Socio-demographic characteristics of study participants during adolescence

Socio-demographic characteristics	n	% or Median (IQR)
Parity		
a) 0	275	50.5
b) 1	181	33.2
c) 2+	89	16.3
Standard of living index (score)	545	39 (34-43)
Maternal education		
a) Illiterate	8	1.5
b) Primary school education	179	32.8
c) Secondary school education	173	31.7
d) Pre-university (undergraduates)	105	19.3
e) Graduates/post-graduates/professionals	80	14.7
Paternal education		
a) Illiterate	22	4.0
b) Primary school education	157	28.8
c) Secondary school education	128	23.5
d) Pre-university (undergraduates)	109	20.0
e) Graduates/post-graduates/professionals	129	23.7
Occupation of the main bread winner categories		
a) Unskilled (eg. labourer, vegetable vendor)	146	26.8
b) Semiskilled (eg. mechanic, construction worker)	85	15.6
c) Skilled (eg. clerk, cashier)	220	40.4
d) Semi-professional (eg. teacher, manager, businessmen)	76	13.9
e) Professional (eg. doctor, engineer, advocate, lecturer)	18	3.3
Income of the main bread winner categories (rupees per month)*		
a) ≤Rs. 3000	111	20.4
b) Rs. 3001-5000	124	22.8
c) Rs. 5001-10000	162	29.7
d) Rs. 10001-15000	60	11.0
e) > Rs. 15,000	88	16.2
Area of residence		
a) Rural	150	27.5
b) Urban	395	72.5
Maternal intelligence (score)	534	81 (69-96)
Home environment (score)	359	45 (41-49)

*Rs 1000= £ 10

Table 3.5 Correlation co-efficient (ρ) between socio-demographic factors

	Parity	SLI	ME	PE	Occup	Income	MIQ	HE	R/U
Parity	1								
SLI	-0.07	1							
ME	-0.14**	0.40***	1						
PE	-0.06	0.49***	0.55***	1					
Occup	-0.08	0.51***	0.49***	0.63***	1				
Income	-0.09	0.63***	0.41***	0.52***	0.56***	1			
MIQ	-0.08	0.24***	0.41***	0.25***	0.22***	0.18***	1		
HE	-0.11*	0.40***	0.45***	0.37***	0.37***	0.37***	0.17**	1	
R/U	0.09	-0.09	0.06	0.02	0.003	0.002	0.09	0.04	1

SLI-standard of living index; ME-maternal education; PE-paternal education; Occup-occupation; MIQ-maternal intelligence; HE-home environment; R/U- rural (0) /urban (1) residence
*p<0.05; **p<0.01; ***p<0.001

Table 3.6 Association between socio-demographic factors and cognitive function during childhood and adolescence

Tests of cognitive function (SD)	Socio-demographic factors (SD)								
	Parity	**SLI**	**ME**	**PE**	**Occupation**	**Income**	**Residence***	**MIQ**	**HE**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
Childhood									
Learning, long-term retrieval	-0.10 (-0.19, -0.02)†	0.22 (0.14, 0.31)§	0.21 (0.12, 0.29)§	0.23 (0.15, 0.31)§	0.15 (0.06, 0.23)‡	0.22 (0.14, 0.30)§	0.40 (0.21, 0.58)§	0.16 (0.10, 0.23)§	0.13 (0.06, 0.19)§
Short-term memory	-0.12 (-0.20, -0.04)‡	0.22 (0.14, 0.31)§	0.28 (0.19, 0.36)§	0.22 (0.13, 0.30)§	0.18 (0.09, 0.27)§	0.15 (0.07, 0.24)§	0.28 (0.09, 0.47)‡	0.15 (0.08, 0.22)§	0.09 (0.02, 0.16)†
Reasoning	-0.13 (-0.21, -0.04)‡	0.27 (0.19, 0.35)§	0.28 (0.20, 0.36)§	0.28 (0.20, 0.36)§	0.22 (0.13, 0.30)§	0.24 (0.16, 0.33)§	0.31 (0.13, 0.50)‡	0.20 (0.13, 0.27)§	0.19 (0.13, 0.26)§
Verbal fluency	-0.07 (-0.15, 0.01)	0.19 (0.10, 0.27)§	0.19 (0.11, 0.28)§	0.17 (0.09, 0.26)§	0.13 (0.04, 0.21)‡	0.09 (0.01, 0.17)†	0.03 (-0.16, 0.22)	0.05 (-0.02, 0.12)	0.11 (0.04, 0.18)‡
Visuo-spatial ability	-0.06 (-0.14, 0.02)	0.21 (0.13, 0.29)§	0.27 (0.19, 0.35)§	0.23 (0.15, 0.32)§	0.16 (0.08, 0.25)§	0.20 (0.12, 0.29)§	0.28 (0.10, 0.47)‡	0.17 (0.10, 0.24)§	0.18 (0.13, 0.25)§
Attention and Concentration	0.002 (-0.08, 0.08)	0.20 (0.12, 0.28)§	0.21 (0.13, 0.29)§	0.20 (0.11, 0.28)§	0.11 (0.02, 0.20)†	0.20 (0.11, 0.28)§	0.11 (-0.08, 0.30)	0.10 (0.03, 0.17)‡	0.10 (0.03, 0.17)‡
Adolescence									
Learning, long-term retrieval	-0.06 (-0.15, 0.02)	0.17 (0.08, 0.25)§	0.21 (0.13, 0.29)§	0.15 (0.06, 0.23)‡	0.11 (0.02, 0.19)†	0.21 (0.13, 0.30)§	0.37 (0.18, 0.56)§	0.11 (0.05, 0.18)‡	0.10 (0.03, 0.17)‡
Short-term memory	-0.06 (-0.14, 0.02)	0.11 (0.03, 0.20)‡	0.25 (0.17, 0.33)§	0.21 (0.12, 0.29)§	0.17 (0.09, 0.25)§	0.18 (0.10, 0.27)§	0.33 (0.14, 0.52)‡	0.14 (0.07, 0.20)§	0.12 (0.05, 0.19)‡
Reasoning	-0.11 (-0.19, -0.02)†	0.28 (0.20, 0.36)§	0.32 (0.23, 0.40)§	0.30 (0.22, 0.38)§	0.21 (0.13, 0.30)§	0.26 (0.18, 0.34)§	0.16 (-0.03, 0.35)	0.20 (0.14, 0.27)§	0.20 (0.13, 0.27)§
Verbal fluency	-0.03 (-0.11, 0.05)	0.08 (-0.004, 0.17)	0.14 (0.06, 0.23)‡	0.18 (0.10, 0.26)§	0.15 (0.07, 0.24)§	0.09 (0.01, 0.17)†	0.03 (-0.16, 0.21)	0.05 (-0.02, 0.12)	0.09 (0.03, 0.16)‡
Visuo-spatial ability	-0.06 (-0.14, 0.02)	0.20 (0.12, 0.28)§	0.27 (0.18, 0.35)§	0.25 (0.16, 0.33)§	0.17 (0.08, 0.25)§	0.22 (0.14, 0.31)§	0.26 (0.07, 0.45)‡	0.20 (0.14, 0.27)§	0.16 (0.10, 0.23)§
Attention and Concentration	-0.06 (-0.14, 0.02)	0.12 (0.03, 0.20)‡	0.25 (0.17, 0.34)§	0.26 (0.18, 0.34)§	0.13 (0.05, 0.22)‡	0.19 (0.11, 0.28)§	0.29 (0.11, 0.48)‡	0. 11 (0.04, 0.17)‡	0.13 (0.06, 0.20)§

SLI-standard of living index; ME: maternal education; PE: paternal education; MIQ: maternal intelligence (imputed); HE: home environment (imputed); *0-rural 1-urban; β is the unadjusted effect size in SD of the cognitive outcome per SD change in the individual socio-demographic factors, derived by linear regression using each socio-demographic factor as a continuous variable; †p<0.05; ‡p<0.01; §p<0.001

Figure 3.4 Scatter plots illustrating associations between a) standard of living index and learning and b) standard of living index and visuo-spatial ability

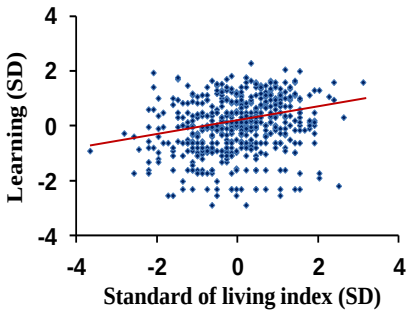


Figure 3.4 a

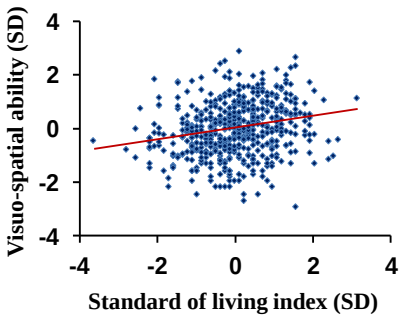
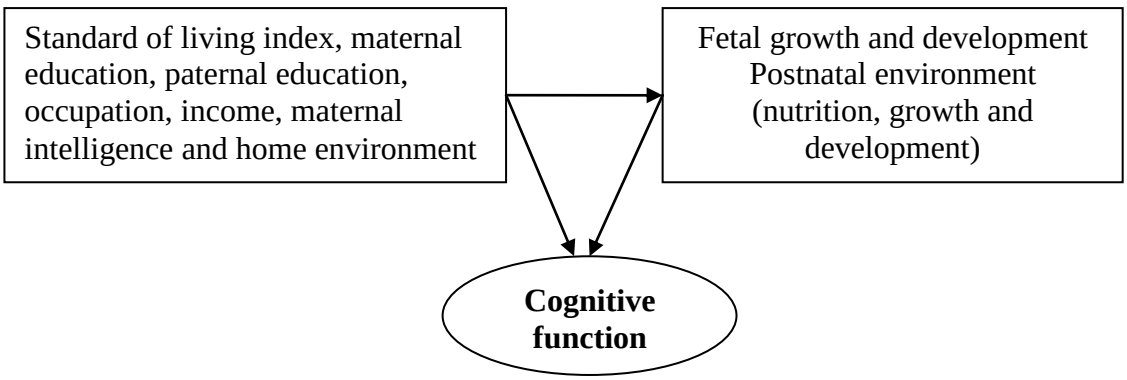


Figure 3.4 b

3.8 Relationship to early life factors

As mentioned in section 1.3.2, socio-economic factors (SLI, parental education, occupation and income), MIQ and home environment can confound, mediate or modify, partly or completely, the association between early life factors and cognitive function by acting not only through prenatal growth but also through the postnatal environment (Figure 3.5). Therefore, for each of the early life factors, I describe its relationship with socio-economic factors, and examine its associations with cognitive function adjusting for socio-demographic factors.

Figure 3.5 Possible pathways linking socio-economic status and its related factors with cognitive function



3.8.1Relationship to birth size

Cognitive test scores were similar in term born and preterm born (n=35) children and therefore all the children were included in the analyses.

Parental education, occupation, income and SLI were positively associated with weight, length, head circumference (HC), mid-upper-arm circumference (MUAC) and sum of skinfolds (SS) at birth ($p<0.05$ for all).

I examined the relationship between birth size and cognitive function, initially adjusting for the child's sex, gestational age and age at the time of cognitive function assessment. I explored this relationship further after controlling for GDM status since neonatal measurements were higher in offspring of GDM mothers[191].

3.8.1.1 Neonatal anthropometry of the study participants (Table 3.7): Boys were longer and had larger HC at birth compared to girls; girls had larger skinfolds and longer gestational age than boys. Other neonatal measurements and prevalence of preterm births were similar in boys and girls.

Table 3.7 Birth characteristics of boys and girls who participated for cognitive function assessment

	Participants during adolescence		
	Boys (n=259)	**Girls (n=286)**	**P**
Neonatal anthropometry	**Mean (SD)**	**Mean (SD)**	
Birthweight (g)	2894 (467)	2832 (432)	0.1
Crown-heel length (cm)	48.9 (2.3)	48.4 (2.2)	0.02
Head circumference (cm)	34.1 (1.4)	33.5 (1.3)	<0.0001
Mid-upper-arm circumference (cm)	10.3 (1.0)	10.2 (1.0)	0.8
Triceps skinfold thickness (mm)*	4.0 (3.5, 4.5)	4.2 (3.7, 4.8)	0.006
Subscapular skinfold thickness (mm)*	4.2 (3.8, 4.7)	4.4 (3.9, 5.0)	0.02
Sum of skinfolds (mm)*	8.2 (7.4, 9.2)	8.6 (7.6, 9.7)	0.007
Gestational age (weeks)	38.9 (1.8)	39.2 (1.6)	0.02
Preterm No (%)	20 (7.7)	15 (5.2)	0.2
Offspring of GDM mothers No (%)	9 (3.6)	23 (8.5)	0.02

*Skewed variable; values are medians (IQR); P value is for the difference between boys and girls

3.8.1.2 Birth size and cognitive performance: Childhood cognitive test scores according to quartiles of birth measurements are described in Table 3.8. Adjusted for the child's sex, gestational age and current age all the cognitive test scores, except verbal fluency and attention and concentration, increased with increasing birthweight, length, HC, and MUAC (Table 3.8). The strongest associations were with learning and visuo-spatial ability and HC was the strongest predictor. Although there was no clear trend of reasoning score across quartiles of HC, there was a positive association between HC (as a continuous variable in regression analysis) and reasoning ability. There were positive associations of SS with learning and visuo-spatial ability.

The effects of these associations adjusted for confounders are presented in Table 3.9. In models that successively added GDM, socio-economic indicators, MIQ and home environment, these associations were attenuated, and coefficients were approximately halved. However newborn HC remained a significant predictor of learning and visuo-spatial ability. Interestingly, of the other birth measurements, MUAC was the most strongly related to cognitive function.

The findings appeared similar for adolescent cognitive function (Table 3.10 and 3.11). Formal testing using generalised estimating equations confirmed that there were no differences in the associations of birth size with cognitive function between the two time points except that the associations of birthweight and birth length with verbal fluency were stronger during adolescence than during childhood ($p<0.05$ for both). During adolescence, newborn HC remained a significant predictor of reasoning and verbal fluency.

Table 3.8 Mean cognitive test scores during childhood according to quartiles of birth measurements

		Mean cognitive test scores according to quartiles of birth measurements					
Birth measurements	**n**	**Learning, long-term retrieval (score)**	**Short-term memory (score)**	**Reasoning ability* (score)**	**Verbal fluency (score)**	**Visuo-spatial ability* (score)**	**Attention and concentration (score)**
Birthweight (kg)							
<2.6	138	66.2	16.2	9.0	16.2	72.8	31.5
-2.86	135	68.7	16.5	10.0	16.3	76.6	33.1
-3.15	134	64.9	16.2	9.5	15.9	77.5	32.8
>3.16	135	70.9	16.7	11.0	16.0	80.0	33.4
P*		**0.006**	0.09	**0.04**	0.7	**0.001**	0.3
Crown-heel length (cm)							
<47.5	144	65.1	16.1	9.0	16.5	72.2	32.3
-48.7	140	69.9	16.5	10.0	16.0	77.6	33.5
-50.0	137	66.7	16.6	11.0	16.0	79.0	32.2
>50.0	121	69.4	16.6	10.0	15.9	74.3	33.0
P*		**0.03**	0.064	0.060	0.9	**0.04**	0.5
Head circumference (cm)							
<33.0	142	66.5	16.1	10.0	16.4	71.8	32.6
-33.9	140	66.2	16.4	11.0	16.4	78.8	33.2
-34.6	132	66.4	16.5	9.0	15.6	76.9	32.3
>34.6	128	72.0	16.5	10.0	16.1	81.3	32.7
P*		**<0.001**	**0.009**	**0.04**	0.7	**0.001**	0.6
Mid-upper-arm circumference (cm)							
<9.6	137	64.1	16.1	10.0	16.0	73.0	31.8
-10.3	155	67.3	16.8	9.0	16.2	76.6	32.6
-10.9	119	67.9	15.9	10.0	15.7	75.6	31.9
>10.9	131	71.8	16.8	11.0	16.5	82.0	34.5
P*		**<0.001**	0.4	**0.03**	0.5	**0.001**	0.1
Sum of skinfold thickness (mm)							
<7.5	136	64.5	16.5	9.0	16.2	74.1	31.2
-8.3	146	68.2	16.1	10.0	16.2	76.9	32.6
-9.4	130	69.1	16.4	10.0	15.2	73.7	33.3
>9.4	130	69.1	16.7	11.0	16.8	81.6	33.9
P*		**0.02**	1.0	0.073	0.5	**0.001**	0.3

*Skewed variable; values are medians; †P value for trend derived (using birth measurements as continuous variable) by multiple linear regression adjusted for the child's sex, gestational age and current age.

Table 3.9 Association between birth size and childhood cognitive function: Multiple regression analyses adjusted for socio-demographic factors

Birth measurements (SD)	n	Cognitive test scores (SD)					
		Learning, long-term retrieval	Short-term memory	Reasoning ability	Verbal fluency	Visuo-spatial ability	Attention and concentration
		β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Birthweight							
Model 1	**542**	0.14 (0.04, 0.24)**	0.09 (-0.01, 0.19)	0.11 (0.01, 0.20)*	-0.02 (-0.05, 0.15)	0.17 (0.07, 0.27)**	0.05 (-0.05, 0.14)
Model 2	**515**	0.09 (-0.01, 0.20)	0.07 (-0.04, 0.18)	0.10 (-0.01, 0.20)	-0.04 (-0.14, 0.06)	0.16 (0.06, 0.26)**	0.04 (-0.06, 0.14)
Model 3	**514**	0.06 (-0.05, 0.16)	0.03 (-0.07, 0.14)	0.04 (-0.06, 0.14)	-0.08 (-0.18, 0.02)	0.12 (0.02, 0.22)*	0.006 (-0.11, 0.09)
Model 4	**514**	0.05 (-0.05, 0.15)	0.03(-0.07, 0.13)	0.03 (-0.06, 0.13)	-0.08 (-0.18, 0.02)	0.11 (0.01, 0.21)*	0.004 (-0.09, 0.10)
Model 5	**514**	0.05 (-0.05, 0.15)	0.03 (-0.07, 0.13)	0.03 (-0.07, 0.12)	-0.08 (-0.18, 0.02)	0.10 (0.005, 0.21)*	-0.001 (-0.10, 0.10)
Crown-heel length							
Model 1	**542**	0.11 (0.01, 0.21)*	0.09 (-0.01, 0.19)	0.09 (-0.004, 0.19)	-0.01 (-0.10, 0.09)	0.10 (0.01, 0.20)*	0.03 (-0.06, 0.12)
Model 2	**515**	0.07 (-0.03, 0.17)	0.08 (-0.02, 0.18)	0.08 (-0.02, 0.18)	-0.02 (-0.12, 0.08)	0.09 (-0.02, 0.19)	0.03 (-0.07, 0.12)
Model 3	**514**	0.05(-0.05, 0.15)	0.07 (-0.03, 0.17)	0.05 (-0.04, 0.15)	-0.04 (-0.14, 0.06)	0.07 (-0.02, 0.17)	0.01 (-0.08, 0.10)
Model 4	**514**	0.05(-0.05, 0.15)	0.07 (-0.03, 0.17)	0.06 (-0.04, 0.15)	-0.04 (-0.14, 0.06)	0.07 (-0.02, 0.17)	0.01 (-0.08, 0.10)
Model 5	**514**	0.05(-0.05, 0.15)	0.07 (-0.03, 0.17)	0.05 (-0.04, 0.14)	-0.05 (-0.15, 0.05)	0.07 (-0.03, 0.16)	0.002 (-0.09, 0.10)
Head circumference							
Model 1	**542**	0.21 (0.10, 0.31)***	0.14 (0.04, 0.24)**	0.11 (0.01, 0.21)*	0.02 (-0.08, 0.12)	0.18 (0.08, 0.28)**	0.03 (-0.07, 0.13)
Model 2	**515**	0.18 (0.07, 0.29)**	0.15 (0.04, 0.25)**	0.13 (0.02, 0.23)*	0.02 (-0.09, 0.12)	0.18 (0.08, 0.29)**	0.03 (-0.07, 0.14)
Model 3	**514**	0.13 (0.03, 0.24)*	0.10 (-0.005, 0.21)	0.06 (-0.05, 0.16)	-0.03 (-0.14, 0.07)	0.13 (0.03, 0.24)*	-0.01 (-0.11, 0.09)
Model 4	**514**	0.14 (0.03, 0.24)*	0.10 (-0.001, 0.21)	0.06 (-0.04, 0.17)	-0.03 (-0.14, 0.07)	0.14 (0.03, 0.24)*	-0.01 (-0.11, 0.09)
Model 5	**514**	0.13 (0.03, 0.24)*	0.10 (-0.001, 0.21)	0.07 (-0.04, 0.17)	-0.03 (-013, 0.08)	0.14 (0.03, 0.24)*	-0.01 (-0.11, 0.09)
Mid-upper-arm circumference							
Model 1	**542**	0.20 (0.11, 0.30)***	0.04 (-0.05, 0.14)	0.11 (0.01, 0.20)*	0.03 (-0.06, 0.13)	0.16 (0.07, 0.26)**	0.07 (-0.02, 0.16)
Model 2	**515**	0.17 (0.07, 0.27)**	0.03 (-0.07, 0.13)	0.10 (-0.004, 0.20)	0.02 (-0.08, 0.12)	0.16 (0.06, 0.26)**	0.07 (-0.03, 0.16)
Model 3	**514**	0.12 (0.03, 0.22)*	-0.02 (-0.12, 0.08)	0.04 (-0.06, 0.14)	-0.02 (-0.11, 0.08)	0.11 (0.01, 0.21)*	0.03 (-0.06, 0.13)
Model 4	**514**	0.12 (0.03, 0.22)*	-0.02 (-0.12, 0.08)	0.04 (-0.06, 0.13)	-0.02 (-0.11, 0.08)	0.11 (0.01, 0.20)*	0.03 (-0.06, 0.13)
Model 5	**514**	0.12 (0.02, 0.21)*	-0.02 (-0.12, 0.08)	0.02 (-0.07, 0.12)	-0.02 (-0.12, 0.08)	0.10 (0.005, 0.20)*	0.03 (-0.06, 0.12)
Sum of skinfold thickness							
Model 1	**542**	0.11 (0.02, 0.20)*	-0.0004 (-0.09, 0.09)	0.08 (-0.01, 0.17)	-0.03 (-0.12, 0.06)	0.15 (0.06, 0.24)**	0.04 (-0.04, 0.13)
Model 2	**515**	0.06 (-0.03, 0.16)	-0.02 (-0.12, 0.07)	0.07 (-0.02, 0.17)	-0.06 (-0.16, 0.03)	0.13 (0.03, 0.23)**	0.04 (-0.05, 0.13)
Model 3	**514**	0.04 (-0.05, 0.14)	-0.05 (-0.14, 0.05)	0.03 (-0.06, 0.13)	-0.09 (-0.18, 0.004)	0.10 (0.004, 0.19)*	0.02 (-0.07, 0.11)
Model 4	**514**	0.05 (-0.05, 0.14)	-0.05 (-0.14, 0.05)	0.03 (-0.06, 0.12)	-0.09 (-0.18, 0.01)	0.10 (0.003, 0.19)*	0.02 (-0.07, 0.11)
Model 5	**514**	0.04 (-0.05, 0.14)	-0.05 (-0.14, 0.05)	0.02 (-0.07, 0.12)	-0.09 (-0.18, 0.003)	0.09 (-0.001, 0.19)	0.02 (-0.07, 0.11)

β is the effect size (SD) of the cognitive test score per SD change in the birth size; *p<0.05; **p<0.01;***p<0.001

Model 1: adjusted for the child's sex, gestational age and current age; **Model 2**: Model 1 + maternal gestational diabetes mellitus status; **Model 3**: Model 2 + SLI, maternal and paternal education, occupation, and income; **Model 4:** Model 3 + maternal intelligence (imputed); **Model 5:** Model 4 + home environment (imputed)

Table 3.10 Mean cognitive test scores during adolescence according to quartiles of birth measurements

		Mean cognitive test scores according to quartiles of birth measurements					
Birth measurements	**n**	**Learning, long-term retrieval (score)**	**Short-term memory (score)**	**Reasoning ability (score)**	**Verbal fluency (score)**	**Visuo-spatial ability (score)**	**Attention and concentration (score)**
Birthweight (kg)							
<2.6	138	78.5	18.2	14.5	20.9	77.7	45.9
-2.86	137	80.4	19.0	15.5	21.0	83.5	48.4
-3.15	135	78.7	19.0	15.2	21.6	82.4	47.1
>3.16	135	82.0	19.6	16.7	21.9	88.5	49.4
P*		**0.04**	**0.004**	**0.02**	**0.03**	**0.007**	0.06
Crown-heel length (cm)							
<47.5	143	80.3	18.2	14.6	21.0	78.0	46.7
-48.7	144	79.3	19.3	15.8	20.9	84.5	48.6
-50.0	122	80.5	19.1	16.0	21.4	86.8	48.0
>50.0	136	79.5	19.2	15.7	22.0	83.3	47.4
P*		0.8	**0.007**	0.065	0.066	0.2	0.3
Head circumference (cm)							
<33.0	148	79.1	18.6	14.9	20.8	79.8	47.7
-33.9	137	78.3	19.1	15.9	21.9	81.5	48.7
-34.6	125	81.4	18.9	15.1	21.1	82.9	45.8
>34.6	135	80.9	19.1	16.2	21.6	88.0	48.3
P*		**0.03**	**0.02**	**0.004**	**0.02**	**0.007**	0.3
Mid-upper-arm circumference (cm)							
<9.6	143	77.2	18.5	14.6	21.2	79.6	45.8
-10.3	156	78.5	18.7	15.3	21.3	83.5	48.4
-10.9	119	80.3	19.1	15.3	20.8	82.2	47.1
>10.9	127	84.	19.5	16.9	21.9	86.9	49.4
P*		**<0.001**	**0.004**	**0.007**	0.4	**0.04**	**0.02**
Sum of skinfold thickness (mm)							
<7.5	139	78.1	18.6	14.9	20.6	80.1	45.4
-8.3	134	80.8	18.9	15.0	21.6	82.5	48.1
-9.4	143	80.2	19.1	16.0	21.1	86.0	48.4
>9.4	129	80.6	19.1	16.0	22.2	83.3	48.9
P*		0.06	0.4	0.1	0.2	0.3	**0.04**

*P value for trend derived (using birth measurements as continuous variable) by multiple linear regression adjusted for the child's sex, gestational age and current age.

Table 3.11 Association between birth size and adolescent cognitive function: Multiple regression analyses adjusted for socio-demographic factors

		Cognitive test scores (SD)					
Birth measurements (SD)	**n**	**Learning, long-term retrieval**	**Short-term memory**	**Reasoning ability**	**Verbal fluency**	**Visuo-spatial ability**	**Attention and concentration**
		β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
Birthweight							
Model 1	**545**	0.10 (0.01, 0.20)*	0.15 (0.05, 0.24)**	0.12 (0.02, 0.22)*	0.10 (0.01, 0.20)*	0.14 (0.04, 0.23)**	0.09 (-0.004, 0.18)
Model 2	**518**	0.07 (-0.04, 0.17)	0.11 (0.003, 0.21)*	0.11 (0.01, 0.22)*	0.11 (0.01, 0.21)*	0.12 (0.02, 0.23)*	0.11 (0.01, 0.21)*
Model 3	**518**	0.04 (-0.07, 0.14)	0.08 (-0.03, 0.17)	0.05 (-0.05, 0.15)	0.09 (-0.01, 0.19)	0.08 (-0.02, 0.18)	0.08 (-0.02, 0.18)
Model 4	**518**	0.04 (-0.07, 0.14)	0.07 (-0.03, 0.17)	0.04 (-0.05, 0.14)	0.09 (-0.01, 0.19)	0.07 (-0.03, 0.17)	0.08 (-0.02, 0.18)
Model 5	**518**	0.03 (-0.08, 0.13)	0.07 (-0.04, 0.17)	0.04 (-0.06, 0.14)	0.09 (-0.01, 0.19)	0.07 (-0.03, 0.17)	0.07 (-0.03, 0.17)
Crown-heel length							
Model 1	**545**	0.01 (-0.09, 0.12)	0.14 (0.04, 0.24)**	0.09 (-0.01, 0.19)	0.09 (-0.01, 0.19)	0.07 (-0.03, 0.17)	0.06 (-0.04, 0.15)
Model 2	**518**	-0.02 (-0.12, 0.09)	0.12 (0.02, 0.22)*	0.08 (-0.02, 0.19)	0.10 (-0.003, 0.20)	0.05 (-0.06, 0.15)	0.06 (-0.04, 0.16)
Model 3	**518**	-0.03 (-0.13, 0.08)	0.11 (0.01, 0.21)*	0.05 (-0.04, 0.15)	0.08 (-0.02, 0.18)	0.03 (-0.07, 0.13)	0.05 (-0.05, 0.14)
Model 4	**518**	-0.03 (-0.13, 0.07)	0.11 (0.01, 0.21)*	0.05 (-0.04, 0.15)	0.08 (-0.02, 0.18)	0.03 (-0.07, 0.13)	0.05 (-0.05, 0.14)
Model 5	**518**	-0.03 (-0.13, 0.07)	0.11 (0.004, 0.21)*	0.05 (-0.05, 0.14)	0.08 (-0.02, 0.18)	0.03 (-0.07, 0.13)	0.04 (-0.06, 0.14)
Head circumference							
Model 1	**545**	0.12 (0.01, 0.22)*	0.13 (0.02, 0.23)*	0.15 (0.05, 0.26)**	0.12 (0.02, 0.23)*	0.14 (0.04, 0.25)**	0.05 (-0.04, 0.15)
Model 2	**518**	0.10 (-0.005, 0.21)	0.11 (0.01, 0.22)*	0.16 (0.05, 0.27)**	0.13 (0.03, 0.23)*	0.14 (0.03, 0.25)*	0.07-0.03, 0.17)
Model 3	**518**	0.07 (-0.04, 0.18)	0.07 (-0.03, 0.18)	0.10 (-0.01, 0.20)	0.10 (-0.004, 0.20)	0.09 (-0.02, 0.19)	0.03 (-0.07, 0.13)
Model 4	**518**	0.07 (-0.03, 0.18)	0.08 (-0.03, 0.18)	0.10 (-0.001, 0.20)	0.10 (-0.003, 0.21)	0.09 (-0.01, 0.20)	0.03 (-0.07, 0.13)
Model 5	**518**	0.07 (-0.04, 0.17)	0.08 (-0.03, 0.18)	0.10 (0.004, 0.20)*	0.10 (0.0004, 0.21)*	0.09 (-0.01, 0.19)	0.03 (-0.07, 0.13)
Mid-upper-arm circumference							
Model 1	**545**	0.20 (0.10, 0.29)***	0.14 (0.04, 0.23)**	0.13 (0.04, 0.22)**	0.04 (-0.05, 0.13)	0.10 (0.01, 0.19)*	0.10 (0.01, 0.19)*
Model 2	**518**	0.17 (0.07, 0.27)**	0.11 (0.01, 0.21)*	0.12 (0.02, 0.22)*	0.05 (-0.05, 0.14)	0.09 (-0.01, 0.19)	0.12 (0.03, 0.22)*
Model 3	**518**	0.15 (0.05, 0.24)**	0.08 (-0.01, 0.18)	0.07 (-0.02, 0.16)	0.03 (-0.07, 0.13)	0.05 (-0.04, 0.15)	0.09 (0.002, 0.19)*
Model 4	**518**	0.14 (0.05, 0.24)**	0.08 (-0.02, 0.17)	0.07 (-0.03, 0.16)	0.03 (-0.07, 0.12)	0.05 (-0.05, 0.14)	0.09 (0.0001, 0.18)*
Model 5	**518**	0.13 (0.04, 0.23)**	0.07 (-0.02, 0.17)	0.06 (-0.03, 0.16)	0.03 (-0.07, 0.12)	0.04 (-0.05, 0.14)	0.09 (-0.001, 0.18)
Sum of skinfold thickness							
Model 1	**545**	0.09 (-0.004, 0.18)	0.04 (-0.05, 0.13)	0.07 (-0.02, 0.17)	0.05 (-0.04, 0.14)	0.05 (-0.04, 0.15)	0.09 (0.01, 0.18)*
Model 2	**518**	0.06 (-0.04, 0.16)	-0.002 (-0.10, 0.10)	0.06 (-0.04, 0.16)	0.06 (-0.04, 0.15)	0.04 (-0.06, 0.14)	0.12 (0.02, 0.21)*
Model 3	**518**	0.04 (-0.06, 0.14)	-0.03 (-0.12, 0.07)	0.02 (-0.07, 0.11)	0.04 (-0.05, 0.14)	0.05 (-0.09, 0.10)	0.09 (0.003, 0.19)*
Model 4	**518**	0.04 (-0.06, 0.14)	-0.03 (-0.12, 0.07)	0.02 (-0.07, 0.11)	0.04 (-0.05, 0.14)	0.005 (-0.09, 0.10)	0.09 (0.003, 0.19)*
Model 5	**518**	0.03(-0.07, 0.12)	-0.03 (-0.12, 0.07)	0.02 (-0.07, 0.11)	0.04 (-0.05, 0.14)	0.001 (-0.09, 0.10)	0.09 (0.001, 0.18)*

β is the effect size (SD) of the cognitive test score per SD change in the birth size; *p<0.05; **p<0.01;***p<0.001

Model 1: adjusted for the child's sex, gestational age and current age; **Model 2**: Model 1 + maternal gestational diabetes mellitus status; **Model 3**: Model 2 + SLI, maternal and paternal education, occupation, and income; **Model 4:** Model 3 + maternal intelligence (imputed); **Model 5:** Model 4 + home environment (imputed)

3.8.2 Relationship to maternal gestational diabetes

Of the 542 children studied during childhood, GDM status was available for 515 children and 32 (6.2%; 11 boys and 21 girls) of them were offspring of GDM mothers. Corresponding figures for the 545 children who were studied during adolescence were 518 and 32 (6.2%; 9 boys and 23 girls). Compared to non-GDM mothers, GDM mothers had better educational attainment (p=0.001) and higher SLI (not significant).

Childhood and adolescent cognitive scores were higher in offspring of GDM mothers compared to offspring of non-GDM mothers; these differences were statistically significant at both ages for learning, long-term retrieval/storage, and reasoning (Table 3.12). These differences were large (up to half a standard deviation) and mostly remained significant after adjusting for the child's sex and birthweight. They were no longer significant after controlling for SLI, parental education, occupation, income, MIQ and home environment (Table 3.12). However, they were only just non-significant for learning, and there was relatively little attenuation of the coefficient. Among controls, either unadjusted or adjusted for potential confounders, maternal fasting and 120-minutes glucose concentrations (used as continuous variables in regression analysis) were unrelated to cognitive function at both time points (data not shown).

Table 3.12 Association between maternal gestational diabetes mellitus and cognitive performance during childhood and adolescence

	Mean cognitive test scores according to maternal GDM status			Multiple regression analyses				
Tests of cognitive function	**Offspring of GDM mothers**	**Controls**	**P**	**β[1] (95% CI)**	**β[2] (95% CI)**	**β[3] (95% CI)**	**β[4] (95% CI)**	**β[5] (95% CI)**
Childhood	**n=32**	**n=483**						
Learning, long-term retrieval	75.9	67.5	0.008	0.49 (0.13, 0.85)**	0.43 (0.07, 0.80)*	0.36 (0.01, 0.72)*	0.38 (0.03, 0.74)*	0.35 (-0.001, 0.71)
Short-term memory	17.0	16.4	0.2	0.18 (-0.18, 0.55)	0.12 (-0.25, 0.49)	0.002 (-0.35, 0.36)	0.02 (-0.34, 0.38)	0.01 (-0.35, 0.37)
Reasoning ability†	12.5	10.0	0.02	0.38 (0.02, 0.74)*	0.28 (-0.09, 0.65)	0.22 (-0.13, 0.57)	0.25 (-0.09, 0.60)	0.19 (-0.15, 0.54)
Verbal fluency	18.2	16.0	0.01	0.37 (0.02, 0.72)*	0.37 (0.02, 0.73)*	0.32 (-0.03, 0.68)	0.32 (-0.03, 0.67)	0.29 (-0.07, 0.64)
Visuo-spatial ability†	85.3	76.2	0.07	0.33 (-0.03, 0.79)	0.19 (-0.17, 0.56)	0.12 (-0.23, 0.48)	0.15 (-0.21, 0.50)	0.11 (-0.24, 0.46)
Attention and concentration	36.8	32.4	0.003	0.46 (0.11, 0.80)**	0.37 (0.02, 0.73)*	0.30 (-0.04, 0.64)	0.31 (-0.03, 0.65)	0.27 (-0.07, 0.61)
Adolescence	**n=32**	**n=486**						
Learning, long-term retrieval	85.9	79.6	0.02	0.45 (0.08, 0.81)*	0.41 (0.04, 0.78)*	0.32 (-0.05, 0.69)	0.30 (-0.06, 0.68)	0.28 (-0.08, 0.65)
Short-term memory	21.0	18.8	0.002	0.54 (0.18, 0.90)**	0.46 (0.09, 0.82)**	0.32 (-0.04, 0.68)	0.32 (-0.03, 0.68)	0.30 (-0.05, 0.66)
Reasoning ability	17.8	15.4	0.04	0.33 (-0.03, 0.70)	0.25 (-0.12, 0.62)	0.15 (-0.20, 0.50)	0.15 (-0.19, 0.50)	0.12 (-0.22, 0.46)
Verbal fluency	22.7	21.2	0.2	0.15 (-0.20, 0.50)	0.05 (-0.31, 0.40)	-0.02 (-0.38, 0.34)	-0.03 (-0.38, 0.33)	-0.05 (-0.41, 0.31)
Visuo-spatial ability	87.1	82.8	0.4	0.19 (-0.17, 0.55)	0.08 (-0.29, 0.45)	-0.03 (-0.39, 0.32)	-0.01 (-0.36, 0.34)	-0.04 (-0.39, 0.32)
Attention and concentration	49.6	47.4	0.3	0.08 (-0.27, 0.43)	-0.04 (-0.39, 0.32)	-0.14 (-0.49, 0.20)	-0.14 (-0.49, 0.21)	-0.18 (-0.52, 0.16)

†Skewed variable; values are medians; GDM-gestational diabetes mellitus; P value for the difference in cognitive test scores between offspring of GDM mothers and non-GDM mothers derived using t test. β (SD) is the difference in cognitive test score between offspring of GDM mothers and non-GDM mothers [1]adjusted for the child's sex; [2]adjusted for the child's sex and birthweight; [3]parameters in 2 + child's current age, SLI, parental education, occupation and income (**n=514 childhood; n=518 adolescence**); [4]parameters in 3 + maternal intelligenc (imputed) (**n=514 childhood; n=518 adolescence**); [5]parameters in 4 + home environment (imputed) (**n=514 childhood; n=518 adolescence**); *p<0.05; **p<0.01

3.8.3 Relationship to breast-feeding

The main breast-feeding exposure was total duration of breast-feeding in months. Of the 542 and 545 participants during childhood and adolescence, breast-feeding duration was available for 514 and 517 respectively. All the children were initially breast-fed, and very few stopped breast-feeding before the age of 3 months (Table 3.13). The mode for duration of breast-feeding was 12-17 months, similar in participants at both time points.

There were no associations between socio-demographic factors and breast-feeding duration.

Duration of breast-feeding (categories or continuous variable) was unrelated to cognitive ability at both time points (Table 3.13). There was some indication of a threshold effect for some tests (for example scores for short-term memory and visuo-spatial ability tended to increase with increase in the duration of breast-feeding until 6-8 months and then decreased with increasing breast-feeding duration beyond 8 months). In others, there was no consistent pattern. However, the findings did not change even when I examined the association between breast-feeding duration and cognitive function by dividing the breast-feeding duration into 2 categories (1-breast-feeding duration ≤8 months and 2-breast-feeding duration >8 months). Formal testing confirmed no non-linear associations between breast-feeding duration and cognitive function.

Since some studies reported stronger associations of breast-feeding duration with later cognitive performance among SGA children (gestation and sex specific birthweight<10th percentile)[137] or LBW children (birthweight<2500 g)[138], I repeated the analyses limiting the sample to SGA children, and separately to LBW children. Once again, there were no associations between breast-feeding duration and cognitive measures in these groups and there were no interactions between LBW/SGA with breast-feeding duration in predicting cognitive function, at either time point.

Table 3.13 Association of duration of breast-feeding with cognitive function during childhood and adolescence

	Mean cognitive test scores according to breast-feeding duration categories						Multiple regression analyses			
Tests of cognitive function	**<3 Months**	**3-5 Months**	**6-8 Months**	**9-11 Months**	**12-17 Months**	**18+ Months**	**β[1] (95% CI)**	**β[2] (95% CI)**	**β[3] (95% CI)**	**β[4] (95% CI)**
Childhood	**n=12**	**n=40**	**n=49**	**n=77**	**n=235**	**n=101**				
Learning, long-term retrieval	62.5	67.8	68.3	67.8	66.9	68.7	0.02 (-0.05, 0.09)	0.01 (-0.05, 0.08)	0.01 (-0.06, 0.08)	0.01 (-0.05, 0.08)
Short-term memory	15.7	16.4	16.9	16.2	16.2	16.6	0.003 (-0.07, 0.07)	0.002 (-0.06, 0.07)	0.002 (-0.06, 0.07)	0.003 (-0.06, 0.07)
Reasoning ability*	7.5	9.0	11.0	11.0	10.0	10.0	-0.0004 (-0.07, 0.07)	-0.007 (-0.07, 0.06)	-0.002 (-0.07, 0.06)	0.002 (-0.06, 0.07)
Verbal fluency	14.3	16.1	15.3	16.8	16.4	15.5	0.01 (-0.06, 0.08)	0.006 (-0.06, 0.07)	0.006 (-0.06, 0.07)	0.007 (-0.06, 0.07)
Visuo-spatial ability*	71.1	84.7	81.8	80.3	75.0	74.3	-0.05 (-0.12, 0.02)	-0.05 (-0.11, 0.02)	-0.04 (-0.11, 0.02)	-0.04 (-0.10, 0.03)
Attention and concentration	29.9	30.8	32.8	31.9	33.2	32.6	0.05 (-0.02, 0.11)	0.04 (-0.02, 0.11)	0.05 (-0.02, 0.11)	0.05 (-0.01, 0.11)
Adolescence	**n=12**	**n=41**	**n=50**	**n=76**	**n=236**	**n=102**				
Learning, long-term retrieval	72.9	82.1	78.5	80.4	80.0	79.5	0.01 (-0.06, 0.08)	0.006 (-0.06, 0.07)	0.001 (-0.07, 0.07)	0.003 (-0.06, 0.07)
Short-term memory	17.8	19.7	19.3	18.5	18.9	19.0	-0.01 (-0.08, 0.06)	-0.02 (-0.09, 0.05)	-0.02 (-0.09, 0.05)	-0.02 (-0.09, 0.05)
Reasoning ability	12.9	13.3	16.5	16.0	15.9	15.1	0.04 (-0.03, 0.11)	0.03 (-0.03, 0.10)	0.03 (-0.03, 0.09)	0.03 (-0.03, 0.10)
Verbal fluency	19.9	20.9	21.1	22.2	21.7	20.2	-0.01 (-0.08, 0.06)	-0.01 (-0.08, 0.05)	-0.02 (-0.08, 0.05)	-0.02 (-0.08, 0.05)
Visuo-spatial ability	86.5	82.4	84.0	84.7	82.3	83.9	-0.008 (-0.08, 0.06)	-0.006 (-0.07, 0.06)	-0.01 (-0.08, 0.06)	-0.01 (-0.07, 0.06)
Attention and concentration	48.1	45.3	49.0	44.9	48.9	47.3	0.04 (-0.03, 0.10)	0.04 (-0.03, 0.10)	0.03 (-0.04, 0.09)	0.03 (-0.03, 0.09)

*Skewed variable; values are medians. β (SD) is the effect size of cognitive test score per category change in breast-feeding duration (assuming a linear trend across breast-feeding duration categories) derived by linear regression; [1]Model 1-unadjusted (**n=514 childhood; 517 adolescence**); [2]Model 2-adjusted for the child's sex, current age, SLI, parental education, occupation and income (**n=513 childhood; 517 adolescence**); [3]Model 3-model 2+ maternal intelligence (imputed) (**n=513 childhood; 517 adolescence**); [4]Model 3 + home environment (imputed) (**n=513 childhood; 517 adolescence)**

3.8.4 Relationship to childhood and adolescent anthropometry

In this section I describe body size and composition (HC, skeletal size (height), muscle mass (MUAC) and adiposity measures (SS, BMI, waist circumference and body fat percentage) of study participants during childhood and adolescence and their relationship to cognitive function in the corresponding period.

3.8.4.1 Definitions and description (Table 3.14): According to WHO age and sex specific growth standards[208], 146 (26.9%), 21 (3.9%) and 37 (6.8 %) children during childhood were underweight, overweight/obese, and stunted respectively. Corresponding figures during adolescence were 104 (19.1%), 59 (10.8%) and 37 (6.8%). The prevalence of overweight/obesity was higher in girls compared to boys ($p<0.05$).

During both childhood and adolescence, boys had larger HC than girls, while girls had larger skinfolds and higher body fat percentage (Table 3.15). I present data relating HC, height, MUAC and SS to cognitive function.

Size and body composition measures at both time points were positively associated with socio-economic factors, parental education, MIQ and home environment ($p<0.05$ for all).

3.8.4.2 Size and body composition and cognitive function: During childhood, the general pattern was that all measures of body size were positively associated with all the cognitive test scores in model 1 (Table 3.16). These associations tended to diminish with adjustment across the various models but remained significant for several tests, especially the associations of all body composition measures with reasoning. During adolescence the findings were similar but generally weaker (Table 3.17). There were similar findings for the other fat indices (BMI, waist circumference and body fat percentage) at both time points.

Table 3.14 Definitions of weight and height status during childhood and adolescence according to WHO age and sex specific growth standards[208]

	Weight status				Height status	
Childhood	**Normal n=375**	**Underweight n=146**	**Overweight n=16**	**Obese n=5**	**Normal n=505**	**Stunted n=37**
Boys	BMI 13.6 – 18.2 kg/m^2	BMI <13.6 kg/m^2	BMI >18.2-<20.9 kg/m^2	BMI>=20.9 kg/m^2	Height>122.8 cm	Height<122.8 cm
Prevalence No (%)	174 (66.7)	79 (30.3)	6 (2.3)	2 (0.8)	246 (94.2)	15 (5.8)
Girls	BMI 13.3 – 18.7 kg/m^2	BMI <13.3 kg/m^2	BMI >18.7-<22.0 kg/m^2	BMI>=22.0 kg/m^2	Height>123.0 cm	Height<123.0 cm
Prevalence No (%)	201 (71.5)	67 (23.8)	10 (3.6)	3 (1.07)	259 (92.2)	22 (7.8)
Adolescence	**n=382**	**n=104**	**n=50**	**n=9**	**n=508**	**n=37**
Boys	BMI 15.2 – 21.3 kg/m^2	BMI <15.2 kg/m^2	BMI >21.3-<25.3 kg/m^2	BMI>=25.3 kg/m^2	Height>144.5 cm	Height<144.5 cm
Prevalence No (%)	182 (70.3)	61 (23.6)	11 (4.2)	5 (1.9)	237 (91.5)	22 (8.5)
Girls	BMI 15.2 – 22.3 kg/m^2	BMI <15.2 kg/m^2	BMI >22.3-<26.8 kg/m^2	BMI>=26.8 kg/m^2	Height>144.4 cm	Height<144.4 cm
Prevalence No (%)	200 (70.0)	43 (15.0)	39 (13.6)	4 (1.4)	271 (94.8)	15 (5.2)

WHO-World Health Organization; BMI-body mass index

Table 3.15 Anthropometry of boys and girls at the time of cognitive function assessment

	Childhood			Adolescence		
	Boys (n=261)	**Girls (n=281)**		**Boys (n=259)**	**Girls (n=286)**	
Anthropometry	**Mean (SD)**	**Mean (SD)**	**P**	**Mean (SD)**	**Mean (SD)**	**P**
Weight (kg)	25.1 (4.10)	25.0 (4.67)	0.7	40.6 (8.8)	43.3 (8.7)	0.0003
Height (cm)	131.1 (5.4)	130.3 (5.9)	0.08	154.2 (8.0)	153.2 (5.9)	0.08
Body mass index (BMI; kg/cm^2)	14.5 (1.7)	14.6 (1.9)	0.5	17.0 (2.7)	18.4 (3.3)	<0.0001
Head circumference (cm)	50.7 (1.4)	50.5 (1.4)	0.04	51.5 (1.4)	51.3 (1.3)	0.03
Mid upper arm circumference (cm)	17.9 (1.9)	18.1 (2.0)	0.1	21.8 (2.8)	22.4 (2.8)	0.03
Waist circumference (cm)	54.1 (4.9)	54.6 (4.7)	0.3	65.3 (8.0)	67.4 (8.0)	0.003
Body fat percentage (%)	24.1 (5.9)	30.2 (5.7)	<0.0001	17.5 (6.7)	25.8 (6.1)	<0.0001
Triceps skin-fold thickness (mm)*	8.1 (6.6, 9.8)	10.4 (8.5, 13.2)	<0.0001	9.7 (7.6, 13.7)	14.3 (11.2, 18.5)	<0.0001
Subscapular skin-fold thickness (mm)*	6.1 (5.3, 7.9)	8.1 (6.4, 10.4)	<0.0001	9.2 (7.3, 13.6)	15.0 (10.7, 19.7)	<0.0001
Sum of skinfolds (mm)*	14.6 (12.1, 17.7)	18.6 (15.0, 24.2)	<0.0001	18.8 (15.0, 27.9)	29.0 (22.1, 39.6)	<0.0001

*Skewed variable; values are medians (IQR); P value for the difference between boys and girls

Table 3.16 Association of childhood head, height, mid-upper-arm circumference and sum of skinfolds with childhood cognitive function

	Mean cognitive test scores according to quartiles of HC (cm)				Multiple regression analyses			
Cognitive tests	**n=136**	**n=134**	**n=138**	**n=132**	**β (95% CI)[1]**	**β (95% CI)[2]**	**β (95% CI)[3]**	**β (95% CI)[4]**
	<49.7	**>49.7-50.6**	**>50.6-51.5**	**>51.5**				
Learning, long-term retrieval	63.6	66.3	67.2	73.5	0.21 (0.13, 0.30)†	0.16 (0.07, 0.24)†	0.15 (0.06, 0.23)‡	0.14 (0.06, 0.23)‡
Short-term memory	15.7	16.5	16.3	17.1	0.22 (0.14, 0.30)†	0.17 (0.09, 0.25)†	0.16 (0.08, 0.25)†	0.16 (0.08, 0.25)†
Reasoning ability*	8.0	10.0	9.0	12.0	0.20 (0.12, 0.29)†	0.13 (0.05, 0.21)‡	0.12 (0.04, 0.20)‡	0.12 (0.04, 0.20)‡
Verbal fluency	15.8	16.1	16.0	16.6	0.11 (0.03, 0.20)‡	0.07 (-0.02, 0.15)	0.07 (-0.02, 0.15)	0.07 (-0.01, 0.15)
Visuo-spatial ability*	72.5	74.0	77.5	83.3	0.17 (0.09, 0.26)†	0.11 (0.03, 0.20)‡	0.11 (0.02, 0.19)§	0.11 (0.02, 0.19)§
Attention and concentration	31.2	34.1	31.4	34.0	0.17 (0.09, 0.25)†	0.12 (0.04, 0.20)‡	0.12 (0.04, 0.20)‡	0.13 (0.05, 0.21)‡
	Mean cognitive test scores according to quartiles of height (cm)							
	< 127.0	**>127.0 – 130.5**	**>130.5- 134.2**	**>134.2**				
Learning, long-term retrieval	64.7	64.8	70.1	71.2	0.16 (0.08, 0.25)†	0.09 (0.01, 0.18)§	0.08 (-0.002, 0.17)	0.08 (-0.01, 0.16)
Short-term memory	16.1	16.0	16.4	17.0	0.16 (0.07, 0.24)†	0.08 (-0.002, 0.17)	0.07 (-0.01, 0.16)	0.07 (-0.01, 0.16)
Reasoning ability*	9.0	9.0	11.0	11.0	0.19 (0.11, 0.27)†	0.11 (0.02, 0.19)§	0.10 (0.01, 0.18)§	0.08 (0.002, 0.16)§
Verbal fluency	15.5	15.6	16.4	16.9	0.10 (0.01, 0.18)§	0.04 (-0.04, 0.13)	0.04 (-0.04, 0.13)	-0.04 (-0.05, 0.12)
Visuo-spatial ability*	70.4	75.0	78.6	81.3	0.18 (0.09, 0.26)†	0.10 (0.02, 0.19)§	0.09 (-0.01, 0.18)§	0.09 (0.003, 0.17)§
Attention and concentration	31.4	31.5	33.5	34.5	0.19 (0.11, 0.27)†	0.14 (0.06, 0.22)‡	0.14 (0.06, 0.22)‡	0.13 (0.05, 0.21)‡
	Mean cognitive test scores according to quartiles of MUAC (cm)							
	<16.8	**>16.8-17.7**	**>17.7-19.1**	**>19.1**				
Learning, long-term retrieval	64.1	65.3	67.9	73.5	0.23 (0.14, 0.32)†	0.17 (0.09, 0.26)†	0.17 (0.09, 0.26)†	0.17 (0.08, 0.25)†
Short-term memory	15.9	16.4	16.4	16.9	0.15 (0.06, 0.23)‡	0.07 (-0.01, 0.16)	0.07 (-0.02, 0.16)	0.07 (-0.02, 0.16)
Reasoning ability*	8.0	9.0	11.0	12.0	0.22 (0.14, 0.31)†	0.14 (0.05, 0.22)‡	0.13 (0.05, 0.22)‡	0.12 (0.04, 0.21)‡
Verbal fluency	15.3	16.0	16.3	16.9	0.13 (0.04, 0.21)‡	0.07 (-0.01, 0.16)	0.08 (-0.01, 0.16)	0.07 (-0.01, 0.16)
Visuo-spatial ability*	72.5	74.0	78.3	83.3	0.14 (0.06, 0.23)‡	0.06 (-0.02, 0.15)	0.06 (-0.03, 0.14)	0.05 (-0.04, 0.14)
Attention and concentration	30.8	32.0	32.9	35.3	0.20 (0.12, 0.28)†	0.15 (0.07, 0.23)†	0.15 (0.06, 0.23)†	0.14 (0.06, 0.23)‡
	Mean cognitive test scores according to quartiles of SS (mm)							
	< 13.3	**>13.3 - 16.3**	**>16.3 - 20.9**	**>20.9**				
Learning, long-term retrieval	66.8	64.4	66.1	73.5	0.18 (0.09, 0.27)†	0.13 (0.04, 0.22)‡	0.13 (0.04, 0.22)‡	0.12 (0.03, 0.21)‡
Short-term memory	16.0	16.1	16.4	17.1	0.13 (0.04, 0.22)‡	0.06 (-0.03, 0.15)	0.06 (-0.03, 0.15)	0.05 (-0.04, 0.15)
Reasoning ability*	9.0	8.0	10.0	12.0	0.20 (0.11, 0.29)†	0.13 (0.04, 0.22)‡	0.12 (0.04, 0.21)‡	0.12 (0.03, 0.20)‡
Verbal fluency-names	14.9	15.8	16.4	17.4	0.10 (0.01, 0.19)§	0.05 (-0.04, 0.14)	0.05 (-0.04, 0.14)	0.05 (-0.04, 0.14)
Visuo-spatial ability*	72.8	75.0	77.6	82.5	0.14 (0.05, 0.24)‡	0.07 (-0.02, 0.16)	0.06 (-0.03, 0.15)	0.06 (-0.03, 0.15)
Attention and concentration	30.5	32.6	32.0	35.8	0.14 (0.05, 0.22)‡	0.09 (-0.001, 0.17)	0.08 (-0.003, 0.17)	0.09 (-0.001, 0.17)

*Skewed variable; values are medians; HC-head circumference; MUAC-mid-upper-arm circumference; SS-sum of skinfolds; β is the effect size in SD of the outcome per SD change in the predictor; [1]adjusted for the child's sex and current age; **n=542**; [2]adjusted for the child's sex, current age + SLI, maternal education, paternal education, occupation and income; **n=539**; [3]adjusted for parameters in 2 + maternal intelligence (imputed); **n=539;** [4]adjusted for parameters in 3 + home environment (imputed); n=**539;** §p<0.05; ‡p<0.01; †p<0.001

Table 3.17 Association of adolescent head, height, mid-upper-arm circumference and sum of skinfolds with adolescent cognitive function

	Mean cognitive test scores according to quartiles of HC (cm)				Multiple regression analyses			
Cognitive tests	**n=136**	**n=136**	**n=136**	**n=135**	**β (95% CI)[1]**	**β (95% CI)[2]**	**β (95% CI)[3]**	**β (95% CI)[4]**
	<50.4	**>50.4 - 51.3**	**>51.3 - 52.2**	**>52.2**				
Learning, long-term retrieval	78.4	77.9	80.5	82.9	0.12 (0.03, 0.20)‡	0.07 (-0.01, 0.16)	0.07 (-0.02, 0.15)	0.05 (-0.03, 0.14)
Short-term memory	18.2	18.8	19.0	19.7	0.16 (0.08, 0.24)†	0.12 (0.03, 0.20)‡	0.11 (0.03, 0.20)‡	0.11 (0.02, 0.19)§
Reasoning ability	13.9	15.3	15.7	17.2	0.22 (0.13, 0.30)†	0.14 (0.06, 0.22)‡	0.13 (0.05, 0.21)‡	0.13 (0.05, 0.21)‡
Verbal fluency	20.4	21.1	21.9	22.0	0.17 (0.09, 0.25)†	0.14 (0.06, 0.23)‡	0.14 (0.06, 0.23)‡	0.14 (0.06, 0.23)‡
Visuo-spatial ability	77.5	83.1	80.3	91.2	0.19 (0.10, 0.27)†	0.13 (0.05, 0.21)‡	0.12 (0.04, 0.20)‡	0.11 (0.03, 0.20)‡
Attention and concentration	45.4	47.9	48.4	49.1	0.15 (0.07, 0.23)†	0.11 (0.03, 0.19)‡	0.11 (0.03, 0.19)‡	0.10 (0.02, 0.18)§
Mean cognitive test scores according to quartiles of height (cm)								
	< 149.0	**>149.0 – 153.4**	**>153.4 – 158.1**	**>158.1**				
Learning, long-term retrieval	79.8	77.9	81.0	80.9	0.05 (-0.03, 0.14)	0.002 (-0.08, 0.09)	-0.003 (-0.09, 0.08)	-0.02 (-0.10, 0.07)
Short-term memory	18.6	18.8	19.1	19.2	0.05 (-0.04, 0.13)	-0.003 (-0.09, 0.08)	-0.01 (-0.10, 0.08)	-0.02 (-0.11, 0.07)
Reasoning ability	14.1	14.2	16.9	16.8	0.17 (0.09, 0.26)†	0.08 (0.002, 0.16)§	0.07 (-0.01, 0.15)	0.06 (-0.02, 0.14)
Verbal fluency	21.0	20.9	22.4	21.1	0.06 (-0.02, 0.14)	0.03 (-0.06, 0.11)	0.03 (-0.06, 0.11)	0.02 (-0.07, 0.10)
Visuo-spatial ability	78.3	78.0	86.2	80.7	0.19 (0.10, 0.27)†	0.13 (0.04, 0.21)‡	0.11 (0.03, 0.20)§	0.10 (0.02, 0.19)§
Attention and concentration	45.5	47.5	49.2	48.6	0.13 (0.05, 0.21)‡	0.09 (0.01, 0.18)§	0.09 (0.01, 0.17)§	0.08 (-0.003, 0.16)
Mean cognitive test scores according to quartiles of MUAC (cm)								
	<20.1	**>20.1- 21.8**	**>21.8 - 23.5**	**>23.5**				
Learning, long-term retrieval	76.9	79.6	80.5	82.6	0.13 (0.05, 0.22)‡	0.11 (0.02, 0.19)§	0.11 (0.03, 0.19)§	0.10 (0.02, 0.18)§
Short-term memory	18.3	19.1	19.1	19.2	0.08 (-0.01, 0.16)	0.05 (-0.04, 0.13)	0.05 (-0.03, 0.13)	0.04 (-0.04, 0.12)
Reasoning ability	13.9	15.1	16.5	16.6	0.15 (0.07, 0.24)†	0.10 (0.02, 0.18)§	0.10 (0.03, 0.18)‡	0.10 (0.02, 0.17)§
Verbal fluency	20.2	21.0	21.7	22.4	0.08 (0.003, 0.17)§	0.06 (-0.02, 0.15)	0.07 (-0.02, 0.15)	0.06(-0.02, 0.14)
Visuo-spatial ability	79.8	83.7	83.7	84.8	0.06 (-0.03, 0.14)	0.02 (-0.07, 0.10)	0.02 (-0.06, 0.10)	0.01 (-0.07, 0.09)
Attention and concentration	45.3	48.1	47.8	49.5	0.09 (0.01, 0.17)§	0.06 (-0.02, 0.14)	0.06 (-0.02, 0.14)	0.05 (-0.03, 0.13)
Mean cognitive test scores according to quartiles of SS (mm)								
	< 17.4	**>17.4 – 24.5**	**>24.5 – 34.2**	**>34.2**				
Learning, long-term retrieval	79.3	78.3	80.0	82.0	0.12 (0.03, 0.21)‡	0.09 (-0.003, 0.18)	0.09 (0.002, 0.18) §	0.08 (-0.01, 0.17)
Short-term memory	18.4	18.6	19.7	19.0	0.05 (-0.04, 0.15)	0.01 (-0.07, 0.10)	0.02 (-0.07, 0.11)	0.01 (-0.07, 0.10)
Reasoning ability	14.7	14.5	15.9	16.9	0.12 (0.03, 0.21) §	0.06 (-0.03, 0.14)	0.07 (-0.01, 0.15)	0.07 (-0.02, 0.15)
Verbal fluency	20.6	19.9	22.6	22.2	0.05 (-0.04, 0.13)	0.02 (-0.06, 0.11)	0.02 (-0.06, 0.11)	0.02 (-0.07, 0.11)
Visuo-spatial ability	82.1	83.0	84.7	82.2	0.05 ((-0.04, 0.14)	-0.001 (-0.09, 0.09)	0.01 (-0.08, 0.10)	0.02 (-0.08, 0.09)
Attention and concentration	44.6	47.0	49.8	49.3	0.07 (-0.02, 0.16)	0.03 (-0.05, 0.12)	0.04 (-0.05, 0.12)	0.03 (-0.06, 0.11)

HC-head circumference; MUAC-mid-upper-arm circumference; SS-sum of skinfolds; β is the effect size in SD of the outcome per SD change in the predictor; [1]adjusted for the child's sex and current age; **n=545;** [2]adjusted for the child's sex, current age + SLI, maternal education, paternal education, occupation and income; **n=545;** [3]adjusted for paramet in 2 + maternal intelligence (imputed); **n=543;** [4]adjusted for parameters in 3+ home environment (imputed); **n=543;** §P<0.05; ‡P<0.01; †P<0.001

3.8.5 Relationship to postnatal growth

In view of the positive associations between body size and cognitive function, I was interested to examine whether growth at specific ages was related to cognitive function. Since CNS development continues rapidly in the early postnatal years, early postnatal growth (which reflects postnatal nutrition), is likely to influence neurodevelopment. I used longitudinal growth data from birth to 13.5 years to investigate the associations of growth during particular time periods with cognitive function.

3.8.5.1 Conditional SD scores: A high correlation between anthropometric measurements in successive years (Figure 3.6a) leads to difficulties in interpreting independent associations between changes in anthropometry in any given age interval (growth) and cognitive function. Therefore I used conditional SD scores to examine these associations. Within cohort SD scores of an anthropometric measurement at each age were regressed on the SD scores of the corresponding measurement at all previous ages. For example, conditional height gain from 9.5 to 13.5 years was obtained by regressing 13.5 year height SD score upon height SD scores at 9.5, 5, 2 and 1 year and length SD score at birth. The residuals obtained give a measure of whether the child has grown more or less than expected during the interval (9.5 to 13.5 years) given its earlier size, and given the growth characteristics of the cohort. These values are normally distributed and the sequence of conditional variables for a measurement is uncorrelated with each other (Figure 3.6b) and therefore they can be simultaneously included in regression models.

Figure 3.6 Scatter plots illustrating association between birth HC SD scores and a) one year HC SD score; b) one year conditional HC SD scores

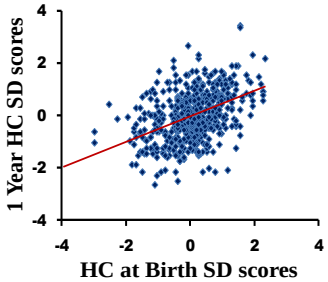


Figure 3.6 a

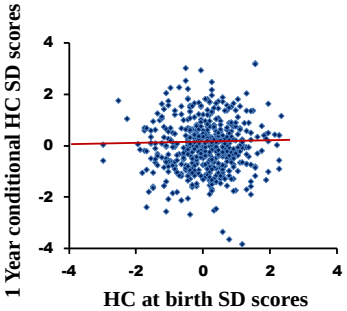


Figure 3.6 b

Conditional growth variables require complete measurement data at all included ages. Of the 542 and 545 children examined during childhood and adolescence complete longitudinal growth data were available for 450 and 465 children respectively.

I constructed conditional variables for 4 body measurements (HC, length/height, MUAC and SS) and used 5 age intervals (birth to 1 year, 1 to 2 years, 2 to 5 years, 5 to 9.5 years and 9.5 to 13.5 years). I define birth-1 year as infancy; 1-2 years as late infancy, 2-5 years as early childhood, 5-9.5 years as late childhood and 9.5-13.5 years as early adolescence.

There were positive associations of SLI, parental education and occupation with conditional height, HC and MUAC during 0-1 year and conditional height during 1-2 years ($p<0.05$ for all). There were also positive associations of home environment with conditional HC during 0-1 year, and of SLI and paternal education with conditional HC during 1-2 years. MIQ was unrelated to postnatal growth. In general, apart from some sporadic associations, there were no significant associations between socio-demographic factors and conditional measurements during 2-5, 5-9.5 and 9.5-13.5 years.

3.8.5.2 Postnatal growth and cognitive function: Higher HC at birth and greater head growth during infancy and childhood predicted most of the cognitive test scores, but gain in HC during adolescence was unrelated to any of the cognitive outcomes (Table 3.18). There were differences in the patterns of associations of HC growth at different ages with different cognitive domains (black line in Figure 3.7a-f). HC at birth was the strongest predictor of learning, short-term memory, reasoning and visuo-spatial ability, and the strength of the associations of head growth tended to decrease with increasing age. There was a small increase in effect with head growth from 2-5 years. In contrast, verbal fluency and attention scores were unrelated to HC at birth and the strength of the associations of head growth tended to increase with increasing age until childhood followed by a reduction with increasing head growth during adolescence. The pattern was similar for other body measurements except that conditional length during 0-2 years, and conditional SS during 5-9.5 years, were generally stronger predictors of cognitive scores than the equivalent conditionals at other ages (Table 3.18 and Figure 3.8a-f).

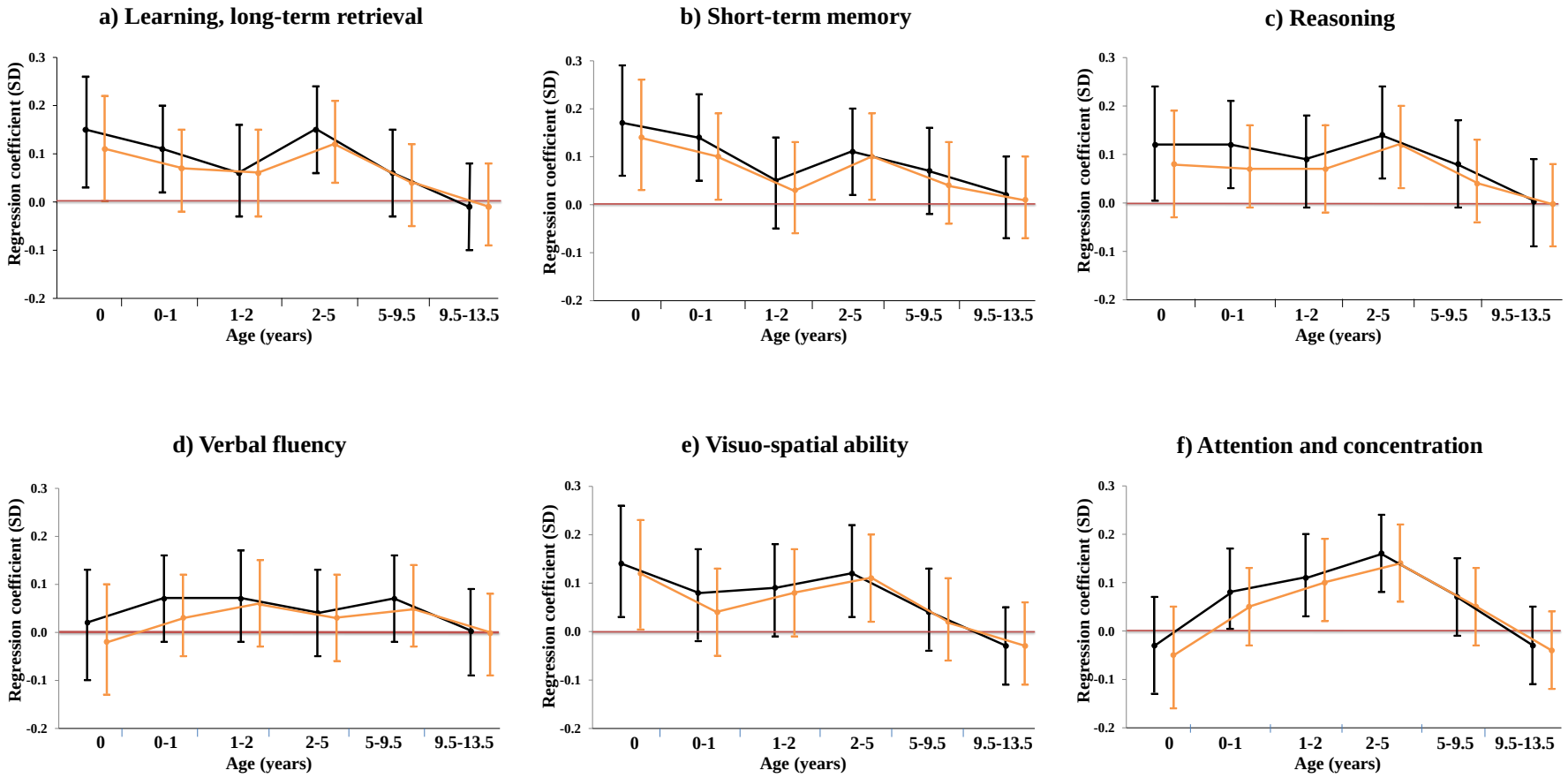
In general the strength of these associations was attenuated after adjusting for confounders, but the pattern remained similar (orange line in Figure 3.7a-f). When the analysis was repeated, controlling for MUAC or SS growth, I found that head growth, but not MUAC or SS growth, independently predicted cognitive function. The findings were similar during adolescence (data not shown).

Table 3.18 Association of postnatal conditional growth with childhood cognitive function

	Birth	0-1 year	1-2 years	2-5 years	5-9.5 years	9.5-13.5 years
Cognitive test scores (SD)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
			Head circumference (SD)			
Learning, long-term retrieval	0.15 (0.03, 0.26)*	0.11 (0.02, 0.20)*	0.06 (-0.03, 0.16)	0.15 (0.06, 0.24)**	0.06 (-0.03, 0.15)	-0.01 (-0.10, 0.08)
Short-term memory	0.17 (0.06, 0.29)**	0.14 (0.05, 0.23)**	0.05 (-0.05, 0.14)	0.11 (0.02, 0.20)*	0.07 (-0.02, 0.16)	0.02 (-0.07, 0.10)
Reasoning ability	0.12 (0.004, 0.24)*	0.12 (0.03, 0.21)**	0.08 (-0.01, 0.18)	0.14 (0.05, 0.24)**	0.08 (-0.01, 0.17)	0.003 (-0.09, 0.09)
Verbal fluency	0.02 (-0.10, 0.13)	0.07 (-0.02, 0.16)	0.07 (-0.02, 0.17)	0.04 (-0.05, 0.13)	0.07 (-0.02, 0.16)	0.002 (-0.09, 0.09)
Visuo-spatial ability	0.14 (0.03, 0.26)*	0.08 (-0.02, 0.17)	0.09 ((-0.01, 0.18)	0.12 (0.03, 0.22)**	0.04 (-0.04, 0.13)	-0.02 (-0.11, 0.07)
Attention and concentration	-0.03 (-0.13, 0.07)	0.08 (0.004, 0.17)*	0.11 (0.03, 0.20)**	0.16 (0.08, 0.24)***	0.07 (-0.01, 0.15)	-0.03 (-0.11, 0.05)
			Length/height (SD)			
Learning, long-term retrieval	0.07 (-0.04, 0.18)	0.13 (0.04, 0.22)**	0.12 (0.03, 0.21)*	-0.003 (-0.09, 0.09)	0.06 (-0.03, 0.14)	0.01 (-0.08, 0.10)
Short-term memory	0.13 (0.02, 0.24)*	0.10 (0.02, 0.19)*	0.11 (0.03, 0.20)*	-0.05 (-0.14, 0.04)	0.06 (-0.03, 0.15)	0.04 (-0.05, 0.13)
Reasoning ability	0.11 (0.001, 0.22)*	0.12 (0.04, 0.21)**	0.10 (0.01, 0.19)*	0.001 (-0.09, 0.09)	0.11 (0.02, 0.19)*	0.06 (-0.03, 0.15)
Verbal fluency	-0.01 (-0.12, 0.10)	0.06 (-0.03, 0.14)	0.10 (0.01, 0.19)*	-0.02 (-0.11, 0.07)	0.06 (-0.02, 0.15)	0.002 (-0.09, 0.09)
Visuo-spatial ability	0.09 (-0.02, 0.21)	0.11 (0.02, 0.19)*	0.07 (-0.02, 0.16)	0.003 (-0.09, 0.09)	0.04 (-0.04, 0.13)	0.02 (-0.07, 0.11)
Attention and concentration	0.02 (-0.08, 0.12)	0.11 (0.04, 0.19)**	0.09 (0.01, 0.17)*	0.05 (-0.03, 0.14)	0.11 (0.03, 0.19)**	0.02 (-0.06, 0.10)
			MUAC (SD)			
Learning, long-term retrieval	0.17 (0.07, 0.28)**	0.12 (0.03, 0.20)**	0.08 (-0.01, 0.16)	0.04 (-0.04, 0.13)	0.16 (0.07, 0.25)***	-0.02 (-0.11, 0.07)
Short-term memory	0.06 (-0.05, 0.17)	0.14 (0.06, 0.23)**	0.04 (-0.05, 0.13)	0.08 (-0.01, 0.17)	0.07 (-0.02, 0.16)	-0.002 (-0.09, 0.09)
Reasoning ability	0.14 (0.03, 0.24)*	0.10 (0.02, 0.19)*	0.04 (-0.05, 0.13)	0.12 (0.03, 0.21)*	0.10 (0.01, 0.19)*	-0.06 (-0.15, 0.03)
Verbal fluency	0.05 (-0.06, 0.15)	0.09 (0.01, 0.18)*	-0.05 (-0.13, 0.04)	0.07 (-0.01, 0.16)	0.06 (-0.03, 0.15)	-0.05 (-0.13, 0.04)
Visuo-spatial ability	0.17 (0.07, 0.28)**	0.07 (-0.02, 0.16)	0.07 (-0.02, 0.16)	0.02 (-0.07, 0.11)	0.05 (-0.04, 0.14)	-0.05 (-0.14, 0.04)
Attention and concentration	0.06 (-0.04, 0.15)	0.07 (-0.01, 0.15)	0.01 (-0.07, 0.09)	0.11 (0.03, 0.19)**	0.09 (0.01, 0.17)*	-0.07 (-0.15, 0.01)
			Sum of skinfolds (SD)			
Learning, long-term retrieval	0.07 (-0.03, 0.18)	0.01 (-0.08, 0.10)	0.02 (-0.07, 0.11)	0.02 (-0.07, 0.11)	0.17 (0.08, 0.26)***	0.01 (-0.08, 0.10)
Short-term memory	0.02 (-0.09, 0.13)	0.10 (0.01, 0.19)*	0.07 (-0.02, 0.15)	0.01 (-0.08, 0.11)	0.08 (-0.01, 0.17)	0.04 (-0.05, 0.13)
Reasoning ability	0.10 (-0.01, 0.20)	0.001 (-0.09, 0.09)	0.01 (-0.08, 0.11)	0.07 (-0.02, 0.17)	0.13 (0.04, 0.22)**	-0.08 (-0.17, 0.01)
Verbal fluency	-0.03 (-0.13, 0.07)	0.06 (-0.03, 0.15)	-0.05 (-0.14, 0.04)	-0.01 (-0.10, 0.09)	0.09 (-0.01, 0.17)	-0.02 (-0.11, 0.07)
Visuo-spatial ability	0.16 (0.05, 0.26)**	0.04 (-0.06, 0.13)	0.06 (-0.03, 0.15)	0.03 (-0.07, 0.12)	0.09 (-0.004, 0.18)	0.003 (-0.09, 0.09)
Attention and concentration	0.04 (-0.06, 0.14)	0.02 (-0.07, 0.10)	0.02 (-0.06, 0.10)	0.03 (-0.06, 0.11)	0.09 (0.005, 0.17)*	-0.05 (-0.13, 0.03)

β is the effect size (SD) of the cognitive outcome per SD change in birth size and conditional postnatal size adjusted for the child's sex, gestational age and age at the time of cognitive function assessment; MUAC-mid-upper-arm circumference; *p<0.05; **p<0.01; ***p<0.001

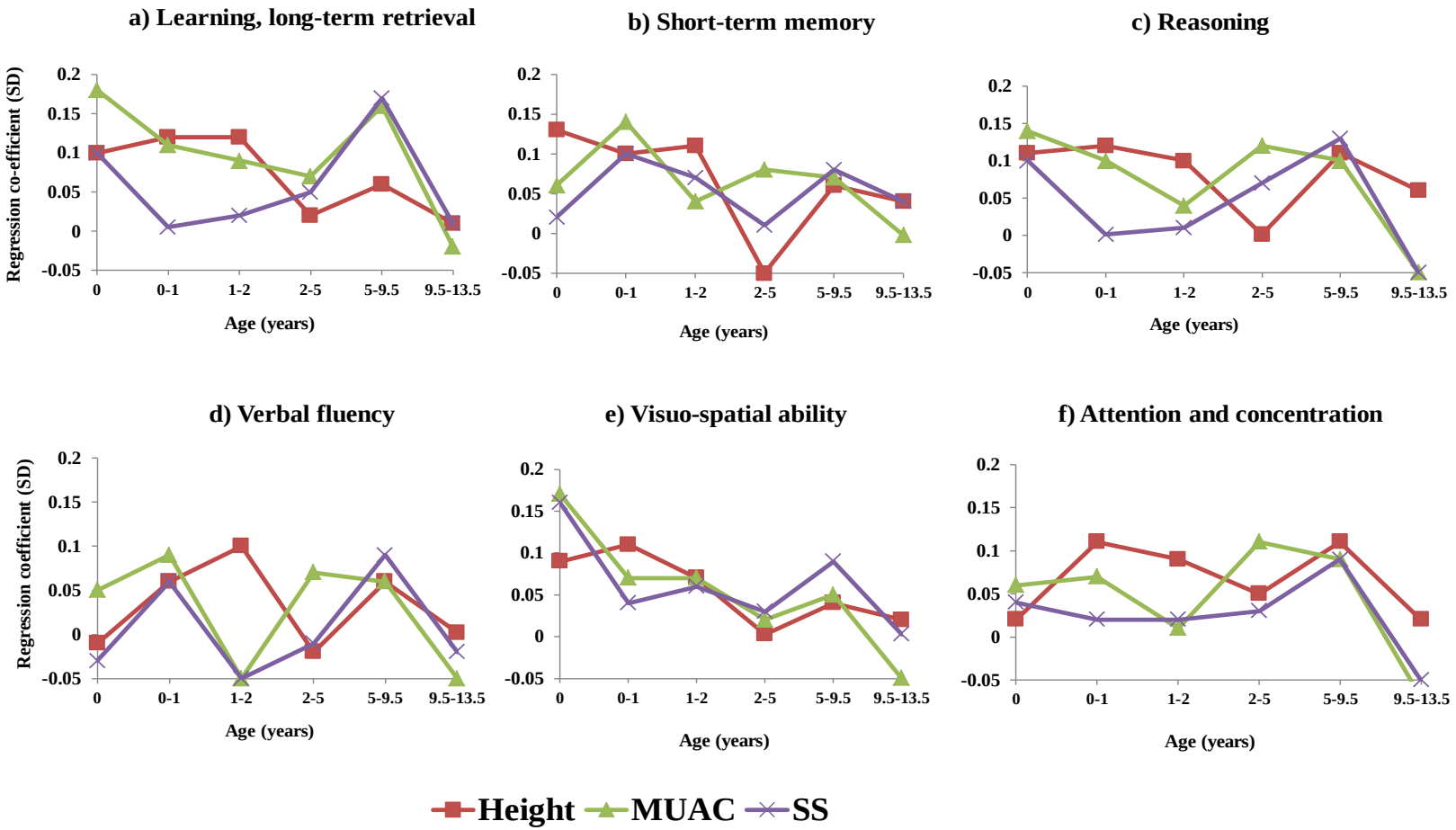
Figure 3.7(a-f) Associations between changes in head circumference (0-13.5 years) and childhood cognitive function



Model 1 Adjusted for the child's sex, gestational age and current age

Model 2 Model 1 parameters + SLI, parental education, occupation, income, maternal intelligence (imputed) and home environment (imputed)

Figure 3.8(a-f) Associations between changes in body size (0-13.5 years) and childhood cognitive function



MUAC-mid-upper-arm circumference; SS-sum of skinfolds
Regression co-efficient adjusted for the child's sex, gestational age and current age;

3.9 Relative strength of associations of socio-demographic and early life factors with childhood cognitive function

I used two simple approaches to compare the relative strength of associations of socio-demographic and early life (prenatal and postnatal) factors with childhood cognitive function: a) by examining the variation explained by each factor individually, and all together, in a linear regression analysis (R^2); and b) by comparing the independent effect size (regression coefficients) of selected socio-demographic and early life factors individually and together as predictors of cognitive function.

3.9.1 R^2 Analysis: The variation in childhood cognitive function explained by socio-demographic and early life factors is presented in Table 3.19. Of the various socio-demographic factors, each individually explained 0.01% to 8% of the variation in cognitive function and, of them, SLI, parental education, maternal intelligence and home environment explained the greatest percentage of variation (4% to 8%). All the socio-demographic factors combined explained 6 to 17% of the variation in cognitive function.

Of the prenatal factors, each factor explained 0.01% to 2% of the variation in cognitive function and HC at birth and GDM explained the greatest percentage of variation (1% to 2%). All the prenatal factors together explained 1 to 3% of the variation in cognitive function.

Of the postnatal factors, each factor explained 0.01% to 2% of the variation in cognitive function and gain in HC during 0-1 year and 2-5 years intervals explained the greatest percentage of variation (1% to 2%). All the postnatal factors together explained 1 to 6% of the variation in cognitive function.

All the above factors together explained 16-25% of the variation in cognitive function. Interestingly, the R^2 for components (socio-demographic factors, prenatal and postnatal factors) did not add up exactly to the total R^2. For example, for learning ability 19% of the variation was explained by a model containing all the factors, of which 12% was due to socio-demographic factors and 7% due to prenatal and postnatal factors, which adds up exactly. In contrast, corresponding figures for visuo-spatial ability were 16%, which is not the exact sum of 13%, and 7%. These differences may be due to interactions between socio-demographic, prenatal and postnatal factors, though I did not formally test this.

Table 3.19 Variation (R^2) in childhood cognitive function explained by sex, socio-demographic, prenatal and postnatal factors

	Learning, long-term retrieval	Short-term memory	Reasoning	Verbal fluency	Visuo-spatial ability	Attention and concentration
a) Sex	0.0000	0.01	0.02	0.07	0.0003	0.09
b) Socio-demographic factors						
Parity	0.01	0.01	0.02	0.005	0.004	0.0000
Standard of living index	0.05	0.05	0.08	0.04	0.05	0.04
Maternal education	0.04	0.08	0.08	0.04	0.08	0.04
Paternal education	0.05	0.05	0.08	0.03	0.06	0.04
Occupation	0.02	0.03	0.05	0.02	0.03	0.01
Income	0.05	0.02	0.06	0.01	0.04	0.04
Rural/urban residence	0.03	0.02	0.02	0.0002	0.02	0.002
Maternal intelligence (imputed)	0.04	0.04	0.06	0.004	0.04	0.01
Home environment (imputed)	0.02	0.01	0.06	0.02	0.05	0.02
Socio-demographic factors together	**0.12**	**0.11**	**0.17**	**0.06**	**0.13**	**0.08**
c) Prenatal factors						
Gestational age	0.002	0.002	0.003	0.008	0.004	0.01
Birth HC	0.02	0.01	0.006	0.0001	0.02	0.0003
GDM	0.01	0.003	0.01	0.01	0.006	0.02
Prenatal factors together	**0.03**	**0.01**	**0.02**	**0.02**	**0.03**	**0.03**
d) Postnatal factors						
0-1 year conditional HC	0.01	0.02	0.01	0.002	0.008	0.006
1-2 years conditional HC	0.004	0.0005	0.007	0.002	0.006	0.01
2-5 years conditional HC	0.02	0.01	0.02	0.002	0.02	0.02
5-9.5 years conditional HC	0.005	0.005	0.006	0.006	0.003	0.006
Breast-feeding duration	0.0004	0.0000	0.0000	0.0003	0.004	0.005
Postnatal factors together	**0.05**	**0.04**	**0.04**	**0.01**	**0.03**	**0.06**
Prenatal and postnatal factors together	**0.07**	**0.07**	**0.08**	**0.04**	**0.07**	**0.09**
All together (a+b+c+d)	**0.19**	**0.18**	**0.25**	**0.18**	**0.16**	**0.21**

Each row represents a separate analysis; HC-head circumference; GDM-gestational diabetes mellitus

3.9.2 Comparison of regression coefficients analysis: For this analysis I used internally Z-standardised values for the exposure and outcome variables to enable comparison of regression coefficients. I excluded rural/urban residence and maternal GDM status, because these could not be Z-standardised. Initially I examined the effects of socio-demographic, prenatal and postnatal factors separately and then combined one selected from each group to compare effect sizes.

In a regression model containing all the socio-demographic factors together, all except parity, occupation and income had some independent effect on at least one measure of cognitive function (Table 3.20a). The strongest and most consistent predictors were maternal education and MIQ score. In a regression model with prenatal factors combined, gestational age and birth HC had some independent effect on several measures of cognitive ability (Table 3.20b). In a model with all the postnatal factors together, all except breast-feeding and gain in HC during 5-9.5 years age interval independently predicted cognitive ability. The strongest and most consistent were gain in HC during 0-1 year and 2-5 years intervals (Table 3.20c).

I then selected maternal education, MIQ, HC at birth and gain in HC during 0-1 year and 2-5 years intervals to compare their relative strength of associations with cognitive function (Table 3.21a and b). All of them independently predicted cognitive function when they were entered in a model together, and the size of the standardized coefficients differed only slightly from those seen in the univariate analyses. The coefficients for the socio-economic variables (range 0.16 to 0.28 SD per SD in univariate analyses, and range 0.15 to 0.25 in multivariate analyses for learning, reasoning and visuo-spatial ability as outcomes) were approximately double those of birth HC (univariate: range 0.10 to 0.15; multivariate: 0.08 to 0.15) and postnatal head growth (univariate: range 0.09 to 0.14; multivariate: 0.07 to 0.15).

Table 3.20a Independent effects of socio-demographic factors on childhood cognitive function

Socio-demographic factors	Learning, long-term retrieval (SD)	Short-term memory (SD)	Reasoning (SD)	Verbal fluency (SD)	Visuo-spatial ability (SD)	Attention and concentration (SD)
	β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
Parity (SD)	-0.07 (-0.14, 0.01)	-0.08 (-0.16, 0.004)	-0.07 (-0.14, 0.01)	-0.03 (-0.11, 0.05)	-0.01 (-0.09, 0.07)	0.04 (-0.04, 0.12)
Standard of living index (SD)	0.07 (-0.03, 0.19)	0.10 (-0.01, 0.21)	0.07 (-0.03, 0.18)	**0.13 (0.02, 0.24)**	0.03 (-0.08, 0.14)	0.09 (-0.02, 0.20)
Maternal education (SD)	0.02 (-0.08, 0.12)	**0.16 (0.06, 0.27)**	0.06 (-0.04, 0.16)	**0.12 (0.01, 0.23)**	**0.12 (0.02, 0.22)**	0.10 (-0.01, 0.21)
Paternal education (SD)	**0.11 (0.004, 0.22)**	0.07 (-0.03, 0.18)	**0.11 (0.004, 0.21)**	0.08 (-0.03, 0.19)	0.09 (-0.01, 0.20)	0.08 (-0.02, 0.19)
Occupation of main breadwinner (SD)	-0.03 (-0.14, 0.07)	0.01 (-0.09, 0.11)	0.01 (-0.09, 0.11)	-0.03 (-0.14, 0.08)	-0.03 (-0.13, 0.08)	-0.07 (-0.18, 0.04)
Income of main breadwinner (SD)	0.09 (0.01, 0.19)	-0.03 (-0.13, 0.07)	0.07 (-0.03, 0.17)	-0.08 (-0.18, 0.03)	0.05 (-0.05, 0.15)	0.07 (-0.03, 0.17)
Maternal intelligence imputed (SD)	**0.11 (0.04, 0.18)**	**0.08 (0.01, 0.15)**	**0.13 (0.06, 0.20)**	-0.004 (-0.08, 0.07)	**0.10 (0.03, 0.17)**	0.04 (-0.03, 0.12)
Home environment imputed (SD)	0.04 (-0.03, 0.12)	-0.01 (-0.09, 0.06)	**0.09 (0.02, 0.16)**	0.04 (-0.03, 0.12)	**0.10 (0.03, 0.17)**	0.03 (-0.04, 0.11)

β is the effect size (SD) of the cognitive outcome per SD change in socio-demographic factors derived using all variables included together in multiple regression
Values in bold are significant (p<0.05 for all)

Table 3.20b Independent effects of prenatal factors on childhood cognitive function

Prenatal factors	Learning, long-term retrieval (SD)	Short-term memory (SD)	Reasoning (SD)	Verbal fluency (SD)	Visuo-spatial ability (SD)	Attention and concentration (SD)
	β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
Gestational age (SD)	**-0.11 (-0.21, -0.02)**	0.01 (-0.09, 0.11)	0.03 (-0.06, 0.13)	**0.11 (0.02, 0.21)**	0.01 (-0.09, 0.11)	**0.14 (0.04, 0.23)**
Birth HC (SD)	**0.19 (0.09, 0.29)**	**0.10 (0.003, 0.20)**	0.07 (-0.03, 0.18)	-0.05 (-0.15, 0.05)	**0.16 (0.07, 0.26)**	-0.03 (-0.14, 0.07)

β is the effect size (SD) of the cognitive outcome per SD change in prenatal factors derived using all variables included together in multiple regression; HC-head circumference
Values in bold are significant (p<0.05 for all)

Table 3.20c Independent effects of postnatal factors on childhood cognitive function

Postnatal factors	Learning, long-term retrieval (SD)	Short-term memory (SD)	Reasoning (SD)	Verbal fluency (SD)	Visuo-spatial ability (SD)	Attention and concentration (SD)
	β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
0-1 year conditional HC (SD)	**0.11 (0.03, 0.20)**	**0.16 (0.07, 0.25)**	**0.12 (0.03, 0.21)**	0.05 (-0.04, 0.14)	**0.10 (0.01, 0.19)**	0.08 (-0.01, 0.16)
1-2 years conditional HC (SD)	0.06 (-0.03, 0.15)	0.01 (-0.08, 0.11)	0.07 (-0.03, 0.16)	0.05 (-0.05, 0.14)	0.07 (-0.03, 0.16)	**0.10 (0.01, 0.19)**
2-5 years conditional HC (SD)	**0.16 (0.07, 0.24)**	**0.12(0.03, 0.21)**	**0.14 (0.05, 0.23)**	0.05 (-0.04, 0.15)	**0.11 (0.02, 0.20)**	**0.16 (0.07, 0.25)**
5-9.5 years conditional HC (SD)	0.07 (-0.02, 0.16)	0.07 (-0.02, 0.16)	0.08 (-0.01, 0.17)	0.08 (-0.02, 0.17)	0.05 (-0.04, 0.14)	0.06 (-0.02, 0.15)
Breast-feeding duration (Categories)	0.05 (-0.03, 0.12)	-0.01 (0.08, 0.06)	0.02 (-0.05, 0.09)	0.02 (-0.05, 0.10)	-0.04 (-0.12, 0.03)	0.08 (-0.01, 0.15)

β is the effect size (SD) of the cognitive outcome per SD change in postnatal factors derived using all variables included together in multiple regression; HC-head circumference
Values in bold are significant (p<0.05 for all)

Table 3.21a Effects of maternal education, head circumference at birth and conditional (0-1 year and 2-5 years) head circumference on childhood learning, long-term retrieval, reasoning and visuo-spatial ability

	ME (SD)	Birth HC (SD)	0-1 year conditional HC (SD)	2-5 years conditional HC (SD)
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
			Learning, long-term retrieval (SD)	
Model 1: Unadjusted (each exposure separately)	0.21 (0.12, 0.29)***	0.14 (0.04, 0.23)**	0.11 (0.03, 0.19)*	0.14 (0.06, 0.23)**
Model 2: ME+ birth head circumference (combined)	0.19 (0.10, 0.28)***	0.12 (0.03, 0.22)*		
Model 3: Model 2+ 0-1 and 2-5 years conditionals HC (combined)	0.18 (0.10, 0.27)***	0.12 (0.03, 0.22)*	0.09 (0.01, 0.18)*	0.15 (0.06, 0.23)**
			Reasoning (SD)	
Model 1: Unadjusted (each exposure separately)	0.28 (0.20, 0.36)***	0.10 (-0.003, 0.20)	0.12 (0.03, 0.21)**	0.13 (0.04, 0.22)**
Model 2: ME+ birth head circumference (combined)	0.25 (0.16, 0.34)***	0.08 (-0.02, 0.18)		
Model 3: Model 2+ 0-1 and 2-5 years conditionals HC (combined)	0.24 (0.15, 0.33)***	0.08 (-0.02, 0.18)	0.10 (0.01, 0.18)*	0.14 (0.05, 0.22)**
			Visuo-spatial ability (SD)	
Model 1: Unadjusted (each exposure separately)	0.27 (0.19, 0.35)***	0.15 (0.06, 0.25)**	0.09 (0.004, 0.18)*	0.12 (0.04, 0.21)**
Model 2: ME + birth head circumference (combined)	0.26 (0.17, 0.34)***	0.14 (0.04, 0.23)**		
Model 3: Model 2+ 0-1 and 2-5 years conditionals HC (combined)	0.25 (0.16, 0.34)***	0.14 (0.04, 0.23)**	0.07 (-0.02, 0.15)	0.13 (0.04, 0.21)**

β is the effect size (SD) of the cognitive outcome per SD change in the exposure; ME-maternal education; HC-head circumference; *** p<0.001; ** p<0.01; *p<0.05

Table 3.21b Effects of maternal intelligence, head circumference at birth and conditional (0-1 year and 2-5 years) head circumference on childhood learning, long-term retrieval, reasoning and visuo-spatial ability

	MIQ (SD)	Birth HC (SD)	0-1 year conditional HC (SD)	2-5 years conditional HC (SD)
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
			Learning, long-term retrieval (SD)	
Model 1: Unadjusted (each exposure separately)	0.16 (0.10, 0.23)***	0.14 (0.04, 0.23)**	0.11 (0.03, 0.19)*	0.14 (0.06, 0.23)**
Model 2: MIQ+ birth head circumference (combined)	0.16 (0.09, 0.23)***	0.13 (0.04, 0.23)**		
Model 3: Model 2+ 0-1 and 2-5 years conditionals HC (combined)	0.15 (0.08, 0.22)***	0.13 (0.04, 0.23)**	0.10 (0.01, 0.18)*	0.15 (0.06, 0.23)**
			Reasoning (SD)	
Model 1: Unadjusted (each exposure separately)	0.20 (0.13, 0.27)***	0.10 (-0.003, 0.20)	0.12 (0.03, 0.21)**	0.13 (0.04, 0.22)**
Model 2: MIQ+ birth head circumference (combined)	0.21 (0.13, 0.28)***	0.09 (-0.006, 0.19)		
Model 3: Model 2+0-1 and 2-5 years conditionals HC (combined)	0.20 (0.13, 0.27)***	0.09 (-0.007, 0.19)	0.10 (0.02, 0.19)*	0.14 (0.05, 0.22)**
			Visuo-spatial ability (SD)	
Model 1: Unadjusted (each exposure separately) (combined)	0.17 (0.10, 0.24)***	0.15 (0.06, 0.25)**	0.09 (0.004, 0.18)*	0.12 (0.04, 0.21)**
Model 2: MIQ+ birth head circumference (combined)	0.17 (0.10, 0.25)***	0.15 (0.05, 0.25)**		
Model 3: Model 2+0-1 and 2-5 years conditionals HC (combined)	0.17 (0.10, 0.24)***	0.15 (0.05, 0.25)**	0.08 (-0.01, 0.16)	0.13 (0.04, 0.21)**

β is the effect size (SD) of the cognitive outcome per SD change in the exposure; MIQ-maternal intelligence (imputed); HC-head circumference; *** p<0.001; **p<0.01; *p<0.05

3.10 Summary of main findings

- Socio-demographic factors (SLI, parental education, occupation, income, MIQ and home environment) were significant predictors of cognitive function during childhood and adolescence.
- Cognitive score increased between childhood and adolescence. There was a strong correlation between childhood and adolescent cognitive test scores showing that cognitive function tracks from childhood to adolescence.
- Generally, associations between early life factors and cognitive function were similar at both ages. Higher birthweight, length, HC, MUAC and SS predicted higher cognitive test scores. The strength of these associations was approximately halved after adjusting for socio-demographic factors, but remained significant for some tests. HC and MUAC at birth were the strongest predictors at both time points.
- Cognitive scores were higher in offspring of GDM mothers compared to offspring of non-GDM mothers at both time points. The size of this difference was large, but it was largely attenuated after controlling for socio-demographic factors.
- There was no association between breast-feeding duration and cognitive function.
- Childhood and adolescent body size and composition (HC, height, muscle mass (MUAC) and adiposity (SS)) predicted cognitive function in the corresponding period. These associations were attenuated, but remained significant for many tests, especially reasoning, after controlling for confounders.
- There were differences in the patterns of associations of head growth at different age intervals with different cognitive domains. Scores for learning, short-term memory, reasoning, and visuo-spatial ability were predicted most strongly by HC at birth and gain in HC during infancy and early childhood. In contrast verbal fluency and attention scores were most strongly predicted by gain in HC during later childhood.
- Head circumference at birth, and postnatal head growth predicted several cognitive domains independently of socio-demographic factors. The effects of, or the variation explained by, head size at birth and postnatal head growth were relatively smaller (approximately half) than the effects of socio-demographic factors.

3.11 Discussion

In this chapter I have described the associations of early life factors (size at birth, gestational diabetes, breast-feeding duration, current body composition and postnatal growth) with cognitive function during childhood and adolescence. It was important to assess the relationship of socio-demographic factors with cognitive function and to examine the associations of early life factors adjusted for these confounders.

3.11.1 Strengths and limitations: The main strength of the study was a high participation rate (~86% at both 9.5 and 13.5 years). This is one of the few studies in India with detailed longitudinal anthropometric data in children. To the best of my knowledge this is the first study in India to report a variety of prenatal and postnatal factors predicting cognitive function in a sample of normal children belonging to a wide range of SES. The quality of the children's anthropometry was high, with regular training of measurers and standardisation of methods. Gestational age was available for all the children. A wide range of cognitive domains were assessed using a validated cognitive battery adopted to suit the local culture; the tests were administered by trained Masters' level psychologists who were unaware of the exposures of interest. A variety of potential confounding factors, including SLI, parental education, occupation, income, MIQ and home environment were documented, which enabled me to examine the extent to which size at birth, gestational diabetes, breast-feeding duration, current body composition and postnatal growth predicted cognitive function independent of these factors.

There are some limitations in my study. The children may not be representative of all Mysore children; they were all born to a group of women booking consecutively into one maternity unit and whose glucose tolerance was examined during pregnancy. However, there is no reason to assume that they were significantly different from other children as their characteristics were similar to those reported for other urban south Indian populations[209,210]. As with all cohort studies, loss to follow-up was a limitation and the main reasons for loss to follow-up were lack of interest in participating, and moving away from the study area. Although follow-up rates were high (~86% at both time points) in the Parthenon cohort, any loss to follow-up carries the risk of introducing bias or limiting the generalizability of the findings. Parent's SES, educational level, occupation and income were higher among non-participants than participants (section 3.3; appendix 6). However, there were no differences in the key exposure variables of interest between these groups.

Losses to follow-up were too small to have a major impact on generalizability. Bias would be introduced only if the relationship between exposures and outcomes differed between those studied and not studied. I cannot think of a mechanism for this and I think it to be unlikely in my study. Another limitation was missing data. This was a problem mainly for maternal glucose tolerance (missing for 5%) and breast-feeding duration (5%), postnatal conditional growth (13%), maternal IQ (7%) and home environment (35%). With missing data there is a risk of reduction in the sample size and the associations found might have differed if a large number have missing data. To overcome this issue I used imputed data, especially for MIQ and home environment but not for others (the reasons for this are explained in section 3.2.3). I used a basic method of imputation (constructing a binary 'missingness' variable and replacing missing values with a constant (the mean value); section 3.2.3) to deal with missing data. There are other methods of imputation. For example, regression substitution, in which missing data is substituted by values predicted by linear regression on the basis of other related variables that are present. However, I did not explore this as it was beyond the scope of my current study. There were significant differences in socio-demographic factors and cognitive scores in participants with and without missing data (described on page 54 and shown in appendix 5). This was particularly true for missing/non-missing home environment scores, because we had to limit this scoring to children living in Mysore. I examined whether missing data could be biasing my results by looking at differences in the slope of predictor variables with cognitive function in those with or without MIQ and home environment data. I tested for interactions between missingness of MIQ and home environment data and predictors (birth size, GDM status, breast-feeding, postnatal growth and maternal nutrition). These analyses showed fewer interactions than expected by chance. This suggests no compelling evidence for the existence of major bias induced by missing data.

Lack of information about the severity and treatment of GDM, and exclusivity and frequency of breast-feeding and quality of breast-milk were other limitations. A further limitation was that I did not adjust for some other potential confounding factors, such as parity, parental age, and the child's pubertal status (the latter especially for adolescent cognitive function). In initial analyses, these factors were related to some cognitive domains, but I decided to omit them in my final analyses, partly because their inclusion did not change my results and partly because of incomplete data (pubertal status was available for ~90%). In retrospect, I think that excluding these factors was a mistake, and that the lack of adjustment is a limitation.

About 50% of the children were first born. This indicates a shift towards smaller family size in India observed in the last 2-3 decades[176]. This is largely attributed to implementation of the family planning programme (two children per family) and also to changing socio-economic conditions, making the families more likely to have one or two children only.

3.11.2 Cognitive test scores; comparison with other populations: Cognitive test scores and inter-correlations between test scores were similar to those in another south Indian population of the same age (personal communication; Dr. Srinivasan, St John's Research Institute, Bangalore). There are studies from western populations assessing cognitive function in children using the same tests as ours but the literature from these studies does not report the raw score of the tests and therefore it was difficult to compare with them. However the inter-correlations among the various cognitive tests used in my study were consistent with an earlier factor analysis of KABC[211].

3.11.3 Socio-demographic factors and cognitive function: I found strong associations of socio-demographic factors (parity, SLI, parental education, occupation, income, rural/urban residence, MIQ and home environment) with cognitive function at both time points. Of these, maternal education and MIQ appeared to have the strongest influence. Furthermore, the effect size was greater than early life factors. Some studies in western populations, similar to my findings, have reported greater effects of socio-demographic factors such as SES (occupation and parental education) [61], birth order[164], MIQ and child rearing practice[212], and parent's occupation[213, 214] compared to the effects of birth size and/or postnatal growth on childhood/adolescent cognitive function.

Socio-demographic factors influence the child's nutrition, parenting practices, parent-child interactions, learning materials and environment[30,149]. Therefore they make an impact on cognitive development and function. These factors, which are linked to one another, can also influence fetal and postnatal growth and development through nutrition and stimulation, and therefore confound the associations of prenatal and postnatal factors with cognitive function. Genetic factors could also play a part in explaining the association between socio-demographic factors and cognitive function. There is evidence (though not consistent) that SES in childhood may modify the influence of genes on cognition[215-217]. There is also some evidence that genetic factors may play a role in the association between

growth and cognition independently or in interaction with environmental factors[218]. However, I have not explored the role of genetic factors as it was beyond the objective of my study.

I found low intelligence scores (<70) in about 26% of mothers. Consistent with this, low intelligence scores (using the same scale) were reported in about 20% of mothers in another south Indian population[219]. I have confidence in the rigour with which the mothers' cognitive performance was assessed, and therefore conclude that this is a 'real' finding. The reasons for these low scores are not known and I can only speculate on the possible reasons. With the existence of the joint family concept in most Indian populations, decisions about household issues are usually made by elders (mostly men), leaving women unexposed to decision-making. Unfamiliarity with tests that require decision-making could be a possible reason for low average MIQ score. Most women would also be unused to doing tasks to a strict time protocol. The tests used in my study capture only non-verbal intelligence as there are no suitable scale for Indians to assess verbal intelligence. However, the finding in my study that MIQ was an independent predictor of cognitive function in the children suggests that the scale ranked women reasonably well, even if their scores are low on average.

3.11.4 Birth size and cognitive function: Cognitive test scores at both time points increased with increasing weight, length and HC at birth. Consistent with my findings, earlier studies also found a positive association of birthweight[41,58,61-66], length[41,60,62-64,66] and HC[41,59,60,62,63] with childhood and adolescent cognitive function (Table 1.1). The effect size in my study was modest and similar to that reported in earlier studies. Visuo-spatial ability score in my study increased by 0.10, 0.07 and 0.14 SD per SD increase in birthweight, length and HC respectively. A study among 10 year old UK children found 0.05 SD increase in cognitive ability per SD increase in birthweight[61]. Another study in Thailand reported 0.09 and 0.14 SD increase in the IQ score per SD increase in weight and length at birth respectively at age 9 years[64]. In Belarus, at age 6.5 years, the IQ score increased by 0.04 to 0.05 SD per SD increase in birthweight and length. For an SD decrease in birthweight, length and HC among Finnish children aged 4 ½ years, general reasoning and visuo-motor integration scores decreased by 0.08 to 0.1 SD, 0.1 SD, and 0.09 to 0.14 SD respectively[60]. Although some other studies have also reported linear associations of birth size with cognitive function[59,62,63,65], I was unable to compare the effect size as they have not reported SD scores for cognitive function. Additionally, I found

increasing scores with increasing MUAC and SS at birth. However, among the measurements of birth size, the effect of HC and MUAC, though small, remained significant for some tests after controlling for confounders, and were the strongest predictors of cognitive function.

Among the birth size measures, consistent with my findings that HC was the strongest predictor of cognitive function, a Finnish cohort also reported that HC had the most robust association with tests of cognitive ability at age 4 ½ years[60]. HC has been used as a surrogate marker of brain volume in newborns and children[18-20] and has been used in previous studies that measured cognitive function[220]. But due to age dependent changes in the relationship between HC and brain volume, a given HC is associated with a range of brain volumes depending on the age at which it is measured[221]. Nevertheless studies on children with attention deficit hyperkinetic disorder, a disorder with prominent cognitive dysfunction, have shown that such children have significantly smaller brain volume compared to healthy controls[222]. Thus better cognitive function associated with larger HC could be due to better functional capacity of the brain.

The association between MUAC and cognitive ability is a new finding. The mechanism for this association is not known. MUAC reflects better nutritional status and muscle mass[223]. Better nutrition helps in neural as well as muscular development. Higher muscle mass possibly reflects better motor function, influencing exploratory behaviour, and possibly better chances of positive reinforcement with caregivers, which enable better cognitive development and thus better cognitive function.

Development of the brain starts during intrauterine life and continues postnatally through infancy, childhood and adolescence. During intrauterine life brain growth is protected and is the last organ to experience growth restriction[48]. Further, the rate of development and maturity of different areas of the brain vary during the prenatal and postnatal period (Figure 1.1)[18-21]. Therefore variations in prenatal and postnatal factors can be associated with variations in brain growth and development. Thus, the impact of prenatal factors on specific aspects of cognitive function, and /or a greater influence of postnatal environmental processes, such as parent's socio-economic factors, MIQ and home environment, are possible explanations for the associations of birth size with some but not with other cognitive domains in my study.

Although all socio-demographic factors (except parity, occupation and income) and birth HC were found to have independent effects on some cognitive domains. I found that the coefficients for the socio-demographic variables were approximately double those of birth HC. In order to compare the size of the effect of socio-demographic factors and birth HC on cognitive function, I selected those socio-demographic factors that were most consistently and strongly predictive of cognitive function and then included them in a model with birth HC. But this crude comparison has some limitations. The mechanisms by which birth size and socio-demographic factors predict cognitive function are different. For example head size reflects neurodevelopment, and maternal education reflects mother-child interactions and also quality of the home environment. Further, maternal education may be a determinant of birth size. Furthermore, head size is a very crude proxy for brain development and similarly maternal education is a crude proxy for mother-child-interaction and quality of the home environment. Therefore it is difficult to estimate the true extent of variation in cognitive function explained by each factor. However, the approach that I used gives an idea of the relative effects (although not absolute) of these factors in predicting cognitive function. Other statistical methods such as structural equation modelling can be used to compare effect sizes and investigate direct and indirect influences of related variables, but is beyond the scope of my current study.

3.11.5 Gestational diabetes and cognitive function: In my study cognitive test scores were higher in children born to GDM mothers compared to children of non-GDM mothers. There are some studies, mainly from developed countries, reporting a negative association between GDM and cognitive function (section 1.3.3.3.5). Further it has been reported that cognitive function was impaired only in those offspring whose mothers had uncontrolled diabetes, suggesting that offspring cognitive performance could be within normal limits in well-controlled GDM. There were no cases of severe or uncontrolled GDM in my study. Confounding effects could be another possible pathway linking GDM and cognitive function. Unlike developed countries, where the prevalence of GDM and poor cognitive ability are higher in low socio-economic populations, in our population GDM and cognitive function were associated with higher SES and maternal education[189]. Reduced associations after controlling for these factors in my study suggest confounding effects of socio-economic factors. A recent report, based on Mendelian randomisation, linking maternal genetic variants related to type 2 diabetes with an increase in the child's IQ, suggests that other than biological or environmental pathways, genetic factors may contribute to the association between GDM and cognitive function[100].

3.11.6 Breast-feeding and cognitive function: Evidence from a large body of research, mainly from developed countries (section 1.3.4.1), suggests that breast-fed children, compared to bottle fed children, score better in cognitive tests. Some studies reported a 'dose-response' effect of duration of breast-feeding on cognitive ability. Other studies did not find any associations. The beneficial effects have been attributed to breast milk constituents (long-chain polyunsaturated fatty acids required for brain development)[126], and/or environmental factors (better mother-baby bonding and sensory stimulation in breast-fed infants[127, 128]. Parents' SES and education/intelligence, which are strongly related to cognitive performance and also to initiation and duration of breast-feeding, may confound the association of breast-feeding with cognitive function. In my study there was no association between breast-feeding duration and cognitive function. One possible explanation is that SES was only weakly related to breast-feeding duration in Mysore[191] and confounding was not an issue, thus revealing a genuine lack of effect of breast-feeding on cognitive ability. Another possibility is that we failed to detect an effect because of a lack of heterogeneity in breast-feeding duration in our population. A striking difference between ours and the studies in high-income countries was that most infants in the latter stopped breast-feeding <6 months[36,133,134], and 'longer duration' could mean anything from 2+ to 8+ months; few of our children were breast-fed for as short a duration as this and 65% were breast-fed for 12 months or more. If the first 6 months of life is a critical period in which breast-feeding can influence cognition, we may have had inadequate power to detect this because almost all our children were breast-fed during that time. It is possible that the contents of breast-milk (fatty acids) are important in this context. Although we do not have the information about breast-milk fatty acids, low levels of these nutrients in breast-milk in our population could be another explanation for our negative findings.

3.11.7 Postnatal growth and cognitive function: As brain development continues in the postnatal years, growth is likely to be related to cognitive skills. In my study, gain in HC in the early postnatal years was associated with higher scores in several cognitive domains. This is consistent with the few studies that have examined this[59, 60]. Two studies from western populations, one from the UK[59] and another from Finland[60], found associations of head growth during infancy (0-1 year in the UK; 0-2 years in Finland) but not during early childhood (1-4 years in the UK; 2-5 years in Finland) with cognitive function. In contrast, in my study HC growth during early childhood (2-5 years) predicted higher cognitive scores in some tests. The reasons for this difference are not clear. A possible reason could

be differences in HC and the velocity of head growth between the western and Indian populations. In my study children, mean HC at birth (33.8 cm) and at one year (44.2 cm) and two years (45.8 cm) are about 2-3 cm smaller than the UK population[224]. Further, a recent study in the UK found that UK infants had rapid head growth (UK-WHO growth standard) during early infancy[224]. It also found that infants of south Asian origin had smaller HC at birth and that the difference persisted during infancy compared to the UK populations. The smaller size in Indian populations at birth, possibly because of maternal undernutrition, and during infancy, possibly because of continued breast-feeding and inadequate complimentary feeding, may delay infant head growth and neurodevelopment. But improvement in nutrition during the childhood years may compensate for the earlier delay, leading to associations of HC growth with cognitive function at a later age than is seen in western populations. I have not adjusted for the children's diet in my analysis and I did not measure quality of schooling, which may have played an important role in the observed associations. Therefore, residual confounding cannot be ruled out as an alternative possible explanation for my results.

In my study there was no association between head growth during later childhood (5-9.5 years) and cognitive test scores. There are inconsistent findings from the few studies that have examined the associations between head growth during later childhood years and cognitive function[59,61,64,225]. Two studies, one from the UK[59] and the other from Thailand[64], found no associations of head growth during later childhood years (4-8 years in the UK; 1-9 years in Thailand) with cognitive function. Two other studies, both from the UK, found positive associations of head growth with cognitive function[61,225]. In one of them, HC at 5 years predicting concurrent cognitive function was partly mediated by HC at 10 years, and HC at 10 years predicted concurrent cognitive function independent of HC at 5 years[61]. In the other, head growth during 9 months-9 years predicted IQ at age 9 years[225]. However, information of HC at birth (in Thailand[64] and in an UK cohort[61]) and head growth after birth and/or infancy in three studies[61,64,225] was unavailable. Therefore, the importance of head growth during later childhood years influencing cognitive development is unclear. In my study head growth continued during later childhood. Head growth in later childhood, compared to early childhood years, may not compensate for poorer growth during infancy and might explain the lack of an association between head growth during later childhood (5-9.5 years) and cognitive function in my study.

I did not find an association of head growth during adolescence (9.5-13.5 years) with any

of the cognitive test scores. Brain volume increases fourfold from birth to 10 years and HC, which correlates with brain volume, rises from birth and peaks at about 5 to 10 years of age[18-20]. Imaging studies in children also show that maximum brain volume is achieved by around age 10 years or earlier. In adolescence, there are changes in the volume of grey relative to white matter but not necessarily in overall volume[226,227]. However, the role of changes in grey/white matter volumes in predicting cognitive development is not well understood. In my study, although head growth continued beyond the late childhood years, the velocity of head growth was reduced. The lack of an association between head growth during adolescence and cognitive function suggest that either changes in overall brain volume during adolescence are not important for cognitive function, or that changes in overall brain volume during adolescence are too small to detect any relationship with cognitive function.

I found differences in the patterns of associations of head growth at different ages with different cognitive domains. HC at birth was the strongest with some domains, and the effect size of head growth tended to decrease with increasing age. Scores of verbal fluency and attention were unrelated to birth HC and the strongest effects were with head growth during later childhood. The reasons for these different patterns are not known. The rate of development and maturity of different areas of the brain, related to different cognitive processes, vary during the prenatal and postnatal period. Areas related to most basic sensory-motor functions (eg. touch, smell, vision, walking) develop rapidly during infancy, areas related to language and spatial orientation continue to develop during childhood and areas related to higher and complex functions like problem solving, memory attain their maturity during late childhood and adolescence (Figure 1.1)[18-21]. Therefore variations in head growth at different ages may relate to different cognitive domains.

In addition to HC, gain in MUAC also predicted higher cognitive function. The mechanism is not known. As already mentioned in section 3.11.4, MUAC reflects nutritional status and muscle mass. Improved muscle mass may enable more physical activity which in turn improves the availability of certain growth factors, like BDNF, which enhances synaptic transmission and function of the brain and therefore contributes for better cognitive function[228]. Additionally, higher muscle mass possibly reflects better motor function, influencing exploratory behaviour, and therefore better chances of positive reinforcement with family, friends and neighbourhood, all of which might be beneficial for cognitive development. Another possibility is that better nutrition helps in better development and

function of the brain. Alternatively, since MUAC and HC are measured more precisely than other measurements, such as skinfolds, they may reflect overall growth and nutrition and therefore better cognitive function rather than specific effects of muscle or brain growth. Findings in my study that the effect of HC on cognitive function was independent of MUAC or SS suggest that the observed associations are likely to be due to specific effects of brain growth. However, the effect of MUAC was not independent of HC suggesting that the effect of MUAC in predicting cognitive function could be due to overall nutrition and growth rather than specific effects of muscle growth.

A role of Insulin-like growth factor (IGF)-1 is another possible pathway linking postnatal growth and cognitive function. IGF-1 has been found to be associated with brain development. By mediating the effects of growth hormone it contributes to somatic growth regulation and organ development[229]. IGF-1 levels in childhood have been found to be related to verbal competence in a population-based sample of children[229] and among children born SGA and treated with a growth hormone[230].

My finding of gain in SS (a measure of adiposity) during late childhood (5-9.5 years), but not during infancy and early childhood or adolescence, predicting higher cognitive scores is new. The causal pathway for this association is not known but there are some possible explanations. Firstly, SS gain may reflect pre-pubertal changes, hence greater maturity and more advanced neurodevelopment. Although, I did not find any association with gain in SS during adolescence it does not necessarily rule out the role of more advanced maturation as an explanation of my findings in late childhood. Secondly, underweight was relatively more prevalent than overweight/obesity in this cohort. Further, the effect of SS was not independent of HC, suggesting that greater gain in adiposity in later childhood years may represent better nutrition and therefore better neurodevelopment and function. Alternatively, confounding by socio-demographic factors is a possible pathway, as some of the associations were reduced after controlling for these factors. However the fact that these associations remained for some tests even after adjusting for these factors suggest that the associations were not completely explained by confounding.

3.12 Conclusion

In this Indian population cognitive function during both childhood and adolescence was independently predicted by some of the early life factors (birth size, especially HC at birth

and postnatal growth, especially head growth during infancy and early childhood). Socio-demographic factors (especially, maternal education and intelligence) also independently predicted cognitive function at both time points. The effects of socio-demographic factors on cognitive function were relatively greater (approximately double) than the effects of early life factors. However, models containing both early life and socio-demographic factors explained a greater percentage of the variance in cognitive function than models containing socio-demographic factors alone, suggesting that both play a role in cognitive development.

The findings from my study suggest that investment to improve maternal education will have an impact on the child's cognitive development and part of this may be through the influence of maternal education on prenatal and postnatal nutrition, growth and development, parenting practice, mother-child interactions and stimulating environment. Since body size in early life reflects organ development including the brain, findings from my study also suggest for investment to improve prenatal and postnatal size to achieve better cognitive capacity.

In the next chapter I will explore the role of maternal nutrition in predicting offspring cognitive function.

CHAPTER 4 MATERNAL NUTRITION AND OFFSPRING COGNITIVE FUNCTION

4.1 Introduction

As already highlighted in the introductory chapter (section 1.3.3.4), maternal nutrition is crucial for fetal growth and development including neurodevelopment. In this chapter I describe maternal nutritional status and its relationship to offspring cognitive function during childhood and adolescence. In the first part I describe a systematic review of published literature that I carried out to explore the evidence linking maternal nutritional status and offspring cognitive function during childhood and adolescence. In the second part I report the findings from my own data. The term maternal nutrition which refers to 'maternal nutritional status during pre-pregnancy and/or pregnancy' means:

- Anthropometric indices (BMI, height and weight)
- Micronutrients: Dietary intake/plasma or serum concentrations/use of supplements/deficiency of vitamins (D, B1, B2, B6, B12 and folate) and iron (Haemoglobin (Hb) level, iron stores or anaemia)
- Macronutrients: Dietary intake of carbohydrate, protein and fat

I chose only the above mentioned micronutrients, and not others such as fatty acids, zinc, for the systematic review in order to compare the findings from my data (which included some of the above micronutrients) with the existing literature. Further, although I do not have data on B1, B2, B6 and iron status, I am interested to examine the status of these nutrients and to explore its link with cognitive function in my population in future.

4.2 Maternal nutritional status and offspring cognitive function during childhood and adolescence; a Systematic Review

4.2.1 Background: Generally policy makers and health professionals across the globe recommend a nutritious diet including micronutrients to pregnant mothers to ensure a healthy pregnancy and its outcome. As maternal nutrition is the only source of nutrition to the growing fetus, maternal macronutrients (carbohydrate, protein and fat) and micronutrients status including vitamins and minerals are likely to influence offspring neurodevelopment[114]. While macronutrients serve as building blocks in overall CNS development, micronutrients enable myelination, synaptogenesis, neurotransmitter

production and transmission[114]. As neurodevelopment occurs rapidly during pregnancy any impairment in the nutritional supply to the growing fetus may interfere with the development and function of the brain. Further, the degree of alteration in the structure and function of the CNS depends on the timing, duration, type and severity of prenatal nutritional deprivation. Taking all these points into consideration one would expect maternal nutritional status to be a potential predictor of offspring cognition later in life.

Studies investigating the association of maternal nutritional status with offspring cognitive function are limited, and there are few systematic reviews. Three systematic reviews were published in 2011. One examined the association of pre-pregnancy and pregnancy obesity with offspring neurodevelopmental outcomes. It included 12 observational studies, of which only 2 investigated cognitive function. It concluded that children of obese women may be at increased risk of cognitive deficits[231]. The second included only 2 studies, both randomized controlled trials, and examined the effect of prenatal folic acid supplementation with other vitamins/minerals on childhood mental performance at different ages (8-21 months, 2 and 6 years). It concluded that prenatal multivitamin supplements containing folic acid do not affect the child's cognitive performance[232]. The third examined the effect of single and multiple micronutrient supplementation (vitamins, minerals, fatty acids, and protein and carbohydrate in different combinations and included 18 randomised control trials of which 17 examined cognitive function) during pregnancy on offspring neurodevelopmental outcomes. It showed no conclusive evidence of benefit and suggested further research[233]. Thus there is no consistent evidence for an association between maternal nutritional status and offspring cognitive function during childhood and/adolescence. There are some new data published following the publication of these reviews. Thus a systematic review is now warranted.

4.2.2 Methods: I used the methods recommended by the Centre for Reviews and Dissemination (CRD), University of York[234] and followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement[235].

4.2.2.1 Eligibility criteria: The following inclusion and exclusion criteria were considered

Inclusion criteria:

- Observational studies
- Macronutrient and/or single micronutrient trials
- Human studies
- English language publication
- Age of study participants 0-18 years

- Year of Publication: 1st January1960-30th April 2014
- Exposure: maternal nutritional status as defined above
- Outcome: childhood or adolescent cognitive function

Exclusion criteria:

- Animal studies
- Studies in adults
- Case reports
- Multiple micronutrient trials
- Articles on maternal obesity (n=2) described in an earlier systematic review

I included trials in my review if it was a single micronutrient trial or if it was a multiple micronutrient trial which included intervention groups that differed by a single micronutrient. I have not described multiple micronutrient trials for the following reasons: a) I was interested in specific single micronutrient effects rather than the multiple micronutrient effects; b) two systematic reviews of multiple micronutrient trials on this topic have been recently published.

4.2.2.2 Search strategy: I consulted my supervisors and a senior researcher trained in conducting systematic review and meta-analyses at the Medical Research Council, Lifecourse Epidemiology Unit, University of Southampton to develop the search strategy (databases, selection of the medical subject headings (MeSH) terms, using keywords, filters to search articles of relevance based on inclusion and exclusion criteria).

I searched Medline/PubMed and the Cochrane Library to retrieve relevant literature using the MeSH terms and text word terms for exposures and outcomes of interest. The MeSH terms and text word terms used are listed in Table 4.1a and b. Additionally, a lateral search (i.e. screening of reference lists of literature retrieved for review) was carried out.

Table 4.1a List of MeSH terms and the text word terms used for exposure

Exposure: Maternal nutritional status during pregnancy	
MeSH terms	**Text word terms**
"exp body weight/ or exp body mass index / or exp anthropometry/ or exp body size/ or exp skinfold thickness/ or exp nutrition assessment/ or exp nutritional status/ or exp mothers/ or exp pregnancy/ or exp malnutrition/ or exp diet vegetarian/ or exp haemoglobin/ or pregnancy complications/ or exp anaemia/ or exp folic acid/ or exp folic acid deficiency / or exp vitamin B12 deficiency/ or exp ferritin/ or exp iron, dietary/ or exp cholecalciferol/ or exp pyridoxine/ or exp vitamin B complex/ or exp riboflavin/ or exp thiamine/ or exp vitamin D/"	"maternal nutrition or maternal anthropometry or pregnancy nutrition or antenatal nutrition or intrauterine nutrition or gestational nutrition or maternal undernutrition or prenatal nutrition or maternal BMI or maternal micronutrients or vegan mothers or vegetarian mothers or macrobiotic mothers or maternal folate or maternal folic acid or maternal vitamin B12 or maternal cobalamin or maternal vitamin D or 25 hydoxy vitamin D or maternal cholecaliciferol or maternal haemoglobin or maternal iron or maternal B vitamins or maternal vitamin B1 or maternal vitamin B6 or maternal vitamin B9 or maternal B vitamins or maternal anaemia or maternal diet"

Table 4.1b List of MeSH terms and the text word terms used for outcome

Outcome: Childhood and adolescent cognitive function	
MeSH terms	**Text word terms**
"exp child/ or exp child development/ or exp adolescent/ or exp neurobehavioral manifestations/ or exp child, preschool/ or exp cognition, physiology/ or exp attention/ or exp memory, long-term/ or exp memory, short-term/ or exp memory / or exp intelligence tests/ or exp psycho motor performance/ or exp child psychology/ or exp decision making/ or exp psychometrics/ or exp intelligence/ or exp mental competence/ or exp cognition/ or exp motor skills/ or exp language development/ or exp learning/ or exp verbal learning/ or exp problem solving/ or exp perception/ or exp thinking/ or exp executive function/ or exp function/ or exp human development/ or exp adolescent development/ or exp speech/ or exp mental processes/	Cognitive function or intelligence or IQ or executive function or psychomotor development or cognitive performance or cognition or educational attainment or cognitive ability or cognitive deficits or intellectual ability or learning or memory or language development.

4.2.2.3 Identification of literature for review: The process of identification and extraction of literature is described in Figure 4.1. From the database search 16,143 articles were identified. The titles and abstracts of all the articles were evaluated to determine whether articles met the eligibility criteria, and 54 articles were selected for full text review. Lateral search of these led to the identification of another 8 articles making a total of 62 citations for full text review. After full text review, excluding 30 for the reasons mentioned in Figure 4.1, 32 studies were finally included for qualitative synthesis.

4.2.2.4 Data extraction and quality assessment: I extracted the following data:

- Study setting
- Population and design
- Selection and baseline characteristics
- Exposure and outcome measurement
- Statistical methods used
- Study results
- Confounding factors adjusted for

Before commencing quality assessment I and a Research Assistant at the Medical Research Council Lifecourse Epidemiology Unit, University of Southampton prepared a standardized form (Table 4.2)[236] and assessed each article independently. The risk of bias was assessed using a set of 22 criteria. Overall there were not many discrepancies between the two quality assessors, and where there were differences they were resolved by discussion. Based on the total score (maximum score 22) and reviewer judgement, risk of bias was defined as: a) **Low:** a total score >16; b) **Medium:** a total score 12-16 and c) **High:** a total score <12.

Figure 4.1 Flow diagram illustrating the selection of literature for inclusion in the qualitative synthesis

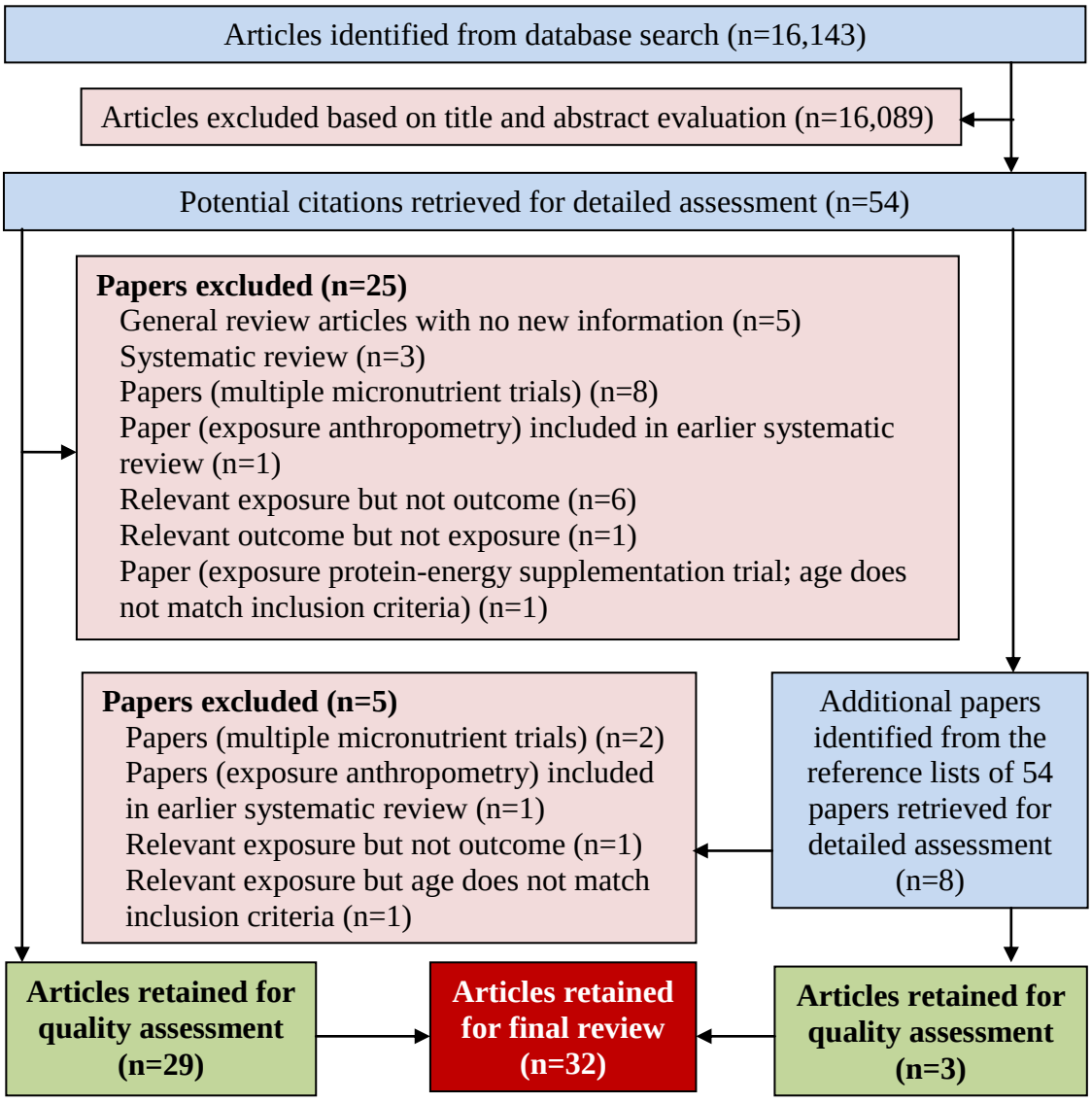


Table 4.2 Quality assessment form for a systematic review

Article ID: **Author and Year of publication:** **Reviewer code:**

Article Title:

Quality	Criteria	Score
Report	1. Is the hypothesis/aim/objective of the study clearly described? **0-No; 1-Yes**	
	2. Are the main outcomes to be measured clearly described in the introduction or methods section? **0-No; 1- Yes**	
	3. Are the characteristics of the patients included in the study clearly described**?** **0-No; 1- Yes**	
	4. Are the main exposures to be measured clearly described in the introduction or methods section? **0-No; 1- Yes**	
	5. Are the main findings of the study clearly described? **0-No; 1- Yes**	
	6. Does the study provide estimates of the random variability in the data for the main outcomes? **0-No; 1- Yes**	
	7. Have the number of patients lost to follow-up been given? **0-No; 1- Yes**	
	8. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001? **0-No; 1- Yes**	
External validity	9. Were the subjects asked to participate in the study representative of the entire population from which they were recruited? **0-No; 0-Unable to determine; 1-Yes**	
	10. Were the subjects who were prepared to take part representative of the entire population from which they were recruited? **0-No; 0-Unable to determine; 1-Yes**	
Internal validity	11. Was an attempt made to blind the assessor of the outcome about the main exposure? **0-No; 0-Unable to determine; 1-Yes**	
	12. If any of the results of the study were based on "data dredging", was this made clear? **0-No; 0-Unable to determine; 1-Yes**	
	13. Do the analyses adjust for different lengths of follow-up of subjects? **0-No; 0-Unable to determine; 1-Yes**	
	14. Were the statistical tests used to assess the main outcomes appropriate? **0-No; 0-Unable to determine; 1-Yes**	
	15. Were the main exposure measures used accurate (valid and reliable; recall bias)? **0-No; 0-Unable to determine; 1-Yes**	
	16. Were the main outcome measures used accurate (valid and reliable; recall bias)? **0-No; 0-Unable to determine; 1-Yes**	
Internal validity confounding (selection bias)	17. Were the subjects in different exposure groups recruited from the same population? **0-No; 0-Unable to determine; 1-Yes**	
	18. Were study subjects in different exposure groups recruited over the same period of time? **0-No; 0-Unable to determine; 1-Yes**	
	19. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn? **0-No; 0-Unable to determine; 1-Yes**	
	20. Were losses of subjects to follow-up taken into account? **0-No; 0-Unable to determine 1-Yes**	
Power	21. Was the power calculation done before the study to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%? **0-No; 0-Unable to determine 1-Yes**	
Conflict of interest	22. Was there a declaration of conflict of interest or identification of funding source **0-No; 1-Yes**	
	Total Score	

4.2.3 Results

Of 32 articles (29 observational studies and 3 double blind randomized controlled trials) included for review there were **9** for anthropometric indicators; **3** for vitamin D, **13** for folate (of which 5 also examined B12 and another examined iron), **6** for vitamin B12 (5 of which also examined folate), and **7** for Iron/Hb/anaemia (of which 1 also examined folate).

4.2.3.1 Maternal anthropometry and offspring cognitive function (Table 4.3): All nine studies were observational and from developed countries[237-245]. One was published in 1982[244] and the remaining during 2011-2013[237-243,245]. Sample size varied from 101 to over 11,000 mother-offspring pairs. The children's age at assessment varied from 2-16 years. Loss to follow-up was high (30-40%) in 5 studies[237,239,241,244,245]. In a study from the USA participants were from the control group of a case (hypothyroid)-control (euthyroid) study[243]. A study in the UK and another three studies, one in Spain and two in the USA excluded children of underweight mothers (BMI<16 and BMI<18.5 kg/m^2 respectively)[239,240,242,243].

In seven studies pre-pregnancy BMI and/or weight was used as categories or as a continuous variable or both[237-243]. In six out of the seven studies pre-pregnancy BMI and/or weight was assessed based on self report[237-239,241-243]and in the other it was measured[240]. Pregnancy weight gain was used in four studies[237,240,244,245] and as a covariate in one study[238]. Pregnancy weight gain was measured in four studies[238, 240,244,245] and self reported in one study[237]. Exposure data was collected at varying periods, for some during antenatal visits[237,240-245] and for others nine months following the index pregnancy or delivery[238,239]. The Bayley Scale of Infant Development (BSID) was used in three studies[238,242,243] and in the others the cognitive domains assessed and the instruments used were different. Assessment was done by trained examiners in all the studies, with the exception of one where it was based on parental report[241]. None of the studies stated whether the outcome assessors were blind to exposure status.

The associations of pre-pregnancy BMI and/or weight or gestational weight gain with cognitive function were mostly consistent, despite some inconsistencies within studies. Six of the seven studies using BMI categories, found negative associations of pre-pregnancy obesity with offspring cognitive function (Table 4.3)[237-240,242,243]. In all the six studies cognitive scores decreased with increasing BMI categories,[237-240,242,243] though in one

cohort with a small sample size (n=412) the association was not significant[242]. In general, the effect size was small. For example, in one study, the apparent adverse effect of maternal obesity on offspring mental development scores at age ~2 years was only evident in the extremely obese category (BMI>35 kg/m^2; effect size: 0.1 SD)[238]. This study also observed a higher risk of delayed mental development among children of underweight (risk ratio 1.36; 95% CI: 1.04, 1.78) mothers compared to normal mothers. In three studies the effect was found in obese (BMI >30 kg/m^2) but not in overweight (BMI 25-29.9 kg/m^2) categories[237, 242,243]. Children of obese rather than normal weight mothers scored ~0.1-0.2 SD lower in reading and mathematics scores at age 5-7 years[237], and mental but not psychomotor development scores at age 1-2 years[242]. Similarly, in a study of two datasets (both with a small sample size, n=~100), in one dataset children of obese rather than normal weight mothers scored 0.6 SD lower in performance IQ but not in full scale and verbal IQ at age 8 years. In the other dataset children of obese rather than normal weight mothers scored 0.2-0.5 SD lower in motor, language and cognitive scores at age 2 years, but the difference was not significant[243]. In this study, the percentage of children who scored below the composite scores in BSID at age 2 years and WISC-III at age 8 years was higher in children of obese compared to normal mothers (BSID: 33% v 13%; WISC-III: 50% v 17%)[243]. In the remaining two studies the effects were found in both overweight and obese categories[239,240]. Children of obese and overweight rather than normal weight mothers scored ~0.1-0.2 SD lower in general intelligence at age 5 and 7 years[239] and performed poorly in a test of executive function at age 7 years[240]. In only one of the seven studies (which included two cohorts), there was no association between maternal overweight/obesity and offspring language and non-verbal skills at age 2-3 years[241]. However, in one of the two cohorts there was an increased risk of lower IQ (OR=0.84; 95% CI: 0.73, 0.98) at age 8 years among children of obese mothers compared to normal mothers.

Three of the seven studies also reported an inverse association between BMI (used as a continuous variable) and cognitive function[239,240,242]. For example, there was a small reduction in offspring general intelligence score (~0.2 SD/10-point increase in maternal BMI; age 5 and 7 years)[239], and cognitive and psychomotor development scores (0.01 SD per unit increase in maternal BMI; age 1-2 years)[242].

In another study, for each kg increase in maternal pre-pregnancy weight there was a small reduction (0.004 SD) in offspring school entry assessment scores at age 4 years and IQ at

age 8 years and odds of achieving adequate final exam results at age 16 years (OR=0.99; 95% CI: 0.98, 0.99)[245].

In three of the seven studies lower cognitive function was found among children of underweight (BMI <18.5 kg/m^2) mothers than normal weight mothers[237-239]. One of these studies found a very small (0.02-0.05 SD) difference in reading and mathematics scores at age 5-7 years that was not significant[237]. In another there was a significant difference in general intelligence (0.2-0.3 SD) at age 5 and 7 years in unadjusted analysis, but no information was provided on the size of the difference after adjustment for potential confounding factors[239]. In the third, there was a significant difference in the risk of delayed mental development at age ~2 years (risk ratio 1.36; 95% CI: 1.04, 1.78), but there was no association when the scores were treated as a continuous variable[238].

The findings were inconsistent among four studies that examined the effect of pregnancy weight gain with offspring cognitive function. One found poorer scores in the Raven coloured progressive matrices test in children of mothers who gained >30 pounds (n=230) compared to those who gained 5-29 pounds (n=1361)[244]. In another study, children of mothers who gained less weight than expected had lower school entry assessment score (0.08 SD) at age 4 years and were less likely to achieve adequate final-exam results (OR=0.88) at age 16 years[245]. This study also found a ~0.07 SD increase in offspring school entry assessment score at age 4 years and also a 0.07 SD increase in IQ at age 8 years for every 400 g/week gain in maternal weight during early and late pregnancy (also during mid-pregnancy for IQ). The other two studies (one with a very small sample size (n=174)) found no association between pregnancy weight gain and offspring cognitive function[237,240].

All studies reported the results adjusted for a number of confounders that they documented. Risk of bias was medium in all of them (score: 13-16). The factors causing a risk of bias were inadequate control for SES, unmeasured confounders such as MIQ and home environment, low validity of the exposure, small sample size and questionable selection, poor reporting about losses to follow-up and observer bias. The confounders adjusted for, and the quality score for each study are presented in Table 4.3.

Table 4.3 Summary of the findings from studies examining associations of maternal BMI and pregnancy weight gain with offspring cognitive function

Author, Year, Sample size, Age, Country, Study design	Maternal anthropometry	Cognitive function	Results after adjustment for confounders	QS and RB
[237]**Tanda R; 2012** N=3412 Age 5-7 years USA Longitudinal	Pre-pregnancy BMI (kg/m^2) and Pregnancy weight gain (kg) BMI 4 categories Underweight (BMI<18.5): 7.2% Normal (BMI 18.5-24.9): 65.6% Overweight (BMI 25.0-29.9): 17.6% Obese (BMI ≥30): 9.6%	Peabody Individual Achievement Test Reading and Mathematics scores	Pre-pregnancy obesity, but not overweight, was negatively associated with cognitive skills Compared to children of normal weight mothers, children of obese mothers scored 3 points lower (0.23 SD) in reading and 2 points lower (0.16 SD) in mathematics score. ↑pregnancy weight gain - ↓ cognitive skills but not significant **Confounders adjusted for:** the child's sex, GA, current age and body size, ethnicity, parity, SES (income), MA, ME, MIQ, HE	**15** **Medium**
[238]**Hinkle SN; 2012** N=6850 Age 2 years USA Population based Longitudinal-Birth cohort	Pre-pregnancy BMI (kg/m^2) and Gestational weight gain (kg) BMI 5 categories Underweight (BMI<18.5): 5% Normal (BMI 18.5-24.9): 56% Overweight (BMI 25.0-29.9): 25% Obese1(BMI 30.0-34.9): 8% Obese2 and 3(BMI>=35.0-39.9): 6%	Bayley Scales of Infant Development –II (Mental Development Index (MDI) and Psychomotor Development Index (PDI))	Compared to the children of normal BMI mothers, children of mothers in all the other categories scored lower MDI, but significant in obese2 and 3 categories (β=2.13 points). Risk of delayed mental development (<-1SD v > 1SD) observed in children of mothers with underweight (RR=1.36) and extreme obese (RR=1.38) categories No association between pre-pregnancy BMI and PDI **Confounders adjusted for:** the child's sex, BWT, GA, BF, MA, ethnicity, marital status, parity, DM, PIH, ME, MS, SES (income)	**16** **Medium**
[239]**Basatemur E; 2012** Age 5 years (n= 11025) Age 7 years (n=9882) UK Prospective population based birth cohort	Pre-pregnancy BMI (kg/m^2) BMI continuous and categories BMI 4 categories Underweight (BMI<18.5): 5.3% Normal (BMI 18.5-24.9): 65.6% Overweight (BMI 25.0-29.9): 20.1% Obese (BMI ≥30): 9% Excluded BMI<16	5 Y-British ability scales-II Expressive language, nonverbal reasoning and spatial visualization 7 Y- British ability scales-II spatial visualization verbal ability, and number skills test (National foundation for educational research progress in Math tests)	Children of underweight, overweight and obese mothers scored lower mean scores (0.1-0.3 SD). Maternal pre-pregnancy BMI is negatively associated with children's general cognitive ability at 5 years (β=-0.075) and 7 years (β=-0.17) 5 years - ↑maternal BMI -↓Spatial visualization but no association with expressive language and nonverbal reasoning 7 years- ↑maternal BMI -↓Spatial visualization, verbal ability and number skills **Confounders adjusted for:** The child's sex, current age, BWT, BMI, ethnicity, MA, ME, PE, SES, income, MS, DM.	**15** **Medium**

[240]**Buss C; 2012** N=174 Age 7.3 years USA Population based prospective Longitudinal-Birth cohort	Pre-pregnancy BMI (kg/m^2) and Gestational weight gain (kg) BMI continuous and categories BMI 3 categories Normal (BMI 18.5-24.9): 58% Overweight (BMI 25.0-29.9): 25.9% Obese (BMI ≥30): 16.1% Excluded underweight mothers	Executive function Continuous Performance Task (Go/No go task)	Higher pre-pregnancy BMI (continuous and categorical) was associated with impaired performance on the Go/No go task ($F_{1.157}$=8.37 and $F_{2.156}$=3.57 respectively). Children of obese mothers scored higher in performance measure (higher score indicates poor performance) compared to children of normal weight mothers. No difference in scores of performance efficiency between children of obese mothers vs children of overweight/normal weight mothers (Chen's *d* effect size 0.62 SD). Gestational weight gain was not associated with child performance on the Go/No go task ($F_{1.157}$=0.27) **Confounders adjusted for:** The child's sex, current age, BMI, ethnicity, GA, parity, BWT, ME, MIQ, depression, obstetric risk (PIH, DM).	**13** **Medium**
[241]**Brion M; 2011** **ALSPAC:** population based prospective cohort UK N=~5000 Age 38 months; Age 8 years **Generation R:** Population based pregnancy cohort Netherlands N=~2500 Age 30 Months	Pre-pregnancy BMI (kg/m^2) Underweight (BMI<18.5) Normal (BMI 18.5-24.9) Overweight (BMI 25.0-29.9) Obese (BMI ≥30) **ALSPAC** Normal BMI: 78.7% Overweight/obese: 21.3% **Generation R** Normal BMI: 77.9% Overweight/obese: 22.1% Excluded obese group (cognitive assessment at age 30-38 months)	**ALSPAC**-Verbal skills-MacArthur Toddler Communication Questionnaire maternal report Non-verbal skills-Diagnostic Analysis of Non-verbal Accuracy Test General intelligence-Wechsler Intelligence Scale for Children -II at 8-y **Generation-R**-Verbal skills-Dutch translation of the Language Development Survey Non-verbal-Dutch version of parent report of children's abilities	ALSPAC: No association of maternal overweight with verbal and non-verbal skills Maternal obesity was associated with ↓IQ (OR=0.84) at 8 years Generation-R: no association between maternal overweight with verbal and non-verbal skills **Confounders adjusted for:** ME, PE, occupation, income, social class (ALSPAC only), MS, BF	**15** **Medium**
[242]**Casas M; 2013** **INMA:** population based prospective birth cohort Spain N=~1967 Age 11-22 months **RHEA:** Population based prospective cohort Greece	Pre-pregnancy BMI (kg/m^2) Underweight (BMI<18.5) Normal (BMI 18.5-24.9) Overweight (BMI 25.0-29.9) Obese (BMI ≥30) **INMA:** 72.9%, 19.2% and 8% normal, overweight and obese respectively **RHEA:** 68.3%, 20.1%, and 11%	INMA: Bayley Scales of Infant Development –I (Mental and Psychomotor scale) RHEA: Bayley Scales of Infant Development –III (Cognitive and fine and gross motor development scale)	Pre-pregnancy obesity, but not overweight, was negatively associated with cognitive skills. Compared to children of normal weight mothers, children of obese mothers scored 2.67 points lower (INMA) and 3.57 points lower (RHEA and not significant) in mental (INMA) and cognitive development (RHEA). Cognitive score ↓ with increasing BMI (INMA -0.17 per kg/m^2; RHEA -0.26 per kg/m^2(not significant) No association of overweight/obesity with motor development in both cohorts.	**14** **Medium**

N=412 Age 17-20 months	normal, overweight and obese respectively Excluded underweight		**Confounders adjusted for:** gender**,** parental education, age, social class (only in INMA), maternal country of birth, breast-feeding duration, MS, employment status during pregnancy and after birth, parity, nursery attendance and main child minder	
[243]Craig WY; 2013 **Study 1**- USA Population based cohort N=101 Age 2 years **Study 2**- USA Population based cohort N=118 Age 8 years In both studies participants were from control group of a case-control study	Pregnancy BMI (kg/m^2; 2nd trimester) Normal (BMI 18.5-24.9) Overweight (BMI 25.0-29.9) Obese (BMI ≥30) **Study 1:** 31.6%, 38.6% and 29.7% normal, overweight and obese respectively **Study 2:** 64.4%, 25.4%, and 10.2% normal, overweight and obese respectively No underweight category	Study 1- Bayley Scales of Infant Development –III Cognitive, language and motor (gross and fine) domains Study 2-Wechsler Intelligence Scale for Children-III Full scale IQ Verbal and performance subscale	Study 1: ↑BMI categories- ↓scores for cognitive, language and motor domains (not significant). Percentage of children with ≥ 1 score below BSID-III score of 85 increased with BMI category and was higher among children of obese mothers compared to children of normal BMI mothers (OR 3.9) Study 2: ↑BMI categories- ↓scores for performance IQ but not for full scale and verbal IQ. Percentage of children with ≥ 1 score below WISC-III score of 85 increased with BMI category and was higher among children of obese mothers compared to children of normal BMI mothers (OR 5.2) **Confounders adjusted for**: gender, maternal age, smoking, number of prior births, SES (based on occupation and education)	**14** **Medium**
[244]Tavris DR; 1982 N=2789 Age 5 years USA Prospective longitudinal	Maternal gestational weight gain (difference in weight between first and last prenatal visits) 3 categories of weight gain 1) -24 to 4 lb, 2) 5 to 29 lb 3) ≥30 lb	Raven's Coloured Progressive Matrices Details of cognitive domains assessed not mentioned	Children of mothers who gained <5 lb and >30 lb scored poorly compared to 2nd category (F=3.23) Compared to 1st and 2nd category – no difference Compared to 2nd and 3rd category- second category scored better (F=4.31) **Confounders adjusted for:** Ethnicity, MA, parity, pre-pregnancy weight/height ratio, GA, ME, PE, income.	**13** **Medium**
[245]Gage SH; 2013 **ALSPAC:** population based prospective cohort-UK N=5832: Age 4 years N=5191; Age 8 years N=7339; Age 16 years	Maternal gestational weight gain (kg) 3 categories of weight gain 1: less than recommended 2: As recommended 3: more than recommended Pre-pregnancy weight (kg)	School Entry Assessment Score-4 years IQ-WISC-III-8 years Adequate final exam results-16 years	Children of women gained weight <expected-↓ school entry assessment score (-0.075 SD) and adequate final-exam results (OR=0.88); ↑Weight gain - early and mid pregnancy -↑school entry assessment score (0.072 and 0.077 SD) ↑ Weight gain in all three periods of pregnancy-↑ IQ at 8 years (0.070 to 0.078 SD) and ↑Pre-pregnancy weight-↓ school entry assessment score (-0.004 SD/kg), IQ (-0.004 SD) and the odds (OR=0.99) of achieving adequate final exam results **Confounders adjusted for:** the child's sex, current age, MA; ME, parity, pre-pregnancy BMI, smoking and mode of delivery	**16** **Medium**

BMI-body mass index; QS-quality score; RB-risk of bias; GA-gestational age; MA-maternal age; BWT-birthweight; SES-socio-economic status; ME-maternal education; PE-paternal education; MIQ-maternal intelligence; HE-home environment; MS-maternal smoking; BF-breast-feeding; DM-maternal diabetes; PIH-pregnancy induced hypertension

4.2.3.2 Maternal vitamin D and offspring cognitive function (Table 4.4): All three studies reviewed were observational and conducted in developed populations[124,125,246]. One was published in 2008[124] and the remaining in 2012. Sample size varied from 178 to over 1800 mother-offspring pairs. The children's age at assessment varied from 14 months-10 years. The proportion of loss to follow-up varied between the studies. It was very high (70%) in one study[124], high in another (30-40%)[125] and small (12%) in the third study[246].

Serum vitamin D concentrations were used in all the studies. It was assessed using stored samples which were collected during the second trimester in two studies[125,246] and during the third trimester in another study[124]. Duration of sample storage was 20+ years in one study[125], 5 years in another[124] and the remaining one did not report this information[246]. In all the studies cognitive function was assessed by trained personnel, though the cognitive domains and the test battery used were different. None of the studies provided information as to whether the outcome assessors were blind to exposure status.

Associations of maternal vitamin D status with cognitive function were mostly consistent. Of the three studies, one with a small sample size (n=178) found no association (unadjusted)[124]. A study in Spain found higher mental and psychomotor development scores (2-3 score points (0.1-0.2 SD)) in children of mothers with normal vitamin D status (>30 ng/ml) compared to children of mothers with low vitamin D status (<20 ng/ml)[246]. It also found a positive association of maternal vitamin D concentrations with offspring cognitive scores (0.8 to 0.9 score points (~0.06 SD) for every 10 ng/ml increase in maternal vitamin D concentrations). A study in Australia found a two fold increase in language impairment in children of mothers with vitamin D deficiency (<46 nmol/L) compared to children of mothers with vitamin D >70 nmol/L[125].

The two studies that found significant associations of maternal vitamin D status with offspring cognitive function adjusted for a variety of confounders. Risk of bias was medium in all three studies (score: 13-16). The confounders used, and the quality assessment score for each study are presented in Table 4.4.

Table 4.4 Summary of the findings from studies examining associations of maternal vitamin D status with offspring cognitive function

Author, Year, Sample size, Age, Country, Study design	Nutrient	Cognitive function	Results after adjustment for confounders	Quality score and risk of bias
[124]**Gale C; 2008** N=178 Age 9 years UK Prospective longitudinal	Serum vitamin D concentrations assessed at 28-42 weeks gestation 21.2 % had <27.5 nmol/L 28.3% had 27.5-50 nmol/L	Wechsler Abbreviated Scale of Intelligence Full scale, verbal or performance IQ	No association between vitamin D concentrations and offspring IQ (full scale, verbal or performance) **Confounders adjusted for:** Unadjusted	**16** **Medium**
[125]**Whitehouse AJO; 2012** Age 5 years (n=534) Age 10 years (n=474) Australia Prospective longitudinal	Serum vitamin D concentrations assessed at 18 weeks gestation 25.2% mothers had insufficiency (≤46 nmol/L) (lowest quartile)	Peabody Picture Vocabulary Test Receptive language	Children of mothers with vitamin D insufficiency (lowest quartile (≤46 nmol/L) were at increased risk (OR=1.97) of language impairment compared to children of mothers without insufficiency (highest quartile (≥72 nmol/L; OR=1.00) **Confounders adjusted for:** MA, MS, parity, family income, season of maternal blood sampling	**13** **Medium**
[246]**Morales E; 2012** N=1820 Age 11-23 Months Spain Prospective population based cohort study	Plasma vitamin D concentrations assessed during 12-23 weeks gestation 19.5 % mothers had deficiency (<20 ng/ml) 31.5% had insufficiency (20-30 ng/ml)	Bayley Scales of Infant Development (mental (MDI) and psychomotor (PDI) developmental score)	A positive linear association between vitamin D concentrations and MDI and PDI Per 10ng/ml increase in vitamin D concentrations MDI and PDI score increased by β=0.79 and β=0.88 points respectively. Compared to infants of deficient mothers, infants of mothers with normal level scored higher MDI (β=2.60) and PDI (β=2.32) points respectively **Confounders adjusted for:** The child's sex, BWT, area of study, maternal country of origin, MA, parity, pre-pregnancy BMI, SES, ME, MS, alcohol and season	**15** **Medium**

QS-quality score; RB-risk of bias; MA-maternal age; BWT-birthweight; ME-maternal education, MS-maternal smoking, BMI-body mass index; SES-socio-economic status

4.2.3.3 Maternal folate and offspring cognitive function (Table 4.5): Of 13 studies (12 observational[118-123, 247-252] and 1 trial[253]), three were conducted in developing countries (all observational)[120-122] and the remaining in developed countries (9 observational[118, 119,123,247-252] and 1 trial in Europeans from three centres (Germany, Spain and Hungary))[253]. With the exception of one (published in 1974)[121], all the other studies were published during 2008-2013. There was a wide variation in the sample size (range 32 to 39000 mother-offspring pairs). In one study in the USA, families were from lower social class and the mothers had participated in a zinc supplementation trial during pregnancy[119]. The children's age at assessment was 1 month to 11 years. In a study among a high risk British population (previous pregnancy complicated by neural tube defect) though the sample size was very low (n=96), the follow-up rate was 99%[252]. Loss to follow-up was high (30-50%) in three studies (2 observational[119,247] and 1 trial[253]) and ~10-20% in four (all observational)[118,123,250,251]. This information was unavailable in the remaining studies[121,122,248,249].

The exposure was measured in different ways, and during different periods of gestation. In the European trial pregnant mothers were supplemented daily with 400 µg of 5-methyltetrahydrofolate alone, or fish oil with/without folate or placebo from the 20th week of gestation until delivery[253]. In a study from Africa folic acid deficiency (based on bone marrow or serum folate level) was used[121]. Four studies (one each from the USA[119], India[122], and Canada[118] and the European trial[253]) used plasma/red cell folate and total homocysteine concentrations (homocysteine not assessed in the trial), assessed during the second and/or the third trimester of gestation and also at the time of delivery in the trial. In three of them it was assessed using frozen samples (~-70^{0}C)[119,122,253] and for another this information was not available[118]. Three studies, one from Mexico and the remaining two from the USA, used daily dietary folate intake during the first and/or the second trimester (calculated from a food frequency questionnaire (FFQ) and/or supplement use) as the predictor[120,123,247]. One of them[123] and the remaining five studies used folic acid supplements (with or without other vitamins and/or minerals)[248-252]. In all of them, except a study in the UK[252], supplement use was self reported and dose was not available. In two of them supplements were used 4 weeks-3 months prior to pregnancy and/or the following 2-3 months[248,249]. In three others there were no details about initiation and duration of supplement use[123,250,251]. In the UK study women used 0.36 mg folic acid with other vitamins daily, a month before planned conception until the second missed menstrual period[252].

Of the 13 studies, three used the Denver development scale[121,248,252], two used BSID[118,120] and others used different batteries to assess cognitive function. Cognitive domains assessed varied between studies and in all the studies, with the exception of two (which relied on parental report)[248,249], cognitive function was assessed by trained investigators. Only two studies reported whether outcome assessors were blind to exposure status[119,120]. In the European trial information about compliance was not reported[253].

Findings from the studies that used plasma folate or homocysteine concentrations as the exposure were consistent. Three studies, all observational, found no association of maternal folate and homocysteine concentrations with offspring mental and psychomotor development at age 1-2 years[118], verbal, non-verbal and general IQ, and gross motor development at age 5 years[119] and non-verbal intelligence, attention and memory at age 9 years[122]. In one of them with a small sample size (n=154), mothers with no folate deficiency tended to be more educated and from a high income background[118]. In another, mothers were from a socially disadvantaged background, and there was not much variation in folate status[119]. In the third study sample size was very small (n=108) and information about folate status was inadequate[122]. The trial, with a small sample (n=154) and ~50% loss to follow-up, also found no association of folate concentrations with mental processing scales at age 6.5 years[253]. In an African case-control study folate deficiency (based on bone marrow examination/serum folate) was associated with abnormal or delayed motor and/or language development at age 6 weeks to 4 years[121]. In this study sample size was very small (n=32) and risk of bias was high (quality score 6).

Findings were fairly consistent in the studies in which dietary folate intake was the exposure. In a Mexican cohort, low maternal folate intake (<400 μg/day) was associated with a lower MDI, but not PDI score (β=-1.8 score points (~0.3 SD); 95% CI: -3.6, -0.04) in the children of mothers who were carriers of the MTHFR677 TT genotype, but not in others, at age 1-12 months[120]. In this study with ~8% loss to follow-up, mothers were from low-medium socio-economic background and the findings were reported for only those who had complete data for dietary intake, genetic polymorphism and outcome and excluded those who had only dietary and outcome data. In an American cohort for each 600 μg/day increment of maternal folate intake from food and supplements during the first trimester, but not during the second trimester and peri-conceptional period, children's receptive language, but not visuo-motor abilities, increased by 1.6 score points (0.1 SD);

95% CI: 0.1, 3.1 at age 3 years[123]. But in the same population, there was no association of maternal folate intake during the first and/or the second trimester with children's receptive language, verbal and non-verbal intelligence at age 7 years[247].

In six studies, including a trial, in which folate supplements were used, findings were fairly consistent. While four out of five observational studies found associations[248-251], one other observational study and the trial found no association between maternal folate and offspring cognitive function[252,253]. Among observational studies, in an American cohort, supplement use was associated with better gross motor development in the children, but not fine motor and language development, at age 3 years (OR=0.51; 95% CI: 0.28, 0.93)[248]. It also found that folic acid use was associated with lower risk of poor psychomotor development among African-American children (OR=0.48; 95% CI: 0.25, 0.94). In a cohort from Norway, with a large sample size, maternal folic acid use was associated with a reduced risk of severe (OR=0.55; 95% CI: 0.35, 0.86) and moderate (OR=0.82; 95% CI: 0.69, 0.97) language delay in the children at age 3 years, but there was no association with gross motor skills[249]. In a Spanish cohort, children of mothers who used folic acid supplements scored ~4-5 score points (0.3 SD) higher in the tests of motor function, verbal ability and verbal-executive function compared to children of non-users at age 4 years, but not in memory and perceptive performance[251]. In the same cohort there was a reduction in the incidence of omission (better attention) but not commission errors at age 11 years (incidence rate ratio:0.80; 95% CI: 0.64, 1.00) in the children of mothers who used supplements compared to non-users[250]. A study among a small sample (n=96) of high risk British population (previous pregnancy complicated by neural tube defect) with no information about confounders found no difference in cognitive scores between the supplemented group and the general population[252]. The European trial found no difference in the scores of mental processing scale between the folate alone supplemented group and other intervention groups at age 6.5 years[253].

All the studies, with the exception of three[121,252,253], reported findings after adjusting for a variety of confounders. Risk of bias was high in two studies (score: 6[121]and 11[248]), low in two (score: 17[249] and 20[253]) and medium in others (score: 13-16). The confounders adjusted for, and the quality assessment score for each study are presented in Table 4.5.

Table 4.5 Summary of the findings from studies examining associations of maternal folate status with offspring cognitive function

Author, Year, Sample size, Age, Country, Study design	Nutrient	Cognitive function	Results after adjustment for confounders	QS and RB
[118]**WU BTF; 2012** N=154 Age 18 Months Canada Prospective	Plasma folate and tHcy concentrations assessed at 16 and 36 weeks gestation No folate deficiency (plasma folate <6.8 nmol/l) High tHcy not reported	Bayley Scales of Infant Development Receptive language, expressive language, cognitive skills, fine motor and gross motor	No association of folate and tHcy with cognitive function **Confounders adjusted for:** The child's sex, BF, ethnicity, MA, MIQ, maternal fatty acid level	**13** **Medium**
[119]**Tamura T; 2005** N=355 Age 5 years USA Prospective	Red cell and plasma folate concentrations – 19, 26 and 37 weeks gestation and tHcy concentrations-26 and 37 weeks Low folate-(plasma folate <11 nmol/L) 19 weeks- 7.4%; 26 weeks- 8.2%; 37 weeks- 14.0% Red cell folate <430 nmol/L) 19 weeks- 7.2%; 26 weeks- 3.8%; 37 weeks- 3.3% High tHcy (tHcy>7 µmol/L) 26 weeks- 8.4%; 37 weeks- 22.1%	Differential Ability Scale (verbal, nonverbal and General IQ), Visual and Auditory Sequential Memory (visual and auditory memory span) Knox Cube (attention span and short-term memory) Gross Motor Scale (Gross motor development and Grooved Pegboard (manipulative dexterity)	No difference in the mental and psychomotor developmental scores between children of mothers with normal and deficient folate and tHcy groups. No difference in test scores even across range of folate status (quartiles) **Confounders adjusted for:** The child's sex, GA, BWT, MA, BMI, MS, MIQ, alcohol and drug use, HE	**15** **Medium**
[122]**Bhate V; 2008** N=108 Age 9 years India Prospective community based birth cohort	Erythrocyte folate and tHcy concentrations assessed at 28 weeks gestation No details about low folate or high tHcy concentrations	Raven's Coloured Progressive Matrices-Intelligence; Visual recognition Colour Trial Test-sustained attention and executive function Digit-span test-short-term or working memory	No association of erythrocyte folate, tHcy with any of the cognitive tests **Confounders adjusted for:** The child's sex, age, education, weight and head circumference, B12 level, SES, education of the head of the family	**14** **Medium**
[121]**Gross RL; 1974** N=32 Age 6 weeks to 4 years Africa Case-control study	Folic acid deficiency (based on bone marrow exam or serum folate level) (Hb 3.2-8.9 g %)	Denver Developmental Screening Test (gross motor, fine motor, language and personal-social)	Folic acid deficiency was associated with abnormal or delayed development on one or more of the 4 areas examined **Confounders adjusted for: No information**	**6** **High**

[120]**Del Rio Garcia; 2009** N=253 Age Infancy (1- 12 months) Mexico Prospective birth cohort	Daily dietary intake of folate (first trimester FFQ) Deficient daily folate intake (<400 µg**) - 70%**	Bayley Scales of Infant Development -II (Mental Development Index(MDI) and Psychomotor Development Index (PDI))	Folate intake deficiency-↓ MDI (β=-1.8) in infants of mothers were carrier of MTHFR677 TT genotype **Confounders adjusted for:** BWT, BF, current age, energy intake at age 6 months, maternal BMI, pregnancy hypertension, ME, HE and MTHFR 1298A>C genotype	**16** **Medium**
[123]**Villamor E; 2012** N=1210 Age 3 years USA Prospective pre-birth cohort	Average daily intake of folate at 1st and 2nd trimester (FFQ + Supplements) Peri-conceptional intake of folate from supplements (LMP-4weeks gestation)	Peabody Picture Vocabulary Test-Receptive Language Wide Range Assessment of Visual Motor Abilities-visual-motor; visual-spatial and fine motor	First but not 2nd trimester folate intake (food + supplement) positively related to receptive language but not with visuo-motor abilities. Every increment of 600 µg/day folate intake -↑1.6 points receptive language. No association of peri-conceptional folate intake with cognitive function **Confounders adjusted for:** MA, parity, ethnicity, MS, pre-pregnancy BMI, ME, PE, MIQ, energy, fish and iron intake, income, the child's sex and English as primary language	**15** **Medium**
[247]**Boeke C; 2013** N=895 Age 7 years USA Prospective pre-birth cohort	Average daily intake of folate at 1st and 2nd trimester (FFQ + Supplements)	Peabody Picture Vocabulary Test-Receptive Language Wide Range Assessment of Memory and Learning-II edition, Design and Picture Memory subtests: visuo-spatial memory Kaufman Brief Intelligence Test-II edition Verbal and non-verbal intelligence	No association of folate intake with cognitive function **Confounders adjusted for:** MA, parity, ethnicity, MS, pre-pregnancy BMI, ME, PE, MIQ, energy, fish and iron intake, income, the child's sex and English as primary language	**16** **Medium**
[248]**Wehby GL; 2008** N=6774 Age 3 years USA Population based longitudinal	Folic acid supplements (3 months prior to pregnancy and/or during the following 3 months) 3% used supplement	Denver developmental screening-language, personal-social, gross motor and fine motor	Folic acid use was associated with improved gross motor development (OR=0.5) **Confounders adjusted for:** The child's sex, age, ethnicity, MA, ME, MS, alcohol, drug abuse, income, maternal health status	**11** **High**
[249]**Roth C; 2011** N=38954 Age 3 years Prospective observational Norway	Folic acid supplements with or without other supplements (4 wks before to 8 wks after conception) 18.9 % used only folic acid 50 % used folic acid + other supplements	Language Grammar Rating scale - Language delay (severe and moderate) Severe-children with minimal expressive language i.e. only 1 word or unintelligible utterances; Moderate-children can produce 2-3 word phrases Gross motor skills-Ages and Stages questionnaire	Use of folic acid resulted in reduced risk of severe (OR=0.55) and moderate language delay (OR=0.80). No association between folic acid intake and delay in gross motor skills **Confounders adjusted for:** Maternal marital status, BMI, parity and education	**17** **Low**

[250]**Forns J; 2012** N=393 Age 11 years Population based prospective birth cohort; Spain	Folic acid supplements with or without other vitamins Dose and duration: No information 66.8 % used folic acid + other supplements	Continuous Performance Test (Attention function) Omission error; Commission error HRT-mean response time (for correct hits)	Supplementation with folic acid reduced the incidence rate ratio (IRR=0.80) of omission errors. No association with commission and HRT **Confounders adjusted for**: Parity, PE, social class, MIQ, maternal mental health, MS, BWT, BF	**14** **Medium**
[251]**Julvez J; 2009** N=420 Age 4 years Population based prospective birth cohort Spain	Folic acid supplements with or without other vitamins Dose and duration: No information 34 % used only folic acid 24 % used folic acid + other supplements	McCarthy Scales of Children's Abilities General cognitive scale and subscales (Verbal, perceptive-performance, memory, quantitative and motor) and executive function (Verbal and perceptive-performance)	Use of maternal folic acid supplement was positively associated with verbal (general cognitive) score (β=3.98) and verbal (executive function (β=3.97)), motor skills (β=4.55). **Confounders adjusted for:** The child's sex, age, school season, area of residence, GA, BF, parity, maternal marital status, MS, use of calcium and iron supplements, ME, PE and social class.	
[252]**Holmes-Siedle; 1992** N=96 Age 2-5 years UK Prospective observational	Peri-conceptional multivitamin containing folic acid (0.36 mg) supplements daily with other vitamins and minerals (Minimum 28 days before conception until the second missed menstrual period)	Denver developmental screening test (DDST) (language, motor and social skills)	No significant difference in development score among supplemented group compared to general population. **Confounders adjusted for: No information**	**12** **Medium**
[253]**Campoy C; 2011** N=154 Age 6.5 years Double blind randomized controlled trial European centres (Germany, Spain and Hungary)	4 supplement (milk based) groups 1. Fish oil (N=37) 2. 5-methyl tetrahydrofolate-400 µg (N=37) 3. Fish oil + 5-methyl tetrahydrofolate (N=35) 4. Placebo (N=45) Daily supplementation from 20th week of gestation until delivery Plasma/erythrocyte folate concentrations during 2nd and 3rd trimester and at the time of delivery; no information about compliance	Kaufman Assessment Battery for Children (KABC): Sequential processing scale Simultaneous processing scale Mental Processing Composite (MPC)	No significant difference in cognitive scores between supplement groups. No association of maternal plasma or erythrocyte folate concentrations during pregnancy and at the time of delivery with cognitive function. **Confounders adjusted for:** Unadjusted	**19** **Low**

QS-quality score; RB-risk of bias; tHcy-total homocysteine; Hb-haemoglobin; LMP-last menstrual period; FFQ-food frequency questionnaire; BMI-body mass index; GA-gestational age; MA-maternal age; BWT-birthweight; BF-breast-feeding; SES-socio-economic status; ME-maternal education; PE-paternal education; MIQ-maternal intelligence; HE-home environment; MS-maternal smoking; MTHFR-methylenetetrahydrofolate reductase

4.2.3.4 Maternal vitamin B12 and offspring cognitive function (Table 4.6): Of six studies (all observational and published during 2008-2013), two were conducted in developing countries[120,122] and the others in developed populations[118,123,247,254]. Sample size varied from 108 to over 6000 mother-offspring pairs. The children's age at assessment varied from 1 month-10 years. Loss to follow-up was about 10-20% in two studies[118,123] and high (~50%) in two studies[247,254].

In two studies the exposure was plasma B12 concentrations[118,122], assessed during the third trimester in one study from frozen (-80^{0}C) samples[122] and during the second and the third trimester in another (which also assessed holotranscobalamin concentrations)[118]. In two studies from the USA, the predictor was average daily dietary B12 intake during the first and the second trimester (calculated from FFQ and supplements use)[123,247]. One of them also used peri-conceptional (last menstrual period to 4 weeks gestation) intake of B12 supplements as the exposure[123]. In two studies, one from Mexico and the other from the UK, daily dietary B12 intake during the first or the third trimester (FFQ) was the predictor[120,254]. Two studies assessed mental and psycho-motor development using a similar test battery (BSID)[118,120] and in others the test instrument and cognitive domains were different. Assessment was done by trained examiners in all the studies. Only one study reported whether outcome assessors were blinded to the exposure[120].

The findings on the relation between maternal vitamin B12 status and offspring cognition were inconsistent. In a rural Indian population, children of mothers with the lowest B12 concentrations performed slowly in Colour Trial A test (sustained attention; 182 vs. 159 seconds) and poorly in Digit Span Backward test (short-term memory; 4.3 vs. 4.4 digits) compared to children of mothers with the highest concentrations at age 9 years[122]. There were no associations in tests of intelligence and visual recognition. This study was conducted in a very small sample (n=108) of children of mothers with lowest and highest plasma B12 concentrations within a larger cohort. In a Canadian cohort there was no association of maternal B12 concentrations with children's language, cognitive and motor skills at age 1.5 years[118]. In this study sample size was small (n=154); mothers had adequate B12 status, probably due to supplement use, and only ~8% had deficiency. In a Mexican cohort, low maternal B12 intake (<2 μg/day) was associated with a lower MDI, but not PDI (β=-1.6 score points (~0.3 SD); 95% CI: -2.8, -0.3) in the children at age 1-12 months[120]. This study with ~8% attrition, reported the findings based on the data from a selective sample (described in the previous section). In an American study, maternal B12

intake from food and supplements during the second trimester, but not during the first trimester and peri-conceptional period, was inversely related to offspring receptive language (-0.4 score points (0.03 SD)/2.6 μg/day; 95% CI: -0.8, -0.1), but not visuo-motor abilities, at age 3 years[123]. But in the same cohort, with ~50% attrition, B12 intake during the first and the second trimester was unrelated to offspring receptive language, verbal and non-verbal intelligence at age 7 years[247]. In a large well-nourished UK population, with ~50% attrition, there was no association between maternal B12 intake and offspring IQ at age 8 years[254]. Further, there were only weak associations between maternal genetic variants linked to plasma vitamin B12 and offspring IQ.

Findings from all the studies were reported after adjustment for a number of confounders. Risk of bias was medium in all (score: 13-16). The confounders adjusted for, and the quality assessment score for each study are presented in Table 4.6.

Table 4.6 Summary of the findings from studies examining associations of maternal vitamin B12 status with offspring cognitive function

Author, Year, Sample size, Age, Country, Study design	Nutrient	Cognitive function	Results after adjustment for confounders	QS and RB
[118]**WU BTF; 2012** N=154 Age 18 Months Canada Prospective	Plasma vitamin B12 and holotranscobalamin concentrations assessed at 16 and 36 weeks gestation 7.8 % low B12 (<148 pmol/l)	Bayley Scales of Infant Development Receptive language, expressive language, cognitive skills, fine motor and gross motor	No association of B12 and holotranscobalamin with cognitive function **Confounders adjusted for:** The child's sex, BF, ethnicity, MA, MIQ, maternal fatty acid level	**13** **Medium**
[122]**Bhate V; 2008** N=108 Age 9 years India Prospective community based	Plasma Vitamin B12 concentrations assessed at 28 weeks gestation B12 status 2 groups 1) Lowest <77 pmol/L 2) Highest >224 pmol/L	Raven's Coloured Progressive Matrices-Intelligence Visual recognition Colour Trial Test-sustained attention and executive function Digit-span test-short-term or working memory	Children in group 1 performed slowly in sustained attention (182 seconds Vs 159) and short-term memory (2.6 digits Vs 2.9). No association with other tests. **Confounders adjusted for:** The child's sex, age, education, weight and head circumference, B12 level, SES, education of the head of the family	**14** **Medium**
[120]**Del Rio Garcia; 2009** N=253 Age Infancy (1- 12 months) Mexico Prospective birth cohort	Daily dietary intake of vitamin B12 (first trimester FFQ) Deficient daily dietary intake (B 12<2.0 µg/day) – 21.3%	Bayley Scales of Infant Development -II (Mental Development Index(MDI) and Psychomotor Development Index (PDI))	B12 intake deficiency-↓mental development (β=-1.6 points) **Confounders adjusted for:** BWT, BF, current age, energy intake at age 6 months, maternal BMI, pregnancy hypertension, ME, HE and MTHFR 1298A>C genotype	**16** **Medium**
[123]**Villamor E; 2012** N=1210 Age 3 years USA Prospective pre-birth cohort	Average daily intake of vitamin B12 - 1st and 2nd trimester (FFQ + Supplements) Peri-conceptional B12 intake from supplements (LMP - 4Wks gestation)	Peabody Picture Vocabulary Test-Receptive Language Wide Range Assessment of Visual Motor Abilities-visual-motor; visual-spatial and fine motor	↑ B12 intake (2.6 µg/day) during 2nd trimester (not 1st trimester) -↓ (0.4 points) receptive language No association of peri-conceptional B12 intake with cognitive function **Confounders adjusted for:** MA, parity, ethnicity, MS, pre-pregnancy BMI, ME, PE, MIQ, energy, fish and iron intake, income, the child's sex and English as primary language	**15** **Medium**

[247]**Boeke C; 2013** N=895 Age 7 years USA Prospective pre-birth cohort	Average daily intake of B12 at 1st and 2nd trimester (FFQ + Supplements)	Peabody Picture Vocabulary Test-Receptive Language Wide Range Assessment of Memory and Learning-II edition, Design and Picture Memory subtests: visuo-spatial memory Kaufman Brief Intelligence Test-II edition Verbal and non-verbal intelligence	No association of B12 intake with cognitive function **Confounders adjusted for:** MA, parity, ethnicity, MS, ME, PE, MIQ, HE, intake of energy, fish and other methyl donors, the child's sex and current age	**16** **Medium**
[254]**Bonilla C; 2012** N=6259 Age 8 years UK Population based prospective birth cohort	Daily dietary vitamin B12 intake (FFQ; 3rd trimester-32 weeks)	Wechsler Intelligence Scale for Children-III- Full scale IQ	No association between maternal B12 intake and child's IQ. **Confounders adjusted for:** The child's sex, GA, BWT, BF, current age, MA, parity, ME, social class, MS, alcohol, maternal energy intake and infections in pregnancy, folate supplementation	**14** **Medium**

QS-quality score; RB-risk of bias; LMP-last menstrual period; BMI-body mass index; FFQ-food frequency questionnaire; BWT-birthweight; SES-socio-economic status; GA-gestational age; MA-maternal age; ME-maternal education; PE-paternal education; MIQ-maternal intelligence; HE-home environment; MS-maternal smoking; BF-breast-feeding; MTHFR-methylenetetrahydrofolate reductase

4.2.3.5 Maternal iron/haemoglobin/anaemia and offspring cognitive function (Table 4.7): All seven studies (published during 2006-2013) were conducted in developed countries (5 observational[248,255-258] and 2 trials one in Australia[259] and another in China[260]). Sample size varied from 63 to over 10,000 mother-offspring pairs. The children's age at assessment varied from 5 months-16 years. In one study that included well educated middle class families, the sample size was small (n=63) and loss to follow-up was high (~30%)[255]. In three studies, including the Chinese trial, loss to follow-up was small (10-20%)[256,257,260], in the Australian trial loss to follow-up was about 30%[259] and there was no information in the remaining two studies[248,258].

The explanatory variable was different in all five observational studies[248,255-258]. An American cohort used iron supplements intake, with or without other vitamins/minerals (self reported; dose not available), 3 months prior to pregnancy and/or the following 3 months, as the exposure[248]. In this study women also used folate supplements (details presented in section 4.2.3.3). The study from Canada used serum ferritin and haemoglobin concentrations assessed during the third trimester as the predictor. In this study, 90% of mothers consumed a diet rich in iron and took iron supplements (27 mg iron) daily in the third trimester[255]. In a Finnish cohort the exposure was Hb concentrations assessed during the third, seventh and ninth gestational months, and anaemia[256]. A study from the Seychelles (mothers exposed to prenatal methyl mercury) used total body iron stores assessed at enrolment as the predictor[257]. In a British cohort Hb level assessed early in pregnancy (before 18 weeks) and late in pregnancy (after 28 weeks) was the exposure[258]. In the Australian trial, pregnant mothers received iron supplements (20 mg/day; supplemented group) or placebo (control group) from 20 weeks of gestation until delivery[259]. In the Chinese trial, pregnant mothers were supplemented daily with 400 µg of folic acid alone (control group), or 400 µg folic acid with 60 mg iron or 400 µg folic acid, 30 mg iron with multiple micronutrient from enrolment (<28 weeks of gestation) until delivery[260]. Cognitive domains and the instruments used to test them were different and were assessed by trained persons in all except one study, in which school scores were self reported at age 14 years and teacher rated at age 16 years[256]. In three studies, including the Chinese trial outcome assessors were unaware about the exposure[256,257,260] and in the remaining studies, including the Australian trial, this information was not reported. In the Chinese trial information about compliance was not adequate[260].

Findings from these studies were fairly consistent. Of the seven studies, four of the five

observational studies and both the trials found no associations of maternal iron status with offspring cognitive function[248,255,257-260]. In an American cohort, despite a large sample size, maternal use of iron supplements was unrelated to mother reported offspring language and motor development at age 3 years[248]. In a very small Canadian cohort (n=63), maternal Hb/serum ferritin was unrelated to mental and psychomotor development at age 6 months[255]. In this study, mothers of the children had adequate iron status (90% of mothers took iron supplements and only 3% had anaemia), were well educated and the majority of them were from a high income background. A well conducted study in Seychelles, with a small sample size (n=229), found no association of maternal iron stores with the offspring MDI and PDI at age 9 and 30 months, visual recognition memory at age 9 and 25 months and working memory, planning and attention at age 25 months[257]. The UK study, well conducted among a large sample, found no association between maternal haemoglobin concentrations and offspring IQ at age 8 years[258]. This study also found no associations of maternal genes linked to iron/Hb concentrations with the offspring IQ. In the Australian trial with ~30% attrition, there was no difference in IQ scores at age 4 years between the iron supplemented and placebo groups[259]. The Chinese trial in which cognitive assessment was done only in a subgroup (one third) of infants of the original trial participants also found no difference in the mental and psychomotor development scores at age one year between the folic acid alone and folic acid with iron groups[260]. The other observational study from Finland, with a large sample size, found a small increase (0.03 to 0.06 SD) in children's school performance score at age 14 and 16 years for each 10 g/L increase in maternal Hb concentrations during the ninth month (but not during the third and the seventh months) of gestation[256]. They also found that the children of non-anaemic mothers scored 0.04 to 0.07 SD higher in school performance than the children of anaemic mothers.

All the studies reported findings after controlling for confounders that they documented. Risk of bias was high in one study (score: 11)[248], medium in two (score: 15-16)[255,258] and low in the other four studies, including the two trials (score: 18-19)[256,257,259,260]. The confounders adjusted for, and the quality assessment score for each study are presented in Table 4.7.

Table 4.7 Summary of the findings from studies examining associations of maternal iron/haemoglobin/anaemia with offspring cognitive function

Author, Year, Sample size, Age, Country, Study design	Nutrient	Cognitive function	Results after adjustment for confounders	QS and RB
[248]**Wehby GL ; 2008** N=6774 Age 3 years USA Population based longitudinal	Prenatal iron supplements (3 months prior to pregnancy and/or during the following 3 months) 36.2% used supplement	Denver developmental screening-language, personal-social, gross motor and fine motor	Iron use was associated with improved performance in personal-social development (OR=0.5) but not with language and motor domains **Confounders adjusted for:** The child's sex, age, ethnicity, MA, ME, MS, alcohol, drug abuse, income, maternal health status	**11** **High**
[255]**Rioux FM; 2011** N=63 Age 6 Months Canada Observational	Hb, serum ferritin at 28-32 weeks gestation 90% mothers took iron supplements (27 mg of iron)	Brunet-Lezine Scale of Psychomotor Development of Early Childhood Bayley Scales of Infant Development	No association between maternal gestational Iron status with mental and psychomotor development **Confounders adjusted for:** ME, PE, MIQ, income, BF, GA, BWT, birth head circumference, infants' current weight and Hb	**16** **Medium**
[256]**Ferarouei. M; 2010** N=9983 14 years N=10474 16 years Finland Prospective Birth cohort study	Hb concentrations at 3rd 7th and 9th gestational months Anaemia	School performance 14 years- Self report 16 years- School report	↑maternal HB at 9months-↑ total school performance score (β=0.03) and theory score at 14 years and total score at 16 years Offspring of mothers with anaemia –low school scores (OR=-0.05 at 14 years and (OR=-0.06) at 16 years **Confounders adjusted for:** The child's sex, BWT, pregnancy wanted or not, ME, social class, parity, marital status, MS, maternal mental health status	**18** **Low**
[257]**Davidson PW; 2008** N=229 Age 5, 9, 25and 30 months Republic of Seychelles Longitudinal cohort study	Iron- total body stores at 14-24 weeks of gestation assessed before the start of iron supplementation	Bayley Scales of Infant Development: Mental Development Index (MDI) and Psychomotor Development Index (PDI) (9 and 30 months) Infant cognition (Fagan Infantest-novelty preference) and Visual Expectation Paradigm –visual recognition memory (9 and 25 months) A-not-B and Delayed Spatial Alternation: inhibition, working memory, planning and attention (25 months)	No association between maternal iron stores and cognitive function at any age **Confounders adjusted for:** The child's sex, BWT, MA, SES, HE, MIQ and both parents living with the child (yes/no)	**19** **Low**

[258]**Lewis SJ; 2013** N=~3,500 Age 8 years UK Population based prospective birth cohort	Hb concentrations Before 18 weeks Hb <11.0 g/dl -8% After 28 weeks Hb <11.0 g/dl -30%	Wechsler Intelligence Scale for Children-III- Full scale IQ	No association between maternal Hb and child's IQ. **Confounders adjusted for:** GA, ME, the child's genotype, iron supplementation, population stratification	**15** **Medium**
[259]**Zhou SJ; 2006** N=302 Age 4 years; Australia Double blind randomized controlled trial	Iron supplements (20mg/day) or placebo from 20 weeks gestation until delivery Compliance-86%	Stanford Binet Intelligence Scale – IQ (verbal reasoning, visual reasoning, quantitative reasoning and short-term memory)	No difference between the children of supplement group and placebo group in the mean score of composite IQ or any subscales IQ or in the proportion of children whose IQ fell 1 or 2 SD below the mean **Confounders adjusted for:** sex, birth order, gestational age, MA, ME, PE, HE, BF	**20** **Low**
[260]**Li Q; 2009** N=1305 Age 3, 6 and 12 months Double blind cluster randomized controlled trial China	3 intervention groups (All received folic acid) 1. Folic acid alone 400 µg (n=471)-control 2. Iron 60 mg + folic acid 400 µg (n=438) 3. Multiple micronutrients ((b vitamins (1,2,3 6 AND 12), vitamin A, D, C, E and minerals (zinc, iodine, copper, selenium) +Iron 30 mg + 400 µg folic acid)) (n=396) Daily supplementation from enrolment until delivery; inadequate information about compliance	Bayley Scales of Infant Development :Mental (MD) and Psychomotor development (PD)	No significant difference in infants MD and PD score at 3 and 6 months and PD score at 12 months between supplement groups. Mean MD score among children of multiple micronutrient group increased by 1 to 1.22 points compared to children of folic acid alone, or folic acid+iron group at 12 months **Confounders adjusted for:** Infants age, sex, gestational age, apgar score, BWT, infant health, maternal age and BMI, parental education, occupation, SES, number of tablets consumed	**19** **Low**

QS-quality score; RB-risk of bias; Hb-haemoglobin; GA-gestational age; MA-maternal age; BWT-birthweight; SES-socio-economic status; ME-maternal education; PE-paternal education; MIQ -maternal intelligence; HE-home environment; MS-maternal smoking; BF-breast-feeding; BMI-body mass index

Since the number of studies in each exposure category was small and cognitive domains assessed varied between studies reviewed, I was unable to compare the effect size or to perform quantitative analysis (meta-analysis).

4.2.4 Discussion

In this systematic review of observational studies and single micronutrient trials, I explored the evidence for a causal link between maternal nutrition during pregnancy and offspring cognitive function during childhood and adolescence. Despite a small number of studies, especially trials, the review found some evidence linking maternal obesity and low micronutrient status with poorer offspring cognitive function. Most of the evidence for this came from observational studies. However, with inconsistent findings especially for micronutrients and the limitations of observational studies such as residual confounding and sample size/selection the evidence is not conclusive.

4.2.4.1 Strength and limitations of the systematic review: The main strength of this systematic review is that it was conducted following CRD recommendations and PRISMA guidelines. Quality assessment was done by two reviewers independently. The majority of the papers assessed were found to have a medium risk of bias and only 2 studies had a high risk of bias. I used several different parameters of maternal nutritional status. However, there are some limitations too. Exclusion of non-English language literature may have resulted in some important studies being missed and biased the conclusions. Although some studies with null findings were published (probably because of emerging interest in recent years in the topic of maternal nutrition and offspring cognitive function), publication bias is another potential limitation, as studies with null finding are less likely to be published. Another limitation is that meta-analyses could not be performed, due to methodological differences in the published research. I did not include multiple micronutrient trials as there were already two published systematic reviews on this topic[232,233]. Another reason is that it would be difficult to know the effect of individual nutrients in these trials.

4.2.4.2 Maternal anthropometry and offspring cognitive function: Six of the seven studies, all from developed countries, showed consistently an association of high maternal BMI with lower cognitive function in the children[237-240,242,243]. The findings are consistent with an earlier systematic review that included two studies, one from a developed and

another from a low-income African-American population[231] and with a recent review (based on five studies published during 2011-2012) which was published after completing my systematic review[261]. The main difference between this recent review and mine is that I included 2 more studies, published in 2013. Although the threshold level at which the effect was observed varied between the studies, there was evidence of a dose response effect in the majority of the studies. For example, compared to the normal maternal weight category the effect was significant in the extremely obese[238] or obese categories, but not in the overweight category[237,242,243] or overweight and obese categories combined[239,240]. The effect size was small, about 0.1-0.2 SD lower IQ/cognitive test scores in children of obese rather than normal weight mothers, and similar in all studies[237-239]. In most of the studies the effect was found for one or more of the mental development domains[237,239,240]. In some the effect was found for mental development but not for motor development[238,242,243]. Only one of the seven studies did not find an association between maternal overweight and offspring verbal and non-verbal skills at age 2-3 years[241].This could be due to the young age at assessment (<3 years); most psychologists think 4 years is probably the earliest age for reliable estimates of cognitive function[262].

The observed associations between maternal adiposity and reduced cognitive function in the children could be biologically plausible due to trans-placental transfer of 'inflammatory factors' from the mother's adipose tissue to the developing fetus. These factors, which cross the blood brain barrier, could lead to inflammation of the brain, a reduction in neurotrophic factors, and have adverse effects on neuronal differentiation, plasticity and function[263,264]. Animal studies in which obesity in rats/mice has been induced during pregnancy using a high fat diet, have demonstrated increased inflammatory cytokines, low levels of BDNF in hippocampal region and poor learning in the offspring[263,264]. Such an experimental approach in humans is clearly impossible.

Confounding is another possible explanation for the findings. This is especially important in studies of cognitive function, which is strongly influenced by the socio-economic environment. Despite appropriate adjustment for SES, the potential issue of residual confounding could not be ruled out since in some cases SES variables were limited to income or occupation. Furthermore, in developed countries cohort studies have shown that lower IQ during childhood is linked with higher BMI/obesity in adulthood[265,266]. Lack of adjustment for MIQ could mean that any link between higher maternal BMI/obesity with offspring cognitive function could be due to confounding. Of the six studies that showed

an association, only two adjusted for MIQ[237,240], and in the remaining studies information about MIQ was unavailable[238,239,242,243].

Three studies from developed countries showed an association of low BMI (maternal undernutrition) with lower cognitive scores in the children, with a difference of 0.02-0.3 SD between children of underweight and normal weight mothers[237-239] but these differences were mainly non-significant possibly due to lack of power as the underweight category tended to be small. However, in one study there was a significantly higher risk of delayed mental development (risk ratio 1.36) in children of underweight mothers compared to normal mothers[238]. This could indicate a causal association between maternal undernutrition and poorer offspring cognitive function. Fetal exposure to nutrient deficiencies or stress following nutritional insults might lead to alterations in the neurotransmitter and neuroendocrine systems, and structural brain development[49,50,114-117] and subsequently reduced cognitive function in later life. Since the home environment, parental care and stimulation, in addition to socio-economic factors, influences cognitive development, inadequate/lack of adjustment for the above unmeasured factors suggests that the findings could be due to residual confounding. With only one 'underweight' category there was no opportunity to look for dose response effects.

Of the four studies that examined maternal weight gain as the exposure, all from developed populations, two studies using different tests found consistent associations of reduced cognitive function in offspring of mothers who gained less weight during pregnancy[244,245]. One also found lower scores in relation to greater gestational weight gain[244]. This could indicate a causal association of maternal undernutrition or overnutrition with poorer cognitive function in the children for the reasons explained above. Further, one of them also found higher scores in relation to greater gestational weight gain in all the three periods (early, mid- and late) of pregnancy[245]. This is biologically plausible as weight gain in pregnancy, indicating better nutritional status, might result in an increased supply of nutrients to the fetus which in turn influences better neurodevelopment and therefore better cognitive function[114]. However, the findings could be due to confounding for the reasons explained above. The effects were found for non-verbal intelligence[244] or overall IQ and academic outcomes[245]. There was no dose response in one study[244] and in the other it could not be interpreted due to lack of weight gain categories[245]. The remaining two studies found no significant association between gestational weight gain and offspring cognitive function[237,240], although the direction of the association was negative.

In conclusion, despite a wide range of study designs there is some evidence of an association between maternal pre-pregnancy obesity and reduced offspring cognitive function. The evidence is not conclusive due to the issues of possible residual confounding by socio-economic factors and lack of adjustment for the potential confounding effect of maternal IQ. There is also some evidence that maternal underweight or low gestational weight gain are associated with reduced cognitive function, but this is not conclusive.

4.2.4.3 Maternal vitamin D status and offspring cognitive function: Two of the three studies, all from developed populations, showed an association of higher maternal vitamin D concentrations with better cognitive function in the children[125,246]. There was evidence of a dose response relationship in both of them. The effect size was modest. For example children of mothers with normal vitamin D status had higher mental and psychomotor development scores by 0.1-0.2 SD compared with children of vitamin D deficient mothers[246]. The percentage with language impairment was double in children of vitamin D deficient mothers compared to normal mothers[125]. The effect was specific to language impairment in one study[125] and in the other the effects were found for both mental and psychomotor development[246]. Findings from these studies are consistent with animal studies which have demonstrated poor learning and memory, alterations in attention in response to maternal transient vitamin D deficiency[267,268].

The observed associations between maternal vitamin D and offspring cognition is plausible due to a variety of biological actions of vitamin D fundamental to neurodevelopment, including a signalling role in cell differentiation and synaptic formation[269], changes in the brain structure and reduced expression of genes[269], neurotrophic actions (regulation in the metabolism of neurotrophic and neurotoxic factors)[270] and a protective role during brain inflammation[271]

Although both studies adjusted appropriately for a number of confounders, there are some limitations. Limited adjustment for socio-economic variables (income or occupation), the lack of data on other socio-demographic variables influencing cognitive function such as MIQ, home environment, parental care and stimulation, and the child's vitamin D status suggests that the findings could be due to confounding by unmeasured factors. Another limitation was that in both the studies maternal vitamin D status was available only during the second trimester but not during different periods of gestation. Since vitamin D status is

known to fluctuate depending on sunlight exposure, the main source of vitamin D, and the rate of development of different areas of the brain underpinning various cognitive processes varies throughout the intra-uterine period, the timing of deficiency may be crucial in determining cognitive development.

One study, although well conducted with appropriate exposure and outcome measures, did not find evidence linking maternal vitamin D and offspring cognition[124]. It could be due to a small sample size (n=178) that reduced the power to find an association.

Thus, based on a limited number of observational studies, there is evidence that maternal vitamin D deficiency is associated with reduced cognitive function in the children. However, with a lack of data from developing countries, a lack of trial data and for a variety of above mentioned limitations, the evidence is not conclusive.

4.2.4.4 Maternal folate status and offspring cognitive function: Among the 13 studies reviewed, the findings were mixed. While some found an association, others did not. Of the 12 observational studies, mainly from developed populations, six cohort studies and a case-control study in an African population using different tests consistently showed positive associations of maternal dietary folate intake or supplement use with offspring cognition[120,121,123,248-251]. Specificity varied between the studies. For example, in one there were associations with both mental and psychomotor development[251], while in another it was specific to mental development especially in children of mothers who were carriers of MTHFR677 TT genotype[120] and in others it was found for one or more of motor or mental development domains[123,248-250]. It was impossible to evaluate dose response effects, due to lack of information about categories of dietary folate intake or dose/duration of supplements used. There was quite a large effect. For example, children of mothers who used folic acid supplements rather than non-users had ~0.3 SD higher mental and psychomotor development scores[251]. Children of mothers with lower dietary folate intake (<400 μg/day) scored 0.3 SD lower in the mental development index[120].

The observed findings could indicate a causal link for a variety of biological actions of folate influencing neurodevelopment such as myelination, maintaining tissue levels of neurotransmitters like neurotrophic and neurotoxic cytokines[272,273]. However, confounding is always a concern in observational studies. Higher dietary intake and/or use of supplements may be an indicator of higher SES or higher maternal education and/or MIQ.

Although, the majority of the studies adjusted for a variety of confounders, SES was limited to income or occupation or education. Further, information about MIQ, home environment, level of adherence (among supplement users) and the child's current folate status were generally unavailable. Other limitations of these studies included self reported exposures (eg. dietary intakes), self reported outcomes (eg. outcomes reported by parents), potential observer bias and selective reporting. A variety of methodological limitations are also possible explanations for the null findings in the remaining studies[118,119,122,252,253]. Most had low power due to a small sample size[118,122,252]. Sample selection is another reason. For example, mothers had little variation in folate status in one study[119] and no folate deficiency in another[118]. A double blind randomised controlled trial overcomes many of these methodological issues, and the European trial was resoundingly negative[253]. The trial was of reasonable quality, but compliance was not reported and it had a high attrition rate. Maternal folate status was not reported, and a trial in Europe, where women are likely to be folate replete, does not rule out an effect in populations with high rates of folate deficiency.

In summary, there is some evidence from observational studies linking maternal folate (dietary/supplements intake) with offspring cognitive function. However, due to a lack of evidence from the trial and high chances of confounding in the observational studies, a causal link between maternal folate and offspring cognitive function seems unlikely. More trial evidence is required from folate-deficient populations.

4.2.4.5 Maternal vitamin B12 status and offspring cognitive function: The six studies reviewed were inconsistent. Two of them, both from developing countries, consistently found reduced cognitive function in children of mothers with deficient vitamin B12 concentrations or dietary intake[120,122]. It was impossible to evaluate any dose response effect. There was quite a big effect. For example, children of mothers with lower dietary B12 intake (<2 μg/day) scored 0.3 SD lower in MDI[120]. The effect was found for one or more mental development domains. The findings linking lower maternal vitamin B12 with poorer offspring cognitive ability may indicate a causal relationship, which would be biologically plausible (mechanisms are similar to those of folate reported in section 4.2.4.4). However, residual confounding, as discussed already, remains a concern. Further, interpretation of the findings is difficult due to limitations, such as a highly selective sample (children of mothers with extremely low or high B12 status[122]).

Of the remaining four studies, from developed countries, in two studies, conducted in the same cohort at different ages, there was an association between maternal B12 dietary intake and offspring cognition at age 3 years[123] but not at age 7 years[247]. Such transient findings could be due to inadequate power or different domains assessed at both ages. In the remaining two there was no evidence of an association between maternal vitamin B12 concentrations/dietary intake and offspring cognition[118,254]. This could be due to a young age at assessment (<2 years), a small sample size, and insufficient variation in the maternal B12 status in one[118], and the use of self reported FFQ-based dietary B12 intake in the other[254]. Recent research has found that different foods can yield very different plasma concentrations of vitamin B12[274]. Hence dietary data may fail to reflect nutritional status.

In conclusion, there is some evidence for an association between maternal vitamin B12 deficiency and reduced cognitive function. However, the lack of trial data, high likelihood of confounding, inconsistent findings and a variety of methodological limitations in the observational studies mean that the evidence is not conclusive.

4.2.4.6 Maternal iron status and offspring cognitive function: Of the seven studies, only one observational study found a positive association between maternal Hb concentrations and offspring cognitive function (school performance score)[256]. The effect size was small, with a difference of 0.04-0.07 SD between the children of non-anaemic and anaemic mothers. The remaining studies, including the trials, found no associations of maternal iron (supplements/haemoglobin/ferritin levels) status with offspring cognition[248,255, 257-60]. A causal link between maternal iron status and offspring cognitive ability is biologically plausible because iron contributes to neurodevelopment and function (cell differentiation, myelination and neurotransmitter synthesis)[275]. Iron uptake by the brain is high during the third trimester of gestation, corresponding to the peak of myelinogenesis. However, my review of the available data provides little support for this.

This might have been due to methodological limitations. For example, in an American cohort, maternal supplement use was based on non-random self prescription, the dose of the supplement was not available, outcome data were based on maternal report, and cognitive tests were designed to screen for developmental delay rather than capture variations within the normal range[248]. In two studies, the sample size was small and the children were young (6 months-to-<3 years)[255,257]. Additionally, in one of them there was little variation in maternal iron status[255]. In a well conducted study with a large sample, Hb

concentrations might not have been low enough to have a significant negative impact on neurodevelopment[258]. Neither of the trials showed an effect of iron supplementation on children's cognition, providing even stronger evidence for a lack of effect of maternal iron status on childhood cognitive function. The low dose of iron in the Australian trial[259], and high losses to follow-up in the Chinese trial[260] mean that there is still a need for more high-quality trial-based evidence, especially in populations with high rates of iron deficiency.

4.2.4.7 Limitations of evidence from current review: The primary and the most important limitation was the small number of studies from developing countries. This is very important for a variety of reasons. Nutritional deficiencies tend to be more common and more extreme in developing countries than in developed countries due to poverty and poor diets. The confounding structure in developing countries is often different from developed countries. Studies in developing countries may reveal associations between nutritional status and cognitive function that are not detectable in developed populations. Lack of experimental evidence and too many observational studies, many with inadequate sample sizes and inadequate data on confounders are other potential limitations, along with possible measurement error due to self report of the exposure and a young age at the time of outcome assessment. Cognitive function after the age of 4 years, when it is more stable, may provide a better indicator of the potential influence of maternal nutrition during pregnancy. Although the quality score was medium in the majority of the studies reviewed, inadequate reporting about loss to follow-up, power calculations, details of sample selection, observer bias and validity of exposure was noticed in this review. Wide variability in the sample size, exposures and outcomes as well as small effect size and inconsistent findings limited the interpretation of adequacy of sample size.

Experimental studies might be an answer for better evidence. However, ethical issues are an important barrier to such studies. For example, in a population with widespread multiple micronutrient deficiency, it is unethical to supplement with only one nutrient and/or multiple nutrients for some groups and not for others. Although trials are likely to overcome the methodological limitations of observational studies, factors such as adequacy of dose and compliance are potential limitations.

4.2.5 Conclusion

Interest in the area of maternal nutrition and cognitive outcomes in the offspring has

increased in recent years. It is evident from my review that most of the studies were published recently, especially in the last decade. This review found some evidence linking maternal obesity and micronutrient status, in particular, folate, vitamin D and B12 during pregnancy with offspring cognitive function suggesting that maternal nutrition is important for optimal neurodevelopment and might have long-lasting effect on cognitive function later in life. However, due to a lack of data from developing populations, a lack of trial data and other limitations of evidence described in the previous section, findings from my review are not conclusive and could not be generalized to a broader population. Taking into consideration the limitations of existing evidence, I suggest that there is a need for more experimental research in this area especially from developing countries.

4.3 Maternal nutrition and offspring cognitive function; findings from my study

In this section I present the findings from my data. I describe maternal nutritional status (anthropometry (BMI, height and skinfolds), and micronutrient concentrations (vitamin D, vitamin B12 and folate) during pregnancy (between 28-32 weeks of gestation) and examine their associations with offspring cognitive function during childhood and adolescence. I also examine associations between maternal homocysteine concentrations, a marker of vitamin B12 and folate status, and offspring cognitive function. In the course of the analysis, I examine whether the associations of maternal body size and micronutrient status with offspring cognitive function are independent of potential confounders including the child's current body size and micronutrient status, and test the following hypothesis:

- Maternal overweight/obesity and/or underweight, and shorter height during pregnancy are associated with poorer cognitive ability in the offspring.
- Lower maternal vitamin D, vitamin B12 and folate concentrations during pregnancy are associated with poorer cognitive ability in the offspring.
- Higher homocysteine concentrations (an indicator of vitamin B12 and folate deficiency) during pregnancy are associated with poorer cognitive ability in the offspring.

4.3.1 Data preparation

4.3.1.1 Data cleaning: Before analysis, missing values were identified and coded appropriately. Range checks were done to ensure that the values were within the expected range.

4.3.1.2 Statistical methods: All analyses were carried out using Stata (version 10.0). Histograms were plotted to determine whether measurements were normally distributed and the most appropriate transformations were used for variables with skewed distributions. Maternal weight, BMI, triceps, biceps and subscapular skinfold thicknesses, vitamin D, vitamin B12 and homocysteine and the child's vitamin B12 concentrations were log transformed (Figure 4.2a and b; Figure 4.3a and b). For suprailiac skinfold and sum of skinfolds, square root transformation was the most appropriate (Figure 4.4a and b).

Differences in the means of exposures (maternal nutritional characteristics) between two groups, such as between mothers of boys and girls, and differences in the means of cognitive scores between children of mothers with and without overweight/obesity or with low and normal micronutrient concentrations were compared using t-tests. Chi-Squared tests were used to test differences in proportions between groups. Correlations between continuous variables (for example vitamin B12, folate and homocysteine concentrations) were examined using Pearson's correlation. Linear regression analyses were used to examine associations between maternal nutritional status (either as a binary variable, such as overweight/obesity (0-no; 1-yes) or as a continuous variable across the entire range of BMI and micronutrient concentrations) and offspring cognitive function, adjusting for potential confounders. I generated 3 dummy variables for the 3 main religion groups, (Hindu, Muslim and Christian) and for season at the time of blood sampling (summer, rainy season and winter). These variables were used as adjustors in multiple regression analyses, where appropriate. In order to facilitate interpretation, internally standardized *Z*-scores of outcome and exposure variables (expressed in units of SD) were used in the regression model where appropriate. In order to examine whether there were non-linear effects of maternal nutritional status with cognitive scores, I tabulated the means and used quadratic terms in the linear regression analyses.

Interaction terms were used to test for differences in associations between maternal nutritional status and cognitive function according to sex, and to examine interactions between maternal vitamin B12 and folate status in relation to cognitive scores. After ensuring that there was no interaction between maternal nutritional status and sex in predicting cognitive ability, sexes were pooled in all analyses with adjustment for sex. Generalised estimating equation models were used to compare associations of maternal nutritional status with childhood and adolescent cognitive performance.

Figure 4.2a Distribution of maternal body mass index (BMI)

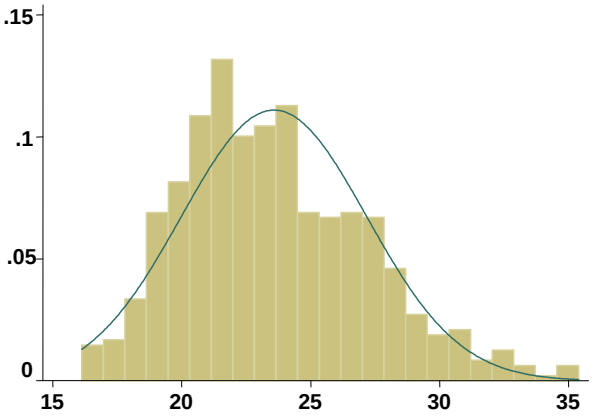


Figure 4.2b Log transformed body mass index (BMI)

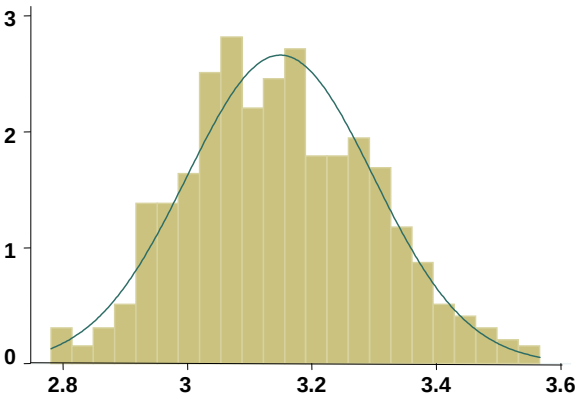


Figure 4.3a Distribution of maternal vitamin D concentrations

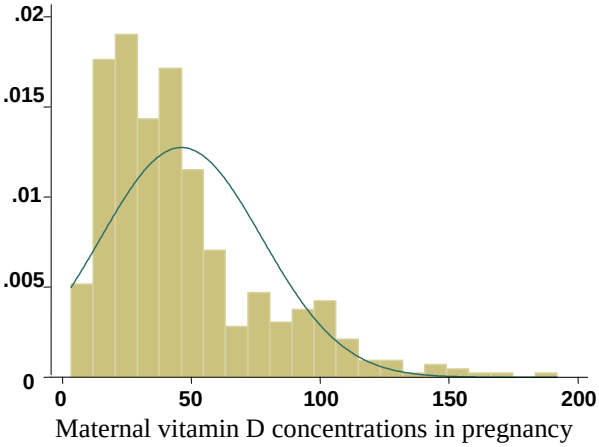


Figure 4.3b Log transformed vitamin D concentrations

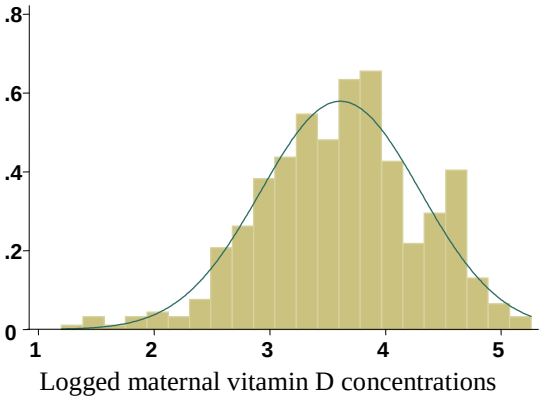


Figure 4.4a Distribution of maternal sum of skinfolds

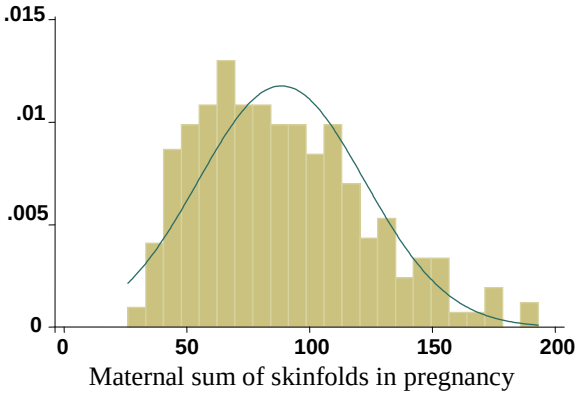
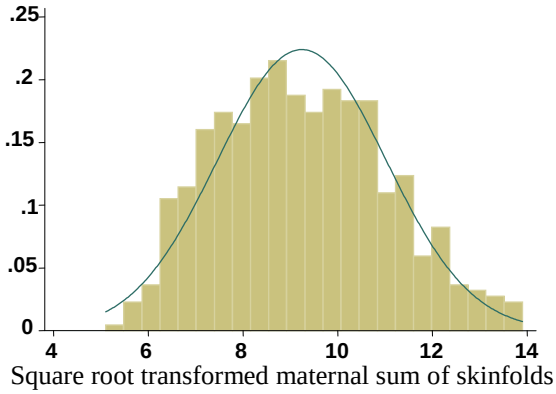


Figure 4.4b Square root transformed sum of skinfolds



4.3.1.3 Missing data: Maternal anthropometric data were available for all the children who participated, at both time points. Maternal vitamin D, vitamin B12, folate and homocysteine concentrations were not available for ~14%, 1%, 1% and 1% of the children respectively. Since these variables were main exposures of interest and also birth size, socio-demographic characteristics and cognitive scores were similar among those who had these data compared to those who did not, I did not consider imputing missing data, and analyses were done using all the available data. However, for the reasons described in section 3.2.3, I used imputed MIQ and home environment data while examining associations of socio-demographic factors with maternal nutritional status, and the confounding effect of socio-demographic factors in associations of maternal nutritional status with offspring cognition.

4.3.2 Study population

As I have already described the differences in birth measurements and socio-demographic characteristics between participants and non-participants in section 3.3 and appendix 6, in this section I describe the differences in maternal characteristics between participants and non-participants. At data collection when the children were aged 9.5 and 13.5 years, proportion of mothers in each religious category, mean/median maternal age, anthropometric parameters and micronutrient concentrations and the prevalence of underweight, overweight/obesity, low micronutrient status and hyperhomocysteinemia were similar between the mothers of participants and non-participants except that the mothers of non-participants were taller and had higher folate concentrations compared to the mothers of participants (data presented in appendix 6).

4.3.3 Description and definitions of maternal nutritional status

Since the sample of children studied at 9.5 years (n=542) was almost identical to that studied at 13.5 years (n=545) and since maternal nutritional parameters of the children studied at both ages were very similar, I describe maternal characteristics only of those studied during adolescence. Maternal vitamin D, vitamin B12, folate and homocysteine concentrations were not available for 73, 6, 6 and 4 children respectively, usually due to an inadequate serum sample. All the characteristics of the mothers of boys and girls were similar except that the mothers of girls had larger skinfold and lower vitamin B12 concentrations compared to mothers of boys (Table 4.8; $p<0.05$ for all). The child's folate and vitamin B12 concentrations at 9.5 years were similar in boys and girls (Table 4.8).

Table 4.8 Maternal characteristics in pregnancy of study participants

		Boys (n=259)	Girls (n=286)	All (n=545)
Maternal Characteristics		**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
Age (years)		23.9 (4.2)	23.8 (4.3)	23.8 (4.2)
Religion (No (%))				
a)	Hindu	141 (54.4)	165 (57.7)	306 (56.2)
b)	Muslim	95 (36.7)	101 (35.3)	196 (36.0)
c)	Christian	23 (8.9)	20 (7.0)	43 (7.8)
Anthropometry				
Weight (kg)*		55.0 (50.0, 60.0)	55.3 (49.5, 63.5)	55.0 (49.5, 61.5)
Height (cm)		154.3 (5.5)	154. 6 (5.2)	154.5 (5.4)
Body mass index (kg/m^2)*		22.9 (21.0, 25.4)	23.4 (20.8, 26.3)	23.2 (20.9, 25.9)
Triceps skinfold (mm)*		16.3 (11.6, 22.2)	17.5 (12.8, 25.8)	16.9 (12.2, 24.0)
Biceps skinfold (mm)*		8.2 (6.2, 12.4)	9.4 (6.6, 13.3)	8.7 (6.5, 12.9)
Subscapular skinfold (mm)*		24.3 (17.7, 30.6)	25.6 (18.5, 34.5)	24.6 (17.9, 33.1)
Suprailiac skinfold (mm)*		30.1 (23.3, 39.0)	34.5 (23.2, 44.5)	31.8 (23.3, 42.2)
Sum of skinfolds (mm)*		78.7 (60.6, 104.6)	88.9 (62.6, 113.4)	84.0 (61.4, 109.7)
Serum micronutrient concentrations				
Vitamin D (nmol/L)*[†]		37.5 (23.0, 54.0)	39.0 (23.8, 60.0)	38.1 (23.5, 56.8)
Vitamin B12 (pmol/L)*[‡]		179.0 (126.0, 224.0)	155.0 (122.0, 208.0)	163.0 (123.0, 221.0)
Folate (nmol/L) [‡]		34.5 (19.1)	35.0 (19.3)	34.8 (19.2)
Homocysteine (µmol/L)[§]		6.0 (5.0, 6.9)	6.0 (5.1, 7.0)	6.0 (5.1,7.0)
Child's micronutrient concentrations[¶]		**n=240**	**n=268**	**n=508**
Vitamin B12 (pmol/L)*		302.5 (246.0, 400.0)	316.0 (257.0, 410.5)	313.0 (250.5, 405.5)
Folate (nmol/L)		28.4 (15.1)	28.8 (14.4)	28.6 (14.8)

*Skewed variable; values are medians (IQR); [†]n=226 boys, 246 girls and 472 all; [‡]n=258 boys, 281 girls and 539 all; [§]n=258 boys, 283 girls and 541 all; [¶]Child's micronutrient concentrations measured at age 9.5 years

Maternal weight status (based on BMI) was defined using the WHO classification[276]. Accordingly 4.6%, 26.2 % and 5.1 % mothers were underweight, overweight and obese respectively (Table 4.9a), similar in mothers of boys and mothers of girls.

Table 4.9a Weight status in pregnancy of mothers of study participants

	Prevalence of maternal weight status (No (%))			
	Normal BMI 18.5-24.9 kg/m^2	**Underweight** BMI <18.5 kg/m^2	**Overweight** BMI 25.0-29.9 kg/m^2	**Obese** BMI≥30.0 kg/m^2
	n=349	**n=25**	**n=143**	**n=28**
Mothers of boys	179 (69.1)	9 (3.5)	61 (23.6)	10 (3.9)
Mothers of girls	170 (59.4)	16 (5.6)	82 (28.7)	18 (6.3)

BMI-body mass index

As there is no specified cut-off for pregnancy, based on the values generally used in a normal population, I defined low vitamin D, vitamin B12 and folate status as concentrations <50 nmol/L[277,278], <150 pmol/L[279], and <7 nmol/L[280] respectively, and hyperhomocysteinemia as a concentration of >10 μmol/L[281]. Accordingly, 67.8% of mothers had low vitamin D, 42.5% had low vitamin B12, 3.9% had low folate status and 3.3% had hyperhomocysteinemia (Table 4.9b). There were no differences in the prevalence of low micronutrient status/hyperhomocysteinemia between the mothers of boys and girls, except that the prevalence of low vitamin B12 was higher in the mothers of girls compared to the mothers of boys ($p<0.05$).

Table 4.9b Micronutrient status in pregnancy of mothers of study participants

	Prevalence of maternal micronutrient status (based on serum concentrations) (No (%))							
	Vitamin D (nmol/L)		**Vitamin B12 (pmol/L)**		**Folate (nmol/L)**		**Homocysteine (μmol/L)**	
	Normal (>50)	**Low (<50)**	**Normal (>150)**	**Low (<150)**	**Normal (>7)**	**Low (<7)**	**Normal (<10)**	**High (>10)**
	n=472		**n=539**		**n=539**		**n=541**	
Mothers of boys	72 (31.9)	154 (68.1)	160 (62.0)	98 (38.0)	250 (96.9)	8 (3.1)	252 (97.7)	6 (2.3)
Mothers of girls	80 (32.5)	166 (67.5)	150 (53.4)	131 (46.6)	268 (95.4)	13 (4.6)	271 (95.8)	12 (4.2)

Maternal vitamin B12 and folate concentrations were positively correlated ($r=0.1$; $p=0.02$) and homocysteine concentrations were inversely correlated with vitamin B12 and folate concentrations ($r=-0.2$ and -0.3 respectively; $p<0.001$ for both).

4.3.3.1 Religion and maternal micronutrient concentrations (Table 4.10): Since animal products are the main source of vitamin B12, and Hindus are mainly lacto-vegetarians (with milk and milk products being the main non-vegetarian food source) I examined maternal micronutrient concentrations according to religion. Hindu women had a higher prevalence of low vitamin B12 status than Muslim and Christian women ($p<0.001$). The prevalence of low folate status was higher among Muslim than Hindu women ($p<0.001$). Hindu women had a higher prevalence of hyperhomocysteinemia than Muslim women ($p<0.001$). The prevalence of low vitamin D status was similar in all religious groups.

4.3.3.2 Season and vitamin D concentrations: Sunlight exposure is the main source of vitamin D. Exposure to sunlight varies with season and we have previously shown marked seasonal variation in vitamin D levels[282]. Vitamin D concentrations were higher among mothers whose blood sample was collected during winter (November-February; median

(IQR) 47.2 (31.1, 77.7 nmol/L) compared to those whose samples were collected during the rainy (July-October; 38.9 (21.6, 62.0)); p=0.01 or summer season (March-June; 29.0 (20.9, 43.0)); p<0.001.

Table 4.10 Micronutrient concentrations in pregnancy of mothers of study participants according to religion

	Religion		
Maternal micronutrients	**Hindu**	**Muslim**	**Christian**
Vitamin D			
N	**265**	**168**	**39**
Serum vitamin D concentrations (nmol/L)*	40.0 (25.5, 56.0)	34.6 (21.6, 55.1)	35.9 (22.5, 76.3)
Low vitamin D (No (%))	**182 (68.7)**	**114 (67.8)**	**24 (61.5)**
Vitamin B12			
N	**303**	**193**	**43**
Serum vitamin B12 concentrations (pmol/L)*	147.0 (115.0, 207.0)	184.0 (136.0, 227.0)	187.0 (132.0, 229.0)
Low vitamin B12 (No (%))	**157 (51.8)**	**57 (29.5)**	**15 (34.9)**
Folate			
N	**303**	**193**	**43**
Serum folate concentrations (nmol/L)†	37.9 (19.0)	28.5 (17.7)	40.2 (19.9)
Low folate (No (%))	**11 (3.6)**	**10 (5.2)**	**0 (0.0)**
Homocysteine			
N	**305**	**193**	**43**
Serum homocysteine concentrations (µmol/L)*	6.2 (5.4, 7.3)	5.6 (4.8, 6.7)	5.8 (5.0, 6.8)
Hyperhomocysteinemia (No (%))	**14 (4.6)**	**1 (0.5)**	**3 (6.9)**

*Skewed variable; values are medians (IQR); †Values are mean (SD)

4.3.3.3 Supplement use and micronutrient concentrations: Data on supplement use was collected at recruitment (<32 weeks of gestation) but not at the time of blood sampling. At recruitment 131 (28%) women reported taking supplements containing calcium and vitamin D3 and 157 (29%) were taking multivitamin supplements containing both vitamin B12 and folic acid, 46 (9%) were taking folic acid alone. There were no associations of supplement use at recruitment with vitamin D, vitamin B12 and folate concentrations at 30±2 weeks of gestation. This was true among women recruited early (<24 weeks of gestation) and those recruited later (24-32 weeks).

4.3.3.4 Socio-demographic factors and maternal nutrition: Parity, parental education and income of the main breadwinner were positively associated with maternal BMI ($p<0.05$ for all). There was no association between SLI and maternal BMI (Table 4.11).

Table 4.11 Maternal nutritional status according to quartiles of standard of living index

		Maternal nutritional status (mean/median) according to quartiles of SLI					
Quartiles of SLI	**N**	**BMI (kg/m^2)**	**Height (cm)**	**Vitamin D (nmol/L)**	**Vitamin B12 (pmol/L)**	**Folate (nmol/L)**	**Homocysteine (µmol/L)**
<35 score	**155**	22.7	152.9	36.6	171.5	26.2	6.1
35-39 score	**133**	23.2	153.0	37.6	158.0	27.9	6.1
40-43 score	**137**	23.4	154.9	39.0	171.5	37.3	6.0
44-64 score	**120**	23.3	156.1	38.4	157.0	46.3	5.9
P*		0.2	<0.001	0.7	0.3	<0.001	0.3

*P value for trend derived by linear regression using standard of living index (SLI) as a continuous variable; BMI-body mass index

While SLI, parental education, occupation and income of the main breadwinner, MIQ and home environment were positively associated with maternal height, parity was negatively related to maternal height ($p<0.05$ for all). Urban mothers tended to have higher BMI and larger skinfolds compared to rural mothers ($p<0.05$ for all). Maternal education, and income of the main breadwinner, were negatively associated with vitamin B12 concentrations ($p<0.05$ for both). There were positive associations of all the socio-demographic factors (except area of residence), and a negative association of parity, with maternal folate concentrations ($p<0.05$ for all). There were no associations of socio-demographic factors with vitamin D and homocysteine concentrations.

4.3.3.5 Birth size, postnatal growth and maternal nutrition: Maternal BMI was positively associated with birthweight and HC at birth and head growth during 5-9.5 years (negatively with head growth during 1-2 years) ($p<0.05$ for all). Maternal height was positively associated with birthweight and head growth from 0-1 and 1-2 years) ($p<0.05$ for all). There were positive associations of maternal folate with birthweight, head growth during 2-5 and 5-9.5 years ($p<0.05$ for all) but it was only weakly related to HC at birth ($p=0.07$). Maternal homocysteine was negatively associated with birthweight ($p=0.002$) and positively with head growth during 1-2 years ($p=0.04$). There were no associations of maternal vitamin D and vitamin B12 with birth size and postnatal growth. There were positive correlations of maternal vitamin B12 and folate concentrations with the child's vitamin B12 and folate concentrations at 9.5 years ($r=0.2$; $p<0.001$ for both).

4.3.4 Association between maternal nutrition and offspring cognitive function

I examined the relationship of maternal nutrition with offspring cognitive function, initially adjusted for the child's sex and age at the time of cognitive function assessment. I explored this relationship further adjusted for GDM status (if the maternal exposure was BMI and height), season (if the maternal exposure was vitamin D) and religion (if the maternal exposure was vitamin B12, folate or homocysteine). In later models I adjusted for socio-demographic variables as in Chapter 3, and for the child's current BMI or height (if the maternal exposure was BMI or height), the child's vitamin B12 concentrations (if the maternal exposure was vitamin B12), the child's folate concentrations, birthweight and post-natal head growth (if the maternal exposure was folate) and the mother's vitamin B12 and folate concentrations, birthweight and postnatal head growth (if the maternal exposure was homocysteine).

4.3.4.1 Maternal anthropometry and offspring cognitive function: In general there were no associations of maternal overweight/obesity or of BMI across the entire range, with offspring cognitive function at either age. However, during childhood there was some indication of lower scores for learning in offspring of overweight/obese mothers compared to offspring of normal weight mothers and the strength of this difference became stronger and significant after adjustment for GDM status, socio-demographic factors and the child's current BMI (Table 4.12). There was also some indication of a non-linear (quadratic) association between maternal BMI and offspring visuo-spatial ability during childhood (scores tended to be lower in the lowest and highest quartiles of BMI) (Table 4.12). However, formal testing showed no non-linear association. There were no associations between maternal sum of skinfolds and offspring cognitive function at either time point (data not shown).

The only association between maternal height and cognitive function was a positive association with offspring short-term memory during childhood (Table 4.12) and verbal fluency during adolescence (data not shown). These associations were attenuated and lost significance after controlling for confounders.

Table 4.12 Associations of maternal body mass index (BMI) and height in pregnancy with offspring cognitive performance during childhood

		Cognitive function tests					
Maternal BMI and height	**N**	**Learning, long-term retrieval**	**Short-term memory**	**Reasoning ability**	**Verbal fluency**	**Visuo-spatial ability**	**Attention and concentration**
Weight status (WHO classification)		**Cognitive test scores (Mean (SD) / Median (IQR)) according to maternal weight status**					
Underweight (BMI<18.5 kg/m^2)	**23**	62.4 (17.2)	16.7 (2.2)	9.0 (3.0, 13.0)	15.8 (4.8)	65.2 (61.4, 82.7)	31.5 (8.1)
Normal (BMI 18.5 – 24.9 kg/m^2)	**347**	68.2 (16.6)	16.3 (2.6)	10.0 (5.0, 14.0)	16.1 (4.9)	77.4 (63.5, 88.4)	32.3 (7.8)
Overweight/obesity (BMI≥25.0 kg/m^2)	**172**	67.4 (18.3)	16.6 (2.6)	10.0 (5.5, 14.0)	16.2 (5.0)	76.8 (63.7, 89.3)	33.7 (8.6)
P*		0.1	0.6	0.3	0.8	**0.04**	0.6
P**		0.7	0.3	0.6	0.9	0.7	0.06
Overweight/obesity (0-no; 1-yes)		**Multiple regression analyses; β (95% CI)**					
Model 1	**519**	-0.05 (-0.24, 0.13)	0.06 (-0.12, 0.25)	0.01 (-0.17, 0.19)	-0.05 (-0.23, 0.13)	0.06 (-0.12, 0.24)	0.08 (-0.10, 0.25)
Model 2	**494**	-0.11 (-0.31, 0.08)	0.03 (-0.16, 0.23)	-0.02 (-0.21, 0.18)	-0.09 (-0.27, 0.10)	0.01 (-0.19, 0.20)	0.04 (-0.13, 0.23)
Model 3	**493**	**-0.22 (-0.41, -0.04)‡**	-0.04 (-0.23, 0.15)	-0.15 (-0.33, 0.03)	-0.14 (-0.33, 0.05)	-0.11 (-0.30, 0.08)	-0.01 (-0.19, 0.17)
Model 4	**491**	**-0.27 (-0.46, -0.09)§**	-0.06 (-0.25, 0.13)	**-0.19 (-0.37, -0.01)‡**	-0.17 (-0.36, 0.02)	-0.12 (-0.31, 0.07)	-0.05 (-0.23, 0.13)
Quartiles of BMI (kg/m^2)		**Cognitive test scores (Mean (SD) / Median (IQR)) according to quartiles of maternal BMI**					
< 20.7	**127**	67.5 (16.5)	16.4 (2.6)	9.0 (4.0, 14.0)	16.0 (5.4)	72.4 (62.5, 84.9)	31.8 (8.4)
>20.7 – 22.8	**131**	66.7 (17.1)	16.4 (2.0)	10.0 (5.0,14.0)	16.1 (4.2)	79.0 (65.7, 89.3)	32.4 (7.7)
>22.8 – 25.6	**135**	68.5 (17.4)	16.4 (2.8)	10.0 (5.0,13.0)	16.4 (4.9)	76.9 (63.0, 88.4)	33.1 (7.5)
>25.6	**149**	68.0 (17.8)	16.5 (2.6)	10.0 (6.0,14.0)	15.9 (5.0)	76.6 (62.0, 89.2)	33.4 (8.7)
P***		0.5	0.9	0.4	0.7	0.1	0.1
		Multiple regression analyses; †β (95% CI)					
Model 1	**542**	0.03 (-0.05, 0.11)	0.002 (-0.08, 0.09)	0.04 (-0.05, 0.12)	-0.01 (-0.09, 0.07)	0.06 (-0.02, 0.15)	0.06 (-0.01, 0.14)
Model 2	**515**	-0.01 (-0.10, 0.08)	-0.01 (-0.10, 0.08)	0.03 (-0.06, 0.12)	-0.03 (-0.11, 0.06)	0.05 (-0.04, 0.14)	0.05 (-0.03, 0.14)
Model 3	**514**	-0.07 (-0.16, 0.02)	-0.05 (-0.14, 0.04)	-0.03 (-0.12, 0.05)	-0.06 (-0.14, 0.03)	-0.005 (-0.09, 0.08)	0.02 (-0.06, 0.11)
Model 4	**512**	**-0.11 (-0.20, -0.02)‡**	-0.07 (-0.16, 0.02)	-0.07 (-0.16, 0.02)	-0.09 (-0.18, 0.005)	-0.01 (-0.10, 0.08)	-0.01 (-0.10, 0.08)

Quartiles of Height (cm)		**Cognitive test scores (Mean (SD) / Median (IQR)) according to quartiles of maternal height**					
< 151	**143**	67.1 (16.2)	16.1 (2.7)	9.0 (4.0, 13.0)	15.8 (4.7)	75.0 (61.5, 84.7)	31.8 (8.2)
>151 – 154.5	**143**	67.6 (17.1)	16.4 (2.4)	10.0 (5.0,14.0)	16.3 (4.7)	76.2 (62.6, 88.5)	33.2 (8.6)
>154.5 – 158	**134**	66.0 (19.7)	16.6 (2.5)	10.0 (5.0,14.0)	15.7 (5.0)	77.2 (63.7, 87.5)	32.9 (8.0)
>158	**122**	70.4 (15.7)	16.6 (2.6)	10.0 (5.0,14.0)	16.8 (5.1)	80.3 (65.4, 91.9)	33.1 (7.6)
P* **		0.2	**0.04**	0.2	0.3	0.4	0.4
		Multiple regression analyses; †β (95% CI)					
Model 1	**542**	0.05 (-0.03, 0.14)	**0.09 (0.002, 0.17)‡**	0.06 (-0.03, 0.14)	0.04 (-0.04, 0.13)	0.04 (-0.04, 0.13)	0.04 (-0.04, 0.12)
Model 2	**515**	0.07 (-0.02, 0.16)	**0.11 (0.02, 0.20)‡**	0.07 (-0.02, 0.16)	0.06 (-0.03, 0.14)	0.05 (-0.04, 0.14)	0.05 (-0.03, 0.14)
Model 3	**514**	0.02 (-0.07, 0.11)	0.05 (-0.04, 0.14)	-0.01 (-0.09, 0.07)	0.01 (-0.08, 0.10)	-0.01 (-0.10, 0.08)	0.001 (-0.08, 0.08)
Model 4	**512**	-0.01 (-0.10, 0.08)	0.03 (-0.07, 0.12)	-0.04 (-0.13, 0.04)	-0.0004 (-0.09, 0.09)	-0.05 (-0.14, 0.05)	-0.06 (-0.14, 0.03)

P values for the difference in cognitive test scores *between children of normal and underweight mothers; ** between children of normal and overweight/obese mothers derived using t test; *** P for trend adjusted for the child's sex and current age derived by multiple linear regression using BMI or height as a continuous variable;

β (SD) is the difference in cognitive test score between children of mothers with and without overweight/obesity and †β is the effect size (SD) of the cognitive test score per SD change in BMI or height (used as a continuous variable) derived by multiple linear regression

Model 1: adjusted for the child's sex and current age

Model 2: Model 1 + maternal gestational diabetes mellitus status

Model 3: Model 2+ SLI, maternal and paternal education, occupation, income, maternal intelligence (imputed) and home environment (imputed)

Model 4: Model 3 + the child's current BMI or height for maternal height); $^{‡}P<0.05$; $^{§}p<0.01$

4.3.4.2 Maternal vitamin D and offspring cognitive function: During childhood maternal vitamin D status (low as well as across the entire range) was unrelated to offspring cognitive performance (data not shown). Formal testing confirmed no non-linear associations. The findings were similar during adolescence, but there was a positive association between vitamin D concentrations and verbal fluency which became stronger and significant after adjusting for season and socio-demographic factors (Table 4.13).

4.3.4.3 Maternal vitamin B12 and offspring cognitive function: During childhood, all the cognitive test scores were higher in the B12 deficient group, significant for verbal fluency (Table 4.14). On further analysis this pattern was more obvious in Hindus than Muslims, significant for reasoning, verbal fluency and visuo-spatial ability ($p<0.05$ for all). There were no differences in cognitive scores between B12 deficient and non-deficient groups and no associations of vitamin B12 concentrations, across the entire range, with any of the cognitive outcomes, after full adjustment (Table 4.14). There was an indication of a non-linear (quadratic) association between maternal vitamin B12 concentrations and offspring visuo-spatial ability (scores tended to be higher in the lowest and highest quartiles of vitamin b12 concentrations) (Table 4.14). However, formal testing showed no non-linear association.

During adolescence, in addition to verbal fluency, reasoning ability scores were higher in offspring of mothers with low vitamin B12 status compared to offspring of mothers with normal status (Table 4.15). These differences were attenuated after adjustment for socio-demographic factors and the child's vitamin B12 concentrations. There were inverse associations of vitamin B12 concentrations, across the whole range, with learning and reasoning. These associations were attenuated after adjustment but remained significant for learning (Table 4.15). This association remained significant even when I tried more exhaustive SES adjustment, using all the measures that constitute the SLI instead of a composite score in the regression model.

Table 4.13 Associations of maternal serum vitamin D concentrations in pregnancy with offspring cognitive performance during adolescence

		Cognitive function tests					
Maternal vitamin D concentrations	**N**	**Learning, long-term retrieval**	**Short-term memory**	**Reasoning ability**	**Verbal fluency**	**Visuo-spatial ability**	**Attention and concentration**
Vitamin D status		**Cognitive test scores (Mean (SD)) according to maternal vitamin D status**					
0-Normal (>50 nmol/L)	**152**	80.3 (15.7)	19.1 (4.0)	16.4 (7.0)	21.7 (5.8)	85.5 (25.3)	48.6 (12.1)
1-Low (<50 nmol/L)	**320**	79.9 (14.0)	19.0 (3.7)	15.3 (6.4)	21.1 (5.7)	83.0 (26.1)	47.5 (10.4)
***P**		0.7	0.8	0.1	0.3	0.3	0.3
		Multiple regression analyses; β (95% CI)					
Model 1	**472**	-0.02 (-0.21, 0.18)	-0.01 (-0.21, 0.18)	-0.14 (-0.34, 0.05)	-0.09 (-0.29, 0.10)	-0.08 (-0.28, 0.11)	-0.07 (-0.25, 0.12)
Model 2	**472**	0.06 (-0.14, 0.27)	0.02 (-0.18, 0.22)	-0.07 (-0.28, 0.13)	-0.14 (-0.33, 0.06)	-0.08 (-0.28, 0.12)	-0.11 (-0.30, 0.08)
Model 3	**472**	0.05 (-0.15, 0.24)	0.02 (-0.17, 0.22)	-0.07 (-0.25, 0.12)	-0.12 (-0.32, 0.08)	-0.07 (-0.26, 0.12)	-0.08 (-0.26, 0.11)
Vitamin D quartiles		**Cognitive test scores (Mean (SD) according to maternal vitamin D quartiles**					
< 23.5 nmol/L	**123**	80.9 (13.4)	19.2 (3.7)	15.4 (6.4)	20.7 (5.3)	82.3 (26.1)	47.7 (10.6)
23.6 – 38.9 nmol/L	**118**	79.0 (13.7)	18.7 (3.7)	14.8 (6.6)	20.8 (6.1)	83.6 (26.1)	46.5 (11.0)
39.0 – 57.0 nmol/L	**116**	80.0 (15.0)	19.0 (3.8)	16.2 (6.6)	22.1 (5.7)	83.5 (25.3)	48.9 (10.6)
>57.0 nmol/L	**115**	79.8 (16.2)	19.1 (4.1)	16.3 (7.1)	21.8 (5.8)	86.0 (26.0)	48.3 (11.8)
****P**		0.7	0.7	0.7	0.08	0.6	0.9
		Multiple regression analyses; ***β (95% CI)					
Model 1	**472**	-0.02 (-0.11, 0.08)	-0.02 (-0.11, 0.07)	0.02 (-0.07, 0.11)	0.08 (-0.01, 0.17)	0.03 (-0.07, 0.12)	0.003 (-0.08, 0.09)
Model 2	**472**	-0.05 (-0.15, 0.04)	-0.04 (-0.13, 0.06)	-0.01 (-0.11, 0.08)	**0.10 (0.01, 0.19)†**	0.02 (-0.07, 0.12)	0.02 (-0.07, 0.11)
Model 3	**472**	-0.03 (-0.12, 0.06)	-0.03 (-0.13, 0.06)	0.002 (-0.08, 0.09)	**0.10 (0.01, 0.19)†**	0.03 (-0.06, 0.12)	0.02 (-0.07, 0.11)

*P value for the difference in cognitive test scores between children of mothers with normal and low vitamin D concentrations derived using t test;
**P for trend adjusted for the child's sex and current age derived by multiple linear regression using vitamin D concentrations as a continuous variable;
β(SD) is the difference in cognitive test score between children of mothers with normal and low vitamin D concentrations and ***β is the effect size (SD) of the cognitive test score per SD change in vitamin D concentrations (used as a continuous variable) derived by multiple linear regression;
Model 1: adjusted for the child's sex and current age; **Model 2**: Model 1 + season at the time of blood sampling; **Model 3**: Model 2+ SLI, maternal and paternal education, occupation, income, maternal intelligence (imputed) and home environment (imputed); †P<0.05

Table 4.14 Associations of maternal serum vitamin B12 concentrations in pregnancy with offspring cognitive performance during childhood

		Cognitive function tests					
Maternal vitamin B12 concentrations	**N**	**Learning, long-term retrieval**	**Short-term memory**	**Reasoning ability**	**Verbal fluency**	**Visuo-spatial ability**	**Attention and concentration**
Vitamin B12 status		**Cognitive test scores (Mean (SD) / Median (IQR)) according to maternal vitamin B12 status and religion**					
0-Normal (>150 pmol/L)-**All**	**308**	**66.3 (17.5)**	**16.3 (2.5)**	**9.0 (4.0, 13.0)**	**15.7 (4.5)**	**76.8(63.6, 87.7)**	**32.3 (8.0)**
Hindu	**153**	66.1 (17.2)	16.1 (2.4)	10.0 (6.0, 14.0)	16.7 (4.8)	77.0 (63.7, 87.2)	33.3 (8.0)
Muslim	**127**	64.9 (17.4)	16.3 (2.6)	8.0 (3.0, 11.0)	14.2 (3.6)	75.0 (59.2, 85.5)	30.6 (8.0)
1-Low (<150 pmol/L)-**All**	**228**	**69.3 (16.7)**	**16.6 (2.6)**	**10.5 (6.0, 14.0)**	**16.7 (5.4)**	**76.5 (63.1, 89.3)**	**33.2 (8.2)**
Hindu	**156**	68.8 (16.6)	16.8 (2.7)	11.0 (6.0, 14.0)	17.6 (5.5)	76.9 (64.5, 89.3)	33.3 (7.8)
Muslim	**55**	67.7 (17.5)	16.1 (2.6)	9.0 (4.0, 11.0)	13.8 (4.0)	66.9 (61.4, 83.3)	31.5 (8.6)
***P**		0.052	0.1	0.07	**0.02**	0.8	0.2
		Multiple regression analyses; β (95% CI)					
Model 1	**536**	0.17 (-0.001, 0.34)	0.12 (-0.05, 0.29)	0.14 (-0.03, 0.31)	0.16 (-0.001, 0.33)	0.03 (-0.14, 0.20)	0.07 (-0.10, 0.23)
Model 2	**536**	0.17 (-0.01, 0.34)	0.11 (-0.06, 0.29)	0.08 (-0.08, 0.25)	0.06 (-0.10, 0.22)	-0.01 (-0.18, 0.17)	0.02 (-0.14, 0.18)
Model 3	**535**	0.13 (-0.04, 0.30)	0.08 (-0.09, 0.25)	0.04 (-0.12, 0.20)	0.04 (-0.12, 0.20)	-0.05 (-0.21, 0.12)	0.01 (-0.15, 0.17)
Model 4	**500**	0.11 (-0.07, 0.28)	0.05 (-0.13, 0.23)	0.02 (-0.15, 0.19)	0.08 (-0.09, 0.25)	-0.05 (-0.22, 0.13)	0.01 (-0.16, 0.18)
Vitamin B12 quartiles		**Cognitive test scores (Mean (SD) / Median (IQR)) according to maternal vitamin B12 quartiles**					
≤122 pmol/L	**128**	69.7 (17.1)	16.5 (2.7)	11.0 (6.0, 15.0)	16.6 (5.1)	76.9 (62.8, 89.6)	33.4 (7.4)
123-162 pmol/L	**140**	68.9 (16.9)	16.5 (2.5)	10.0 (6.0, 13.0)	16.3 (5.4)	72.8 (62.8, 86.9)	33.1 (9.0)
163-220 pmol/L	**134**	64.6 (16.8)	16.3 (2.4)	9.0 (5.0, 13.0)	15.4 (4.3)	72.2 (66.3, 88.9)	31.8 (8.3)
>220 pmol/L	**134**	67.2 (17.7)	16.3 (2.6)	10.0 (4.0, 14.0)	16.1 (4.7)	77.2 (62.5, 88.4)	32.4 (7.6)
****P**		0.06	0.6	0.08	0.4	0.6	0.3
		Multiple regression analyses; ***β (95% CI)					
Model 1	**536**	-0.08 (-0.17, 0.003)	-0.03 (-0.11, 0.06)	-0.07 (-0.16, 0.01)	-0.04 (-0.13, 0.04)	0.02 (-0.11, 0.06)	-0.05 (-0.12, 0.03)
Model 2	**536**	-0.08 (-0.17, 0.0004)	-0.02 (-0.11, 0.06)	-0.06 (-0.14, 0.02)	-0.0005 (-0.08, 0.08)	-0.02 (-0.10, 0.07)	-0.03 (-0.11, 0.05)
Model 3	**535**	-0.07 (-0.16, 0.01)	-0.01 (-0.10, 0.07)	-0.05 (-0.13, 0.03)	0.01 (-0.07, 0.09)	-0.02 (-0.10, 0.07)	-0.03 (-0.11, 0.05)
Model 4	**500**	-0.06 (-0.15, 0.03)	0.01 (-0.09, 0.10)	-0.03 (-0.12, 0.06)	-0.01 (-0.10, 0.07)	-0.004 (-0.09, 0.09)	-0.03 (-0.12, 0.05)

*P value for the difference in cognitive test scores between children of mothers with normal and low vitamin B12 concentrations derived using t test;
** P for trend adjusted for the child's sex and current age derived by multiple linear regression using vitamin B12 concentrations as a continuous variable;
β (SD) is the difference in cognitive test score between children of mothers with normal and low vitamin B12 concentrations and ***β is the effect size (SD) of the cognitive test score per SD change in vitamin B12 concentrations (used as a continuous variable) derived by multiple linear regression; **Model 1:** adjusted for the child's sex and current age; **Model 2**: Model 1 + religion; **Model 3**: Model 2 + SLI, maternal and paternal education, occupation, income; maternal intelligence (imputed) and home environment (imputed); **Model 4**: Model 3 + the child's vitamin B12 concentrations at 9.5 years

Table 4.15 Associations of maternal serum vitamin B12 concentrations in pregnancy with offspring cognitive performance during adolescence

		Cognitive function tests					
Maternal vitamin B12 concentrations	**N**	**Learning, long-term retrieval**	**Short-term memory**	**Reasoning ability**	**Verbal fluency**	**Visuo-spatial ability**	**Attention and concentration**
Vitamin B12 status		**Cognitive test scores (Mean (SD) / Median (IQR)) according to maternal vitamin B12 status and religion**					
0-Normal (>150 pmol/L)-**All**	**310**	**78.9 (15.2)**	**18.8 (3.8)**	**14.7 (6.5)**	**20.7 (5.4)**	**83.5 (25.7)**	**47.3 (10.9)**
Hindu	**146**	76.5 (15.5)	18.7 (3.5)	15.6 (6.7)	21.7 (5.4)	83.4 (25.1)	48.0 (12.1)
Muslim	**136**	80.1 (15.1)	18.9 (4.1)	13.1 (6.0)	19.5 (5.5)	82.5 (26.3)	45.7 (9.6)
1-Low (<150 pmol/L)-**All**	**229**	**81.1 (13.3)**	**19.0 (3.8)**	**16.6 (6.4)**	**22.3 (5.8)**	**82.3 (26.0)**	**48.0 (11.1)**
Hindu	**157**	80.8 (13.4)	19.1 (3.9)	17.6 (6.5)	23.0 (5.9)	84.7 (27.6)	48.6 (10.7)
Muslim	**57**	80.8 (14.1)	18.5 (3.8)	13.0 (5.1)	20.0 (5.5)	73.7 (19.9)	45.4 (11.2)
***P**		0.08	0.6	**0.001**	**0.001**	0.6	0.5
		Multiple regression analyses; β (95% CI)					
Model 1	**539**	0.15 (-0.03, 0.32)	0.02 (-0.15, 0.19)	**0.27 (0.10, 0.44)‡**	**0.24 (0.07, 0.41)‡**	-0.04 (-0.21, 0.13)	-0.003 (-0.17, 0.16)
Model 2	**539**	**0.18 (0.01, 0.36)†**	0.02 (-0.16, 0.19)	**0.17 (0.0002, 0.33)†**	0.14 (-0.02, 0.31)	-0.08 (-0.25, 0.10)	-0.05 (-0.22, 0.11)
Model 3	**539**	0.14 (-0.03, 0.31)	-0.03 (-0.21, 0.14)	0.11 (-0.05, 0.26)	0.12 (-0.05, 0.29)	-0.13 (-0.30, 0.04)	-0.09 (-0.25, 0.07)
Model 4	**502**	0.14 (-0.04, 0.33)	-0.09 (-0.27, 0.09)	0.12 (-0.04, 0.29)	0.15 (-0.03, 0.33)	-0.15 (-0.33, 0.02)	-0.10 (-0.27, 0.07)
Vitamin B12 quartiles		**Cognitive test scores (Mean (SD) / Median (IQR)) according to maternal vitamin B12 quartiles**					
≤122 pmol/L	**131**	81.6 (12.6)	19.0 (4.0)	17.2 (6.7)	22.0 (5.6)	82.9 (27.5)	48.1 (10.6)
123-162 pmol/L	**136**	82.3 (13.7)	18.8 (3.4)	15.5 (5.8)	22.0 (5.9)	81.2 (23.9)	47.8 (11.6)
163-220 pmol/L	**136**	76.7 (15.3)	18.7 (3.5)	14.4 (6.4)	20.2 (5.2)	82.4 (25.4)	47.7 (11.7)
>220 pmol/L	**136**	78.9 (15.4)	19.0 (4.2)	14.9 (6.9)	21.1 (5.7)	85.3 (26.6)	46.8 (9.8)
****P**		**0.02**	0.7	**0.004**	0.2	0.5	0.7
		Multiple regression analyses; ***β (95% CI)					
Model 1	**539**	**-0.10 (-0.18, -0.02)†**	0.02 (-0.07, 0.10)	**-0.12 (-0.20, -0.04)‡**	-0.05 (-0.13, 0.03)	0.03 (-0.05, 0.11)	-0.01 (-0.09, 0.07)
Model 2	**539**	**-0.12 (-0.20, -0.03)‡**	0.02 (-0.07, 0.10)	-0.08 (-0.16, 0.001)	-0.01 (-0.09, 0.07)	0.04 (-0.04, 0.13)	0.004 (-0.08, 0.08)
Model 3	**539**	**-0.10 (-0.18, -0.02)†**	0.04 (-0.04, 0.12)	-0.05 (-0.12, 0.03)	-0.003 (-0.08, 0.08)	0.07 (-0.01, 0.15)	0.02 (-0.06, 0.10)
Model 4	**502**	**-0.11 (-0.19, -0.02)†**	0.07 (-0.02, 0.15)	-0.06 (-0.14, 0.02)	-0.02 (-0.10, 0.07)	0.08 (-0.01, 0.16)	0.03 (-0.06, 0.11)

*P value for the difference in cognitive test scores between children of mothers with normal and low vitamin B12 concentrations derived using t test;
** P for trend adjusted for the child's sex and current age derived by multiple linear regression using vitamin B12 concentrations as a continuous variable;
β (SD) is the difference in cognitive test score between children of mothers with normal and low vitamin B12 concentrations and ***β is the effect size (SD) of the cognitive test score per SD change in vitamin B12 concentrations (used as a continuous variable) derived by multiple linear regression; **Model 1:** adjusted for the child's sex and current age; **Model 2**: Model 1 + religion; **Model 3**: Model 2 + SLI, maternal and paternal education, occupation, income; maternal intelligence (imputed) and home environment (imputed); **Model 4**: Model 3 + the child's vitamin B12 concentrations at 9.5 years; †P<0.05; ‡p<0.01

4.3.4.4 Maternal folate and offspring cognitive function: During childhood low maternal folate status was not significantly related to any of the cognitive outcomes, although cognitive scores were slightly lower in the small group of children of mothers with low folate status compared to children of mothers with normal status (Table 4.16). However, adjusted for the child's sex and age there were positive associations, across the whole range of folate concentrations, with all the cognitive scores (Table 4.16). The strength of these associations remained similar after adjustment for religion. After additional adjustment for socio-demographic factors, the child's birthweight, postnatal head growth and folate status, the effects of these associations reduced but remained significant for learning (Table 4.16).

The findings appeared similar, but generally weaker for adolescent cognitive function (Table 4.17). Formal testing using generalised estimating equations showed that there were no differences in the associations of maternal folate concentrations with cognitive function between the two time points except that the association of maternal folate concentrations with offspring learning was stronger during childhood than during adolescence (p=0.03).

Since a study among elderly Americans and another recent study among elderly Australians reported that higher folate status in the presence of vitamin B12 deficiency was associated with impaired cognitive function[283,284] and also a study in India reported that children of mothers with high folate and low vitamin B12 status in pregnancy had increased insulin resistance[285], I was interested in looking at possible interactions between maternal folate and vitamin B12 status in relation to offspring cognitive function in my study. There were no significant interactions between maternal folate and vitamin B12 groups in relation to cognitive scores. All the cognitive test scores tended to increase with increasing thirds of folate concentrations in children of mothers with low as well as normal vitamin B12 status (Figure 4.5).

Table 4.16 Associations of maternal serum folate concentrations in pregnancy with offspring cognitive performance during childhood

		Cognitive function tests					
Maternal folate concentrations	**N**	**Learning, long-term retrieval**	**Short-term memory**	**Reasoning ability**	**Verbal fluency**	**Visuo-spatial ability**	**Attention and concentration**
Folate status		**Cognitive test scores (Mean (SD) / Median (IQR)) according to maternal folate status**					
0-Normal (>7 nmol/L)	**514**	67.9 (17.0)	16.4 (2.5)	10.0 (5.0, 14.0)	16.1 (4.9)	76.9 (63.7, 88.3)	32.7 (8.1)
1-Low (<7 nmol/L)	**22**	60.7 (20.4)	16.4 (3.0)	5.0 (3.0, 13.0)	17.5 (5.5)	71.0 (57.2, 90.3)	31.3 (7.8)
***P**		0.06	1.0	0.07	0.2	0.4	0.4
		Multiple regression analyses; β (95% CI)					
Model 1	**536**	-0.40 (-0.83, 0.03)	0.01 (-0.41, 0.44)	-0.37 (-0.80, 0.05)	0.32 (-0.10, 0.73)	-0.22 (-0.64, 0.21)	-0.10 (-0.50, 0.29)
Model 2	**536**	-0.37 (-0.79, 0.06)	0.04 (-0.39, 0.47)	-0.30 (-0.71, 0.12)	**0.43 (0.03, 0.82)↑**	-0.15 (-0.57, 0.27)	-0.04 (-0.43, 0.35)
Model 3	**535**	-0.32 (-0.73, 0.09)	0.004 (-0.41, 0.42)	-0.30 (-0.69, 0.09)	**0.43 (0.04, 0.82)↑**	-0.17 (-0.57, 0.23)	-0.02 (-0.41, 0.37)
Model 4	**457**	-0.38 (-0.79, 0.03)	0.02 (-0.41, 0.45)	-0.28 (-0.69, 0.13)	0.30 (-0.10, 0.71)	-0.18 (-0.60, 0.24)	0.02 (-0.37, 0.41)
Folate quartiles		**Cognitive test scores (Mean (SD) / Median (IQR)) according to maternal folate quartiles**					
≤17 nmol/L	**129**	63.5 (17.5)	16.1 (2.4)	8.0 (4.0, 13.0)	14.9 (4.7)	74.3 (62.0, 85.2)	30.6 (7.6)
17.1-32.6 nmol/L	**129**	67.0 (16.9)	16.2 (2.4)	9.0 (4.0, 13.0)	15.9 (4.3)	73.1 (58.7, 83.4)	32.4 (8.5)
32.7-50.7 nmol/L	**146**	67.3 (16.9)	16.5 (2.5)	10.0 (6.0, 14.0)	16.6 (4.9)	75.9 (63.1, 88.7)	33.4 (7.9)
≥50.8 nmol/L	**132**	72.5 (16.4)	16.8 (2.9)	11.0 (7.0, 14.0)	16.9 (5.4)	81.9 (71.0, 92.6)	34.2 (8.1)
****P**		**<0.001**	**0.02**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
		Multiple regression analyses; ***β (95% CI)					
Model 1	**536**	**0.17 (0.08, 0.25)§**	**0.10 (0.02, 0.18)†**	**0.16 (0.07, 0.24)§**	**0.16 (0.07, 0.24)§**	**0.17 (0.08, 0.25)§**	**0.17 (0.09, 0.25)§**
Model 2	**536**	**0.16 (0.07, 0.24)§**	**0.09 (0.004, 0.18)†**	**0.12 (0.03, 0.20)‡**	**0.09 (0.01, 0.17)↑**	**0.14 (0.06, 0.23)‡**	**0.14 (0.06, 0.22)‡**
Model 3	**535**	**0.09 (0.01, 0.18)†**	0.03 (-0.06, 0.11)	0.05 (-0.04, 0.12)	0.05 (-0.03, 0.14)	**0.09 (0.0003, 0.17)†**	**0.09 (0.01, 0.17)↑**
Model 4	**457**	**0.13 (0.03, 0.22)‡**	0.01 (-0.08, 0.11)	0.05 (-0.04, 0.14)	0.04 (-0.05, 0.13)	0.09 (-0.003, 0.18)	0.07 (-0.01, 0.16)

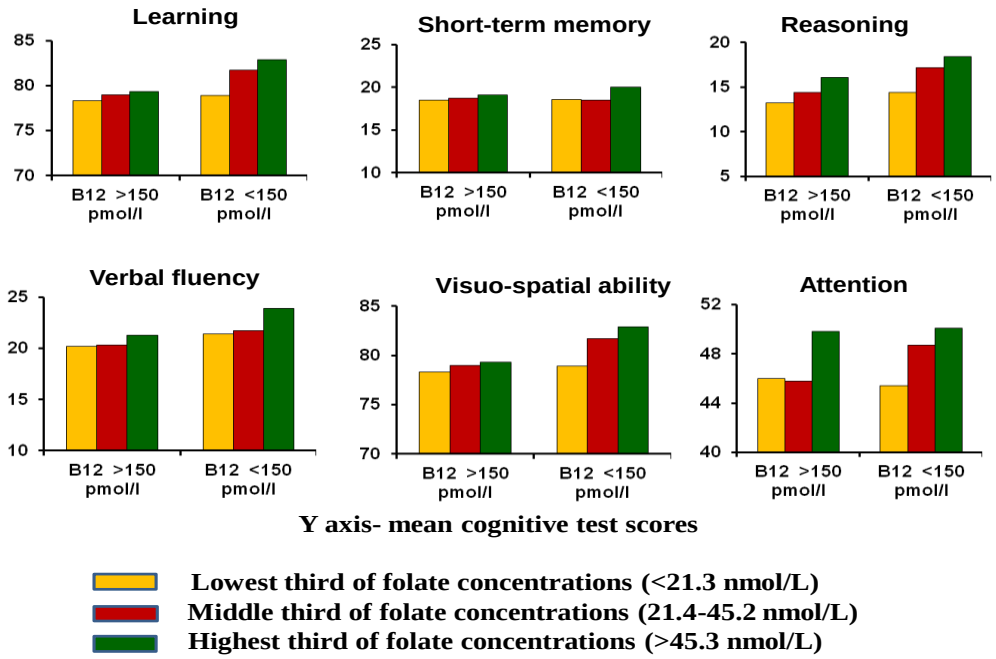
*P value for the difference in cognitive test scores between children of mothers with normal and low folate concentrations derived using t test;
** P for trend adjusted for the child's sex and age derived by multiple linear regression using folate concentrations as a continuous variable;
β (SD) is the difference in cognitive test score between children of mothers with normal and low folate concentrations and ***β is the effect size (SD) of the cognitive test score per SD change in folate concentrations (used as a continuous variable) derived by multiple linear regression;
Model 1: adjusted for the child's sex and current age; **Model 2**: Model 1 + religion; **Model 3**: Model 2 + SLI, maternal and paternal education, occupation, income; maternal intelligence (imputed) and home environment (imputed); **Model 4**: Model 3 + the child's birthweight, conditional head circumference during 2-5 and 5-9.5 years and folate concentrations at 9.5 years; §P<0.001; ‡P<0.01; †p<0.05

Table 4.17 Associations of maternal serum folate concentrations in pregnancy with offspring cognitive performance during adolescence

		Cognitive function tests					
Maternal folate concentrations	**N**	**Learning, long-term retrieval**	**Short-term memory**	**Reasoning ability**	**Verbal fluency**	**Visuo-spatial ability**	**Attention and concentration**
Folate status		**Cognitive test scores (Mean (SD) / Median (IQR)) according to maternal folate status**					
0-Normal (>7 nmol/L)	**518**	78.9 (14.5)	18.9 (3.8)	15.5 (6.5)	21.3 (5.6)	83.3 (25.9)	47.7 (10.9)
1-Low (<7 nmol/L)	**21**	78.2 (12.4)	18.3 (3.7)	14.2 (6.3)	22.2 (6.6)	74.4 (21.3)	45.8 (11.8)
***P**		0.6	0.5	0.4	0.5	0.1	0.4
		Multiple regression analyses; β (95% CI)					
Model 1	**539**	-0.11 (-0.55, 0.33)	-0.18 (-0.62, 0.25)	-0.23 (-0.66, 0.21)	0.10 (-0.32, 0.53)	-0.33 (-0.77, 0.11)	-0.22 (-0.63, 0.20)
Model 2	**539**	-0.08 (-0.52, 0.35)	-0.16 (-0.60, 0.27)	-0.13 (-0.54, 0.29)	0.16 (-0.26, 0.57)	-0.29 (-0.73, 0.14)	-0.16 (-0.57, 0.25)
Model 3	**539**	-0.05 (-0.47, 0.38)	-0.12 (-0.55, 0.30)	-0.04 (-0.43, 0.35)	0.19 (-0.22, 0.61)	-0.22 (-0.63, 0.19)	-0.09 (-0.49, 0.30)
Model 4	**455**	-0.02 (-0.45, 0.40)	-0.01 (-0.44, 0.42)	0.08 (-0.32, 0.48)	0.30 (-0.13, 0.73)	-0.12 (-0.54, 0.30)	0.04 (-0.36, 0.44)
Folate quartiles		**Cognitive test scores (Mean (SD) / Median (IQR)) according to maternal folate quartiles**					
≤17 nmol/L	**130**	79.0 (13.8)	18.5 (3.8)	13.7 (5.9)	20.7 (5.9)	77.2 (23.5)	45.3 (10.5)
17.1-32.6 nmol/L	**129**	79.3 (14.9)	18.2 (3.5)	14.8 (6.1)	20.9 (5.6)	82.5 (26.0)	46.1 (10.9)
32.7-50.7 nmol/L	**146**	78.7 (15.0)	19.3 (3.9)	16.2 (6.7)	21.4 (5.2)	84.8 (26.0)	49.1 (10.0)
≥50.8 nmol/L	**134**	82.4 (13.8)	19.5 (3.8)	17.2 (6.9)	22.2 (5.9)	86.9 (26.8)	49.6 (11.9)
****P**		0.06	**0.002**	**<0.001**	**0.02**	**0.001**	**<0.001**
		Multiple regression analyses; ***β (95% CI)					
Model 1	**539**	0.08 (-0.003, 0.17)	**0.14 (0.05, 0.22)‡**	**0.21 (0.12, 0.29)§**	**0.10 (0.02, 0.18)†**	**0.15 (0.06, 0.23)‡**	**0.15 (0.07, 0.24)§**
Model 2	**539**	0.09 (-0.0004, 0.18)	**0.13 (0.05, 0.22)‡**	**0.14 (0.06, 0.22)‡**	0.05 (-0.03, 0.13)	**0.13 (0.04, 0.21)‡**	**0.12 (0.04, 0.21)‡**
Model 3	**539**	0.04 (-0.05, 0.13)	0.08 (-0.01, 0.16)	0.07 (-0.01, 0.15)	0.02 (-0.07, 0.10)	0.06 (-0.03, 0.14)	0.07 (-0.01, 0.16)
Model 4	**455**	0.02 (-0.07, 0.11)	0.07 (-0.02, 0.16)	0.04 (-0.05, 0.13)	-0.01 (-0.10, 0.09)	0.07 (-0.02, 0.17)	0.05 (-0.04, 0.14)

*P value for the difference in cognitive test scores between children of mothers with normal and low folate concentrations derived using t test;
** P for trend adjusted for the child's sex and age derived by multiple linear regression using folate concentrations as a continuous variable;
β (SD) is the difference in cognitive test score between children of mothers with normal and low folate concentrations and ***β is the effect size (SD) of the cognitive test score per SD change in folate concentrations (used as a continuous variable) derived by multiple linear regression;
Model 1: adjusted for the child's sex and current age; **Model 2**: Model 1 + religion; **Model 3**: Model 2 + SLI, maternal and paternal education, occupation, income; maternal intelligence (imputed) and home environment (imputed); **Model 4**: Model 3 + the child's birthweight, conditional head circumference during 2-5 and 5-9.5 years and folate concentrations at 9.5 years; §P<0.001; ‡P<0.01; †p<0.05

Figure 4.5 Offspring cognitive test scores during adolescence according to maternal vitamin B12 and folate status during pregnancy



4.3.4.5 Maternal homocysteine and offspring cognitive function: During childhood all the cognitive scores were higher in the small group of children of mothers with high homocysteine status compared to children of mothers with normal status, but this was significant only for verbal fluency (Table 4.18). Since hyperhomocysteinemia is an indicator of B12 deficiency and also I found religious group effects of B12 on cognitive function, I explored the data to examine the religious group effects of homocysteine on cognitive function. The above findings were more obvious in Hindus than Muslims in the children of mothers with normal as well as high homocysteine status. The differences were significant for reasoning, verbal fluency, visuo-spatial ability and attention ($p<0.01$ for all) in the children of mothers with normal homocysteine status. But in the children of mothers with hyperhomocysteinaemia it was not possible to make a meaningful statistical comparison as the number of Muslims in this group was too small. The difference in verbal fluency between children of mothers with and without hyperhomocysteinemia was large (up to half a standard deviation) and remained similar after adjustment for confounders across all the models (Table 4.18). Similarly, there was a positive association, across the whole range, of homocysteine concentrations with verbal fluency (Table 4.18). There were also positive associations of homocysteine concentrations with short-term memory and visuo-spatial ability which became stronger and significant in the fully adjusted model (Table 4.18). During adolescence the findings were similar but not significant for any of the cognitive outcome (data not shown).

Table 4.18 Associations of maternal serum homocysteine concentrations in pregnancy with offspring cognitive performance during childhood

		Cognitive function tests					
Maternal homocysteine concentrations	**N**	**Learning, long-term retrieval**	**Short-term memory**	**Reasoning ability**	**Verbal fluency**	**Visuo-spatial ability**	**Attention and concentration**
Homocysteine status		**Cognitive test scores (Mean (SD) / Median (IQR)) according to maternal homocysteine status and religion**					
0-Normal (<10 µmol/L)-**All**	**518**	67.8 (16.9)	16.4 (2.5)	10.0 (5.0, 14.0)	16.0 (4.9)	76.9 (63.5, 88.3)	32.8 (8.0)
Hindu	**297**	67.9 (16.6)	16.4 (2.6)	10.0 (6.0, 14.0)	17.1 (5.2)	77.4 (65.2, 88.4)	33.4 (7.8)
Muslim	**180**	66.0 (17.4)	16.2 (2.6)	8.0 (3.0, 11.0)	14.1 (3.7)	72.5 (59.2, 84.8)	31.1 (8.1)
1-High (>10 µmol/L)-**All**	**18**	68.3 (24.1)	17.4 (2.8)	12.0 (8.0, 17.0)	19.0 (5.0)	81.4 (71.1, 93.1)	34.0 (9.2)
Hindu	**12**	66.0 (24.8)	17.6 (3.2)	12.0 (6.5, 16.0)	18.4 (4.3)	76.7 (65.1, 89.6)	33.9 (8.0)
Muslim	**2**	52.0 (33.9)	16.5 (0.7)	7.5 (4.0, 11.0)	17.0 (7.1)	74.4 (56.2, 92.6)	27.0 (8.5)
***P**		0.9	0.1	0.1	**0.01**	0.2	0.5
		Multiple regression analyses; β (95% CI)					
Model 1	**536**	0.05 (-0.43, 0.52)	0.40 (-0.07, 0.88)	0.38 (-0.09, 0.85)	**0.61 (0.15, 1.06)‡**	0.27 (-0.20, 0.74)	0.20 (-0.23, 0.64)
Model 2	**536**	-0.05 (-0.52, 0.42)	0.35 (-0.12, 0.83)	0.24 (-0.22, 0.70)	**0.46 (0.03, 0.90)↑**	0.15 (-0.32, 0.61)	0.09 (-0.34, 0.52)
Model 3	**535**	-0.05 (-0.50, 0.41)	0.30 (-0.15, 0.76)	0.24 (-0.19, 0.67)	0.42 (-0.02, 0.85)	0.11 (-0.33, 0.55)	0.05 (-0.37, 0.47)
Model 4	**483**	0.05 (-0.41, 0.52)	0.40 (-0.08, 0.87)	0.33 (-0.12, 0.78)	**0.51 (0.06, 0.96)†**	0.26 (-0.21, 0.72)	0.18 (-0.25, 0.61)
Homocysteine quartiles		**Cognitive test scores (Mean (SD) / Median (IQR)) according to maternal homocysteine quartiles**					
< 5.1 µmol/L	**144**	65.2 (18.6)	15.9 (2.6)	9.0 (4.0, 12.0)	14.9 (4.6)	75.6 (63.0, 85.8)	32.1 (8.1)
5.1-6.0 µmol/L	**131**	69.5 (17.4)	16.8 (2.7)	11.0 (5.0, 15.0)	16.8 (4.6)	75.0 (61.5, 89.3)	34.1 (8.4)
6.1-7.1 µmol/L	**139**	69.7 (15.8)	16.4 (2.3)	10.0 (6.0, 14.0)	16.3 (4.9)	78.9 (65.4, 90.8)	32.8 (7.2)
>7.1 µmol/L	**122**	67.0 (16.6)	16.6 (2.6)	9.0 (4.0, 14.0)	16.7 (5.3)	78.0 (63.7, 89.3)	32.2 (8.3)
****P**		0.9	0.09	0.1	**0.009**	0.3	0.4
		Multiple regression analyses; ***β (95% CI)					
Model 1	**536**	0.006 (-0.08, 0.09)	0.04 (-0.05, 0.12)	0.04 (-0.05, 0.12)	**0.11 (0.03, 0.19)‡**	0.03 (-0.05, 0.12)	0.01 (-0.06, 0.09)
Model 2	**536**	-0.002 (-0.09, 0.08)	0.03 (-0.06, 0.12)	0.005 (-0.08, 0.09)	0.06 (-0.02, 0.14)	0.01 (-0.07, 0.10)	-0.01 (-0.09, 0.07)
Model 3	**535**	0.02 (-0.07, 0.10)	0.04 (-0.04, 0.12)	0.02 (-0.05, 0.10)	0.07 (-0.01, 0.15)	0.02 (-0.06, 0.10)	0.001 (-0.08, 0.08)
Model 4	**483**	0.05 (-0.04, 0.15)	**0.11 (0.01, 0.20)†**	0.06 (-0.03, 0.14)	**0.12 (0.04, 0.21)‡**	**0.10 (0.01, 0.18)†**	0.04 (-0.04, 0.13)

*P value for the difference in cognitive test scores between children of mothers with normal and high homocysteine concentrations derived using t test; ** P for trend adjusted for the child's sex and age derived by multiple linear regression using homocysteine concentrations as a continuous variable; β(SD) is the difference in cognitive test score between children of mothers with normal and high homocysteine concentrations and ***β is the effect size (SD) of the cognitive test score per SD change in homocysteine concentrations (used as a continuous variable) derived by multiple linear regression; **Model 1:** adjusted for the child's sex and current age; **Model 2**: Model 1 + religion; **Model 3**: Model 2 + SLI, maternal and paternal education, occupation, income; maternal intelligence (imputed) and home environment (imputed); **Model 4**: Model 3 + maternal vitamin b12 and folate concentrations, the child's birthweight and head growth during 1-2 years; †p<0.05; ‡P<0.01

4.4 Relative strength of associations of socio-demographic, early life and maternal nutritional factors with childhood cognitive function

As described in section 3.9, I used similar methods to compare the relative strength of associations of maternal nutrition, birth size, postnatal growth and socio-demographic factors with childhood cognitive function. I considered using maternal vitamin B12, folate and homocysteine in this analysis as BMI, height and vitamin D were either unrelated or only sporadically related to cognitive function.

4.4.1 R^2 Analysis: Since I have already described the variation in childhood cognitive function explained by socio-demographic, and prenatal and postnatal factors in section 3.9, in this section initially I describe the variation in cognitive function explained by maternal nutritional factors (individual and together), and later by combining all the factors together.

Of the various maternal nutritional factors, each individually explained 0.01% to 3% of the variation in cognitive function (Table 4.19). Maternal folate concentrations explained the greatest percentage of variation (3%). All of them together, explained 2 to 5% of the variation in cognitive function. A model including socio-demographic, early life and maternal nutritional factors together explained 18-26% of the variation in cognitive function. Since of the maternal nutritional factors, folate explained the greatest percentage of variation, I examined the additional variation explained by maternal folate over and above that of socio-demographic or early life factors by including all of them in a model together (Table 4.19). The variation in cognitive function explained by maternal folate together with socio-demographic factors (7-18%) or early life factors (6-10%) differed only slightly to those of socio-demographic (6-17%) or early life factors (4-9%) alone. A model including maternal folate, sex, socio-demographic and early life factors together explained 16-25% of the variation in cognitive function. Interestingly, the R^2 for components (socio-demographic factors, prenatal and postnatal factors and maternal folate) did not add up exactly to the total R^2. For example, for learning, long-term retrieval ability 20% of the variation was explained by a model containing all the factors, of which 12% was due to socio-demographic factors, 7% due to prenatal and postnatal factors and 1% due to maternal folate, which adds up exactly. In contrast corresponding figures for visuo-spatial ability were 16%, which is not the exact sum of 13%, 7% and 1%. These differences may be due to interactions between socio-demographic, prenatal and postnatal factors, though I did not formally test this.

Table 4.19 Variation (R^2) in offspring cognitive function during childhood explained by maternal nutritional status during pregnancy, socio-demographic factors and prenatal and postnatal factors

Maternal nutritional status	Learning, long-term retrieval	Short-term memory	Reasoning	Verbal fluency	Visuo-spatial ability	Attention and concentration
Vitamin B12 concentrations	0.007	0.001	0.008	0.004	0.0004	0.006
Folate concentrations	0.03	0.01	0.03	0.03	0.03	0.03
Homocysteine concentrations	0.0000	0.001	0.0009	0.01	0.001	0.0000
a) Maternal nutritional status together	**0.04**	**0.02**	**0.04**	**0.05**	**0.04**	**0.04**
b) Socio-demographic factors together	**0.12**	**0.11**	**0.17**	**0.06**	**0.13**	**0.08**
c) Prenatal and postnatal factors together	**0.07**	**0.07**	**0.08**	**0.04**	**0.07**	**0.09**
d) Sex	**0.0000**	**0.01**	**0.02**	**0.07**	**0.0003**	**0.09**
All together (a+b+c+d)	**0.20**	**0.19**	**0.26**	**0.22**	**0.18**	**0.22**
e) Maternal folate+b together	**0.13**	**0.12**	**0.18**	**0.07**	**0.14**	**0.09**
f) Maternal folate+c together	**0.10**	**0.07**	**0.10**	**0.06**	**0.09**	**0.11**
g) Maternal folate+b+c+d together	**0.20**	**0.18**	**0.25**	**0.18**	**0.16**	**0.22**

Each row represents a separate analysis;

a) Maternal vitamin B12, folate and homocysteine concentrations together

b) Parity, standard of living index, maternal and paternal education, occupation, income, rural/urban residence, maternal intelligence (imputed) and home environment (imputed) together

c) Gestational age, maternal gestational diabetes mellitus, head circumference at birth, postnatal head growth (0-1 year, 1-2 years, 2-5 years and 5-9.5 years) and breast-feeding duration together

4.4.2 Comparison of regression coefficients analysis: In a regression model containing all the maternal nutritional factors together, all had some independent association with some cognitive outcome (Table 4.20). The strongest and most consistent was maternal folate status. I then included maternal folate concentrations along with maternal education, MIQ, HC at birth and gain in HC during 0-1 year and 2-5 years (as they were the strongest and most consistent predictors (Table 3.20a-c)) together in a single model to compare their relative strength of associations on cognitive function. The coefficient for folate reduced slightly in the multivariate model, consistent with a confounding effect of maternal education/intelligence (Table 4.21a and b); the strength of associations were not attenuated much more even on addition of all the other SES variables (Table 4.16). Taken together, the results suggest an independent association with folate. Apart from folate, all the other factors (mentioned above) included in this analysis independently predicted cognitive function and the size of the coefficients differed only slightly from those seen in the univariate analyses (Table 4.21a and b). The coefficients for the maternal folate concentrations (range 0.18 to 0.20 SD per SD in univariate analyses; range 0.12 to 0.16 in multivariate analyses for learning, reasoning and visuo-spatial ability as outcomes) were quite similar to those of socio-economic variables (univariate: range 0.16 to 0.28, multivariate: 0.14 to 0.23), birth HC (univariate: range 0.10 to 0.15; multivariate: 0.07 to 0.13) and postnatal growth (univariate: range 0.09 to 0.14; multivariate: 0.07 to 0.13).

Table 4.20 Independent effects of maternal nutritional status during pregnancy on offspring cognitive function during childhood

	Maternal nutritional status (SD)		
Cognitive function tests (SD)	**Vitamin B12 concentrations**	**Folate concentrations**	**Homocysteine concentrations**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**
Learning, long-term retrieval	**-0.10 (-0.18, -0.01)**	**0.19 (0.10, 0.27)**	0.04 (-0.05, 0.13)
Short-term memory	-0.04 (-0.12, 0.05)	**0.13 (0.04, 0.22)**	0.06 (-0.03, 0.16)
Reasoning	**-0.09 (-0.18, -0.01)**	**0.19 (0.11, 0.28)**	0.06 (-0.02, 0.15)
Verbal fluency	-0.06 (-0.14, 0.03)	**0.21 (0.13, 0.30)**	**0.15 (0.06, 0.24)**
Visuo-spatial ability	-0.02 (-0.11, 0.06)	**0.20 (0.11, 0.29)**	**0.09 (0.003, 0.18)**
Attention and concentration	**-0.09 (-0.18, -0.01)**	**0.19 (0.10, 0.28)**	0.03 (-0.05, 0.12)

β is the effect size (SD) of the cognitive outcome per SD change in maternal nutritional status derived using all variables included together in multiple regression
Values in bold are significant ($p<0.05$ for all)

Table 4.21a Effects of maternal education, head circumference at birth, conditional (0-1 year and 2-5 years) head circumference and maternal folate concentrations on offspring learning, long-term retrieval, reasoning and visuo-spatial ability during childhood

	Maternal education (SD)	Birth HC (SD)	0-1 year conditional HC (SD)	2-5 years conditional HC (SD)	Maternal folate concentrations (SD)
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
			Learning, long-term retrieval (SD)		
Model 1	0.21 (0.12, 0.29)***	0.14 (0.04, 0.23)**	0.11 (0.03, 0.19)*	0.14 (0.06, 0.23)**	0.20 (0.10, 0.29)***
Model 2	0.16 (0.07, 0.24)***	0.11 (0.01, 0.20)*	0.08 (0.01, 0.17)*	0.13 (0.04, 0.22)**	0.15 (0.06, 0.24)**
			Reasoning (SD)		
Model 1	0.28 (0.20, 0.36)***	0.10 (-0.003, 0.20)	0.12 (0.03, 0.21)**	0.13 (0.04, 0.22)**	0.19 (0.10, 0.28)***
Model 2	0.22 (0.13, 0.31)***	0.07 (-0.03, 0.16)	0.10 (0.01, 0.18)*	0.12 (0.03, 0.21)**	0.13 (0.04, 0.22)**
			Visuo-spatial ability (SD)		
Model 1	0.27 (0.19, 0.35)***	0.15 (0.06, 0.25)**	0.09 (0.004, 0.18)*	0.12 (0.04, 0.21)**	0.18 (0.09, 0.27)***
Model 2	0.23 (0.14, 0.32)***	0.12 (0.02, 0.22)*	0.07 (-0.02, 0.15)	0.12 (0.03, 0.20)*	0.12 (0.03, 0.21)*

β is the effect size (SD) of the cognitive outcome per SD change in the exposure; Model 1: Unadjusted; Model 2: Maternal education, birth head circumference (HC), 0-1 year and 2-5 years conditional HC and maternal folate concentrations combined together; *** p<0.001; ** p<0.01; *p<0.05

Table 4.21b Effects of maternal intelligence, head circumference at birth, conditional (0-1 year and 2-5 years) head circumference and maternal folate concentrations on offspring learning, long-term retrieval, reasoning and visuo-spatial ability during childhood

	Maternal intelligence (SD)	Birth HC (SD)	0-1 year conditional HC (SD)	2-5 years conditional HC (SD)	Maternal folate concentrations (SD)
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
			Learning, long-term retrieval (SD)		
Model 1	0.16 (0.10, 0.23)***	0.14 (0.04, 0.23)**	0.11 (0.03, 0.19)*	0.14 (0.06, 0.23)**	0.20 (0.10, 0.29)***
Model 2	0.14 (0.07, 0.21)***	0.11 (0.02, 0.21)*	0.09 (0.004, 0.17)*	0.13 (0.04, 0.22)**	0.16 (0.07, 0.25)***
			Reasoning (SD)		
Model 1	0.20 (0.13, 0.27)***	0.10 (-0.003, 0.20)	0.12 (0.03, 0.21)**	0.13 (0.04, 0.22)**	0.19 (0.10, 0.28)***
Model 2	0.19 (0.12, 0.27)***	0.07 (-0.02, 0.17)	0.10 (0.02, 0.19)*	0.12 (0.03, 0.21)**	0.15 (0.06, 0.24)**
			Visuo-spatial ability (SD)		
Model 1	0.17 (0.10, 0.24)***	0.15 (0.06, 0.25)**	0.09 (0.004, 0.18)*	0.12 (0.04, 0.21)**	0.18 (0.09, 0.27)***
Model 2	0.16 (0.09, 0.23)***	0.13 (0.03, 0.23)**	0.07 (-0.01, 0.16)	0.11 (0.03, 0.20)*	0.14 (0.05, 0.23)**

β is the effect size (SD) of the cognitive outcome per SD change in the exposure; Model 1: Unadjusted; Model 2: Maternal intelligence, birth head circumference (HC), 0-1 year and 2-5 years conditional HC and maternal folate concentrations combined together; *** p<0.001; ** p<0.01; *p<0.05

4.5 Summary of main findings

- In this population there was a very small prevalence of underweight, obesity, low folate status and hyperhomocysteinemia, and a high prevalence of low vitamin D and vitamin B12 status in the mothers during pregnancy.
- There were no consistent associations of maternal underweight, overweight/obesity and BMI across the entire range with offspring cognitive function during childhood and adolescence.
- Maternal height was positively associated with offspring short-term memory during childhood and verbal fluency during adolescence. The strength of these associations was approximately halved and lost significance after adjusting for socio-demographic factors.
- There were no associations of maternal vitamin D concentrations (low as well as across the entire range) with offspring cognitive function.
- Lower maternal B12 concentrations were associated with higher scores in some cognitive outcomes. This pattern was more obvious in Hindus than Muslims. The strength of these associations was reduced after controlling for socio-demographic factors, but the association with adolescent learning remained significant.
- Higher maternal folate concentrations predicted higher cognitive test scores. The strength of these associations was approximately halved after adjusting for socio-demographic factors, but remained significant for some tests. Associations between maternal folate status and cognitive function were similar at both ages.
- There was a positive association between maternal homocysteine and offspring verbal fluency during childhood. The strength of this association remained significant after adjustment for maternal B12 and folate concentrations, birth size and postnatal growth.
- Maternal folate concentrations during pregnancy predicted several cognitive domains independently of socio-demographic and early life factors. The effect of, or the variation explained by, maternal folate differed only slightly in size to those of socio-demographic factors, HC at birth and postnatal growth.

4.6 Discussion

In the second part of this chapter I have described the associations of maternal nutritional status during pregnancy (underweight, overweight/obesity, height and serum vitamin D,

vitamin B12, folate and homocysteine status) with offspring cognitive function during childhood and adolescence. I have also described these associations adjusted for socio-demographic factors, birth size and postnatal growth.

4.6.1 Strengths and limitations: Since I have already described the strengths and limitations of this cohort in section 3.11.1, in this section I am describing the strengths and limitations related to maternal characteristics in my study.

As strengths, maternal anthropometric measurements during pregnancy were measured using standardised procedures by trained investigators and were not based on self report. Another important strength was the measurement of circulating maternal nutrients during pregnancy, a more robust indicator of maternal nutritional status than dietary or supplement intakes.

There are some limitations. The pregnant women studied were recruited from a single maternity unit generally visited by families who could afford basic health care. Therefore they may not be representative of all pregnant women in Mysore, particularly those from very poor or very affluent social backgrounds. Although their characteristics such as age, weight, height and BMI were similar to other Indian pregnant women[285-287], they were a restricted sample in terms of social background. They can probably be considered as representative of urban middle-class Indian women. Despite similarities in most of the maternal characteristics between participants and non-participants, the mothers of non-participants tended to be taller and had higher folate concentrations and therefore the generalizability of findings may be limited. Maternal micronutrient assays were performed using stored serum samples for ~8 years. However, vitamin D, vitamin B12 and folate have been shown to be stable following long-term storage at lower temperatures[288]. Lack of data on maternal vitamin D concentrations in ~14% of the study participants was another limitation. However, birth size, socio-demographic factors and cognitive scores were similar among those who had this data compared to those who did not and therefore chances of bias in my findings are unlikely. Other limitations were lack of information on the maternal diet, sunlight exposure, use of vitamin D, vitamin B12 and folate supplements at the time of blood sampling and the child's vitamin D status.

4.6.2 Supplement use and micronutrients status: I found no significant associations between intake of vitamin supplements and vitamin concentrations. It is possibly due to a

lack of complete information on supplement intake, as the study was not originally designed to examine maternal vitamin D, vitamin B12 and folate status and supplement use was recorded only at the time of recruitment. Among women recruited between 24 and 32 weeks gestation, very few were taking supplements. Women who were taking supplements in early pregnancy might have stopped taking them by 30 weeks and women who were not taking supplements at recruitment may have been prescribed them later in pregnancy. However, despite the common practice of obstetricians prescribing calcium and vitamin D, vitamin B12 and folic acid during the second trimester of pregnancy, many women had low concentrations of vitamin D and vitamin B12.

4.6.3 Seasonal variation of vitamin D concentrations: The finding of seasonal variation in vitamin D concentrations in my study is possibly due to sunlight exposure. As reported earlier, although data on sunlight exposure was not available in my study, vitamin D concentrations were found to be lowest during the rainy season and summer season when people avoid the sun, and highest in the winter season when the weather is cooler and people go out in the sun[282]. Seasonal variations in vitamin D concentrations and correlations with sunlight exposure have been reported in other Indian[289] and Asian populations[290]. Low vitamin D concentrations during winter have been reported among western populations[291,292].

4.6.4 Maternal nutritional status; comparison with other populations: In my study a very small percentage of women were underweight (4%) and obese (5%), consistent with findings from another urban Indian study[287]. The prevalence of underweight was similar to that of western populations[237-239]. But the prevalence of maternal obesity was lower in my study compared to western populations (range 8-25%)[237-243]. The difference could be due to differences in the associations of SES and its related factors with obesity; in developed countries obesity is more common among lower SES populations, but in developing countries it is more prevalent among higher SES populations.

The high prevalence of low maternal vitamin D status in my study is consistent with findings in other Indian[293] and western populations[124,125,246]. South Asians, both in their country of origin and after migration to Europe or the USA, have lower vitamin D concentrations than white Caucasians[289,292]. The main reasons for low vitamin D status are skin pigmentation, dress code (especially in women) and low dietary vitamin D intake. Another possible reason may be differences in vitamin D metabolism in Asian Indians; in

vitro studies have shown that tissue fibroblasts have increased 25-hydroxy-24-hydroxylase activity, leading to increased catabolism of activated vitamin D and therefore are at increased risk of developing vitamin D deficeincy[294].

The high prevalence of low vitamin B12 status (~43%) in my study is consistent with findings in other Indian studies[285,286,295]. The main reason is thought to be dietary habits. Since animal products are the main source of vitamin B12, Hindus who are mainly lacto-vegetarians were found to have the lowest concentrations (51%). However, despite not being vegetarians, ~30% of Muslims and 35% of Christians were also found to have low vitamin B12 status. This might be due to poverty and inability to afford foods rich in vitamin B12. Malabsorption due to tropical enteropathy is another possible reason. Consistent with the findings in another Indian study that <1% of the mothers had low erythrocyte folate concentrations[285], few (4%) women in my study had low serum folate concentrations. The lowest concentrations were found in Muslims, who are mainly non-vegetarians with low vegetable intakes. In contrast, in studies of other Indian populations, 22 to 26% of pregnant women had low erythrocyte/serum folate status[286,296]. The reasons for this difference are not clear. Differences in the dietary intake could be a possible explanation.

4.6.5 Maternal BMI, height and offspring cognitive function: According to my systematic review there is quite consistent evidence from studies in developed countries that maternal obesity is associated with lower cognitive function in the children, which may be due to inflammatory factors reaching the fetus, but could also be due to confounding (page 127). In my study there was no association of maternal overweight/obesity or of BMI across the whole range, with offspring cognition. This could mean there is no biological effect of maternal adiposity on offspring neurodevelopment, or it could be because my study had very few obese mothers, and it is probably not an adequate test. The negative effect of maternal obesity on offspring cognition could be examined by better studies in populations with a higher prevalence of obesity, in which maternal IQ and other important measures are adjusted for. Alternatively, it could be tested by quasi-experimental studies (for example studies of pregnancies before and after obesity reduction surgery).

There is some evidence, though not conclusive, from a small number of studies in developed countries that maternal undernutrition (underweight or lower gestational weight

gain) is associated with lower cognitive function in the children. This could be due to intrauterine exposure of the fetus to undernutrition, but could also be due to confounding (page 127). In my study there was no association between maternal underweight and offspring cognitive function. This suggests that a causal link between maternal underweight and offspring neurodevelopment is unlikely. Alternatively, since my study had very few underweight mothers, inadequate power could be a reason for no association. Cohort studies with a higher number of underweight women belonging to a range of socio-economic strata are needed to examine the negative effect of maternal undernutrition on offspring cognition.

To the best of my knowledge no studies have examined the association of maternal height with offspring cognitive function. There is good evidence linking height to an individual's own cognitive ability[297,298]. In general, taller individuals tend to have better cognitive ability, although the correlation between height and cognitive ability is small (r=0.1 to 0.2). A study in South Africa exploring maternal predictors of intelligence among children with fetal alcohol spectrum disorder, using structural equation modeling, reported that taller maternal stature during pregnancy was associated with better intelligence at age 7 years[299]. In my study there was a positive association of height with some cognitive domains. Given the well established link between height and cognitive function, one would expect taller and brighter mothers on an average to have brighter, taller children, for a combination of genetic and environmental factors. The fact that maternal height in my study was linked to MIQ suggests that the association between maternal height and offspring cognitive function in my study could be due to genetic factors. Alternatively, taller maternal height, reflecting better nutrition, could contribute to better fetal growth and neurodevelopment. However, parents' SES and its related factors, which are strongly related to cognitive performance and also to maternal height, may confound the association of maternal height with cognitive function. Reduced associations after adjustment for these factors in my study suggest confounding effects.

4.6.6 Maternal vitamin D and offspring cognitive function: As described earlier (page 128), vitamin D is important for a variety of biological actions fundamental to neurodevelopment[269-271]. There is some evidence for a causal link from a limited number of observational studies among affluent populations that maternal vitamin D deficiency during early pregnancy is associated with lower cognitive function in the offspring, but this could also be due to confounding (page 129-130). In my study there was no association

between maternal vitamin D status during the third trimester (28-32 weeks) and offspring cognitive function. One possible explanation is that there is no biological effect of maternal vitamin D status on offspring cognitive development. Another is that, with more than two thirds of women having low vitamin D status in my study, there may have been insufficient variability in vitamin D status. If timing in pregnancy is critical, a lack of information about vitamin D status in early gestation might be an alternative explanation. Additional studies, trials in particular or cohort studies with adequate information about vitamin D status during different stages of pregnancy as well as important confounding factors would be more appropriate to examine the negative effect of maternal vitamin D status on offspring cognitive function.

4.6.7 Maternal vitamin B12 and offspring cognitive function: There was no conclusive evidence from observational studies, mainly in developed populations, for a causal association between low maternal vitamin B12 status and lower cognitive function in the children (page 131-132). In my study low maternal serum vitamin B12 status (low vs. normal and across the entire range assessed during 28-32 weeks) was associated with higher scores in some cognitive outcomes, especially of learning. The difference in my findings compared to earlier studies could be due to differences in the sample size and selection, age groups of the children, measures and timing of assessment of nutritional status and of cognitive function.

I cannot explain the inverse associations between maternal vitamin B12 and offspring cognitive function, especially among Hindus, in my study. I can only speculate that it could be due to cultural differences. In the caste-based traditional Indian society the more orthodox Hindus, who are strict vegetarians (and hence have low vitamin B12), also tend to be of upper caste, more highly educated and to have high SES. With a lack of information about caste and dietary habits in my study, I was unable to explore this further. More research, with adequate information about confounding factors such as dietary preferences and caste would be needed to confirm my study findings.

Alternatively, since several statistical tests were performed, chance might be an explanation for the observed findings. However, if the findings are true they may have implications for any ongoing trials of maternal supplementation with vitamin B12. For example, if the trials are aimed to improve birth size, they might show benefit of supplementation in improving birth size. On the other hand supplementation might be

harmful for cognitive development. Although this is speculative, it would be important to assess cognitive function in the children of mothers who participated in the trial.

4.6.8 Maternal folate and offspring cognitive function: Based on my systematic review, a causal link between maternal folate status and offspring cognitive function seemed unlikely (page 130-131). In my study there was no association between folate deficiency and offspring cognitive function, probably because the low folate status group was small. However, cognitive scores increased with increasing folate concentrations across the whole range. These findings suggest a possible biological effect. Other than a causal effect, partial attenuation in strength of the associations in some cognitive domains after adjustment for socio-demographic factors suggests that the observed associations were due at least in part to confounding. Since a study found an association of deficient dietary folate intake with lower cognitive function in infants of mothers who were carriers of MTHFR677 TT genotype[120], genetic factors might explain the observed association in my study. But this explanation seems unlikely as the TT allele of this genetic variant was quite rare (<2%) in my study[300]. Although a high proportion (18%) of mothers were heterozygous carriers of the T allele (CT), the cognitive function of their children, and associations between the mother's folate deficiency or folate concentrations and the child's cognitive function did not differ in this group from women of CC genotype (data not shown). More studies, trials in particular, are needed to confirm the protective effects of maternal folate on offspring cognitive function.

4.6.9 Maternal homocysteine and offspring cognitive function: Since higher homocysteine concentration is an indicator of deficiency of vitamin B12 and/or folate or other B vitamins, one would expect lower cognitive scores in relation to higher homocysteine concentrations. A small number of observational studies, mainly in developed populations, did not show any association between maternal homocysteine status and offspring cognitive function (Table 4.5). In contrast, in my study high maternal homocysteine concentrations (high vs. normal and also across the entire range) were positively associated with offspring verbal fluency. The reasons for different findings are unknown. For the reasons described in section 4.6.7, residual confounding due to inadequate control for unmeasured factors such as caste and dietary habits could be an explanation for my findings. Since several statistical tests were performed, chance might be another reason for the observed associations.

4.7 Conclusion

In this Indian population cognitive function during both childhood and adolescence was independently predicted by maternal folate concentrations during pregnancy. The strength of effects of maternal folate differed only slightly from those of early life (HC at birth and postnatal head growth) and socio-demographic factors. Models containing maternal folate, early life and socio-demographic factors together explained a greater percentage of the variance in cognitive function than models containing socio-demographic factors or early life factors alone, suggesting that all the factors play a role in determining cognitive function.

Given the findings of my systematic review, an association in my study between maternal folate status and the children's cognitive function, indicating a possible causal link, needs to be confirmed by further research, especially trials. The implication of findings in my study for public health and policy and future research in India will be discussed in detail in the final discussion.

CHAPTER 5 FINAL DISCUSSION

5.1 Summary

In this thesis I have described cognitive function during childhood (9-10 years) and adolescence (13-14 years) in a cohort of normal south Indian children, and its associations with early life factors including size at birth and during the postnatal period, gestational diabetes in the mother, breast-feeding duration, maternal nutrition during pregnancy and socio-demographic factors.

In previous chapters I discussed in detail my specific findings and the strengths and limitations of my study. In this final chapter, my discussion mainly focuses on the relevance and implications of my findings for public health and policy in India. I have also suggested priorities for future research in this area. Before that, I briefly review whether or not the findings from my data support the proposed hypotheses (section 1.6.5).

Hypothesis 1: Based on observations from different populations (section 1.3.3.2; Table 1.1), I proposed that smaller size at birth (birthweight, length, head and mid-upper-arm circumferences, and sum of skinfolds) is associated with poorer cognitive ability during childhood and adolescence. Lower birthweight, shorter length and smaller head circumference at birth in my study predicted poorer cognitive scores. Further, consistent with the findings from an earlier study[60], head circumference was the most robust predictor of cognitive function. The findings support possible programming effects of an unfavourable intra-uterine environment (exposure to undernutrition) leading to both poor fetal growth and impaired neurodevelopment.

Hypothesis 2: Because central nervous system development continues rapidly during the early postnatal years, I proposed that postnatal growth, especially from birth to 5 years, is likely to be related to cognitive function. I observed positive associations between growth in body size from birth to 5 years and cognitive function, strongest for head growth during early childhood (2-5 years).

Hypothesis 3: Earlier studies, mainly from developed populations where the prevalence of obesity ranged from 8-25%, reported negative effects of maternal pre-pregnancy BMI on offspring cognitive function (section 4.2.3.1; Table 4.3). I found no association between

maternal overweight/obesity, or underweight, or BMI as a continuous variable and offspring cognitive function. This may have been due to a lack of extreme BMI values (very high and very low BMI) in my study sample. However, my data did not support my hypothesis that maternal overweight/obesity and/or underweight during pregnancy are associated with poorer cognitive function in the offspring.

In view of the well established association of height with an individual's own cognition, attributed to a combination of genetic and environmental factors[296,297], I proposed that shorter maternal height during pregnancy is associated with poorer cognition in the children. In my study, maternal height was positively associated with offspring cognition and the mother's own IQ. However, the associations between maternal height and offspring cognition were lost after adjusting for socio-demographic factors, suggesting that they were not biological associations, but rather were explained by confounding.

Hypothesis 4: My fourth hypothesis proposed that lower maternal vitamin D, vitamin B12 and folate concentrations during pregnancy are associated with poorer cognitive function in the offspring. Observational studies, mainly from developed populations, have shown some evidence for a causal link of maternal vitamin D and vitamin B12 status with offspring cognition (Table 4.4, section 4.2.3.2 and Table 4.6, section 4.2.3.4 respectively). In my study, I found no association between maternal vitamin D and offspring cognitive function. This is unlikely to be due to inadequate variation in vitamin D status, because the mothers had a wide range of values and 68% were deficient. It may have been measured at the wrong time in gestation to see an effect. The data did not, however, support my hypothesis. Vitamin B12 status in my study was inversely related to offspring cognitive function and this association remained significant for some domains of cognition after adjustment for confounders. I am unable to explain this, but speculated that it could be due to confounding due to dietary practices among higher caste Hindus.

Earlier studies, including a trial, mainly from developed populations did not suggest a causal link between maternal folate status during pregnancy and offspring cognition (Table 4.5; section 4.2.3.3). In my study, with only 4% of women having low folate status, there was no significant difference in the cognitive scores between children of mothers with or without folate deficiency. However, offspring cognitive scores increased with increasing maternal folate concentrations across the whole range suggesting that higher maternal folate concentrations could be protective for offspring cognitive development.

Hypothesis 5: Some studies, mainly from developed populations, have reported a negative relationship between gestational diabetes in the mother and offspring cognitive function[98,99]. In my study, cognitive scores were higher in children of mothers with gestational diabetes compared to children of mothers without gestational diabetes. However, the associations were lost after adjusting for socio-demographic factors, suggesting that they were not true biological associations, but rather were explained by confounding, and the fact that women who develop gestational diabetes in India tend to be of higher SES. Also, there were no associations across the entire range of fasting and post glucose load maternal glucose concentrations with offspring cognitive function.

Hypothesis 6: Evidence from a large body of research, mainly from developed populations (section 1.3.4.1), has shown that breastfed children[126,136,137], and those breastfed for longer[36,133,134], have higher cognitive function. In my study, breast-feeding duration was unrelated to children's cognitive function. With almost all the children being breast-fed during the first 6-8 months and nearly two thirds for 12 months and beyond, a lack of heterogeneity in this cohort may explain the absence of an association. Further, unlike developed populations, where breast-feeding duration is related to higher SES[146], SES was only weakly related to breast-feeding duration in my cohort.

I examined the relative strength of associations of maternal nutrition, birth size, postnatal growth and socio-demographic factors, and found that the variance in cognitive function explained by maternal folate status was 1-3%, that explained by newborn head circumference and childhood head growth was 4-9% and that explained by current socio-demographic factors was 6-17%. The effects appeared to be additive (total variance explained 16-25%). I conclude that all these factors have modest but important, and independent, roles in predicting cognitive function in this Indian population.

5.2 Relevance and implications for public health and policy in India

It has been estimated that more than 200 million children <5 years of age in developing countries may fail to achieve their full developmental potential in cognitive, motor and socio-emotional skills[6]. A couple of reviews of research studies in developing countries have shown consistent evidence that intrauterine growth restriction and stunting, iron and iodine deficiency in the child, poor health, poor cognitive stimulation and caregiver

responsiveness (for example due to maternal depression) during early childhood, are risk factors for poor cognitive development[301,302]. They also concluded that some factors, for instance maternal education, are protective for optimal child development[302].

In India low birthweight is widely prevalent (~30%)[177]. The mean birthweight is 2.7 kg, about 800 g lower than in developed populations[178,179], attributed mainly to intrauterine growth restriction, probably due to intrauterine exposure to undernutrition[113] rather than prematurity[180]. According to the 2005-2006 National Family Health Survey-3 report, about 36% of Indian women of reproductive age (15-49 years) are underweight and 12% are stunted (height<147 cm)[176]. About 43% of Indian children below 5 years are underweight and about 48% are stunted, indicating chronic undernutrition[176]. This means that a large proportion of children in India are exposed to prenatal and postnatal undernutrition.

In my study cohort, as reported elsewhere, all the neonatal measurements were smaller than those in the developed populations[179]. The mean birthweight was 2.8 kg and about 18% were of low birthweight. A very small percentage of mothers (4%) were underweight and ~4% were stunted. At age 5 years, 25% of children were underweight and 11% were stunted. These figures are considerably better than the average values for India as a whole, given above. Nevertheless, my study found that smaller head circumference at birth, and slower head growth during early childhood, predicted poorer cognitive test scores. This suggests that many Parthenon children were not optimally nourished in utero or postnatally, and that this had adverse effects on their cognitive development. On the positive side, these risk factors could be modified in future, with appropriate interventions or programmes.

Different interventions, implemented in either trials or programmes, have been tried in developing countries to improve early child development[303,304]. Some examples are: a) food supplementation to pregnant mothers; b) food supplementation to children during the first 2-3 years of life; c) education for pregnant mothers about nutrition, stimulation and child rearing practices; d) provision of learning opportunities to children; and e) integrated interventions including combinations of (a) to (d). A systematic evaluation of the individual and/or integrated interventions has found convincing evidence for a beneficial effect on the child's cognitive function[303,304]. Key factors were thought to be the prevention of stunting in children, through improvements in diets of pregnant women and children during infancy and the early childhood years; the prevention of iron and iodine deficiency

in children; and improvements in parenting practices, cognitive stimulation and preschool enrolment. Further, the authors concluded that a high quality, high intensity and longer duration package, integrated with health, nutrition, educational services and family support, and targeting younger children and disadvantaged families, was more effective than individual interventions.

In India, as a foundation of human development, an integrated social welfare programme termed 'The Integrated Child Development Service (ICDS)' has been in operation since 1975[305]. It is one of the world's largest outreach packages designed to prevent maternal malnutrition as well as to improve the nutrition, health and development of children below 6 years of age, mainly targeting poor families. The scheme provides: daily nutritional (protein-calorie) supplementation to pregnant and lactating mothers and to children aged 3-6 years; health education to pregnant and lactating women about child rearing practices; preschool education to 3-6 year old children; growth monitoring of children aged 0-5 years; and immunization and health services. In recent years, the scheme has been extended to improve the nutrition and health of adolescent girls. The delivery of these services is integrated and managed through primary health centres and Anganwadi (courtyard of a house) centres, and its workers and helpers. The programme is centrally sponsored with assistance from the World Bank and United Nations International Children's Emergency Fund (UNICEF). It has been estimated that the average cost of the ICDS programme is about US$10–22 per child a year with the state governments contributing up to 1.00 rupee (1.7¢ US or 0.01 UK£) per day per child. Around 50% of the cost is incurred in food supplementation. Since its inception, the scheme has been expanded rapidly in scope and coverage, and currently, as of 31st March 2014, the programme serves ~20 million pregnant and lactating women and ~85 million children <6 years of age.

Some studies have examined the effectiveness of the ICDS programme and found a beneficial effect. A study was conducted by the National Institute of Public Cooperation and Child Development among a nationally representative, randomly selected sample of 14000 children from tribal, rural and urban areas participating in the ICDS services. The study also included a control sample of 2000 children from similar areas where ICDS was not implemented, and where the level of development, for example socio-economic status, income, land ownership and health and educational facilities were similar to those of ICDS participants' areas. The study found less likelihood of severe malnutrition and greater

likelihood of school attendance among children participating in ICDS compared to non-ICDS participants[306,307]. In another study mental and motor development among a randomly selected sample of 3-6 year old children (n=2428) residing in rural areas of the three southern Indian states and participating in the ICDS services were examined using WHO motor and mental development scales. The study also included a control group (n=1296) from the same areas not participating in the ICDS services but whose age and socio-demographic profile such as family size, parent's literacy and SES were similar to those of ICDS participants. The study found that the scores for mental and motor development tests were higher (effect size not available) in ICDS participants than non-ICDS participants[308]. The study also found that the effects were greater for younger and malnourished children in two of the three states. Although these findings suggest that the programme can be effective, recent evaluations indicate that the positive effects such as reduction in severe malnutrition, improvement in school attendance, and mental and motor development scores were modest, probably due to a variety of factors affecting the implementation, infrastructure and quality of service delivery[309-311]. These include low funding, service delivery by unskilled, underpaid and overburdened Anganwadi workers, insufficient training, lack of infrastructure and basic amenities, and inadequate functioning of the health education, nutrition and preschool education components of the programme. Nevertheless, the ICDS programme has a huge potential to promote child development. The government is planning to universalize the ICDS programme. Thus, I would support the 11th planning commission report suggesting revision and restructuring of the financial allocations, and improvement in the quality and functioning of the interrelated services of the programme in order to improve its effectiveness[311].

In India ~60% of pregnant women have iron deficiency[176], and deficiencies of vitamin D[293], vitamin B12[285,286,295,296] and folic acid are common[286,296]. Since all these micronutrients have a role to play in various biological processes of neurodevelopment (page 129,130,131), a strategy to prevent these micronutrient deficiencies in women is another promising approach to improve cognitive development of the offspring. The finding in my study of a positive association between maternal folate concentrations and offspring cognitive function suggests that folic acid supplementation during pregnancy might benefit offspring cognitive development. In India, in accordance with government policy to reduce the prevalence of maternal anaemia, women are supplemented with 100 mg iron and 0.5 mg folic acid for at least 100 days during pregnancy from the second trimester, the time pregnant women usually start attending antenatal clinics[312]. Despite this

policy, folate deficiency is common. This is thought to be due to poor compliance; according to the 2007-2008 District Level Household and Facility Survey-3 in India only 47% of pregnant women reported taking these supplements for the recommended duration[313]. To overcome the issue of compliance, food fortification with folic acid or integrating the supplementation via ICDS might be an alternative strategy to improve folate status. In many countries worldwide, in order to prevent neural tube defects, food items are fortified with folic acid and use of folic acid supplements has been recommended during the peri-conceptional period[314]. Recently, the Indian government has implemented weekly supplementation of iron and folic acid to adolescent girls and supplementation during the peri-conceptional period is under consideration[312].

Recently the effect of iron and folic acid supplementation of women during pregnancy on offspring birthweight in India was evaluated. Pooled data, based on self reported questionnaires, from a nationally representative sample collected during the second (1998-1999) and the third (2005-2006) National Family Health Surveys was used for this evaluation. Despite limitations such as recall bias and incomplete information about the duration of supplement use, there was a 41g increase in the mean birthweight and a reduction in the risk of low birthweight (OR=0.77) in offspring of women who took supplements compared to those who did not take supplements[315]. However, the effect of supplementation on cognitive development of the offspring is not known. Investigating the effect of supplementation on cognitive development would be helpful to address the adequacy of the existing policy issues related to folic acid supplementation.

My study showed no evidence that poorer maternal vitamin D and vitamin B12 status was associated with lower offspring cognition. All biological processes, including neurodevelopment, involve a chain of biochemical reactions, requiring a number of different micronutrients[114]. Individuals who are deficient for one micronutrient are often at risk of multiple deficiencies. Thus the apparent importance of a particular nutrient can appear small if other 'limiting' nutrients are deficient. For the same reasons, a single micronutrient will only appear effective in a trial if all other essential micronutrients are available. Therefore it is difficult to identify individual nutrients important for cognitive development in human populations. Sometimes essential nutrients for neurodevelopment have been discovered because of an accident of nature, for example iodine which is absent in the soil of hilly and remote inland areas, or a man-made accident, for example the removal of thiamine from rice by polishing (removal of the outer husk), leading to the

absence of specific nutrients from the diet. A meta-analysis of mainly observational studies in children showed that the IQ scores of individuals in iodine-sufficient regions were on average 13.5 IQ points higher than individuals in iodine-deficient regions[316]. Another recent meta-analysis of observational and intervention studies in Chinese children showed a similar estimate of 12.5 IQ points difference between those living in iodine-sufficient and iodine-deficient regions[317]. An example of man-made thiamine deficiency and its relationship to cognitive development has been reported in a study in Israel. In Israel a manufacturer by mistake stopped adding thiamine to infant formula. The doctors who discovered this followed up the children who had been fed on this formula and monitored their growth and development. These children, although not showing any cognitive impairment during infancy, showed deficits in language ability compared to controls at age 5-7 years[318]. This is the opposite of supplementation, but has sometimes been successful in identifying essential nutrients for particular processes. It is, therefore, important to consider strategies to improve intakes of all nutrients in women before and during pregnancy.

Apart from the micronutrients normally included in supplements, there may be other important nutrients. Maternal intake of essential fatty acids, for example docosahexanoic acid and arachidonic acid, which are structural components of the central nervous system, could potentially influence offspring cognitive development[114]. An observational study in the UK found higher verbal IQ scores in 9 year old children of mothers who ate oily fish (a rich source of these fatty acids) in late pregnancy compared to children of mothers who did not[319]. Another observational study in Canada found no association between maternal docosahexanoic acid status during pregnancy and infants' mental and psychomotor development[255]. A recent randomized controlled trial among Europeans found a beneficial effect of fish oil supplementation during pregnancy on offspring cognitive scores at age 6-7 years[253]. However, a systematic review of randomized controlled trials of fatty acid supplementation during pregnancy, mainly in developed countries, found mixed results[233]. The author reported that the evidence was not conclusive, due to the methodological limitations of published trials, and suggested that more research into the potential role of essential fatty acids in pregnancy on offspring cognition was needed especially in developing countries. A recent review of studies in developing countries and a study in India has observed that the dietary intakes of essential fatty acids during pregnancy were lower than the recommended average daily intake[320,321]. To my knowledge, no studies in India have examined the relation between maternal fatty acid status in pregnancy and offspring cognitive function.

In India, illiteracy is another public health concern. According to a report from the 2005-2006 National Family Health Survey-3, only around 55% of women of reproductive age are literate and between 5 and 37% of women complete 12 or more years of education[176]. Lower maternal educational level is likely to affect child development independent of poverty and undernutrition. My study showed a strong and independent relationship between maternal education and cognitive function in children. Additionally, maternal education explained a greater variance in cognitive function than early life factors (Table 3.19; page 82). These findings thus emphasise the need for investment to improve the literacy of girls. Higher maternal education is likely to improve offspring cognitive function not only through its influence on prenatal and postnatal nutrition, growth and development, parenting practice and stimulating environment, but also on the use of and compliance with available intervention services.

The Government of India has made education free and compulsory for children aged 6-14 years since 2000-2001, through a programme called "Sarva Shiksha Abhiyan" (Education for All Movement)[322]. School teachers visit house-to-house once every year campaigning about compulsory primary education. Following this, school enrolment has substantially improved[176]. According to the latest 2011 census report, the literacy rate has increased by about 9% in the last decade and interestingly, the increase in the female literacy rate has been greater than the increase in the male literacy rate (12% vs.7%)[323]. Some of the factors related to this increase, although not formally evaluated, may be community involvement, better funding for infrastructure and teachers, and provision of free mid-day meals in schools to prevent drop-outs. I would suggest that this programme to increase access to education is continued and that campaigning about the importance of female literacy is included in the existing ICDS programme.

To conclude, despite a long way to go, it is possible to improve cognitive function over time in India by improving nutrition and education. In this context, I would like to briefly discuss the Flynn effect and its relevance in India. Improvement in IQ scores of a population, termed 'the Flynn effect', has been shown in developed countries over years[324]. In many developed countries test scores of adults taking identical versions of cognitive tests 10-30 years apart have been compared to assess the Flynn effect[324]. They show an increase of about 5-25 IQ points over a generation or 5-9 IQ points per decade. The IQ gains are generally observed for culturally reduced tests (tests of fluid or non-verbal

intelligence), for example Raven's Progressive Matrices, but IQ gains have also been observed in tests of crystallized intelligence. Further, a rise in the fluid intelligence scores of about 12 IQ points over a period of 50 years or 2 IQ points per decade has also been observed in 9-10 year old British children[325]. The reasons for the IQ gains, though not fully understood, have been attributed to improvement in nutrition, schooling, educational standards and increased environmental complexity. Thus, the presence of a Flynn effect, although this cannot be completely proved, suggests that IQ is modifiable by the environment and is not a fixed genetic attribute.

In India, where undernutrition and illiteracy are highly prevalent, my finding that cognitive scores increase with increasing maternal folate status, HC at birth, head growth during early childhood years and maternal educational level suggests that IQ may be modifiable by better nutrition and education. To the best of my knowledge the Flynn effect has not been studied in India. Evidence for a Flynn effect in other developing countries is scarce. Two studies in 1984 and 1998 among ~8 year old rural Kenyan children, using Raven's Progressive Matrices test, showed a 4.5 point gain in fluid intelligence scores over 14 years[326]. They also found a 2.5 point rise in crystallized intelligence scores (the Verbal Meaning Test). The authors reported that the gain in IQ scores was related to a reduction in family size and improvement in parents' literacy, and children's nutrition and health. Two studies in China during 1985-86 and 2011-12 among ~12 year old urban children, using a Chinese version of the WISC-revised test, showed an increase of 6.2, 1.9 and 6.5 points in the full scale, and verbal and performance IQ respectively over 26 years[327]. The authors speculated that the gain in IQ was due to improved family income, standard of living, literacy, nutrition, health and preschool education.

5.3 Gaps in previous research linking early life factors and cognitive function

A lack of **s**tudies, either observational studies or trials, investigating the role of nutrition in cognitive development in populations where there are major nutritional deficiencies is an important gap in previous research. There are too few studies in India that examine the importance of parenting practices and cognitive stimulation for child development. A case-control study found that infants of depressed mothers scored lower on a test of mental development compared to controls[328]. The authors attributed this to direct effects of maternal depressive symptoms on infant mental development. Although not evaluated, they also speculated that interference in infant nutrition or psychosocial stimulation were

alternative explanations, since breast-feeding problems were reported by depressed mothers during the early post-partum review (<6-8 weeks following delivery), and bottled milk feeding was more common in infants of depressed mothers. In a randomized controlled trial in south India, children in the intervention group, who received early home based stimulation therapy, scored higher in tests of mental and motor development at age one (5.8 units) and two years (2.8 units) than controls who received routine postnatal care[329].

If I were to start the Parthenon cohort study again in order to identify key early life factors predicting cognitive function, I would change a number of aspects of the study and data collection. I would recruit a sample representing a much wider range of social sectors by enrolling women visiting the antenatal clinics of government, private and corporate hospitals. I would assess a larger range of micronutrients in the mothers such as iron, folate, vitamin B12, vitamin D and fatty acids using a food frequency questionnaire and biochemical assays. I would also collect details of supplement use before and during different stages of pregnancy. Collecting this information is not easy for a variety of reasons. Firstly, in our experience, generally women do not remember the names of tablets or syrup and do not bring the pills or the prescriptions with them to the research clinic. Secondly, women tend to consult multiple practitioners at several places, and as a result either they take medicine prescribed by one practitioner or multiple practitioners. To overcome this problem, I would develop strategies to collect the details of supplement use. For example, I would ask women to keep a diary, with details of medicine that are prescribed and consumed by them, including the duration and/or to bring the tablets and/or the prescription when they attend the research clinic. Alternatively, I would contact these women by telephone or personal visits at regular intervals, for instance fortnightly or once in a month. I would collect detailed information about caste and religious groups in order to understand their dietary habits. I would also assess maternal postnatal depression and collect information about psycho-social stimulation and other parenting practices by standard scales or questionnaires validated for this population. I would also collect information about the child's diet and nutrient status such as exclusive breast-feeding and details about complementary foods during infancy, by follow-up of the cohort at regular intervals, for instance once every 3 months in the first year. During the annual follow-up, at age 2-3 years, I would also assess the child's dietary pattern and micronutrient status by food frequency questionnaire and/or biochemical assays.

5.4 Final comment and future plans in the Parthenon cohort

This study provided an insight into the role of some of the early life, maternal nutritional and socio-demographic factors influencing cognitive development in Indians. Since the risk factors related to poor cognitive development are also linked to behavioural problems and emotional states[6], the psychological wellbeing of children as measured by their behaviour and feelings of stress may be related to cognitive function. Alternatively, poor cognitive function may influence behaviour. Since neurodevelopment continues during later childhood and adolescence (Figure 1.1), dietary pattern and nutrient status during these periods may influence cognitive function, both currently and in later life. Some of the immediate plans for further research in this cohort are:

- To investigate the association of stress and stress responsiveness with cognitive performance. We have collected data on stress responses at 13.5 years using a validated and standardised stress test, 'The Trier Social Stress Test for Children'[330].

- To examine the association of the psychological wellbeing of the children with cognitive function. We also plan to examine the role of adolescent cognitive function as a determinant of psychological wellbeing in the corresponding period. We have assessed adolescent behaviour by administering the Strength and Difficulties Questionnaire[331] and Temperament Measurement Questionnaire[332] to parents and children.

- To examine the association of the child's own diet and micronutrient concentrations with cognitive function. At 9.5 years, we assessed the child's diet through a food frequency questionnaire and a 24-hour recall administered to the parents and children[333]. We also measured the child's plasma vitamin B12 and folate status at age 5 and 9.5 years. The status of other micronutrients such as iron, vitamin D, fatty acids and iodine, can be assessed using stored samples of plasma and urine collected at age 9.5 years.

Other than the immediate plans, I would also like to examine the association of maternal iron and fatty acids status with offspring cognitive function. This is possible by measurement of iron and fatty acid concentrations in stored maternal serum samples.

Finally, further follow-up of this cohort into adult life will determine whether the factors covered in my thesis, and the other factors mentioned here, are related to later cognitive ability and other measures of human capital outcomes such as educational attainment, occupation and income, and health and quality of life.

APPENDICES

APPENDIX 1- PROTOCOLS

1 Neonatal Anthropometry

1.1 Weight

The baby was placed naked on the digital weighing scale (Seca, Germany) and a single reading to the nearest 0.5 g was taken.

1.2 Length (Crown-heel; Crown-rump)

These lengths were measured using Harpenden infant stadiometer and three readings were taken to the nearest 0.1 cm.

1.2.1 Crown-heel length

The baby's head was held against the end of the head plate and the legs extended until they were flat. The foot plate was brought up to the heels ensuring that the feet and knees were flat and the length was read off the dial.

1.2.2 Crown-rump length

The baby's head was held against the end of the head plate; both knees and hip were flexed ensuring that the back is still flat on the mat. The foot plate was brought up to the buttocks and the length was read off the dial.

1.3 Circumferences

Circumferences were measured initially by marking on a blank tape and later measured the marked tape against a fixed ruler. Three readings were taken to the nearest 0.1 cm.

1.3.1 Head

This measurement was taken with the baby's head on one side, so that the maximum occipito-frontal circumference could be found. The tape was placed on the forehead, on the most anterior point (just above the eyebrows) and passed around the head to the most posterior part of the head making sure that the maximum circumference was found.

1.3.2 Mid-upper-arm (MUAC)

This measurement was made with the arm bent, allowing the measurement to be taken with the baby in its natural position rather than having to straighten out and hold the arm.

Preferably, the arm should be relaxed rather than held in position. The mid-point should be eyeballed and marked accordingly. Readings are taken at this point.

1.3.3 Abdomen

This measurement was taken by placing the tape around the abdomen immediately above the umbilicus ensuring that it was horizontal and marked at the end of expiration.

1.3.4 Chest

This measurement was taken by placing the tape around the chest at the level of xiphisternum ensuring that it was horizontal and marked at the end of expiration.

1.4 Skinfold thickness

Measurements were made on the left side of the body using the 'Harpenden' John-Bull calipers (CMS Instruments, London). The readings were taken at exactly 5 seconds after the application of the calipers jaws. Three readings to the nearest 0.1 mm were taken unless this caused too much distress, in which case, a single measurement was taken.

1.4.1 Triceps

The tape was placed round the upper arm at the level of the mark done while measuring MUAC. With the tape in position, a horizontal line was drawn on the skin posteriorly at the level of the mark. Another vertical line was marked on this line at the most dorsal part of the upper arm. This level was determined by 'eyeballing' the mid-point. The point at which the fold is to be measured was now marked by a cross. The skin was picked up over the posterior surface of triceps muscle, above the cross, on a vertical line passing upward from the olecranon to the acromion. The calipers were applied below the fingers such that the marked cross was at the apex of the fold.

1.4.2 Subscapular

Inferior angle of the scapula was identified and the skin was marked immediately below the angle. The skinfold was picked up above the mark with the fold slightly inclined downward and laterally, in the natural cleavage of the skin. The caliper jaws were applied below the fingers, such that the marked cross was at the apex of the fold.

2 Anthropometry - Childhood and early adolescence

2.1 Weight

Weight was measured using the electronic digital weighing scale (Seca, Germany or Salter, UK). The scale was placed on the most level and stable part of the ground. The dial was checked for 'zero' reading. The child was asked to stand bare-feet on the scale with minimal clothing and look straight ahead. One reading to the nearest 100 g was taken.

2.2 Height

Height is measured using the Microtoise wall-mounting stadiometer (CMS Instruments, London). The subject was asked to remove shoes and stand as tall and straight as possible with feet together, arms held loosely by the side and shoulders relaxed with his/her back, including the posterior surface of the head and heels applied to the wall. The head was positioned in the Frankfurt plane, such that an imaginary line joining the upper margin of the external auditory meatus and the lower border of the orbit of the eye is horizontal. An assistant was present to check that the child's position was correct. The head plate of the stadiometer, fixed to the wall was pulled down and placed firmly on the top of the head in a horizontal position. The examiner aimed to read the scale from as level a position as possible to minimise the errors due to parallax. One reading to the nearest 0.1 cm was taken.

2.3 Sitting height

The child was asked to sit erect, with straight back, and head in the Frankfurt plane, on a wooden stool kept under the stadiometer, applied to the wall. The sitting height was measured once to the nearest 0.1 cm and the stool height was subtracted from the obtained value.

2.4 Circumferences

Circumferences were measured using a fibreglass anthropometric tape. Three readings were taken to the nearest 0.1 cm.

2.4.1 Head

The child was asked to stand erect and look straight ahead. A measuring tape was passed firmly around the head such that it passes around the most posterior part on the back (at the level of external occipital protuberance) and just above the eyebrows anteriorly, and thus

the maximum antero-posterior circumference was measured.

2.4.2 Mid-upper-arm

The child was asked to stand with his/her back to the measurer, arm being flexed at 90^{o}. The tip of the acromion (the point of the shoulder) and the olecranon were palpated and a point midway between them (measured with a tape) marked on the skin. This marks the vertical level at which the skinfold will be made. The child was then asked to relax, with the arm hanging by the side. The tape was placed around the upper arm such that its upper border is at the level of the marking. Before taking the reading ensure that the tape should be horizontal all round, should be firmly resting on the skin, but should not be pulled too tight.

2.4.3 Abdomen

After ensuring that the abdominal wall is relaxed, the tape was placed horizontally around the abdomen at the level of the umbilicus, taking care not to pull the tape too tight as to indent the skin. Readings were taken at the end of the expiration.

2.4.4 Chest

The tape was placed firmly and horizontally at the level of the xiphisternum. Readings were taken at the end of expiration.

2.4.5 Waist

The child was asked to stand straight facing the measurer with arms folded over chest. The iliac crests (highest points of the hipbone) on both sides were palpated and marked at the mid-axillary line. The lower borders of the 12^{th} rib on both sides were marked at the mid-axillary line. With a measuring tape, midpoint between these two levels was marked on either side. Ensuring that the tape is horizontal all round, the circumference was measured at this level and readings were taken at the end of expiration.

2.4.5 Hip

The subject was asked to empty the hip pockets and stand sideways with arms folded over chest. The tape was passed around the hip area (at the level of greater trochanter) and adjusted upwards and downwards until the maximum circumference was achieved. Measurements were taken at this level after ensuring that the tape is horizontal and firmly placed all around.

2.5 Skinfold thickness

It was ensured that the child was as relaxed as possible while doing these measurements. Measurements were made with the 'Harpenden' John-Bull calipers (CMS Instruments, London).

2.5.1 Triceps

The tape was placed round the upper arm at the level of the mark done while measuring MUAC. With the tape in position, a horizontal line was drawn on the skin posteriorly at the level of the mark. Another vertical line was marked on this line at the most dorsal part of the upper arm. This level was determined by 'eyeballing' the mid-point or by a pen held vertically with one end on the olecranon process and the other end pointing towards the acromion. The point at which the fold is to be measured was now marked by a cross, formed by a horizontal line indicating the vertical level, and a vertical line marking the lateral level. The skin was picked up over the posterior surface of triceps muscle, above the cross, on a vertical line passing upward from the olecranon to the acromion. The calipers were applied below the fingers such that the marked cross was at the apex of the fold. The readings were taken at exactly 5 seconds after the application of the calipers jaws. Three readings to the nearest 0.1 mm were taken.

2.5.2 Subscapular

Inferior angle of the scapula was identified and the skin was marked immediately below the angle. The skinfold was picked up above the mark with the fold slightly inclined downward and laterally, in the natural cleavage of the skin. The caliper jaws were applied below the fingers, such that the marked cross was at the apex of the fold. Three readings were taken.

3. Bioimpedance

Bioimpedance was measured using a Bodystat, Quadscan 4000 analyser. The child was made to lie supine on a couch for 5 minutes before starting the measurements, and any heavy jewellery and metal accessories on the body were removed. The child was asked to lie quietly and as comfortable as possible. After cleaning the area with the surgical spirit, on the hand, one electrode was attached at the level of the ulnar head at the wrist and the other just behind the knuckles. On the foot, the two electrodes were attached at the level of the medial and lateral malleoli and just behind the toes, respectively. Two sets of the main

leads connected to the back panel of the machine were attached to the electrodes. After ensuring that no parts of the body were touching one another, measurements were done by pressing the ENTER button on the machine.

4. Cognitive function assessment

4.1 Children

Cognitive function was assessed in the morning (before 12 noon) after ensuring that the child has completed the blood test and finished breakfast.

4.2 Mothers

Mother's intelligence test was administered when the child visited the research unit for the blood test and cognitive function assessment.

5. Home environment assessment

The data on home environment was collected by visiting the home where the participant is residing. The questionnaire was administered preferably to mother or to father/guardian of the child if the mother is dead or if the child is not staying with his/her parents. Presence of the child is mandatory for collecting data.

APPENDIX 2- QUESTIONNAIRES AND LETTERS

MYSORE PARTHENON BIRTH COHORT

Cortisol and Cognitive Function Study: 2011 - 2012

Study Number: ---------- ***Follow-Up Number:*** ----------

Child's Name: ---------- ***Sex:*** --------

Date of Birth: ---------- ***Date of Investigation:*** ----------

Area of Residence: …… **1.** Rural **2.** Urban

Mother's Name: ----------

Father's Name: ----------

Current Address: ----------

Phone: ----------

CHECK LIST

No	DESCRIPTION	YES	NO	COMMENTS
1	Consent			
2	Photograph			
3	Medical history and examination			
4	Anthropometry			
5	Pubertal staging			
6	Bioimpedance			
7	Blood pressure			
8	Blood collection			
9	Cognitive function assessment			
10	Maternal intelligence assessment			
11	Bradley's Home Inventory			
12	Feedback form			
13	Report			
14	Blood/Cognitive Gift			
15	Blood/Cognitive TA			

MOTHER AND CHILD CONSENT FORM

Study Number: ---------- ***Child's Name:*** ----------

I/we have been fully informed about the new study conducted by the Epidemiology Research Unit, C/O Holdsworth Memorial Hospital, Mysore and have been told about all the points as listed below:

1. Performance test- child's body reaction to story and math's performance as explained by the research staff
2. Frequent saliva samples on the day of the performance test for hormones
3. Body measurements, body fat measurement, maturity level and general health check-up
4. Blood pressure (BP) measurement
5. Single blood sample for the child after overnight fasting for measuring blood sugar level, Hb% (for anaemia), and some health indicators
6. Storage of plasma and blood samples from this test for future use
7. Mental function assessment using some playful activities
8. Questions to understand child's general health, behaviour, mood, diet, physical activity, socio-economic status etc.
9. Questionnaires and simple mental function activities for the mother

- I confirm that I have read and understood the information sheet for the above study and have had the opportunity to ask questions.
- I understand that our participation is voluntary and that we are free to withdraw at any time, without giving any reason, and without our medical care and future participation being affected.
- I understand that the stored samples may be used to **study new genes, not yet discovered, in the future**. I have been informed that the results will not be of immediate use or relevance to the child's health or treatment, but the parents will be kept fully informed if anything of importance for the individual child is clearly found. I have also been informed that the study is intended only for research purpose and not for any commercial use.
- I have been informed that for some of the tests, the samples will need to be sent out of Mysore, to a different laboratory that specializes in specific biochemical and genetic studies, but they will remain in India.
- I have been informed that all the information collected will be kept in the **strictest confidence**
- **I agree that our child takes part in the above study.**
- **I agree that I (mother) take part in the above study.**

Mother's Name: ----------

Signature: ---------- ***Date:*** ----------

Name (if not mother): ---------- ***Date:*** ----------

Relationship to Child: ----------

Interviewer: ---------- ***Signature:*** ----------

<u>CHILD'S ASSENT</u>

I agree to take part in the study: ----------

(Please write your name)

Health/ Morbidity Data

1**.** Has the child had any major health problems during the past one year? --------

0. No 1.Yes

If yes ----------

Code | | | |

2. Any present/recent health problems? ----------

0. No 1.Yes

If yes ----------

Code | | | |

3. Is the child taking any medications currently? ----------

0. No 1.Yes

If yes ----------

Code | | | |

4. Did the child face any severe stressful situation in the past 1week? ----------

0. No 1.Yes 9.NA.

If yes ----------

Code | | | |

General examination: Comments and Advice

.....

.....

BLOOD PRESSURE

Instructions: 1. Child should be seated quietly for 5 minutes before the measurements.
2. Measurement taken on the left side
3. In case of overlapping MUAC ranges, always use the higher sized cuff
4. 2 minutes of rest between 2 measurements

Time AM/PM	**Room** Temperature	**Side** 1. Left 2. Right	**Cuff Size** 1. Child 2. Small adult 3. Adult 4. Large Adult

	Systolic (mm Hg)	**Diastolic (mm Hg)**	**Pulse (beats/ min)**
1			
2			

BLOOD TEST

Date	**Fasting 0. No. 1. Yes**	**Fasting Sample Time**

BLOOD TEST RESULTS :

Sample	**Results**
Fasting Glucose (mg/dl)	
Haemoglobin (g%)	
Others	

Fasting Glucose Status: _____ **0. Normal 1. Abnormal 2. Not done**

Comments:

_____

_____

_____

MYSORE PARTHENON 13.5 YEARS FOLLOW-UP STUDY : 2011 - 2012

Study Number _____ **Follow-up Number** _____

Date of Birth _____ **Date of Interview** _____

Child's Name _____

Address: _____

_____

_____

_____

_____

Phone _____

ANTHROPOMETRY

Measurer's Name _____ **Code** _____

Weight (kg) _____

Height (cm) _____

Sitting Height (cm) _____

CIRCUMFERENCES

MUAC (cm)	_____	_____	_____
Head (cm)	_____	_____	_____
Waist (cm)	_____	_____	_____
Hip (cm)	_____	_____	_____

SKIN FOLDS

Triceps (mm)	_____	_____	_____
Subscapular (mm)	_____	_____	_____

PELVIC MEASUREMENTS ***(Girls Only)***

Intercristal (cm)	_____	_____	_____
Interspinous (cm)	_____	_____	_____
External conjugate (cm)	_____	_____	_____

Comments: _____

BIO-IMPEDANCE

Investigator's Name _____ **Code** _____

Test No _____ Time _____ AM/PM Activity level Selected **Medium**

Bio-impedance Complete: _____ 0. No 1. Yes If No, Code _____

Fat _____ **%** **Fat weight** _____ **kg**

Lean _____ **%** **Lean weight** _____ **kg**

TBW_____ **%** **TBW** _____ **lt**

Est. metabolic rate at rest _____ kcal **Energy required** _____ kcal

Impedance 5 kHz _____ 50 kHz _____100 kHz _____200 kHz _____

Comments: _____

PUBERTAL GROWTH

a) Menarche achieved ☐ 0. No 1. Yes 9. Not Applicable

b) If yes, Date of menarche ☐☐☐☐☐☐

Age at Menarche **…… Years**

c) Axillary Hair ☐ 0. Absent 1. Present

d) SMR Staging Done ☐ 0. No 1. Yes

If not done, Reason: _____ Code ____

SMR done by Name: _____ **Code**____

Breast Stage		Pubic Hair Stage	Testicular Volume		Genitals
Right	Left		Right	Left	

Comments_____

REPORT

Study number:…… **Date: ----------**

Child: _____

Height _____ cm

Weight _____ kg

Body fat: _____ kg _____ %

Lean weight: _____ kg _____ %

Blood Pressure: Systolic: _____ mm Hg

Diastolic: _____ mm Hg

Haemoglobin (Hb %): _____ g%

Fasting Blood sugar: ---------- mg/dl

Cognitive function: Score **…….**

Remarks: ----------

Comments and Advice:

_____

_____

_____

Signature: ----------

MYSORE PARTHENON STUDY

COGNITIVE FUNCTION TEST - 2011- 2012

Kaufman Assessment Battery for Children (Second Edition) – Indian Adaptation

SUMMARY SHEET

Study Number: _____ Follow-up Number: _____

Child's Name: _____

Child's Education Level: _____

Date of Birth: _____ Date of Testing: _____

Time of Testing: _____

Recorded by: _____

Test Name	Score
Atlantis	
Koh's Block Design-IQ	
Word Order	
Pattern Reasoning	
Verbal Fluency-First Names	
WISC-III-Coding B – Concentration Correct items	

☐ **Checked for Completeness by _____**

Study Number _____ **Child Name** _____

Atlantis

Item	Score			Response
1	0	1	2	CHAI (fish)
2	0	1	2	TRA (fish)
3	0	1	2	CHAI (fish)
4	0	1	2	TRA (fish)
5	0	1	2	DHO (fish)
6	0	1	2	DHO (fish)
7	0	1	2	TLE (fish)
8	0	1	2	CHAI (fish)
9	0	1	2	TRA (fish)
10	0	1	2	GIBA (plant)
11	0	1	2	TLE (fish)
Sum of 1-11 (max.22) STOP if 15 or less				
12	0	1	2	DHO (fish)
13	0	1	2	GIBA (plant)
14	0	1	2	CHAGE (plant)
15	0	1	2	CHAI (fish)
16	0	1	2	CHAI (fish)
17	0	1	2	CHAGE (plant)
18	0	1	2	TRA (fish)
19	0	1	2	MAJU (plant)
20	0	1	2	TLE (fish)
Sum of 1-20 (max.40) STOP if 24 or less				
21	0	1	2	TLE (fish)
22	0	1	2	KAYO (plant)
23	0	1	2	CHAI (fish)
24	0	1	2	MAJU (plant)
25	0	1	2	DHO (fish)
26	0	1	2	GIBA (plant)
27	0	1	2	PLATAKU (shell)
28	0	1	2	TLE (fish)
29	0	1	2	KAYO (plant)
Sum of 1-29 (max.58) STOP if 38 or less				

Item	Score			Response
30	0	1	2	CHAI (fish)
31	0	1	2	CHAGE (plant)
32	0	1	2	PLATAKU (shell)
33	0	1	2	TRA (fish)
34	0	1	2	GIBA (plant)
35	0	1	2	TAJAGI (shell)
36	0	1	2	no name (fish)
37	0	1	2	DHO (fish)
38	0	1	2	LINAYO (shell)
39	0	1	2	TRA (fish)
40	0	1	2	TAJAGI (shell)
41	0	1	2	MAJU (plant)
42	0	1	2	no name (fish)
Sum of 1-42 (max.84) STOP if 63 or less				
43	0	1	2	CHAGE (plant)
44	0	1	2	LINAYO (shell)
45	0	1	2	CHAI (fish)
46	0	1	2	WOKALA (shell)
47	0	1	2	KAYO (plant)
48	0	1	2	no name (shell)
49	0	1	2	GIBA (plant)
50	0	1	2	MAJU (plant)
51	0	1	2	WOKALA (shell)
52	0	1	2	TLE (fish)
53	0	1	2	PLATAKU (shell)
54	0	1	2	no name (plant)

Stopping Point:

Item	**11**	**20**	**29**	**42**	**54**

Cumulative Score: _____

→ Raw score (max.108): _____

Instructions: There is no time limit. **Discontinue** at Item 11, 20, 29, or 42 if cumulative score is below the specified value; add the scores of answered items to get the cumulative score. Raw score is the value corresponding to the cumulative score mentioned in the table in the manual.

Atlantis Score: _____

SCORING PAD FOR KOH'S BLOCK DESIGN TEST

Name: _____ Educational Level: _____

Father's Name: _____ Rural / Urban: _____

Date of Birth: _____ Age: _____ (years) _____ (months)

Place: _____Date: _____ Time _____

Design No.	Time (in seconds)	Movement	Behavioural clues	Remarks	Score point
A (practice)					
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					

Discussion:

Total Score Point =
Mental Age =
I.Q = M.A. × 100 =
C.A.

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4/230, KACHERI GHAT, AGRA – 282004 (U.P.) INDIA

Instructions: For **designs 1-4** time limit is **1minute**; for **designs 5-9** time limit is **3 minutes**; for **designs 10-17** time limit is **5 minutes**. Stop the test if there are 2 consecutive failures. Score for each design depends on time taken and number of movements. For scoring each design refer the table in the manual. Mental age is the value corresponding to the total score point mentioned in the table in the manual. Chronological age is calculated by subtracting the date of birth from the date of testing.

Koh's Block Design (IQ) Score: _____

Study Number _____ **Child Name** _____

Word Order

Discontinue : 3 consecutive scores of 0

Item	**Score**				Response
# 1	**…**	**…**	**2**	**Trial 1**	Flower
	0	**1**	**….**	**Trial 2**	
# 2	**…**	**…**	**2**	**Trial 1**	Glass
	0	**1**	**….**	**Trial 2**	
3	**0**	**1**			Book
# A					House – Glass
# 4	**…**	**…**	**2**	**Trial 1**	Flower - Glass
	0	**1**	**….**	**Trial 2**	
# 5	**…**	**…**	**2**	**Trial 1**	Bird - Book
	0	**1**	**….**	**Trial 2**	
6	**0**	**1**			Book – House
7	**0**	**1**			House - Bird – Flower
8	**0**	**1**			Flower - Glass – House
9	**0**	**1**			Glass – Book - Bird
10	**0**	**1**			Book – Bird – Flower – House
11	**0**	**1**			House – Flower - Book - Glass
12	**0**	**1**			Sun – Tree - Cat – Chair
13	**0**	**1**			Cat - Leg – Bus - Ball
14	**0**	**1**			Cat - Ball – Bus - Sun – Leg
15	**0**	**1**			Bus – Tree - Ball – Chair - Sun
16	**0**	**1**			Tree - Cat - Ball – Leg - Chair
17	**0**	**1**			Leg – Chair - Sun – Tree - Cat - Ball
18	**0**	**1**			Ball - Cat - Bus – Tree – Leg – Chair
# B					Leg - Bus
# C					Chair - Cat
19	**0**	**1**			Ball – Sun
20	**0**	**1**			Tree – Leg
21	**0**	**1**			Sun – Tree - Cat
22	**0**	**1**			Cat - Ball – Tree
23	**0**	**1**			Chair - Bus – Leg
24	**0**	**1**			Cat – Tree - Ball – Leg
25	**0**	**1**			Sun - Cat – Leg – Tree
26	**0**	**1**			Leg – Sun – Tree - Ball - Cat
27	**0**	**1**			Cat - Bus – Chair – Leg – Ball

Add the scores of answered items **Word Order Score: _____**

Study Number_____ **Child Name** _____

Pattern Reasoning

Discontinue: 4 scores of 0 in 5 consecutive items **Time limit:** No time limits

Item	Score		Response	
# S			A	
# 1	**0**	**1**	B	
# 2	**0**	**1**	C	
3	**0**	**1**	D	
4	**0**	**1**	D	
5	**0**	**1**	B	
6	**0**	**1**	D	
7	**0**	**1**	A	
8	**0**	**1**	C	
9	**0**	**1**	B	
10	**0**	**1**	B	
11	**0**	**1**	F	
12	**0**	**1**	C	
13	**0**	**1**	D	
14	**0**	**1**	B	
15	**0**	**1**	F	
16	**0**	**1**	A	
17	**0**	**1**	E	
18	**0**	**1**	C	
19	**0**	**1**	C	
20	**0**	**1**	E	
21	**0**	**1**	B	
22	**0**	**1**	B	
23	**0**	**1**	A	
24	**0**	**1**	B	
25	**0**	**1**	E	
26	**0**	**1**	B	
27	**0**	**1**	F	
28	**0**	**1**	E	
29	**0**	**1**	C	
30	**0**	**1**	F	
31	**0**	**1**	B	
32	**0**	**1**	A	
33	**0**	**1**	E	
34	**0**	**1**	C	
35	**0**	**1**	D	
36	**0**	**1**	A	

Add the scores of answered items **Pattern Reasoning Score: _____**

Study Number_____ **Child Name** _____

Verbal Fluency **-Answer sheet-**

First Names:

Time limit: 1 minute

1	16	31
2	17	32
3	18	33
4	19	34
5	20	35
6	21	36
7	22	37
8	23	38
9	24	39
10	25	40
11	26	41
12	27	42
13	28	43
14	29	44
15	30	45

Verbal Fluency - Total Number of First Names mentioned (excluding duplicates)	

☐ **Checked for Completeness by** _____

Study Number _____ **Child Name _____**

Concentration Test

Time limit: 2 minutes

-Answer sheet-

Recorder: _____

Date of Testing: _____

Number of correct items	
Number of in-correct items	

Coding B

☐ **Checked for Completeness by** _____

Coding B

Ages 8–16

SAMPLE ITEMS

2	1	4	6	3	5	2	1	3	4	2	1	3	1	2	3	1	4	2	6	3

1	2	5	1	3	1	5	4	2	7	4	6	9	2	5	8	4	7	6	1	8

7	5	4	8	6	9	4	3	1	8	2	9	7	6	2	5	8	7	3	6	4

5	9	4	1	6	8	9	3	7	5	1	4	9	1	5	8	7	6	9	7	8

2	4	8	3	5	6	7	1	9	4	3	6	2	7	9	3	5	6	7	4	5

2	7	8	1	3	9	2	6	8	4	1	3	2	6	4	9	3	8	5	1	8

Mysore Parthenon Study

DR. Bhatia's Battery of Performance Tests of Intelligence 2011-2012

Summary Sheet

Study Number: _____ **Follow-up Number**: _____

Mother's Name: _____

Date of rating: _____ **Time of Rating**: _____

Scored by: _____

Any particular emotional tendency noticed during the test: ☐ 0. No 1. Yes

If **Yes**, give details: _____

_____

Koh's Block Design Test Score: _____

Passalong Test Score: _____

Prorated Raw Score: _____

IQ: _____

☐ **Checked for Completeness by**_____

TEST 1- KOH'S BLOCK DESIGN TEST

Instructions:

1. Ask the subject to replicate the design shown on the card using 4 (designs 1-5), 9 (design 6 and 7) and 16 (design 8-10) coloured blocks.
2. Time limit: 2 minutes for each design for designs 1-5 and 3 minutes for each design for designs 6-10
3. Stop the test after 2 successive failures.
3. Scoring:

For designs 1-5: 2 marks for success within a minute; 1 mark for success between 1 and 2 minutes; 0 mark for failure or success after the time limit

For designs 6-10: 3 marks for success within a minute; 2 mark for success between 1 and 2 minutes (excluding 2 minutes); 1 mark for success between 2 and 3 minutes; 0 mark for failure or success after the time limit

Design No.	Time taken (in minutes & seconds)	Success or Failure	Score	Any remarks
1 (Practice)				
2.				
3.				
4.				
5				
6.				
7.				
8.				
9.				
10.				

Total Koh's block design test score: _____

TEST 2- PASSALONG TEST

Instructions:

1. Ask the subject to replicate the design shown on the card by moving (sliding and not lifting) the coloured blocks kept in the box. .
2. Time limit: 2 minutes for each design for designs 1-4 and 3 minutes for each design for designs 5-8
3. Stop the test after 2 successive failures.
3. Scoring:

For designs 1-4: 2 marks for success within a minute; 1 mark for success between 1 and 2 minutes; 0 mark for failure or success after the time limit

For designs 5-8: 3 marks for success within a minute; 2 mark for success between 1 and 2 minutes (excluding 2 minutes); 1 mark for success between 2 and 3 minutes; 0 mark for failure or success after the time limit

Design No.	Time taken (in minutes & seconds)	Success or Failure	Score	Any remarks
1 (Practice)				
2.				
3.				
4.				
5				
6.				
7.				
8.				

Total Passalong test score: _____

Prorated Raw Score = ----------
(Test1 score + Test 2 score)*2.5

IQ = ----------
(Score corresponding to the raw score mentioned in the manual (**Table 15 if literate or Table 16 if illiterate**))

Mysore Parthenon Study

Bradley's Early Adolescent Home Inventory Questionnaire 2011-2012

SUMMARY SHEET

Study Number: _____ Follow-up Number: _____

Date of Visit: _____ Visitor: _____

Child's name: _____

Informant: _____ 1. Mother 2. Father 3. Others

If other than parent, relationship to child _____

Family composition:

_____

(Persons living in household, including sex and age of children)

Children	Sex	Age
1		
2		
3		
4		
5		

Language Spoken: _____

Is mother employed? _____ 0. No 1. Yes

If yes, Type of work when employed? _____

Hrs/Wk_____

Is father employed? _____ 0. No 1. Yes

If yes, Type of work when employed? _____

Hrs/Wk_____

Is Child employed? _____ 0. No 1. Yes

If yes, Type of work when employed? _____

Hrs/Wk_____

Is child in school?_____ 0. No 1. Yes

If yes, Grade_____

Where?_____

Comments:

_____

_____

Subscale	Actual Score
1. Physical environment	
2. Learning materials	
3. Modeling	
4. Fostering self-sufficiency	
5. Regulatory activities	
6. Family companionship	
7. Acceptance	
TOTAL HOME ENVIRONMENT SCORE (Sum of 1+2+3+4+5+6+7)	

☐ **Checked for completeness by: _____**

Instructions:

1. Place **1** or **0** in the box alongside each item depending on whether the behaviour is observed during the visit or if the parent reports that the conditions or events are characteristic of the home environment.

2. Enter the total of each subscale and the total including all the subscales in the summary sheet.

1. PHYSICAL ENVIRONMENT	
1. Adolescent's room has at least 2 pictures or decorations appealing to an adolescent. **E**	
2. House or apartment has no potentially dangerous structural or health hazards. **O**	
3. Home has adequate/comfortable amount of living space per person. **O**	
4. Home and immediate surroundings are not overly noisy. **O**	
5. House or apartment is clean. **O**	
6. The interior of the house or apartment is not dark or perceptually monotonous. **O**	
7. Immediate external environment is aesthetically pleasing and contains no obvious health or safety hazards. **O**	
Total	
2. LEARNING MATERIALS	
8. Adolescent has access to materials for arts and crafts and/or collections. **E**	
9. Adolescent has library card or name on library list. **I**	
10. Adolescent has access to at least 20 developmentally appropriate books. **E**	
11. Home has at least 2 types of reference materials (eg. dictionary, encyclopaedia, CD).**E**	
12. Adolescent has access to a musical instrument. **E**	
13. Adolescent has access to desk or other suitable place for reading or studying. **E**	
14. Adolescent has access to home computer. **E**	
15. Adolescent has access to at least 2 appropriate board games. **E**	
16. Adolescent has access to at least 2 pieces of appropriate equipment for physical development or organized sports activities. **E**	
17. At least one full shelf of books is visible in the home. **E**	
Total	
3. MODELING	
18. Parent has read at least four books during past year. **I**	
19. Parent obtains and reads a newspaper daily or a weekly news magazine. **I**	
20. Parent regularly participates in religious activities (any religious affiliation). **I**	
21. Parent participates in an adolescent-oriented organization. **I**	
22. Parent has friends with whom s/he regularly interacts outside of work. **I**	
23. Parent regularly engages in fitness activities at least 2 days a week. **I**	
24. Parent has not lost temper with adolescent more than once during past week. **I**	
25. None of the adults in the home displays obvious signs of recent alcohol or non-prescriptive drug consumption **O**	

26. Parent uses complex sentence structure and some long words in conversing **O**	
27. Parent does not violate rules of common courtesy (ignoring visitors, derogatory comments, or hitting child) during the visit. **O**	
Total	
4. FOSTERING SELF-SUFFICIENCY	
28. Parent has discussed current events with adolescent during past 2 weeks. **I**	
29. Parent teaches adolescent basic cooking or cleaning skills. **I**	
30. Parent has taught adolescent how to deal with health and safety emergencies. **I**	
31. Parent has arranged for special instruction outside of school for adolescent. **I**	
32. Parent has assisted adolescent with home work and school assignments during past 2 weeks. **I**	
33. Parent has established rules about home work and checks to see if home work is completed. **I**	
Total	
5. REGULATORY ACTIVITIES	
34. Family has a TV, and it is used judiciously, not left on continuously. **I**	
35. Parent periodically discusses the hazards of alcohol and tobacco consumption (smoking, eating pan/supari/gutka) with adolescent. **I**	
36. Parent has provided guidance or advice to adolescent during the past year concerning responsible sexuality and physical hygiene. **I**	
37. Adolescent has weekly routine household responsibilities. **I**	
38. Family has a fairly regular and predictable daily schedule. **I**	
39. Parent requires adolescent to sleep at home on school nights. **I**	
40. When Parent is not available to adolescent at home, reasonable procedures have been established for check in with parents, or their designee, on weekends and after school. **I**	
41. Parent establishes rules for adolescent's behaviour with peers and asks questions to determine whether the rules are being followed. **I**	
42. Parent has had contact with at least 2 of the adolescent's friends in the last month. **I**	
43. Parent knows signs of usage of alcohol, smoking, eating pan/supari/gutka and remains alert to possible experimentation or abuse. **I**	
Total	
6. FAMILY COMPANIONSHIP	
44. Family member has arranged for adolescent to go to a scientific, historical, or art museum during the past year. **I**	
45. Family member has arranged for adolescent to attend some type of live musical or theatre performance during the past year. **I**	
46. Family member has arranged for adolescent to go on a trip of more than 50 miles from home during the past year. **I**	
47. Father regularly engages in outdoor activity with the adolescent at least once every two weeks. **I**	
48. Adolescent spends some time with father (or father figure) 4 day a week. **I**	
49. Adolescent eats at least one meal per day, on most days, with mother and father. **I**	
50. Family visits or receives visits from relatives or friends at least once a month. **I**	
51. Family member has taken adolescent to a live organized athletic or sporting event during the past year. **I**	
Total	

7. ACCEPTANCE	
52. Parent mentions a particular skill, strength, or accomplishment of adolescent during interview. **O**	
53. Parent shows some positive emotional response to praise of adolescent by visitor. **O**	
54. Parent does not ridicule or express hostility or refer to the adolescent in a derogatory manner during the visit. **O**	
55. Parent talks to adolescents during the visit (Beyond correction and introduction). **O**	
56. During the visit, when speaking of or to the child, the parent's voice conveys positive feeling. **O**	
57. Parent allows adolescent to have some privacy. **I**	
58. Parents encourages adolescent to contribute to the conversation during visit. **O**	
59. Parent responds appropriately and positively to adolescent's questions or comments during the visit. **O**	
60. Adolescent can have a disagreement with parent without harsh reprisals. **I**	
Total	

Standard of Living Index

Study Number:_____ **Follow-up Number:_____**

Child Name: _____ **Date: _____**

Informant: _____ **1. Mother 2. Father 3. Guardian 4. Others**

1) Family type:

1) Nuclear 2) Joint 3) Other ☐

2) Number of persons (specify): _____ Score

3) What is the main source of drinking water for members of your household?

1) Piped water
2) Hand pump
3) Well
4) Public tap/p. hand pump/p. well
5) River/stream
6) Tanker
7) Other

☐

4) What kind of toilet facility does your household have? ☐

1) Own flush toilet
2) Shared flush toilet
3) Public flush toilet
4) Own pit toilet/Latrine
5) Shared pit toilet/Latrine
6) Public pit toilet/Latrine
7) No facility/Bush/Field
8) Others

5) What is the main source of lighting for your household? ☐

1) Electricity
2) Kerosene
3) Oil
4) Gas
5) Other (specify): _____

6) How many rooms are there in your household?

Rooms No: _____

7) Do you have a separate room that is used as a kitchen? ☐

1) Yes 2) No

8) What type of fuel does your household mainly use for cooking? ☐

1) Electricity
2) Wood
3) Crop residues
4) Liquid petroleum gas
5) Biogas
6) Coal/Charcoal/Coke
7) Kerosene
8) Others specify):_____

9) Does this household own this house or any other house? ☐

1) Yes 2) No

10) Type of house (record observation) ☐

Roof _____
Walls _____
Floor _____

1) Pucca
2) Semi-pucca
3) Kachha

11) Does this household own any agriculture land? ☐

1) Yes (specify): _____ acres

2) No

12) Out of this how much is irrigated land? ☐

1) _____ acres 2) None

13) Does this household own any livestock? ☐

1) Yes (specify): _____ Number: _____

2) No

14) Does this household own any of the following? ☐

1) Mattress	1) Yes 2) No	2) Pressure cooker	1) Yes 2) No
3) Chair	1) Yes 2) No	4) Cot/Bed	1) Yes 2) No
5) Table	1) Yes 2) No	6) Clock/Watch	1) Yes 2) No
7) Electric fan	1) Yes 2) No	8) Bicycle	1) Yes 2) No
9) Radio/Transistor	1) Yes 2) No	10) Television (B&W)	1) Yes 2) No
11) Television (colour)	1) Yes 2) No	12) Moped/Scooter	1) Yes 2) No
13) Car/Jeep	1) Yes 2) No	14) Water pump	1) Yes 2) No
15) Bullock cart	1) Yes 2) No	16) Thresher	1) Yes 2) No
17) Tractor	1) Yes 2) No	18) Refrigerator	1) Yes 2) No
19) Telephone	1) Yes 2) No	20) Sewing machine	1) Yes 2) No

Total SLI Score ☐

Kuppuswamy Score

15) Education Level of Mother: _____ Score _____

16) Education Level of Father: _____ Score _____

17) Occupation of Main breadwinner Score _____

_____

18) Income of the head of the family Score _____

_____ Rs / Month

Total Kuppuswamy Score ☐

MRC No: _____ **Date**: _____

Dear Parents,

You and your child _____ are an integral part of our research project (effects of mother's glucose metabolism on the growth and development of children), and have been kindly participating in the routine growth follow-up for the past 13 years. As you are aware, we have examined your child frequently since birth, and have done detailed check-ups including blood tests at 5 and 9.5 years of age.

We are writing to invite you and ---------- to help us with a new study. This is to find out how a child's birth size and childhood growth influences his/her responses to everyday tasks in later childhood/ adolescence. As these responses affect health and wellbeing, we hope to identify important new ways to help parents and their children.

As part of this study, ---------- will be asked to tell a short story and do some maths (similar to school work). To measure his/her body's response to these tasks, we will check his/her pulse, blood pressure and hormone levels in his/her saliva. We will also collect ----------'s fasting blood sample the next day to measure blood sugar, cholesterol etc., and assess his brain function using school-work type of questions/ tasks. This will also help us to know whether his/her body's response to the task given is related to bodily and mental changes. For taking part in this study the child and an accompanying adult should stay in our research centre from approximately 10:30 in the morning till 5 in the evening. The child is also required to come to our centre the next day morning for approximately 2 hours.
We will provide lunch for the day of the stress test (day 1) and breakfast for the day of the blood test (day 2). We will refund your travel costs as usual.

If you think you might be able to help with the study please let us know. Our research team members will contact you to describe the study and give full details of the performance test. If you need more information on the study, please feel free to contact us.

We look forward to your kind co-operation.

With regards,

Dr. SR Veena **Dr. GV Krishnaveni**

MRC OFFICE

CHILDREN'S BLOCK, MISSION HOSPITAL, MYSORE
Phone No. – 2529347

MRC No: ———————— **Date**:————————————

Dear Parents,

You and your child _____ are an integral part of our research project (effects of mother's glucose metabolism on the growth and development of children), and have been kindly participating in the routine growth follow-up for the past 13 years. As you are aware, we have examined your child frequently since birth, and have done detailed check-ups including blood tests at 5 and 9.5 years of age.

We are writing now to invite you and ----------
to help us with another round of tests. This round of check-up includes:

1. Blood test for the child - We will take **ONLY ONE** blood sample after overnight fasting for measuring sugar level, Hb% (for anaemia), and some health indicators in the blood
2. Plasma and blood samples will be stored for future analysis
3. Mental function assessment using some playful activities
4. Routine body measurements, maturity level check-up and measurement of body fat
5. Blood Pressure (BP) measurements
6. Some questionnaires to understand your child's behaviour, moods etc.
7. Questionnaires and simple mental function activities for the mother

The total time of all these tests is **approximately 3 hours**.

Therefore, we request you to kindly bring the child on _____ at **8 AM** to the **MRC centre** at the **Mission Hospital**. To avoid useless results, please follow the instructions given below before coming.

- Make sure that your child has eaten properly for three days prior to the test day.
- Make sure that the child **DOES NOT eat or drink anything except water** after **9 PM** during the previous night.
- Make sure that the child **DOES NOT eat or drink anything except water** (**Not even coffee/tea/milk etc**) in the morning before coming to the hospital; i.e. the child should come to the hospital after **fasting overnight. We will provide breakfast after the blood test.**

The travel expenses will be refunded as in the previous years

NOTE: Blood taking procedure will be made **PAINLESS** for the child by applying a cream

We will assure you that all these tests are safe and not harmful to your child. **Kindly co-operate as in the previous years and make this project a success.**

Date of Clinic: ---------- **Time: ----------**

PLACE: **MRC OFFICE, CHILDREN'S BLOCK, MISSION HOSPITAL, MYSORE**

Phone: 0821 – 2529347

Thanks in advance for your co-operation

Dr. SR Veena **Dr. Krishnaveni GV**

APPENDIX 3- INTER OBSERVER VARIATION STUDIES

IOV for children's anthropometry

Table I Inter observer variation studies of children's anthropometry; 20 subjects and 5 observers

	Weight	Height	Sitting height	Head	MUAC	Waist	Hip	Subscapular	Triceps
Mean	37.8	151.9	119.8	51.2	20.5	64.5	71.1	12.1	10.7
Subject range	-12.05 24.98	-10.51 10.28	-5.58 6.47	-1.54 2.72	-4.05 8.14	-11.87 21.69	-10.39 18.66	-7.71 23.12	-6.34 14.13
Observer sum of square	0.012	1.535	0.581	0.191	0.407	2.332	2.148	11.539	0.703
Subject sum of square	6348.19	2607.17	805.72	105.88	833.24	6093.48	3992.99	5178.87	1852.15
% Variation due to Observer	0.0002	0.6	0.07	0.2	0.05	0.04	0.05	0.2	0.04
SD (Observer difference)	0.018	0.19	0.12	0.07	0.10	0.24	0.23	0.54	0.13
SD/Mean (%)	0.05	0.1	0.1	0.1	0.5	0.4	0.3	4.5	1.2
Observer range	-0.025 0.020	-0.310 0.235	-0.205 0.090	-0.075 0.085	-0.110 0.120	-0.340 0.260	-0.305 0.315	-0.795 0.695	-0.120 0.210
Ratio of SD between observer difference to SD between subject difference	0.003	0.05	0.06	0.09	0.05	0.04	0.05	0.10	0.04

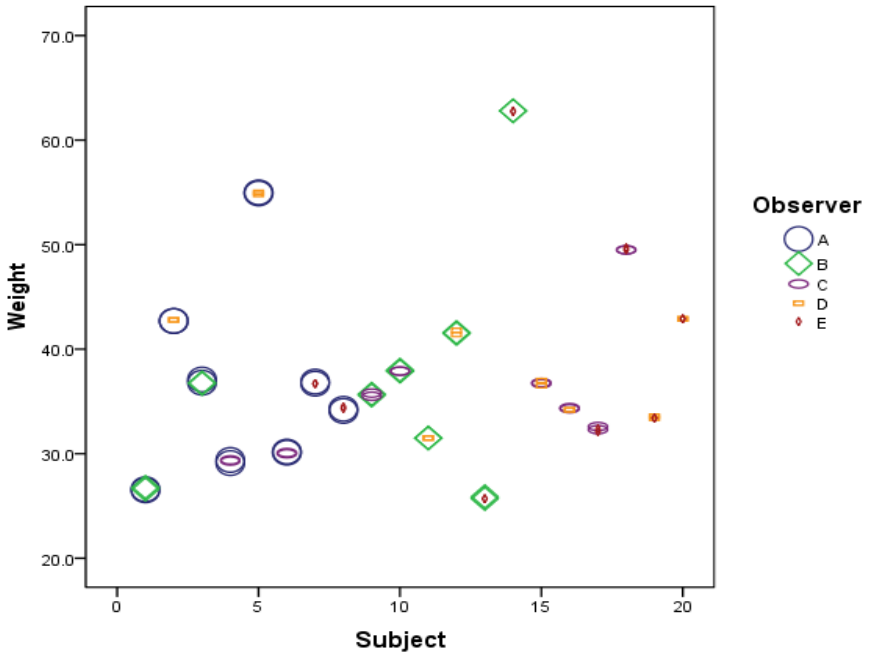

Figure I Plot of weight with subject by observer

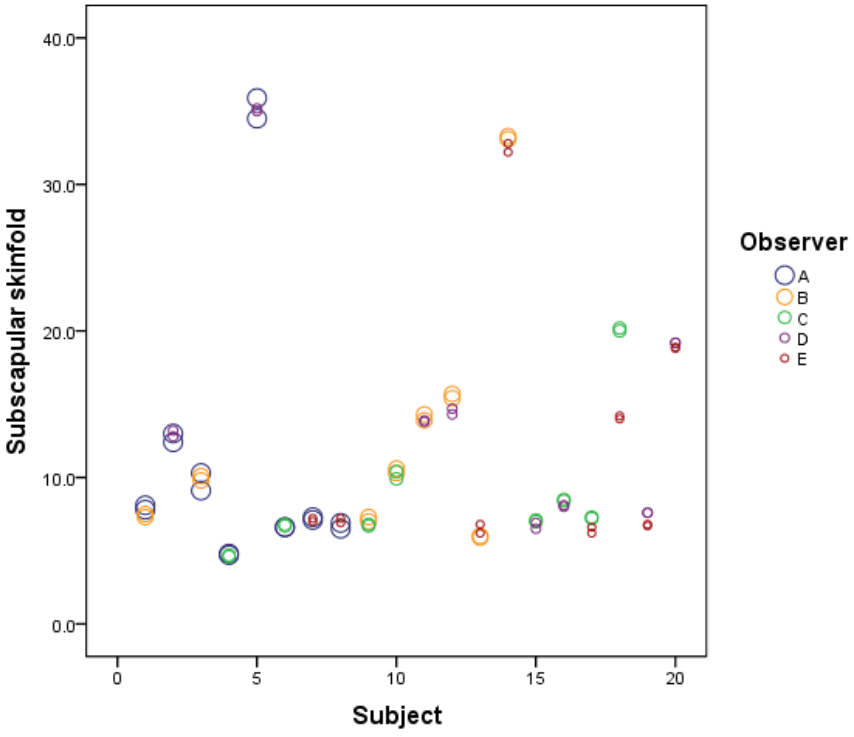

Figure II Plot of subscapular skinfold with subject by observer

IOV for children's cognitive function tests

Table II Inter observer variation studies of children's cognitive function tests; 30 subjects and 3 observers

	Atlantis	Koh's block design	Word order	Pattern reasoning	Verbal fluency	Coding-WISC
Mean	84.3	84.4	18.1	16.3	19.4	43.8
Subject range	-46.3 18.7	-33.8 48.1	-5.1 8.9	-12.3 14.7	-5.4 6.6	-19.8 26.2
Observer sum of square	0.000	0.007	0.000	0.000	7.467	0.600
Subject sum of square	18162.9	52956.2	1190.1	4410.9	1268.3	14440.4
% Variation due to Observer	0.0	0.0001	0.0	0.0	0.6	0.004
SD (Observer difference)	0.00	0.01	0.00	0.00	0.35	0.10
SD/Mean (%)	0.00	0.01	0.00	0.00	1.8	0.2
Observer range	-0.00 0.00	-0.12 0.009	-0.00 0.00	-0.00 0.00	-0.40 0.27	-0.10 0.10
Ratio of SD between observer difference to SD between subject difference	0.00	0.001	0.00	0.00	0.29	0.02

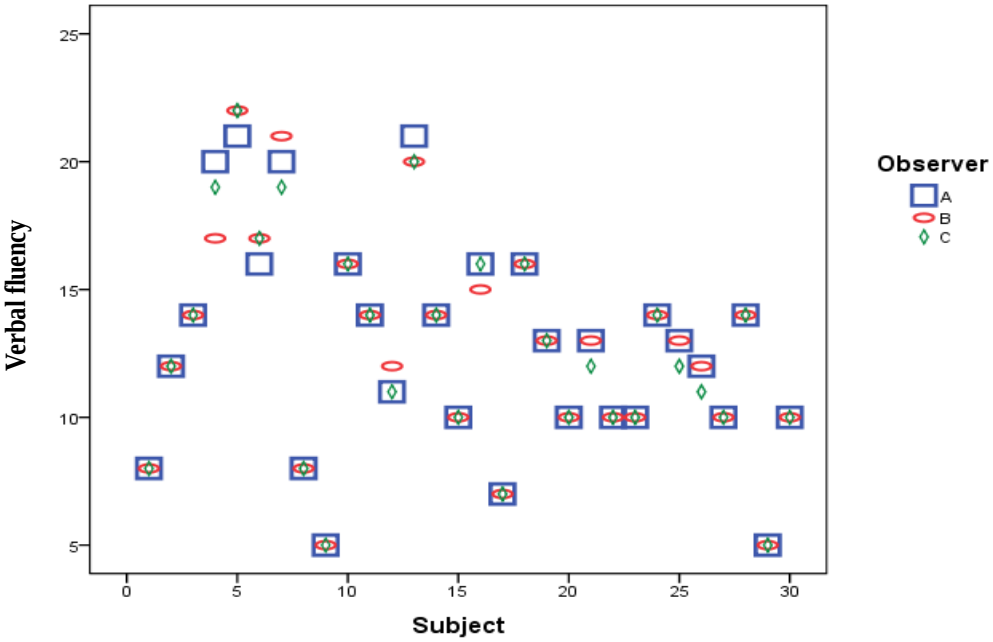

Figure III Plot of verbal fluency with subject by observer

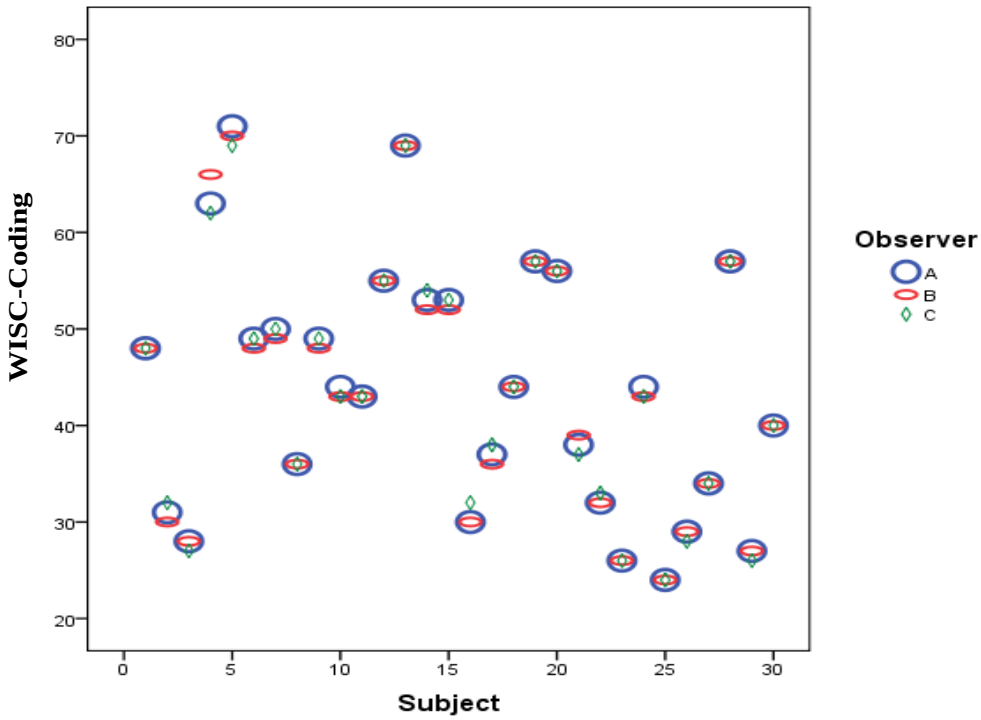

Figure IV Plot of WISC-Coding with subject by observer

APPENDIX 4-DESCRIPTION OF FISHER-YATES TRANSFORMATION

Fisher-Yates transformation is an alternative useful transformation to create normal scores. This process maps the variable to be transformed to one that is exactly normally distributed. It therefore loses all information about scale from the original variable, but preserves the ranking. The resulting variable will have a mean of 0 and a standard deviation of 1, so a unit change in the variable can be interpreted as a standard deviation change.

The following steps describes how a Fisher-Yates transformed variable is created

1. Rank function is used to generate ranks of the variable (x) to be transformed
   generate variable rankx=rank(x)
2. Generate a variable that indicates the maximum number of observations
   generate variable obs = max(rankx)
3. Generate a variable that indicates the maximum number of observations + 1
   generate maxobsplus1 = obs + 1
4. Generate a variable that describes how far through the distribution of each value is.
   generate percentile = rankx/maxobsplus1
5. Finally, create a variable using the inversenorm function, which maps from the percentile to the associated normal scores.
   gen zx= invnorm(percentile)
6. Drop all the extra variables (rankx, obs, maxobsplus1, percentile) created along the way.
7. Retain the variable zx (Fisher-Yates z-score) and check this variable has a mean of approximately 0 and a standard deviation of approximately 1.
8. This variable (zx) can now be used in analyses, such as an unpaired t-test, requiring a normally distributed variable

APPENDIX 5- BASELINE CHARACTERISTICS AND COGNITIVE SCORES OF PARTICIPANTS WITH AND WITHOUT MISSING DATA

Table 1IIa Comparison of socio-demographic factors, birth size, current body size and cognitive scores between study participants with and without maternal intelligence data during childhood

	Non-missing (n=508)	Missing (n=34)	
	Mean	**Mean**	**P**
Socio-demographic characteristics			
Standard of living index (score)	36.0	39.2	0.03
Maternal education (No (%))			
a) Illiterate	6 (1.1)	0 (0.0)	
b) Primary school education	180 (35.4)	8 (26.5)	
c) Secondary school education	162 (31.9)	5 (14.7)	0.002
d) Pre-university (undergraduates)	99 (19.5)	8 (23.5)	
e) Graduates/post-graduates/professionals	61 (12.0)	12 (35.3)	
Paternal education (No (%))			
a) Illiterate	18 (3.5)	0 (0.0)	
b) Primary school education	178 (35.0)	10 (29.4)	
c) Secondary school education	113 (22.0)	5 (14.7)	0.2
d) Pre-university (undergraduates)	89 (17.5)	6 (17.7)	
e) Graduates/post-graduates/professionals	111 (21.9)	13 (38.2)	
Occupation of the main breadwinner categories (No (%))			
a) Unskilled (eg. labourer, vegetable vendor)	106 (20.9)	5 (15.1)	
b) Semiskilled (eg. mechanic, construction worker)	102 (20.1)	4 (12.1)	
c) Skilled (eg. clerk, cashier)	240 (47.2)	15 (45.4)	0.04
d) Semi-professional (eg. teacher, manager)	45 (8.9)	5 (15.1)	
e) Professional (eg. doctor, engineer, advocate)	15 (2.9)	4 (12.1)	
Income of the main breadwinner categories (No (%)) †			
a) ≤Rs. 3000	142 (27.9)	8 (24.2)	
b) Rs. 3001-5000	114 (22.4)	4 (12.1)	
c) Rs. 5001-10000	91 (17.9)	6 (18.1)	0.4
d) Rs. 10001-15000	69 (13.6)	5 (15.2)	
e) > Rs. 15,000	92 (18.1)	10 (30.3)	
Area of residence (No (%)) a) Rural	141 (27.8)	7 (20.6)	0.4
b) Urban	367 (72.2)	27 (79.4)	
Home environment (Score)	44.4	39.8	0.1
Neonatal anthropometry			
Birthweight (g)	2865	2953	0.3
Crown-heel length (cm)	48.6	48.3	0.4
Head circumference (cm)	33.8	33.8	0.9
Mid-upper-arm circumference (cm)	10.3	10.4	0.4
Sum of skinfolds (mm)*	8.3	9.1	0.03
Current body composition			
BMI (kg/m^2)	14.6	15.1	0.07
Height (cm)	130.7	130.9	0.8
Head circumference (cm)	50.6	50.6	0.9
Mid-upper-arm circumference (cm)	18.0	18.6	0.07
Sum of skinfolds (mm)*	16.4	16.5	1.0
Cognitive function			
Learning, long-term retrieval/storage (Score)	67.8	66.2	0.6
Short-term memory (Score)	16.4	17.0	0.2
Reasoning ability (Score)*	10.0	12.0	0.01
Verbal fluency (Score)	16.1	16.6	0.5
Visuo-spatial ability (Score)*	75.9	86.7	0.048
Attention and concentration (Score)	32.6	34.6	0.2

*Skewed variable; values are medians; P value for the difference between study participants with and without maternal intelligence data derived using t-test or Chi2 test

†Income in Rupees per month; Rs.1000=£ 10

Table 1IIb Comparison of socio-demographic factors, birth size, current body size and cognitive scores between study participants with and without home environment data during childhood

	Non-missing (n=345)	Missing (n=197)	
	Mean	**Mean**	**P**
Socio-demographic characteristics			
Standard of living index (score)	36.4	35.9	0.5
Maternal education (No (%))			
a) Illiterate	1 (0.3)	5 (2.5)	
b) Primary school education	111 (32.2)	78 (39.6)	
c) Secondary school education	117 (33.9)	50 (25.4)	0.03
d) Pre-university (undergraduates)	68 (19.7)	39 (19.8)	
e) Graduates/post-graduates/professionals	48 (13.9)	25 (12.7)	
Paternal education (No (%))			
a) Illiterate	10 (2.9)	8 (4.1)	
b) Primary school education	123 (35.7)	65 (33.0)	
c) Secondary school education	77 (22.3)	40 (20.3)	0.7
d) Pre-university (undergraduates)	62 (17.9)	33 (16.8)	
e) Graduates/post-graduates/professionals	73 (21.2)	51 (25.9)	
Occupation of the main breadwinner categories (No (%))			
a) Unskilled (eg. labourer, vegetable vendor)	77 (22.3)	34 (17.4)	
b) Semiskilled (eg. mechanic, construction worker)	83 (24.1)	23 (11.7)	
c) Skilled (eg. clerk, cashier)	142 (41.2)	113 (57.7)	0.001
d) Semi-professional (eg. teacher, manager)	33 (9.6)	17 (8.7)	
e) Professional (eg. doctor, engineer, advocate)	10 (2.9)	9 (4.6)	
Income of the main breadwinner categories (No (%)) [†]			
a) ≤Rs. 3000	73 (21.2)	77 (39.3)	
b) Rs. 3001-5000	79 (22.9)	39 (19.9)	
c) Rs. 5001-10000	66 (19.1)	31 (15.8)	<0.001
d) Rs. 10001-15000	58 (16.8)	16 (8.2)	
e) > Rs. 15,000	69 (20.0)	23 (16.8)	
Area of residence (No (%)) a) Rural	31 (9.0)	117 (59.4)	<0.001
b) Urban	314 (91.0)	80 (40.6)	
Maternal intelligence (Score)	85.6	85.3	0.8
Neonatal anthropometry			
Birthweight (g)	2879	2857	0.6
Crown-heel length (cm)	48.6	48.7	0.6
Head circumference (cm)	33.8	33.7	0.8
Mid-upper-arm circumference (cm)	10.3	10.2	0.4
Sum of skinfolds (mm)*	8.3	8.4	0.7
Current body composition			
BMI (kg/m^2)	14.6	14.5	0.8
Height (cm)	130.8	130.4	0.4
Head circumference (cm)	50.7	50.5	0.2
Mid-upper-arm circumference (cm)	18.1	17.9	0.3
Sum of skinfolds (mm)*	16.9	15.9	0.1
Cognitive function			
Learning, long-term retrieval/storage (Score)	69.4	64.8	0.003
Short-term memory (Score)	16.5	16.3	0.4
Reasoning ability (Score)*	10.0	9.0	0.08
Verbal fluency (Score)	16.1	16.1	0.9
Visuo-spatial ability (Score)*	77.9	73.7	0.1
Attention and concentration (Score)	32.7	32.8	0.9

*Skewed variable; values are medians; P value for the difference between study participants with and without home environment data derived using t-test or Chi2 test

[†]Income in Rupees per month; Rs.1000=£ 10

APPENDIX 6- BASELINE CHARACTERISTICS OF STUDY PARTICIPANTS AND NON-PARTICIPANTS

Table 1Va Comparison of baseline characteristics between study participants and non- participants during childhood

Baseline characteristics		Study participants (n=542)	Non-participants (n=88)	P
Neonatal anthropometry		**Mean**	**Mean**	
Gestational age (weeks)		39.1	39.2	0.4
Birthweight (g)		2871	2875	0.9
Crown-heel length (cm)		48.6	48.8	0.5
Head circumference (cm)		33.8	33.7	0.4
Mid-upper-arm circumference (cm)		10.3	10.3	0.9
Triceps skinfold thickness (mm)*		4.1	4.3	0.9
Subscapular skinfold thickness (mm)*		4.3	4.3	0.5
Sum of skinfolds (mm)*		8.4	8.5	0.7
Sex (No (%))	Male	261 (48.1)	40 (45.4)	0.6
	Female	281 (51.9)	48 (54.6)	
Socio-demographic characteristics				
Parity (No (%))	a) 0	275 (50.7)	46 (52.3)	
	b) 1	178 (32.8)	35 (39.8)	0.1
	c) 2+	89 (16.4)	7 (8.0)	
Standard of living index (score)		14.8 (4.9)	17.1 (5.4)	0.0001
Maternal education (No (%))				
a) Illiterate		11 (2.03)	2 (2.3)	
b) Primary school education		112 (20.7)	12 (13.6)	
c) Secondary school education		220 (40.6)	26 (29.5)	0.02
d) Pre-university (undergraduates)		115 (21.2)	24 (27.3)	
e) Graduates/post-graduates/professionals		84 (15.5)	24 (27.3)	
Paternal education (No (%))				
a) Illiterate		13 (2.4)	2 (2.3)	
b) Primary school education		119 (22.0)	8 (9.1)	
c) Secondary school education		164 (30.3)	27 (30.7)	0.04
d) Pre-university (undergraduates)		93 (17.2)	15 17.1)	
e) Graduates/post-graduates/professionals		153 (28.2)	36 (40.8)	
Occupation of the main breadwinner categories (No (%))				
a) Unskilled (eg. labourer, vegetable vendor)		38 (7.0)	6 (6.8)	
b) Semiskilled (eg. mechanic, construction worker)		87 (16.1)	8 (9.1)	
c) Skilled (eg. clerk, cashier)		376 (69.4)	53 (60.2)	<0.001
d) Semi-professional (eg. teacher, manager)		35 (6.5)	17 (19.3)	
e) Professional (eg. doctor, engineer, advocate)		6 (1.1)	4 (4.6)	
Income of the main breadwinner categories (No (%)) †				
a) ≤Rs. 3000		73 (13.5)	7 (8.0)	
b) Rs. 3001-5000		147 (27.1)	19 (21.6)	
c) Rs. 5001-10000		181 (33.4)	25 (28.4)	0.04
d) Rs. 10001-15000		73 (13.5)	20 (22.7)	
e) > Rs. 15,000		68 (12.6)	17 (19.3)	
Area of residence (No (%))	a) Rural	148 (27.3)	19 (21.6)	0.3
	b) Urban	394 (72.7)	69 (78.4)	

*Skewed variable; values are medians; P value for the difference between study participants and non-participants derived using t-test or Chi2 test

†Income in Rupees per month; Rs.1000=£ 10

Table 1Vb Comparison of maternal characteristics in pregnancy between the mothers of study participants and non-participants during childhood

Maternal characteristics	Study participants (n=542)	Non-participants (n=88)	P
	Mean/No (%)	Mean/No (%)	
Age (years)	23.9	24.5	0.2
Religion (No (%))			
a) Hindu	312 (57.6)	43 (48.9)	
b) Muslim	185 (34.1)	38 (43.1)	0.2
c) Christian	45 (8.3)	7 (8.0)	
Anthropometry			
Weight (kg)*	55.0	57.0	0.06
Height (cm)	154.3	156.6	0.0003
Body mass index (kg/m^2)*	23.2	23.1	0.7
Normal (BMI 18.5-24.9 kg/m^2)	347 (64.0)	53 (60.2)	
Underweight (BMI <18.5 kg/m^2)	23 (4.2)	4 (4.6)	0.9
Overweight (BMI 25.0-29.9 kg/m^2)	144 (26.6)	25 (28.4)	
Obesity (BMI≥30.0 kg/m^2)	28 (5.1)	6 (6.8)	
Triceps skinfold (mm)*	17.0	18.4	0.1
Biceps skinfold (mm)*	8.7	9.7	0.2
Subscapular skinfold (mm)*	24.9	25.0	0.2
Suprailiac skinfold (mm)*	31.8	34.9	0.08
Sum of skinfolds (mm)*	83.3	87.9	0.1
Serum micronutrient concentrations			
Vitamin D (nmol/L)*†	38.9	39.9	0.5
Low vitamin D status (<50 nmol/L)			
0-No	155 (33.1)	24 (30.8)	0.7
1-Yes	313 (66.9)	54 (69.2)	
Vitamin B12 (pmol/L)*‡	162.5	179.0	0.1
Low vitamin B12 status (<150 pmol/L)			
0-No	308 (57.5)	56 (65.1)	0.2
1-Yes	228 (42.5)	54 (34.9)	
Folate (nmol/L) ‡	34.7	39.2	0.050
Low folate status (<7.0 nmol/L)			
0-No	514 (95.9)	83 (96.5)	0.8
1-Yes	22 (4.1)	3 (3.5)	
Homocysteine (µmol/L)*‡	6.01	6.01	0.3
Hyperhomocystenemia (>10.0 µmol/L)			
0-No	518 (96.6)	81 (94.2)	0.3
1-Yes	18 (3.4)	5 (5.8)	

*Skewed variable; values are medians (IQR); P value for the difference between study participants and non-participants derived using t-test or Chi2 test

†participants n=468; non-participants n=78; ‡participants n=536; non-participants n=86

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