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Associations of Early-life Nutrition with Motor, Cognitive, and Social-emotional Development
during Early and Middle Childhood in Ghanaian Children

By

KATHERINE YONG KWON
DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

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Dedication

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Abstract

Adequate nutrition during the first 1000 days of life is critical for infant survival and health. The early-life benefits of breastfeeding and nutrient adequacy, as indicated by dietary diversity (DD), on infant growth are well established. However, the long-term impacts of breastfeeding and diet diversity, particularly on developmental outcomes of children in low- and middle-income countries, are less clear. This dissertation aimed to fill this gap using data from the International Lipid-based Nutrient Supplements (iLiNS)-DYAD-Ghana randomized controlled trial, in which 1320 pregnant women received one of three nutrient supplements [iron folic acid (IFA) and placebo, multiple micronutrients (MMN), or small-quantity lipid-based nutrient supplements (SQ-LNS)] until 6 months postpartum. Dietary data were collected at multiple time points from birth to 18 months of age using 24-hr recalls and comprehensive child development assessments were conducted during the follow-up studies when children were 4-6 years and 9-11 years old using a battery of tests. This dissertation includes three secondary analyses that explore the associations between early-life nutrition and neurodevelopmental outcomes in middle and late childhood.

The first analysis (Chapter 2) examined the association of exclusive and predominant breastfeeding (EBF and PBF) with motor, cognitive and social-emotional outcomes. In this sample (n=1020), 61% were EBF and 77% were EBF or PBF ≥ 5 months. At 4-6 years, EBF ≥ 5 months was positively associated with cognitive factor z-score ($\beta=0.13$; 95% CI: 0.01, 0.26), head-to-toe inhibition z-score (0.04; 0.01, 0.07), and pre-academic skills z-score (0.18; 0.05, 0.32). At 9-11 years, EBF ≥ 5 months was associated with lower total difficulties as reported by parents (-0.55 points; -1.07, -0.04) and teachers (-0.95; -1.74, -0.16), as well as lower conduct

problems as reported by parents (-0.22; -0.40, - 0.04) and teachers (-0.34; -0.59, -0.09). There were no associations with social-emotional outcomes reported by parents at 4-6 years or by the child at 9-11 years of age.

The second analysis (Chapter 3) investigated the association of DD with motor, cognitive and social-emotional development in the IFA and MMN groups (n=737). With the dietary data collected at 6, 9, 12, 15, 18 months, a cumulative DD score (range: 0-40) was calculated based on the eight defined food groups of the World Health Organization (WHO) minimum dietary diversity indicator. Mean (SD) cumulative DD scores were 21.7 (3.9) and ranged from 9 to 32. DD was positively associated with language z-scores at 18 months ($\beta=0.11$; 95% CI: 0.04, 0.17; $p=0.002$) and 4-6 years (0.09; 0.02, 0.15; $p=0.006$). Higher DD score was also associated with lower hyperactivity/inattention scores as reported by parents at 4-6 years (-0.16; -0.32, 0.00; $p=0.048$) and 9-11 years (-0.20; -0.35, -0.05; $p=0.010$). No other associations were significant.

The third analysis (Chapter 4) examined the association of early-life anemia and iron status with motor, cognitive and social-emotional development in the IFA and MMN groups (n=657). Child hemoglobin (Hb) and zinc protoporphyrin (ZPP) were measured at 6 and 18 months of age. Mean $\pm$ SD Hb was 113.3 ± 9.9 g/L at 6 months of age and 112.0 ± 10.7 g/L at 18 months of age. Poorer iron status (higher ZPP) at 6 months and lower Hb at 18 months of age were associated with lower pre-academic skills at 4-6 years ($p<0.035$). An inverse-U shaped relationship was found between Hb at 18 months and intellect z-scores at 9-11 years ($p=0.045$). Poorer iron status was associated with more conduct problems as reported by parents at 9-11 years ($p<0.040$).

Overall, these findings suggest that early-life nutrition, including breastfeeding, dietary

diversity, and iron status, has lasting associations on cognitive and behavioral outcomes extending into preadolescence in this Ghanaian population. These results underscore the importance of optimal infant and young child feeding (IYCF) practices in the first 1000 days for healthy neurodevelopment, particularly in low- and middle-income countries where the burden of undernutrition is greatest.

Table of Contents

Acknowledgements.....	ii
Dedication.....	v
Abstract.....	vi
Table of Contents.....	vii
 Chapter 1. Literature Review.....	 1
1.1 Introduction.....	2
1.2 Nutrition and brain development.....	2
1.2.1 Breastfeeding.....	3
1.2.2 Complementary feeding.....	4
1.3 Early-life nutrition and child development.....	6
1.4 Summary and research gaps.....	7
1.5 References.....	10
 Chapter 2: A longitudinal analysis of breastfeeding and psychological development at 4-6 y and 9-11 y in Ghanaian children.....	 13
2.1 Abstract.....	14
2.2 Introduction.....	15
2.3 Methods.....	17
2.4 Results.....	23
2.5 Discussion.....	25
2.6 Conclusion.....	30
2.7 References.....	31
2.8 Tables and Figures.....	37
2.9 Supplementary Material.....	44

Chapter 3: A longitudinal analysis of early-life dietary diversity and developmental outcomes at 18 mo, 4-6 y and 9-11 y in Ghanaian children.....	46
3.1 Abstract.....	47
3.2 Introduction.....	48
3.3 Methods.....	49
3.4 Results.....	55
3.5 Discussion.....	57
3.6 Conclusion.....	62
3.7 References.....	63
3.8 Tables and Figures.....	68
3.9 Supplementary Materials.....	74
 Chapter 4: Associations of early-life anemia and iron status with developmental outcomes at 4-6 y and 9-11 y: a longitudinal study of Ghanaian preadolescents.....	75
4.1 Abstract.....	76
4.2 Introduction.....	77
4.3 Methods.....	78
4.4 Results.....	84
4.5 Discussion.....	88
4.6 Conclusion.....	91
4.7 References.....	92
4.8 Tables and Figures.....	96
4.9 Supplementary Materials.....	101

Chapter 1

Literature Review

1.1 Introduction

Early childhood development refers to the physical, cognitive, social-emotional, and linguistic development of children from before birth until age eight years (1). Many policies and programs supporting early childhood development focus on the first three years of life because this is a critical period of rapid growth and development of the brain. A child's experience during these early years establishes their foundations for health, learning and behavior later in life (2). To guide global efforts in supporting young children, the World Health Organization (WHO) and United Nations Children's Fund (UNICEF) developed the Nurturing Care Framework, which identifies five interrelated components essential for optimal child development: good health, adequate nutrition, responsive caregiving, security and safety, and opportunities for early learning (1). While all five elements are important, the emphasis placed on each will vary depending on context and available resources. In low- and middle-income countries (LMICs), where child undernutrition is highly prevalent (3), ensuring adequate nutrition during the early years is particularly essential to prevent nutrient deficiencies that can impair child growth and development. For this reason, many nutrition programs and initiatives targeting maternal and child health focus on the "first 1000 days," the period from conception to two years of life, as a critical window for intervention.

1.2 Nutrition and brain development

Adequate nutrition during the first 1000 days is critical not only for infant survival and health, but also for optimal brain development. During this window of rapid brain growth, key processes including neuronal cell migration, dendritic outgrowth, synaptic production, and myelination occur in an orchestrated sequence to establish the structural and functional

architecture of the brain (4). Many of these processes begin in utero and continue throughout the postnatal years and because these processes are interdependent, disruptions at any stage can have cascading consequences for later development (4).

Nutrition plays a critical role in supporting each of these processes and ensuring adequate intake of key nutrients during the first 1000 days is necessary for healthy brain development. Optimal infant and young child feeding (IYCF) practices, which encompasses breastfeeding and complementary feeding, are the primary means by which these nutritional needs are met in early life. The WHO recommends exclusive breastfeeding for the first six months of life, followed by the introduction of diverse complementary foods alongside continued breastfeeding for up to two years (5).

1.2.1 Breastfeeding

Human milk is a dynamic and nutritionally complete food source uniquely suited to meet the needs of the growing infant for the first six months of life. It contains a combination of macro- and micro-nutrients, including long-chain polyunsaturated fatty acids such as docosahexaenoic acid (DHA), which is critical for nervous system and cognitive development (6). It also contains bioactive compounds that support immune function and the establishment of a healthy gut microbiome (7-9). This balance of nutrients and bioactive compounds supports the rapid physical growth and brain development during the first months of life, and longer breastfeeding duration has been associated with better cognitive performance in childhood and a reduced risk of overweight and obesity later in life (10).

Beyond its nutritional composition, breastfeeding also confers important protective benefits. By replacing potentially contaminated water or foods, breastfeeding minimizes infants' exposure to pathogens, which is particularly consequential in low-resource settings where environmental enteric dysfunction (EED) is common. EED is a condition which adversely alters the small intestine structure and function and can lead to gut inflammation, nutrient malabsorption, growth faltering, and impaired neurodevelopment (11, 12). Breastfeeding has been identified as a potential intervention to reduce its prevalence and severity particularly in children living under unsanitary conditions (13).

The act of breastfeeding itself has a nurturing dimension as it is inherently a response to an infant's hunger cues. The skin-to-skin contact and the infant's suckling stimulate the release of the stress-regulating hormones oxytocin and prolactin, fostering mother-infant bonding and emotional regulation (14). The frequent and close physical interaction inherent to breastfeeding may also promote parent-child engagement in ways that support social-emotional development well beyond infancy.

1.2.2 Complementary feeding

After six months of age, children need to be introduced to diverse, nutrient-dense foods alongside continued breastfeeding to meet their growing nutritional requirements. During this complementary feeding period, children's metabolic needs are high yet their stomach capacity is limited, making it especially important to provide nutrient-dense foods, or foods with high amounts of nutrients per 100 kilocalories (15). Iron-rich foods are particularly critical, as infants' iron stores become depleted around six months of age and breast milk alone does not provide sufficient iron to meet increasing demands (15, 16). The iron requirement for infants ages 6 to 12

months is 11 mg/day, far exceeding the amount they receive from breastmilk (about 0.2-0.4 µg/mL) (17). Infants with inadequate iron intakes are at high risk of iron deficiency and iron-deficiency anemia, both of which can impair brain development through disrupted neurogenesis and myelination (18). Iron is also essential for hemoglobin synthesis and oxygen transportation to the brain, among other physiological functions (19). In addition to iron, adequate intake of other nutrients essential for healthy brain development, such as B-vitamins, zinc and essential fatty acids, is equally important during this period (20).

Diet quality during the complementary feeding period is commonly assessed using the minimum dietary diversity (MDD) indicator, which measures the proportion of children aged 6-23 months who consumed food from at least five of eight defined food groups in the previous day (21). These groups include: 1) breast milk; 2) grains, roots, and tubers; 3) legumes and nuts; 4) dairy products; 5) flesh foods; 6) eggs; 7) vitamin A-rich fruits and vegetables; 8) other fruits and vegetables. Dietary diversity is a proxy for nutrient adequacy, as children who consume more diverse diets are more likely to meet their requirements for critical micronutrients (22, 23). Despite this evidence, poor complementary feeding practices remain common and can lead to nutrient deficiencies and growth faltering that adversely affect child development (16, 24, 25). To highlight the need to improve early childhood diets, UNICEF has introduced the concept of “child food poverty,” which is measured using the UNICEF and WHO dietary diversity score (26). Depending on the number of food groups children are fed per day, they are categorized as living in severe child food poverty (0-2 food groups/day), moderate food poverty (3-4 food groups/day) or not living in child food poverty (≥ 5 food groups/day). Unlike traditional metrics of poverty, which focus on household level monetary poverty, child food poverty is a distinct metric reflecting the multiple dimensions of poverty and its effects on children.

While a commonly used indicator for nutrient adequacy, dietary diversity may also reflect responsive caregiving, a nurturing dimension of infant feeding. Complementary feeding involves regular caregiver-child interactions, and mealtimes in particular may expose infants to diverse vocabulary and narrative that supports language development (27, 28). Furthermore, when caregivers provide attentive and reciprocal responses to a child's hunger and satiety cues, this is a form of responsive parenting associated with healthy social-emotional development (29-31) and higher language scores in children in Ethiopia (32) and Kenya (33).

1.3 Early-life nutrition and child development

The preceding sections highlight the well-established role of breastfeeding and complementary feeding in supporting infant health and growth (2, 5). However, the long-term effects of these early-life nutrition practices on neurodevelopmental outcomes are less clear. Studies examining the relation between breastfeeding and later-life cognitive and behavioral development have had mixed results, possibly due to many confounding factors, such as maternal education level, socioeconomic status, and home stimulation (34). A WHO meta-analysis of 13 studies found that breastfeeding was associated with increased performance on intelligence tests, of 3.5 points on average, in childhood and adolescence (35). There was significant heterogeneity among the studies, however, and adjusting for maternal intelligence accounted for most of the heterogeneity. A recent review on the benefits of breastfeeding on child intelligence and executive function also found that breastfeeding likely has a positive effect on IQ in later childhood, but the magnitude is uncertain. There were only three studies examining breastfeeding and executive function and the results showed a negative association, positive correlation and no benefit (34). In addition to cognitive outcomes, the nurturing

dimension of breastfeeding may influence a child's social-emotional development, though this remains an area of ongoing investigation.

The association between dietary diversity during the complementary feeding period and neurodevelopmental outcomes has also been examined, though evidence remains limited and mixed. Focusing on specific nutrient-dense foods such as animal source foods, longitudinal studies in LMICs have generally found that animal source food consumption or protein and fat intake are positively associated with total development and cognition scores (36-39). Studies examining domain-specific outcomes found inconsistent results. Diverse diets were positively and negatively associated with fine motor and language scores but not other outcomes in Uganda (40) and Singapore (41).

The long-term neurodevelopmental consequences of iron deficiency and iron deficiency anemia have been more consistently demonstrated. Evidence suggests that iron deficiency and iron deficiency anemia during infancy are associated with a range of poorer outcomes, including lower IQ scores in childhood (42), more frequent grade repetition in preadolescence (43), and impaired executive functioning and memory in early adulthood (44). These findings underscore the importance of preventing iron deficiency during infancy and toddlerhood, not only for immediate health but also for long-term cognitive and behavioral development.

1.4 Summary and research gaps

The evidence reviewed above highlights the critical role of early-life nutrition in supporting neurodevelopmental outcomes, yet significant gaps remain, particularly in LMICs where the burden of undernutrition is greatest. While the short-term benefits of breastfeeding for infant health and survival are well established, studies examining its long-term associations with

cognitive and behavioral outcomes have been mixed and largely conducted in high-income settings. Similarly, although dietary diversity during the complementary feeding period has been associated with neurodevelopmental outcomes in some LMICs, most studies have focused on toddlerhood, with little evidence extending into later childhood or preadolescence. Finally, while anemia and iron deficiency during infancy have been linked to poorer cognitive and behavioral outcomes, few studies have examined this association specifically in geographical areas at highest risk of iron deficiency, such as in sub-Saharan Africa (45).

Ghana presents an important and underexamined context in which to address these gaps. In Ghana, breastfeeding prevalence is relatively high across socioeconomic groups (10), yet the long-term neurodevelopmental implications of early feeding practices remain poorly understood. The burden of poor diet quality, anemia, and iron deficiency is also high. An estimated 31% of Ghanaian children 6 to 59 months of age live in severe child food poverty and 43% in moderate child food poverty (26), while approximately 35% of children 12-23 months old in Ghana had anemia, 34% had iron deficiency, and 22% had iron deficiency anemia in 2017 (46).

To address these gaps, this dissertation uses data from the International Lipid-based Nutrient Supplements (iLiNS) trial, conducted from 2009-2014 in semiurban districts of the Eastern Region of Ghana, with follow-up studies assessing motor, cognitive, and social-emotional outcomes when children were 4-6 years and 9-11 years of age. The cohort is particularly well-suited to examine these research questions because dietary data including breastfeeding and complementary feeding practices were collected prospectively at multiple time points from birth to 18 months. In addition to diet and nutrition, child development is influenced by other factors including maternal education and mental health, household socio-economic

status, home stimulation and school quality, which were measured through early and middle childhood.

The three analyses of this dissertation aimed to address these research gaps about early-life nutrition and long-term neurodevelopmental outcomes using longitudinal data from the iLiNS-DYAD-Ghana trial and its follow up studies. In the first analysis, I examined the association of exclusive and predominant breastfeeding with motor, cognitive and social-emotional outcomes at 4-6 years of age and 9-11 years of age. In the second analysis, I investigated the relation between dietary diversity from 6-18 months of age and motor, cognitive and social-emotional outcomes at 18 months, 4-6 years and 9-11 years of age. In the third analysis, I examined associations between anemia and iron deficiency at 6 and 18 months of age and motor, cognitive and social-emotional outcomes at 4-6 year and 9-11 years of age. Collectively, these studies provide insight into the long-term neurodevelopmental impacts of early-life breastfeeding, dietary diversity, and iron status in a setting with a high burden of child undernutrition.

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Chapter 2

A longitudinal analysis of breastfeeding and psychological development
at 4-6 y and 9-11 y in Ghanaian children

2.1 Abstract

While the early-life benefits of breastfeeding are well established, the long-term impacts are less clear, particularly on children's psychological development. Using data from a longitudinal study in Ghana, we examined the associations of exclusive or predominant breastfeeding (EBF/PBF) durations with developmental outcomes at 4-6 y and 9-11 y of age. Maternal reports of breastfeeding were collected at birth and monthly through 6 mo. Motor, cognitive, and social-emotional outcomes were assessed using a battery of behavioral tests and surveys. Associations of EBF/PBF for ≥ 5 mo (yes/no) or EBF/PBF duration with developmental outcomes were examined using linear regression controlling for maternal, child, and household factors. In this cohort (n=1020 children), 61% were EBF and 77% were EBF or PBF ≥ 5 mo. At 4-6 y, EBF ≥ 5 mo was associated with higher cognitive factor scores ($\beta=0.13$; 95% CI: 0.01, 0.26), inhibitory control (0.04; 0.01, 0.07), and pre-academic skills (0.18; 0.05, 0.32). At 9-11 y, EBF ≥ 5 mo was associated with fewer total social-emotional problems as reported by parents (-0.55 points; -1.07, -0.04) and teachers (-0.95; -1.74, -0.16), as well as lower conduct problem scores as reported by parents (-0.22; -0.40, -0.04) and teachers (-0.34; -0.59, -0.09). Longer duration of PBF was associated with lower cognitive factor scores (-0.03; -0.05, 0.00) and lower literacy scores (-0.04; -0.07, -0.01) at 9-11 y. There were no associations with social-emotional outcomes reported by parents at 4-6 y or by child at 9-11 y of age. In a population with high breastfeeding rates, longer duration of EBF may positively influence cognitive and social-emotional development in later childhood.

2.2 Introduction

The World Health Organization (WHO) recommends exclusive breastfeeding (EBF) for an infant's first six months of life and continued breastfeeding (BF) for up to two years and beyond (World Health Organization [WHO], 2001). BF helps prevent infant mortality, infections, and malnutrition as human milk provides an essential combination of macro- and micronutrients in addition to various bioactive components (Brockway, Daniel, Reyes, Gauglitz, et al., 2024; Brockway, Daniel, Reyes, Granger, et al., 2024; Reyes et al., 2024). It promotes healthy brain development and helps protect children from developing obesity and other chronic diseases later in life (Pérez-Escamilla et al., 2023). Mothers also benefit from breastfeeding as it reduces their risk of developing ovarian and breast cancers, cardiovascular disease and type 2 diabetes (Victora et al., 2016).

While the substantial early-life benefits of BF are well established, the long-term impacts of BF, particularly on psychological development, are less clear. Studies examining the relation between BF and cognition in later life have had mixed results, possibly due to the many confounding factors, such as socioeconomic status and home stimulation, that could affect child development (McGowan & Bland, 2023). A meta-analysis of 13 studies published by the WHO found that BF was associated with increased performance on intelligence tests, of 3.5 points on average, in childhood and adolescence (Horta, 2013). There was significant heterogeneity among the studies, however, and adjusting for maternal intelligence accounted for 77.3% of the heterogeneity. A review on the benefits of BF on child intelligence, behavior and executive function also found that BF likely has a positive effect on IQ in later childhood, but the magnitude is uncertain; there were only three studies examining BF and executive function and

the results showed a negative association, positive correlation and no benefit (McGowan & Bland, 2023).

The majority of studies examining BF and development have been conducted in resource-rich settings, where mothers who breastfeed their children tend to have a higher education level, higher socioeconomic status, and provide a more stimulating home environment compared to mothers who do not breastfeed (Horta, 2013). Very few studies have investigated this relation in low and middle-income contexts where there tends to be a high prevalence of breastfeeding regardless of socioeconomic status (Victora et al., 2016). In a systematic review of studies examining breastfeeding and cognitive development and educational achievement in sub-Saharan Africa, Mohammed et al. (2022) found no significant associations. However, only four of the included 17 studies sufficiently adjusted for key confounders such as maternal education or family socioeconomic status and there was considerable variability in the measurements of cognitive development and analytic approaches across the studies. The authors concluded that there is a need for high-quality research on the educational benefits of breastfeeding in sub-Saharan Africa (Mohammed et al., 2022).

Our analysis will address this evidence gap by examining the associations of breastfeeding duration and exclusivity with motor, cognitive, and social-emotional outcomes in Ghanaian children at 4-6 y and 9-11 y of age. Given the extensive data collected during the original iLiNS-DYAD-Ghana trial and its follow-up studies, we were able to adjust for confounding factors including maternal education and mental health, socioeconomic status, home stimulation and school characteristics.

2.3 Methods

Study design and participants

This is a secondary data analysis study using data from the iLiNS DYAD-Ghana trial and its follow-up studies at 4-6 y and 9-11 y. The iLiNS DYAD-Ghana trial was a randomized controlled trial conducted in the semi-urban Yilo and Lower Manya Krobo districts of the Eastern Region of Ghana between 2009 and 2014. Details of the original trial design and methods ([clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00970866); NCT00970866) have been published previously (Adu-Afarwuah et al., 2016; Adu-Afarwuah et al., 2015). Briefly, 1320 women who were <20 weeks pregnant were randomized to receive one of three supplements: 1) iron and folic acid (IFA) supplements from enrollment until birth and then daily 200 mg/d calcium as a placebo from birth to 6 mo postpartum; 2) multiple micronutrient (MMN) supplements from enrollment to 6 mo postpartum; or 3) small-quantity lipid-based nutrient supplements (SQ-LNS) from enrollment to 6 mo postpartum and SQ-LNS for their children from 6 to 18 mo of age. For this analysis, mothers and children from all three groups were included because none of the infants received supplementation from 0 to 6 mo of age. Follow-up visits were conducted in 2016, when children were 4-6 y old, and 2020 to 2021, when children were 9-11 y old.

Ethical approvals for the original trial and follow-up studies were obtained from the Institutional Review Board of the University of California Davis, the Ghana Health Service Ethical Review Committee, the University of Ghana Noguchi Memorial Institute for Medical Research Institutional Review Board, and the Ethics Committee for the College of Basic and Applied Sciences of the University of Ghana.

Data collection

Maternal and household information, including maternal age, education, household asset index and food insecurity, were obtained using questionnaires at enrollment into the original iLiNS-DYAD-Ghana trial. The household asset index indicator was constructed using principal components analysis based on ownership of a set of assets and household characteristics (Vyas & Kumaranayake, 2006). Food insecurity was measured using the household food insecurity access scale (HFIAS), which captures perceptions and experiences of food insecurity (Coates et al., 2007). Early BF practices were first assessed around day 8 postpartum. At the 1 mo, 2 mo, 3 mo, 4 mo, 5 mo, and 6 mo time points, information about BF and complementary feeding practices was collected using a food frequency questionnaire. At each monthly visit, the mother or caregiver was asked about BF frequency and exclusivity (i.e., if the infant consumed any liquids or foods other than breast milk).

Developmental outcome assessments were conducted during the 4-6 y and 9-11 y follow-up studies by trained data collectors who were blinded to the treatment group. Detailed descriptions of these assessments have been published previously (Ocansey et al., 2019; Prado et al., 2023). In brief, fine motor function at 4-6 y was measured using the National Institutes of Health (NIH) Toolbox 9-hole pegboard test (Gershon et al., 2013). Cognition at 4-6 y was assessed using a battery of tests. Language ability was assessed using the Developmental Neuropsychological Assessment-II (NEPSY-II) body part naming and identification and comprehension of instructions subtests (Brooks et al., 2009). Visuospatial ability was measured using the block design test (Elliott et al., 1996; Wechsler, 2002). We assessed declarative memory using the paired-associate task (Baddeley et al., 1995). Executive function was assessed using the International Development and Early Learning Assessment (IDELA) head-toe subtest

for inhibitory control and visual search task for attentional control (Pisani, 2015). Pre-academic skills were measured using the pre-academic subscale of the Parent's Evaluation of Developmental Status (PEDS) pre-academic subscale (Brothers et al., 2008).

An additional battery of tools was used to assess cognition at 9-11 y. We measured general intellectual ability using a block design test based on the British Ability Scales II pattern construction subtest (Elliott et al., 1996), the Wechsler Primary and Preschool Scale of Intelligence block design subtest (Wechsler, 2002) and the NEPSY-II (Brooks et al., 2009). Literacy skills were assessed with a letter, sentence, and word recognition test (Alcock et al., 2000; Baddeley et al., 1995). We measured arithmetic ability through a test based on the Wechsler Intelligence Scale for Children (WISC) arithmetic task (Wechsler, 2002). Declarative memory, procedural memory, and executive function were assessed using the Rapid Assessment of Cognitive and Emotional Regulation (RACER) tasks (Ford et al., 2019). The RACER declarative memory task was developed based on the Paired-Associate Learning (PAL) paradigm. The RACER procedural memory task was developed based on the "Serial Reaction Time" (SRT) paradigm (Nissen & Bullemer, 1987). The RACER inhibitory control task was based on the "Simon Task," which is widely used to measure executive function (O'Leary & Barber, 1993).

Social-emotional development was assessed using the parent-reported Strengths and Difficulties Questionnaire (SDQ-P) at 4-6 y and 9-11 y, as well as teacher and child self-reports (SDQ-T, SDQ-C) at 9-11 y. The SDQ consists of five subscales: 1) emotional symptoms, 2) conduct problems, 3) hyperactivity/inattention, 4) peer relationship problems, and 5) prosocial behavior. We summed the scores from subscales 1-4 to generate a "total difficulties score" (Goodman & Goodman, 2009; Goodman, 1997).

Additionally, caregiving and stimulation available to children were assessed using locally adapted versions of the Family Care Indicator (FCI) (Kariger et al., 2012) at 18 mo and the Early Childhood and Middle Childhood versions of the Home Observation for the Measurement of the Environment (HOME) Inventory (Caldwell & Bradley, 2003) at the 4-6 y and 9-11 y follow-ups. We assessed maternal depression using the Edinburgh Postnatal Depression Scale (EPDS) at 6 mo postpartum (Cox et al., 1996) and the Center for Epidemiological Studies Depression Scale at the 9-11 y follow-up (Radloff, 1977). Maternal cognition was assessed using a mental rotation test and digit span test at the 4-6 y follow-up. Child pubertal development at 9-11 y of age was measured using the youth self-reported Petersen Pubertal Development Scale (Petersen et al., 1988).

Sample size and statistical analyses

A statistical analysis plan was posted to Open Science Framework (<https://osf.io/bmv9d/files/89feq>) before conducting analyses using Stata (version 18). We used data from children in all three groups (SQ-LNS, MMN, IFA). Among the children with 4-6 y and 9-11 y outcome data, 1020 had complete dietary data from 0 to 5 mo. This sample size provided 80% power to detect associations between BF and continuous outcomes with a strength of correlation of 0.09 or larger at a significance level of $p < 0.05$.

Our primary exposure variables were duration of EBF (continuous) and EBF ≥ 5 mo (binary). EBF was defined as the infant receiving no other liquids or foods other than breast milk (with the exception of medicines and vitamin and mineral supplements). Duration of EBF was defined as the first age at which the child reported not being EBF. Our secondary exposure

variables were duration of PBF and $\text{PBF} \geq 5$ mo, defined as the infant receiving breast milk as the predominant source of nourishment and allowing for water and water-based drinks.

We created the continuous EBF/PBF variables based on food frequency questionnaires administered at 8 days, 1 mo, 2 mo, 3 mo, 4 mo, 5 mo postpartum. Although we collected breastfeeding data when children were 6 mo of age, many were introduced to complementary foods by this time so we did not include this time point when constructing the exposure variables. At each time point, caregivers were asked whether the baby was breastfed and if the baby consumed any other foods or liquids the previous day. We used the first report of non-EBF as the child's age of cessation of EBF, which determined the child's duration of EBF. For missingness, we imputed data in the following ways. If the child stopped EBF prior to a missed visit, we assumed non-EBF at their missed visit. If they reported EBF both before and after the missed visit, then we assumed EBF during the missed visit. If they reported EBF before the missed visit and non-EBF after the missed visit, then we took the mean age between the two time points as their age of cessation. Once this age of cessation (or duration of EBF) was calculated, we created a dichotomous variable indicating children who were EBF for at least 5 mo. For the PBF variable, we followed the same logic as we did for EBF but allowed for water and water-based drinks in the variable definition.

Primary outcomes for this analysis were motor score at 4-6 y, cognitive factor score at 4-6 y and 9-11 y, and social-emotional total difficulties score at 4-6 y and 9-11 y. The cognitive factor score at 4-6 y was calculated using the principal-axis factoring method including the following outcome measures: language, visuospatial ability, declarative memory, executive, pre-academic skills. The cognitive factor score at 9-11 y was also calculated using the principal-axis factoring method including the following outcome measures: general intellectual, literacy,

arithmetic, declarative memory, procedural memory, and executive function. Secondary outcomes for this study included the 5 subscale scores of the SDQ-P (emotional symptoms, conduct problems, hyperactivity/inattention, peer relationship problems, prosocial behavior) at 4-6 y and 9-11 y and the SDQ-T and SDQ-C at 9-11 y, in addition to the previously mentioned cognitive scores at 4-6 y and 9-11 y.

For motor and cognitive outcome scores, we calculated the age-adjusted z-score as the standardized residual of age predicting the score. This z-score represents the deviation of the child's score in units of SD from the score expected for the child's age. For all social-emotional outcomes, we reported unadjusted mean scores, as the SDQ is often used as a screening tool for assessing psychosocial problems in children (Goodman & Goodman, 2009).

Linear regression models were used for all continuous outcomes. All models were minimally adjusted for child age at time of development assessment, child sex, and outcome assessment enumerator. In fully adjusted models, we included potential confounders including maternal education, maternal cognition, parity, gestational age, birth weight, household assets, food insecurity, HOME scores, school quality and child pubertal development scores if they were associated ($p < 0.1$) with the outcome in bivariate testing (Figure S1). For covariates that were highly correlated (e.g., $r > .50$) with each other and were associated with the outcome, we only included the covariate that was most strongly correlated with the outcome. For moderation testing, we included an additional interaction term of exposure with the pre-specified modifiers (i.e., maternal education, depression, cognition, household asset index, FCI score) in both the minimally adjusted and fully adjusted models.

2.4 Results

For the 4-6 y follow-up, we re-enrolled 1014 of the 1222 eligible children and obtained developmental data from 966 children. For the 9-11 y follow-up, we re-enrolled 979 of the 1217 eligible children and obtained developmental data from 966 children (Figure S2); 883 were assessed at both time points and 1063 were assessed at one or both time points. Of these 1063 children, 1020 had breastfeeding data up to 6 mo.

At enrollment, women were 26.8 ± 5.4 y old (mean $\pm$ SD) and had attained 7.7 ± 3.6 y of education; 32% were nulliparous (Table 1). Infant gestational age at delivery was 39.3 ± 1.8 wk and birthweight was 3.0 ± 0.4 kg. Durations of EBF and PBF were 5.3 ± 2.2 mo and 6.2 ± 2.1 mo, respectively. EBF ≥ 5 mo was reported by 61% of women and EBF or PBF ≥ 5 mo by 77% of women. Any BF was reported by all (100%) women at 6 mo and 98.8% at 12 mo. Children were on average 4.9 y old at the 4-6 y follow-up and 9.9 y old at the 9-11 y follow-up assessments.

Cognitive and motor outcomes

Results from fully adjusted linear regression models are shown in Tables 2 and 3. Children who were EBF ($p=0.033$) or PBF ($p=0.049$) ≥ 5 mo had higher cognitive factor z-scores at 4-6 y compared to those who were EBF or PBF < 5 mo (Figure 1). Longer duration of EBF ($p=0.021$) and EBF ≥ 5 mo ($p=0.044$) were positively associated with inhibitory control at 4-6 y of age (Table 2). Children who were EBF ≥ 5 mo had higher pre-academic skills ($p=0.006$) at 4-6 y of age compared to those who were EBF < 5 mo (Table 2). Dichotomous EBF and PBF measures were not associated with cognitive measures at 9-11 y of age. However, longer duration of PBF was associated with lower cognitive factor z-score ($p=0.047$) and lower literacy

($p=0.021$) (Table 3). No other associations of EBF or PBF with cognitive outcomes at 4-6 y or 9-11 y were statistically significant. EBF and PBF were not associated with motor outcomes at 4-6 y of age (Table 2).

Social-emotional outcomes

In fully adjusted models, EBF and PBF were not associated with any parent-reported social-emotional outcomes at 4-6 y of age (Table 2). However, at 9-11 y of age, EBF and PBF ≥ 5 mo were associated with better social-emotional outcomes as reported by both parents and teachers (Table 3). Children who were EBF or PBF ≥ 5 mo had fewer total difficulties, as reported by parents (EBF $p=0.033$; PBF $p=0.014$) and teachers (EBF $p=0.017$; PBF $p=0.006$), compared to those who were EBF or PBF < 5 mo (Figure 2). Longer duration of EBF was also associated with lower total difficulties scores as reported by parents ($p=0.029$) and teachers ($p=0.020$) (Table 3).

A similar pattern was seen with SDQ conduct problems at 9-11 y. Compared to those who were EBF or PBF < 5 mo, children who were EBF or PBF ≥ 5 mo had fewer conduct problems as reported by parents (EBF $p=0.017$; PBF $p=0.013$) and teachers (EBF $p=0.008$; PBF $p=0.003$) (Figure 3). Longer duration of EBF also predicted fewer parent ($p=0.009$) and teacher-reported ($p=0.005$) conduct problems (Table 3). Additionally, PBF ≥ 5 mo was associated with fewer teacher-reported emotional symptoms ($p=0.018$) (Table 3). EBF and PBF were not associated with any other SDQ-P or SDQ-T outcomes, nor with any child-reported (SDQ-C) outcomes at 9-11 y of age (Table 3).

We examined potential interactions with maternal education, maternal depression at 6 mo postpartum, maternal cognition, baseline household asset index, and FCI score at 18 mo. Of the 780 tests that were conducted, 30 (3.85%) were statistically significant (p for interaction < 0.05) with no consistent pattern.

2.5 Discussion

Our study provides evidence that longer duration and exclusivity of BF are associated with certain aspects of children's psychological development many years later. In this Ghanaian cohort of children with high BF rates during early life, EBF and PBF ≥ 5 mo were associated with better cognitive outcomes at 4-6 y of age and better social-emotional outcomes at 9-11 y of age compared to EBF/PBF for < 5 mo. Although longer duration of EBF was associated with better social-emotional outcomes at 9-11 y of age, as reported by both parents and teachers, longer duration of PBF was associated with worse cognitive outcomes at 9-11 y of age. EBF/PBF were not significantly associated with motor or social-emotional outcomes at 4-6 y of age or child-reported social-emotional outcomes at 9-11 y of age.

Overall, the body of literature examining associations of BF with developmental outcomes is mixed, with many studies showing no relation (Belfort et al., 2016; Fergusson et al., 1987; Horta, 2013; Lind et al., 2014; McGowan & Bland, 2023). This inconsistency may be due to the complex relations between BF and child development, which may be context specific. For example, in high-income countries, BF rates tend to correlate with household income or maternal education whereas in low- and middle-income countries, BF rates are relatively high across income or education levels (Horta, 2013; Victora et al., 2016). Additionally, in contexts with low

breastfeeding rates, breastfed children are often compared to never breastfed (or formula-fed) children, which could also lead to overall inconsistent findings.

Our observations that EBF and PBF are associated with better cognitive and social-emotional outcomes in later childhood are consistent with several longitudinal studies in the UK, Australia, and Spain (Heikkilä et al., 2011; Julvez et al., 2007; Oddy et al., 2010; Quigley et al., 2012). However, counter to this overall pattern, we also observed that duration of PBF was associated with lower cognitive and literacy scores at 9-11 y of age. One possible explanation is that BF may confer cognitive benefits detectable in early childhood that attenuate over time, while other factors such as school quality may have a stronger influence on cognitive development in preadolescence. Studies investigating associations of BF with developmental outcomes in low- and middle-income settings similar to that of our cohort have also shown mixed results. In Malawi, children who were EBF for 6 mo were less likely to be over-age for grade (i.e., they had better educational attainment) compared to those who were EBF for less than 3 mo (Mohammed et al., 2023). In a South African cohort, EBF for 6 mo was associated with fewer conduct disorders, but not with overall cognition or executive function outcomes, at 7-11 y of age (Rochat et al. 2016).

In this analysis, EBF and PBF for ≥ 5 mo were associated with fewer social-emotional difficulties and conduct problems at 9-11 y as reported by both parents and teachers. If BF was associated with better social-emotional outcomes by parent-report only, this could have been due to reporting bias from mothers who BF their children for longer durations and have a stronger attachment to their child (McGowan & Bland, 2023). However, these associations were consistent with teachers' reports of the same social-emotional outcomes, who presumably had no knowledge of the children's BF experiences as infants. Very few studies have examined the

relations between BF and social-emotional outcomes based on different reporters. A randomized controlled trial of a breastfeeding intervention in Belarus measured both parent and teacher reports of developmental outcomes and although the intervention significantly increased duration of BF, there was no intervention effect on mother or teacher-reported SDQ (Kramer, Aboud, et al., 2008; Kramer, Fombonne, et al., 2008).

When considering potential mechanisms underlying these associations, it is important to consider the nourishing, protective, and nurturing effects of BF on children. It is well established that optimal nutrition, particularly during the first 1000 days, is critical for healthy brain development (Prado & Dewey, 2014). Human milk is a rich source of nutrients and bioactive compounds essential for an infant's growth, immunity, and establishment of a healthy gut microbiota (Brockway, Daniel, Reyes, Gauglitz, et al., 2024; Brockway, Daniel, Reyes, Granger, et al., 2024; Reyes et al., 2024). It contains long-chain polyunsaturated fatty acids such as docosahexaenoic acid (DHA), which is critical for nervous system and cognitive development (Lauritzen et al., 2001). In our study, however, it is difficult to attribute the observed associations solely to the nutrients in human milk because the majority of non-EBF or PBF infants were also breastfed.

In addition to providing nourishment, EBF protects infants from potential harmful exposures to pathogens present in contaminated water or foods. Children living under unsanitary conditions have a high prevalence of environmental enteric dysfunction (EED), which alters the small intestine structure and function, and can ultimately result in gut inflammation and nutrient malabsorption (Owino et al., 2016). Given the significant disturbance in gastrointestinal function and often chronicity of pathogen exposure, EED during early-life can lead to growth failure, stunting, and impaired neurodevelopment (Gilmartin & Petri, 2015; Jiang et al., 2014;

Prendergast & Kelly, 2012). BF is considered a potential intervention to address EED in infants as it minimizes the risk of exposures to pathogens and has been associated with lower levels of gut inflammation (Moodley-Govender et al., 2015; Owino et al., 2016). In this study, which was conducted in a semi-urban area in Ghana, nearly all participants had access to potable water (Adu-Afarwuah et al., 2016), so they were likely at low risk of EED. Nonetheless, exclusivity of BF in this cohort may have prevented early exposure to any pathogens in contaminated complementary foods introduced earlier than 6 mo, thus mitigating potential adverse impacts on developmental outcomes.

Finally, the act of BF fosters mother-infant bonding and stress management through skin-to-skin contact and the infant's suckling from the breast, which releases the stress-regulating hormones oxytocin and prolactin (Uvnäs Moberg et al., 2020). Among children who are EBF or PBF, there may be a greater frequency of parent-child interaction, which may influence social-emotional development. Although the group-wise differences in the SDQ total difficulties and conduct problems scores were small and may not have been clinically meaningful, it is still notable that there were significant associations between EBF ≥ 5 mo, a distal predictor, and these outcomes at 9-11 y of age. Mother-infant interactions, or the nurturing dimension of BF, during the first months of life may have a long-lasting influence on a child's social-emotional development, even into preadolescence.

For all of the associations in this analysis, we examined interactions with maternal education, maternal depression at 6 mo postpartum, maternal cognition, household asset index, and FCI score at 18 mo, as these variables could modify the relation between BF practices and child development (Horta, 2013). Of the 780 interaction tests that were conducted, only 30 were

statistically significant and, among those, there was no discernible pattern. Thus, these results are likely due to chance.

A major strength of our study was the prospective collection of BF data from our participants at birth and at each month until infants were 6 mo of age. Questions about BF practices are often asked cross-sectionally with short-term recall (e.g. “did you breastfeed your baby yesterday?”) or retrospectively (e.g., “How long did you breastfeed your baby?”) and are prone to recall and social desirability bias (Stewart et al., 2025). By constructing our BF exposure variables using prospectively collected data from six different time points, we attempted to mitigate the degree of recall bias in this analysis. Another strength was the multi-method assessment of developmental outcomes, with behavioral tests and parent reports used for cognitive assessment at 4-6 y and with social-emotional outcomes at 9-11 y assessed from multiple reporters. Having teacher reports of children’s conduct problems and total difficulties in agreement with parent reports provides additional validation to the findings. Finally, given the extensive amount of maternal, child and household data collected during the original iLiNS-DYAD trial and the follow-up studies in Ghana, we were able to control for numerous potentially confounding factors, such as maternal education, maternal cognition, HOME scores, and household asset index. However, our ability to detect differences between EBF/PBF groups may have been limited by very high BF rates in our cohort. Formula feeding was uncommon and the EBF/PBF <5 mo group was still breastfed, which may have limited our ability to detect differences between groups. This may also limit the generalizability of our findings in populations with a more diverse range of infant feeding practices.

2.6 Conclusion

The practice of BF is a physiologically and emotionally complex process that shapes and influences the mother-infant relationship. In our study, exclusivity and longer durations of EBF were generally associated with better cognitive outcomes at preschool age and better social-emotional development at preadolescence in this Ghanaian cohort. Examining this relationship in other settings could provide context-specific insights about BF and child development. Further investigation into potential mechanisms that could explain these findings is also warranted. This would contribute valuable information for public health programs that promote and support BF, particularly in low- and middle-income settings.

2.7 References

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2.8 Tables and Figures

Table 2.1: Characteristics among participants included in the analysis (n=1020)

	Mean (SD)
Maternal characteristics	
Age at enrollment (y)	26.8 (5.4)
Height (cm)	159.0 (5.7)
Pre-pregnancy BMI (kg/m ²)	24.6 (4.5)
Education (y)	7.7 (3.6)
Nulliparous (%)	32
Depression score at 6 mo	5.3 (4.7)
Married or co-habiting (%)	93
Child characteristics	
Gestational age at delivery (wk)	39.3 (1.8)
Birthweight (kg)	3.0 (0.4)
Male (%)	49
Household characteristics at 6 mo	
Household asset index	0.0 (1)
Food insecurity score	2.5 (4.1)
Breastfeeding indicators	
EBF $\geq$ 5 mo (%)	61
Duration of EBF (mo)	5.3 (2.2)
EBF or PBF $\geq$ 5 mo (%)	77
Duration of PBF (mo)	6.2 (2.1)
Any BF at 6 mo (%)	100
Any BF at 12 mo (%)	98.8
Follow-up measurements	
Child age at 4-6 y follow-up (y)	4.9 (0.6)
Child age at 9-11 y follow-up (y)	9.9 (0.5)
FCI score at 18 mo	8.3 (2.7)
EC-HOME score at 4-6 y follow-up	28.0 (4.4)
MC-HOME score at 9-11 y follow-up	35.2 (5.9)

BF: breastfeeding; EBF: exclusive breastfeeding; PBF: predominant breastfeeding; FCI: Family Care Indicators; EC-HOME: Early Childhood Home Observation for the Measurement of the Environment Inventory; MC-HOME: Middle Childhood Home Observation for the Measurement of the Environment Inventory.

Table 2.2: Associations of exclusive and predominant breastfeeding with developmental outcomes at 4-6 y

	Exclusively breastfed				Predominantly breastfed			
	≥5 mo (N=568)	<5 mo (N=362)	≥5 mo vs. <5 mo	Duration (per mo)	≥5 mo (N=706)	<5 mo (N=211)	≥5 mo vs. <5 mo	Duration (per mo)
	Mean (SD)		β (95% CI) †		Mean (SD)		β (95% CI) †	
Motor z-score	-0.04 (0.91)	0.05 (0.88)	-0.09 (-0.21, 0.04)	-0.01 (-0.03, 0.02)	-0.04 (0.89)	0.10 (0.95)	-0.08 (-0.22, 0.07)	-0.01 (-0.04, 0.02)
Cognitive outcomes								
Cognitive factor z-score	0.07 (0.90)	-0.07 (0.90)	0.13 (0.01, 0.26) ^a	0.01 (-0.02, 0.04)	0.06 (0.89)	-0.11 (0.92)	0.14 (0.00, 0.29) ^a	-0.01 (-0.04, 0.02)
Language	0.03 (0.75)	-0.02 (0.79)	0.06 (-0.05, 0.17)	0.00 (-0.03, 0.02)	0.04 (0.76)	-0.08 (0.78)	0.12 (-0.01, 0.24)	-0.01 (-0.03, 0.02)
Visuospatial ability	-0.02 (0.57)	0.02 (0.56)	-0.04 (-0.11, 0.04)	0.00 (-0.02, 0.01)	-0.02 (0.55)	0.04 (0.58)	-0.06 (-0.15, 0.03)	0.00 (-0.02, 0.02)
Declarative memory	0.04 (0.90)	-0.05 (0.91)	0.05 (-0.08, 0.17)	0.01 (-0.02, 0.04)	0.04 (0.91)	-0.08 (0.92)	0.10 (-0.05, 0.24)	0.01 (-0.02, 0.04)
Visual search test*	-0.06 (0.86)	0.03 (0.85)	-0.08 (-0.20, 0.04)	0.00 (-0.03, 0.02)	-0.07 (0.82)	0.11 (0.92)	-0.11 (-0.25, 0.04)	0.00 (-0.03, 0.03)
Head-to-toe inhibition*	0.07 (1.02)	-0.09 (0.97)	0.15 (0.00, 0.30) ^a	0.04 (0.01, 0.07) ^a	0.04 (1.01)	-0.10 (0.97)	0.13 (-0.04, 0.30)	0.02 (-0.02, 0.05)
Pre-academic skills	0.09 (0.97)	-0.11 (1.01)	0.18 (0.05, 0.32) ^a	0.01 (-0.02, 0.04)	0.05 (0.97)	-0.09 (1.04)	0.04 (-0.11, 0.20)	-0.02 (-0.05, 0.01)
Social-emotional outcomes (SDQ-P)								
Total difficulties	13.32 (4.36)	13.71 (4.47)	-0.28 (-0.82, 0.27)	-0.06 (-0.19, 0.06)	13.43 (4.31)	13.66 (4.68)	-0.27 (-0.90, 0.37)	-0.02 (-0.15, 0.11)
Emotional symptoms	2.99 (1.95)	3.18 (2.15)	-0.08 (-0.31, 0.15)	0.00 (-0.05, 0.05)	3.05 (1.97)	3.09 (2.23)	-0.01 (-0.28, 0.25)	0.02 (-0.04, 0.07)
Conduct problems	2.74 (1.63)	2.81 (1.78)	-0.05 (-0.26, 0.17)	-0.03 (-0.07, 0.02)	2.76 (1.62)	2.84 (1.90)	-0.08 (-0.34, 0.17)	-0.02 (-0.07, 0.03)
Hyperactivity/ inattention	5.26 (1.82)	5.27 (1.74)	0.05 (-0.21, 0.30)	0.01 (-0.05, 0.07)	5.25 (1.80)	5.34 (1.75)	0.08 (-0.22, 0.37)	0.03 (-0.03, 0.09)
Peer relationship problems	2.33 (1.55)	2.44 (1.49)	-0.15 (-0.34, 0.04)	-0.03 (-0.08, 0.01)	2.37 (1.54)	2.40 (1.43)	-0.09 (-0.31, 0.13)	-0.02 (-0.06, 0.03)

Prosocial behavior	7.92 (1.86)	7.80 (1.93)	0.09 (-0.16, 0.34)	0.04 (-0.01, 0.10)	7.88 (1.87)	7.86 (1.94)	0.00 (-0.30, 0.29)	0.05 (-0.01, 0.11)
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^ap -value < 0.05

*For visual search test and head-to-toe inhibition outcomes: EBF $\geq$ 5 mo (n=517), EBF < 5mo (n=333), PBF $\geq$ 5 mo (n=645), PBF < 5mo (n=193).

†Estimates were obtained from linear regression models of EBF/PBF predicting indicated development outcome and adjusted for child age at assessment, child sex, outcome enumerator, maternal age, maternal education, maternal cognition, parity, gestational age, birth weight, intervention group, household asset index, household food insecurity, FCI score.

Table 2.3: Associations of exclusive and predominant breastfeeding with developmental outcomes at 9-11 y

	Exclusively breastfed				Predominantly breastfed			
	≥5 mo (N=566)	<5 mo (N=366)	≥5 mo vs. <5 mo	Duration (per mo)	≥5 mo (N=703)	<5 mo (N=215)	≥5 mo vs. <5 mo	Duration (per mo)
	Mean (SD)		β (95% CI) [†]		Mean (SD)		β (95% CI) [†]	
Cognitive outcomes								
Cognitive factor z-score	0.03 (0.78)	-0.04 (0.80)	0.03 (-0.08, 0.14)	-0.01 (-0.03, 0.02)	0.02 (0.77)	-0.04 (0.83)	-0.02 (-0.15, 0.10)	-0.03 (-0.05, 0.00) ^a
Intellect	0.02 (1.00)	-0.02 (0.99)	0.02 (-0.12, 0.16)	0.00 (-0.04, 0.03)	0.01 (0.99)	-0.03 (1.02)	-0.04 (-0.20, 0.13)	-0.03 (-0.06, 0.00)
Literacy	0.05 (1.00)	-0.05 (0.95)	0.05 (-0.08, 0.19)	-0.01 (-0.05, 0.02)	0.03 (0.99)	-0.04 (0.93)	-0.05 (-0.21, 0.11)	-0.04 (-0.07, -0.01) ^a
Arithmetic	0.04 (0.96)	-0.04 (1.05)	0.02 (-0.12, 0.16)	0.00 (-0.04, 0.03)	0.03 (0.96)	-0.05 (1.09)	0.01 (-0.16, 0.17)	-0.02 (-0.05, 0.01)
Declarative memory*	-0.02 (1.00)	0.02 (1.01)	-0.03 (-0.21, 0.14)	0.01 (-0.03, 0.05)	-0.01 (1.01)	-0.01 (1.00)	-0.06 (-0.26, 0.15)	0.01 (-0.03, 0.05)
Procedural memory	0.00 (0.99)	0.01 (1.02)	-0.01 (-0.15, 0.12)	0.00 (-0.03, 0.03)	0.00 (1.00)	-0.03 (1.03)	0.02 (-0.14, 0.17)	0.00 (-0.03, 0.03)
Executive function	0.03 (0.90)	-0.04 (0.99)	0.07 (-0.05, 0.19)	0.00 (-0.03, 0.03)	0.01 (0.93)	-0.06 (0.97)	0.07 (-0.07, 0.21)	0.00 (-0.03, 0.03)
Social-emotional outcomes (SDQ-P)								
Total difficulties	7.13 (3.86)	7.71 (4.20)	-0.56 (-1.07, -0.05) ^a	-0.13 (-0.24, -0.01) ^a	7.23 (3.95)	7.89 (4.19)	-0.75 (-1.35, -0.16) ^a	-0.12 (-0.24, 0.00)
Emotional symptoms	1.69 (1.44)	1.82 (1.41)	-0.12 (-0.30, 0.05)	-0.03 (-0.07, 0.01)	1.75 (1.44)	1.74 (1.42)	0.00 (-0.20, 0.21)	0.00 (-0.04, 0.04)
Conduct problems	1.41 (1.29)	1.61 (1.44)	-0.22 (-0.41, -0.04) ^a	-0.05 (-0.10, -0.01) ^a	1.45 (1.30)	1.67 (1.49)	-0.27 (-0.49, -0.06) ^a	-0.05 (-0.09, 0.00) ^a
Hyperactivity/inattention	2.69 (1.90)	2.85 (2.08)	-0.08 (-0.35, 0.18)	-0.02 (-0.08, 0.04)	2.69 (1.95)	2.96 (2.06)	-0.21 (-0.52, 0.10)	-0.05 (-0.11, 0.01)
Peer relationship problems	1.35 (1.22)	1.41 (1.22)	-0.03 (-0.19, 0.13)	0.00 (-0.04, 0.03)	1.34 (1.21)	1.49 (1.23)	-0.15 (-0.34, 0.03)	-0.01 (-0.04, 0.03)
Prosocial behavior	8.67 (1.48)	8.51 (1.65)	0.15 (-0.06, 0.36)	0.04 (-0.01, 0.08)	8.61 (1.53)	8.56 (1.64)	0.07 (-0.18, 0.31)	0.01 (-0.04, 0.06)

Social-emotional outcomes (SDQ-T)								
Total difficulties	8.72 (5.21)	9.63 (5.62)	-0.96 (-1.75, -0.17) ^a	-0.21 (-0.38, -0.03) ^a	8.75 (5.30)	10.25 (5.54)	-1.29 (-2.22, -0.36) ^a	-0.15 (-0.33, 0.04)
Emotional symptoms	2.24 (2.10)	2.42 (2.14)	-0.24 (-0.54, 0.07)	-0.04 (-0.10, 0.03)	2.23 (2.11)	2.65 (2.14)	-0.43 (-0.79, -0.07) ^a	-0.07 (-0.14, 0.00)
Conduct problems	1.54 (1.80)	1.90 (1.95)	-0.34 (-0.59, -0.09) ^a	-0.08 (-0.14, -0.02) ^a	1.58 (1.83)	2.06 (1.99)	-0.44 (-0.74, -0.15) ^a	-0.06 (-0.11, 0.00)
Hyperactivity/inattention	3.21 (2.25)	3.47 (2.28)	-0.21 (-0.54, 0.13)	-0.05 (-0.13, 0.02)	3.21 (2.26)	3.64 (2.27)	-0.31 (-0.71, -0.08)	-0.02 (-0.10, 0.06)
Peer relationship problems	1.73 (1.41)	1.85 (1.51)	-0.12 (-0.31, 0.08)	-0.02 (-0.07, 0.02)	1.75 (1.42)	1.89 (1.53)	-0.16 (-0.39, 0.07)	-0.01 (-0.06, 0.03)
Prosocial behavior	7.77 (1.93)	7.75 (2.02)	0.07 (-0.21, 0.34)	0.02 (-0.04, 0.08)	7.80 (1.94)	7.66 (2.06)	0.19 (-0.14, 0.51)	0.04 (-0.02, 0.11)
Social-emotional outcomes (SDQ-C)								
Total difficulties	11.68 (4.20)	11.58 (3.89)	0.10 (-0.43, 0.64)	0.08 (-0.04, 0.20)	11.61 (4.10)	11.68 (3.97)	0.00 (-0.62, 0.62)	0.09 (-0.03, 0.22)
Emotional symptoms	3.61 (1.52)	3.58 (1.48)	-0.01 (-0.21, 0.19)	0.00 (-0.05, 0.04)	3.59 (1.50)	3.60 (1.52)	-0.03 (-0.26, 0.21)	0.01 (-0.04, 0.06)
Conduct problems	1.95 (1.41)	1.91 (1.36)	0.05 (-0.13, 0.23)	0.03 (-0.01, 0.07)	1.92 (1.38)	1.97 (1.42)	-0.03 (-0.24, 0.19)	0.03 (-0.02, 0.07)
Hyperactivity/inattention	3.62 (1.72)	3.63 (1.53)	0.00 (-0.21, 0.21)	0.01 (-0.03, 0.06)	3.61 (1.69)	3.66 (1.49)	-0.02 (-0.27, 0.23)	0.03 (-0.02, 0.08)
Peer relationship problems	2.49 (1.29)	2.47 (1.29)	0.03 (-0.14, 0.20)	0.03 (0.00, 0.07)	2.49 (1.28)	2.46 (1.28)	0.05 (-0.15, 0.25)	0.03 (-0.01, 0.07)
Prosocial behavior	6.73 (1.45)	6.69 (1.46)	0.02 (-0.17, 0.22)	0.00 (-0.04, 0.05)	6.70 (1.44)	6.79 (1.49)	-0.08 (-0.30, 0.14)	-0.02 (-0.07, 0.02)

^ap -value < 0.05

*For declarative memory outcome: EBF ≥ 5 mo (n=420), EBF < 5mo (n=275), PBF ≥ 5 mo (n=524), PBF < 5mo (n=159).

†Estimates were obtained from linear regression models of EBF/PBF predicting indicated development outcome and adjusted for child age at assessment, child sex, outcome enumerator, maternal age, maternal education, maternal cognition, parity, gestational age, birth weight, intervention group, household asset index, household food insecurity, FCI score, HOME score at 10 y, school quality.

Figure 2.1: Cognitive factor z-score at 4-6 y by EBF/PBF

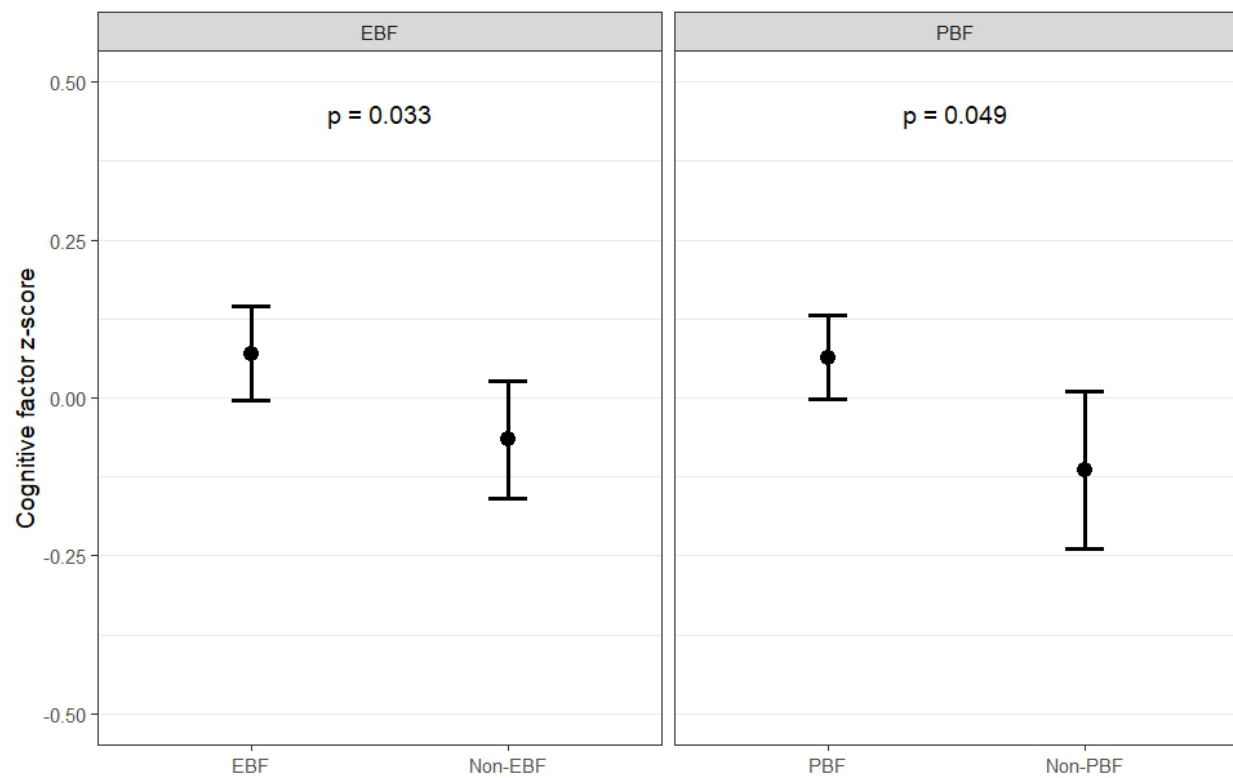
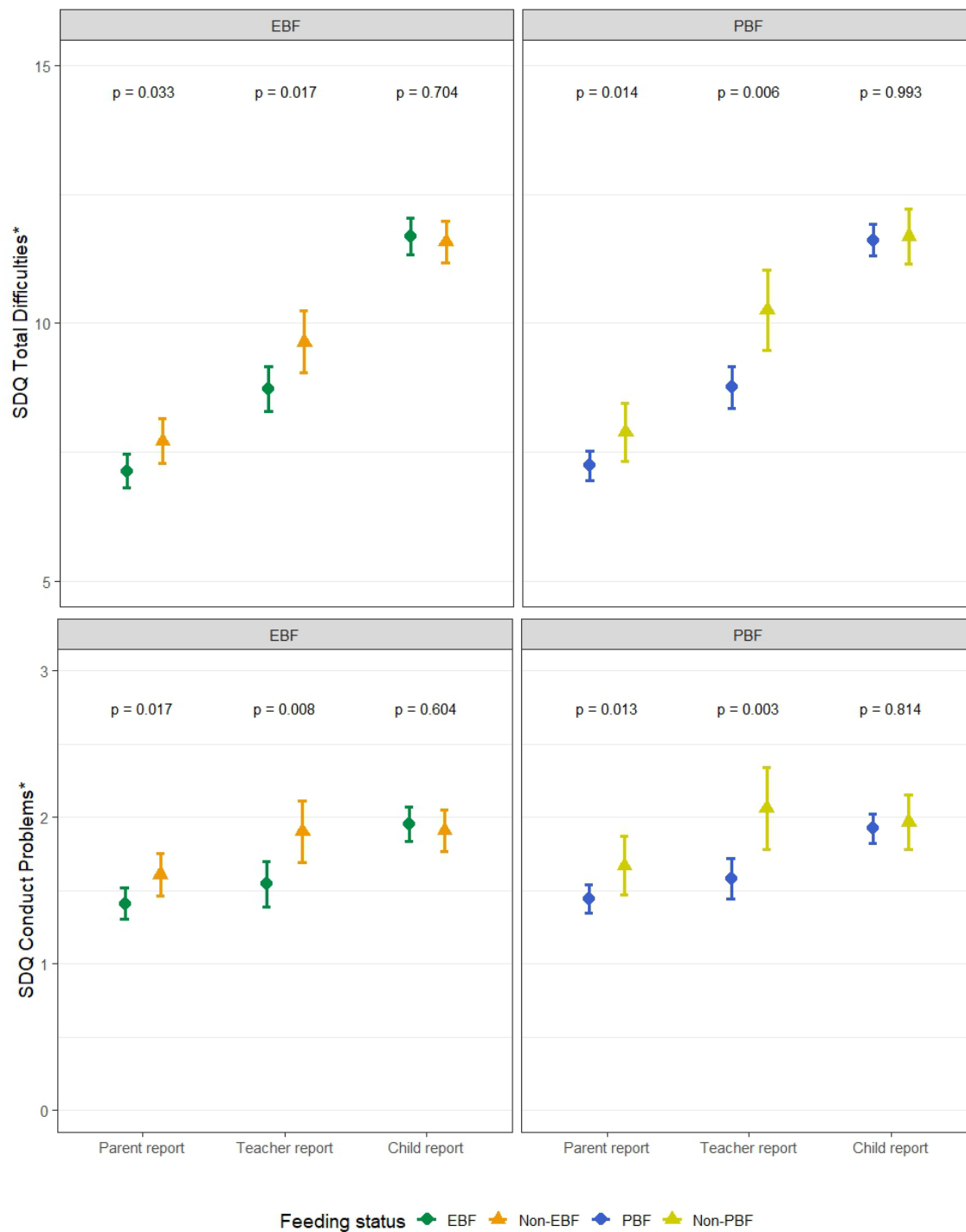


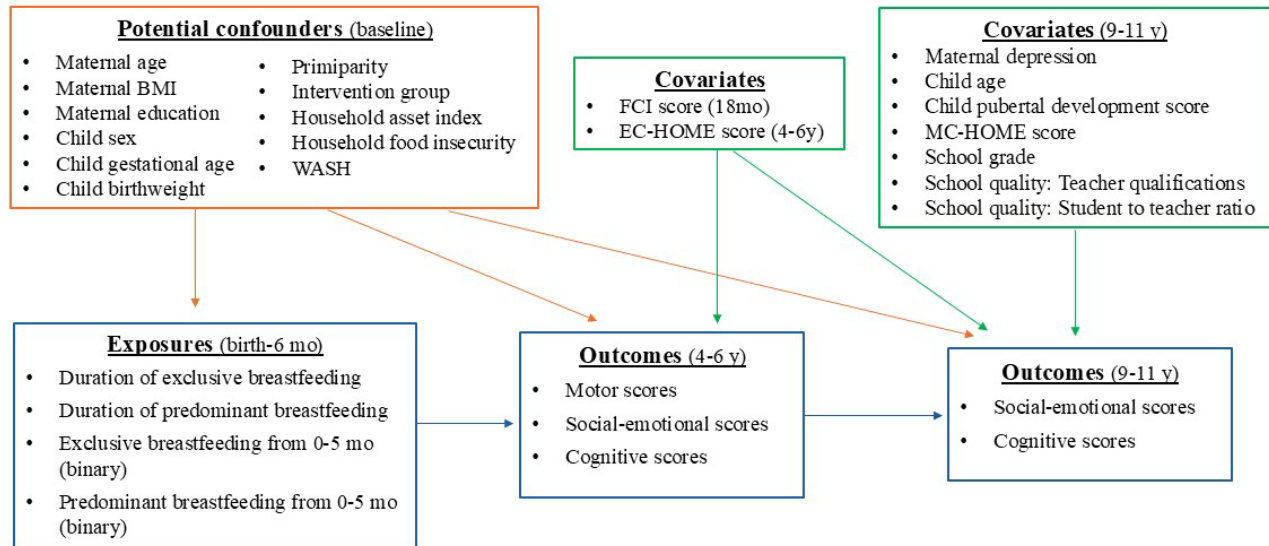
Figure 2.2: Mean SDQ scores by EBF/PBF and reporter



*SDQ total difficulties scores range from 0 to 40 and SDQ conduct problems subscale scores range from 0 to 10.

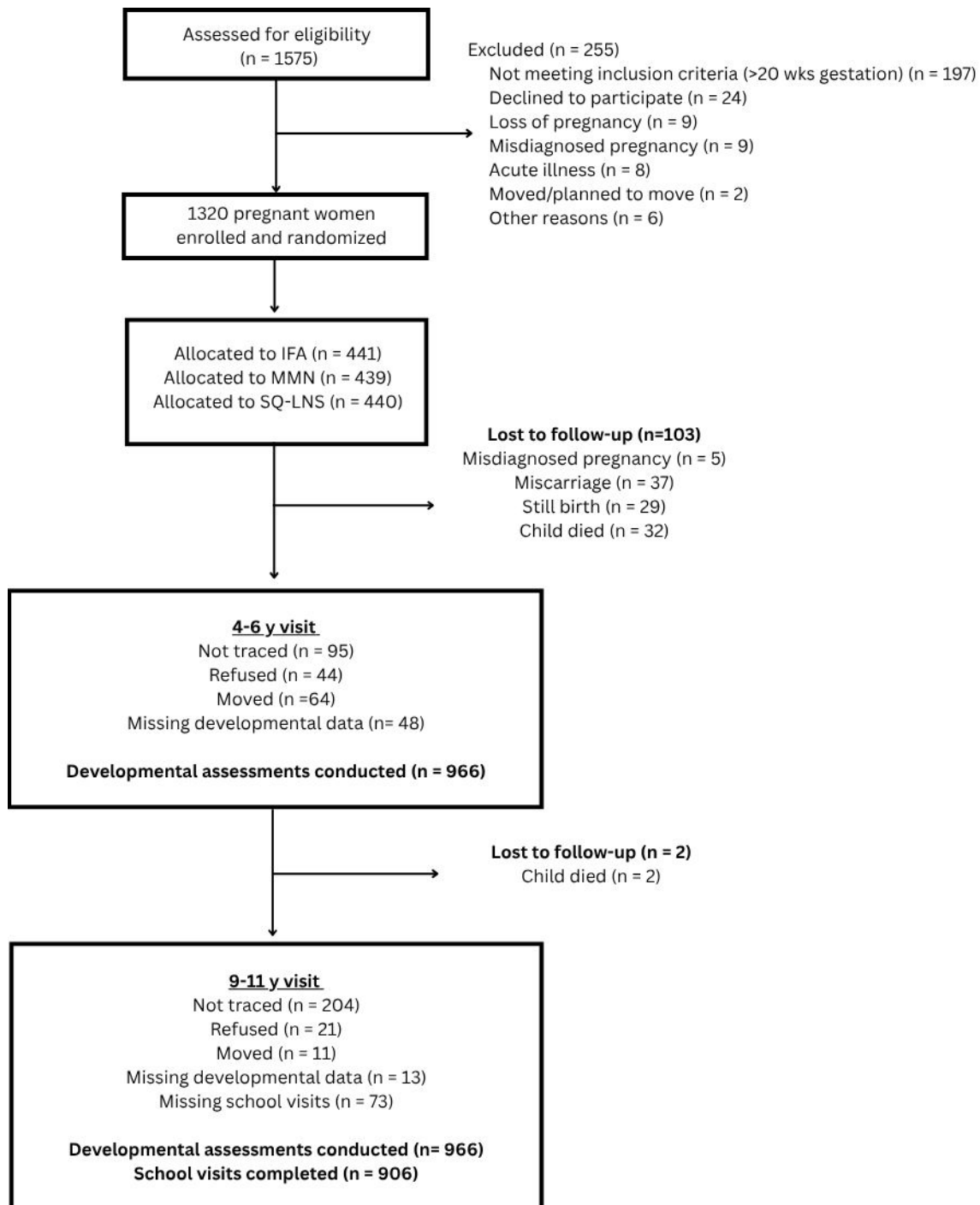
2.9 Supplementary Material

Figure S2.1: Conceptual model illustrating relationships among variables including potential confounders and covariates



FCI: Family Care Indicators; EC-HOME: Early Childhood Home Observation for the Measurement of the Environment; MC-HOME: Middle Childhood Home Observation for the Measurement of the Environment

Figure S2.2: CONSORT diagram showing infants whose mothers were enrolled into the trial, and the reasons some infants were lost to follow-up



Chapter 3

A longitudinal analysis of early-life dietary diversity and developmental outcomes
at 18 mo, 4-6 y and 9-11 y in Ghanaian children

3.1 Abstract

Background: After six months of age, children need diverse, nutrient-dense foods in addition to breast milk to meet nutrient requirements. Poor complementary feeding practices impair physical growth, but little is known about impacts on mental development.

Objective: We investigated the associations of dietary diversity (DD) from 6-18 mo of age with motor, cognitive, and social-emotional outcomes in Ghanaian children at 18 mo, 4-6 y, and 9-11 y of age.

Methods: This secondary analysis uses data from the control group (n=737) of the International Lipid-Based Nutrient Supplements (iLiNS)-DYAD-Ghana trial and its follow-up studies.

Caregiver report of child dietary intake was collected at 6, 9, 12, 15, 18 mo, and a cumulative 6-18 mo DD score (range: 0-40) was calculated based on the eight defined food groups of WHO's minimum dietary diversity indicator. This cumulative score was used to create a binary "low" (DD score ≤ 24) and "adequate" (DD score 25-40) exposure variable. Motor, cognitive, and social-emotional outcomes were assessed at 18 mo, 4-6 y, and 9-11 y. Associations of DD score (continuous and binary) with 44 developmental outcomes were examined using linear regression controlling for maternal, child, and household factors.

Results: Mean (SD) cumulative DD scores were 21.7 (3.9) and ranged from 9 to 32. DD was positively associated with language z-scores at 18 mo ($p=0.002$) and 4-6 y ($p=0.006$). Higher DD score was also associated with lower hyperactivity/inattention scores at 4-6 y ($p=0.048$) and 9-11 y ($p=0.010$). Comparing children with low versus adequate DD produced significant differences, but these were not consistent over time or across reporters.

Conclusion: In this Ghanaian cohort, greater dietary diversity early in life was associated with better language development in early to middle childhood, and fewer hyperactivity/inattention problems in late childhood, signaling the enduring importance of optimal complementary feeding for infants and young children.

3.2 Introduction

After six months of age, children need diverse, nutrient-dense foods in addition to breast milk in order to meet their nutrient requirements. The importance of optimal complementary feeding for infant growth and development is well established (1, 2). Poor complementary feeding practices can lead to nutrient deficiencies and growth faltering, which in turn may adversely impact development such as poor school performance and low work productivity in adulthood (3-5). Current evidence suggests that poor nutrition can impair cognitive development particularly during the early childhood years (6-8). However, scant data exists about long-term development, particularly in the social-emotional domain, in relation to measures of early diet quality, including dietary diversity (DD).

In large-scale population surveys, nutrient adequacy in diets of children 6-23 months of age is measured using the minimum dietary diversity (MDD) indicator, which is the percentage of children who consumed foods and beverages from at least five out of eight defined food groups during the previous day (9). During this time, it is especially important to ensure adequate intake of nutrients necessary for healthy brain development such as B-vitamins, iron, zinc and essential fatty acids (10). Since DD is an indicator of diet quality for infants and young children in low- and middle-income countries, children who consume diverse diets are more likely to be receiving adequate quantities of these critical micronutrients (11, 12).

In recent years, UNICEF introduced the concept of “child food poverty,” which is defined as children’s inability to access and consume a nutritious and diverse diet in early childhood and is measured using the UNICEF and WHO dietary diversity score (13). Depending on the number of food groups children are fed per day, they are categorized as living in severe child food poverty (0-2 food groups/day), moderate food poverty (3-4 food groups/day) or not living in child food poverty (≥ 5 food groups/day). As both the MDD and the child food poverty metric are derived from the same food groups, the UNICEF child food poverty metric is thus an extension and reframing of the MDD indicator.

In Ghana, an estimated 31% of children under five years of age live in severe child food poverty and an additional 43% live in moderate child food poverty (13). Children who do not meet the MDD for their age range are at higher risk for faltered growth and while this association of MDD and linear growth has been investigated in low- and middle-income countries (5), few studies have examined associations of MDD and child development. Given the extensive child dietary data and developmental assessments collected during the iLiNS-DYAD-Ghana trial and its follow-up studies, we have a unique opportunity to investigate the relation between early-life dietary diversity and child development in Ghanaian preadolescents.

3.3 Methods

Study design and participants

This secondary analysis used data from the International Lipid-Based Nutrient Supplements (iLiNS) DYAD-Ghana trial and the follow-up studies at 4-6 y and 9-11 y. Details of the trial design and methods (clinicaltrials.gov; NCT00970866) have been published elsewhere

(14, 15). In brief, 1320 pregnant women 18 years or older residing in the semiurban Yilo and Lower Manya Krobo districts of the Eastern Region of Ghana were enrolled in the iLiNS-DYAD-Ghana trial between 2009 and 2014. Participants were randomized to receive one of three nutrition supplements: 1) daily iron and folic acid (IFA) capsule during pregnancy and a daily placebo (200 mg of calcium) from birth to six months postpartum; 2) daily multiple micronutrient capsule (MMN), containing one to two times the Recommended Dietary Allowance (RDA) of 18 vitamins and minerals, during pregnancy and six months postpartum; 3) daily lipid-based nutrient supplements (LNS), with 118 kcal, macro- and micronutrients, during pregnancy and six months postpartum. Infants in the LNS group also received daily LNS supplementation from 6 to 18 mo of age, while infants in the IFA and MMN groups did not receive any supplementation during the trial. This analysis is restricted to the two groups that did not receive LNS. A comprehensive developmental assessment was conducted at the end of the trial when children were 18 mo of age. Follow-up visits were conducted in 2016, when children were 4-6 y old, and in 2021, when children were 9-11 y old. Ethical approval for the original trial and follow-up studies was obtained from the Institutional Review Board of the University of California Davis, the Ethics Committee for the College of Basic and Applied Sciences of the University of Ghana and the Ghana Health Service Ethical Review Committee.

Data collection

Information about breastfeeding and complementary feeding practices was collected using an interviewer-administered 24-hour dietary recall at 6, 9, 12, 15, and 18 mo, respectively. Mothers or caregivers were asked to recall any foods, including breast milk, the child consumed the previous day. Foods were then categorized into one of the eight food groups comprising the

WHO MDD indicator: 1) breast milk; 2) grains, roots, tubers and plantains; 3) pulses, nuts, and seeds; 4) dairy products (milk, infant formula, yogurt, cheese); 5) flesh foods (meat, fish, poultry, organ meats); 6) eggs; 7) vitamin A rich fruits and vegetables; 8) other fruits and vegetables (9). Each food category was assigned a DD score of 1 or 0 indicating whether or not the child consumed at least one food from the group. The sum of this DD score (range 0-8) reflects the child's DD score for one specific time point; we then summed the DD scores from all 5 time points to create a cumulative DD score (range 0-40), which was our primary exposure variable. Using this cumulative DD score, we created 3 dietary intake levels for group comparisons: "Very low" (cumulative DD score ≤ 10); "Low" (cumulative DD score 11-24); "Adequate" (cumulative DD score 25-40). These cutoffs were selected to align with UNICEF's definition of severe child food poverty (children fed 0-2 food groups/day), moderate food poverty (children fed 3-4 food groups/day) or not living in child food poverty (children fed ≥ 5 food groups/day) (13).

Developmental outcomes were assessed at 18 mo, 4-6 y, and 9-11 y by trained data collectors who were blinded to the intervention group. Details of these assessments have been published previously (16-18). At 18 mo, motor development was assessed using the Kilifi Developmental Inventory (KDI), a tool developed in Kenya that scores a child's performance of 34 fine motor and 35 gross motor skills (19). Language development was assessed using a 100-word vocabulary checklist based on the MacArthur-Bates Communicative Development Inventory (CDI) (20) and adapted to the local languages. Social-emotional skills were assessed using the Profile of Social and Emotional Development (PSED), in which caregivers were asked about the child's emotional and behavioral regulation, aggression, and social competence. Executive function was assessed using a version of the A not B task, which has previously been

used in Kenya (21). Scores were 1) total correct trials, out of 10 trials administered; and 2) perseverative errors, which is the total number of errors committed after the first switch to the alternate location.

At 4-6 y, fine motor function was measured using the National Institutes of Health (NIH) Toolbox 9-hole pegboard test (22). We assessed cognition at 4-6 y with a battery of tests: language skills using the Developmental Neuropsychological Assessment-II (NEPSY-II) body part naming and identification and comprehension of instructions subtests (23); visuospatial ability using the block design test (24); declarative memory using the paired-associate task (25); executive function using the International Development and Early Learning Assessment (IDELA) head-toes subtest and visual search task (26); and pre-academic skills using the Parent's Evaluation of Developmental Statusy (PEDS) pre-academic subscale (27).

A similar battery of tests was used to measure cognitive development at 9-11 y. General intellectual ability was assessed using a block design test that was based on the Wechsler Primary and Preschool Scale of Intelligence block design subtest (24), the British Ability Scales II pattern construction subtest (28), and the NEPSY-II (23); literacy through a letter, sentence, and word recognition test (25, 29); arithmetic ability with a test similar to the Wechsler Intelligence Scale for Children (WISC) arithmetic task (24) that was adapted to the local context; declarative memory, procedural memory, and executive function using various Rapid Assessment of Cognitive and Emotional Regulation (RACER) tasks (30).

Social-emotional development was assessed using the Strengths and Difficulties Questionnaire (SDQ) as reported by parents/caregivers (SDQ-P) at 4-6 y and 9-11 y, and by teachers (SDQ-T) and children (SDQ-C) at 9-11 y. The SDQ consists of 5 subscales: 1) emotional symptoms, 2) conduct problems, 3) hyperactivity/inattention, 4) peer relationship

problems, and 5) prosocial behavior. We summed the scores from subscales 1-4 to create a “total difficulties” score, with higher scores indicating more social-emotional problems (31, 32).

Additionally, we measured caregiving and stimulation from the home environment at each time point using the Family Care Indicator (FCI) score at 18 mo (33) and the Home Observation for the Measurement of the Environment (HOME) Inventory (Early Childhood version at 4-6 y; Middle Childhood version at 9-11 y) (34). All three assessments were adapted to the local context in Ghana. Maternal depression was assessed at 9-11 y with the Center for Epidemiological Studies Depression Scale (35).

Sample size and statistical analyses

A statistical analysis plan for this study was posted on Open Science Framework before conducting analyses (<https://osf.io/bmv9d/files/q59hd>). The iLiNS DYAD-Ghana study enrolled 1320 participants and we have dietary data for 1196 infants. We re-enrolled about 1,010 children at age 4-6 years and 979 at 9-11 years (Figure S1). For this analysis, we used data from children in the non-LNS groups (i.e., IFA and MMN groups) because children in these two groups did not receive any nutritional supplements during the trial. Children in the LNS group received daily SQ-LNS containing ~120 kcal and approximately the recommended dietary allowance of more than 20 vitamins and minerals from 6 to 18 months of age. DD is a proxy indicator for micronutrient adequacy (36), and since children in the LNS group were able to likely achieve micronutrient adequacy in the absence of DD, we decided to exclude the LNS group from this analysis.

For this analysis, we only included children with dietary data from at least 3 of the 5 time points. We had complete data for 85% of the sample, while 9% were missing data from 1 time

point, and 3% were missing data from 2 time points. For missingness, we imputed missing data by modeling the relationship between the available datapoints, child's age at the time of missingness, child sex, as well as household asset index and food insecurity score. This sample size provided 80% power to detect associations between DD scores and continuous outcomes with a strength of correlation of 0.12 or larger at a significance level of $p < 0.05$.

Our primary exposure variables were cumulative DD score and DD adequacy (categorical). Using the cumulative DD score, we created 3 dietary intake levels for group comparisons: "Very low" (cumulative DD score ≤ 10); "Low" (cumulative DD score 11-24); "Adequate" (cumulative DD score 25-40). However, due to the low number of participants in the "very low" category ($n=3$), we decided to combine the "very low" and "low" groups, which resulted in a binary "low" and "adequate" variable.

Primary outcomes for this analysis were motor scores at 18 mo and 4-6 y; social-emotional score at 18 mo and social-emotional SDQ-P total difficulties scores at 4-6 y and 9-11 y; language score and executive function (total correct) score at 18 mo and cognitive factor scores at 4-6 y and 9-11 y. The cognitive factor score at 4-6 y was calculated using the principal-axis factoring method and included the following outcome measures: language, visuospatial ability, declarative memory, executive, pre-academic skills. The cognitive factor score at 9-11 y was also calculated using the principal-axis factoring method with general intellectual ability, literacy, arithmetic, declarative memory, procedural memory, and executive function. Secondary outcomes for this study included executive function (perseverative errors) at 18 mo, each of the 5 subscale scores of the SDQ-P (emotional symptoms, conduct problems, hyperactivity/inattention, peer relationship problems, prosocial behavior) at 4-6 y and 9-11 y and the SDQ-T and SDQ-C, as well as the cognitive scores of the assessments listed above.

For the cumulative DD score, we calculated the z-score by standardizing it with a mean of 0 and SD of 1. This would improve the interpretation of our results such that the beta coefficient would reflect a change in one SD of DD score over time rather than an increase of one food group at one time point. For all developmental outcomes at 18 mo and all cognitive outcomes at 4-6 y and 9-11 y, we calculated age-adjusted z-scores as the standardized residual of age predicting the score. This z-score represents the deviation of the child's score in units of SD from the score expected for the child's age. For social-emotional outcomes at 4-6 y and 9-11 y, we reported unadjusted mean scores since the SDQ is a common screening tool for measuring psychosocial problems in children (31).

We used multivariable linear regression models for all continuous outcomes. All models were minimally adjusted for child age at time of development assessment, child sex and outcome assessment enumerator. In fully adjusted models, we included covariates including maternal education, maternal depression, parity, child gestational age and birthweight, household assets at 6 mo, household food insecurity at 6 mo, FCI score, HOME scores, and school quality if they were associated with outcome in bivariate testing ($p < 0.1$). For effect modification testing, we included an additional interaction term of exposure with the pre-specified modifier (i.e., child sex) in both the minimally adjusted and fully adjusted models.

3.4 Results

Among children in the IFA and MMN groups, 683 children (83% of eligible) participated in the comprehensive developmental assessment at 18 mo of age (Figure S1). We re-enrolled 662 and obtained developmental data from 626 children (77% of eligible) at 4-6 y and re-enrolled 648 and obtained developmental data from 635 children (78% of eligible) at 9-11 y (Figure S1);

518 were assessed at all three time points and 762 were assessed at any time point. Of these 762 children, 737 had dietary data from 6 to 18 mo and this resulting analytic sample size represented children with exposure and at least one outcome measurement combination.

Baseline characteristics of participants in this analytic sample are presented in Table 1. Mean $\pm$ SD cumulative DD score from 6-18 mo was 21.7 ± 3.9 (range 0-40) and the proportion of children achieving MDD (consuming ≥ 5 food of eight defined food groups in a day) at each time point was 12% at 6 mo, 61% at 9 mo, 71% at 12 mo, 73% at 15 mo and 72% at 18 mo. Figure 1 depicts the mean DD score at each time point and the proportion of the sample reporting consumption of each of the eight food groups.

Cognitive outcomes

Cumulative DD z-score from 6-18 mo was positively associated with language z-score at 18 mo ($\beta=0.11$; 95% CI: 0.04, 0.17; $p=0.002$) and at 4-6 y ($\beta=0.09$; 95% CI: 0.02, 0.15; $p=0.006$) (Figure 2). No other associations between cumulative DD scores and developmental outcomes at 18 mo, 4-6 y, or 9-11 y were significant (Tables 2 and 3). Compared to children with low DD, those with adequate DD had higher language z-scores at 18 mo ($\beta=0.19$; 95% CI: 0.03, 0.34; $p=0.019$), yet lower scores for declarative memory ($\beta= -0.37$; 95% CI: -0.60, -0.13; $p=0.002$) and procedural memory ($\beta= -0.25$; 95% CI: -0.44, -0.05; $p=0.014$) at 9-11 y. No other significant group differences were found for cognitive outcomes at 18 mo, 4-6 y or 9-11 y.

Social-emotional outcomes

Higher cumulative DD score was associated with fewer hyperactivity/inattention problems as reported by parents at 4-6 y ($\beta = -0.16$; 95% CI: -0.32, 0.00; $p=0.048$) and 9-11 y ($\beta = -0.20$; 95% CI: -0.35, -0.05; $p=0.010$), but not with child or teacher reports at 9-11 y (Figure 3). Compared to the parent report, the associations of cumulative DD score with teacher reported hyperactivity/inattention problems trended in the opposite direction, although non-significantly, whereas the child report trended in the same direction. Similarly, children with adequate DD, compared to children with low DD, had fewer self-reported hyperactivity/inattention problems at 9-11 y ($\beta = -0.43$; 95% CI: -0.75, -0.10; $p=0.011$) (Table 3). Adequate DD was associated with worse parent-reported prosocial behavior at 4-6 y ($\beta = -0.47$; 95% CI: -0.84, -0.10; $p=0.013$), yet better prosocial behavior at 9-11 y as reported by both parents ($\beta=0.31$; 95% CI: 0.02, 0.61; $p=0.037$) and children ($\beta=0.30$; 95% CI: 0.03, 0.57; $p=0.030$) Furthermore, adequate DD was associated with fewer child-reported hyperactivity/inattention problems at 9-11 y ($\beta = -0.43$; 95% CI: -0.75, -0.10; $p=0.013$) (Tables 2 and 3). Children with adequate DD, had more emotional symptoms at 9-11 y as reported by teachers ($\beta=0.43$; 95% CI: 0.03, 0.83; $p=0.034$). We did not find significant associations of DD score (cumulative or binary) with any other social-emotional outcomes at 18 mo, 4-6 y or 9-11 y.

We examined effect modification by child sex and did not find significant interaction for any of the associations between DD score and motor, cognitive or social-emotional outcomes at any time point.

3.5 Discussion

Overall, our study provides insights about the diversity of complementary foods consumed by Ghanaian children during the complementary feeding period and its associations

with developmental outcomes in early and middle childhood. In our analysis, greater cumulative DD score from 6-18 mo of age was associated with better language development at 18 mo and preschool age, as well as fewer parent-reported hyperactivity/inattention problems at preschool age and preadolescence. Cumulative DD score was not associated with any other motor, cognitive or social-emotional outcomes at any time point. Associations with the binary “low” vs. “adequate” DD indicator and developmental outcomes yielded mixed results with no discernible pattern, likely because the sample lacked participants experiencing severe food deprivation. Few studies have examined the relation between DD and social-emotional development in later childhood, which makes our finding that greater early-life DD is associated with fewer hyperactivity problems at 4-6 y and 9-11 y of age unique. In a prospective cohort study of children in the Democratic Republic of the Congo, DD score at 6-23 mo of age was positively associated with total, communication, and personal social (but not motor or problem solving) Ages and Stages Questionnaire (ASQ) scores 6 mo later (37). A cross-sectional study in China found that meeting the MDD was associated with a reduced risk of developmental delays in personal social subscales of the ASQ in children aged 6-23 mo (8).

Potential mechanisms of our findings may be attributable to two different dimensions of complementary feeding: diet quality and feeding approaches. Diet quality, which is often measured by DD, meal frequency or diet adequacy (9), is critical to ensure the child’s nutrient needs are being met, particularly during 6-23 mo of age (2). Nutrients such as iron, zinc, B-vitamins and essential fatty acids are necessary for healthy brain development (10) and inadequate intake may lead to structural and functional neurodevelopmental deficits (38). Previous studies found that DD predicted the micronutrient density of young children’s diets and child nutritional status in low- and middle-income countries (LMICs) (36, 39, 40). Thus, our

finding that greater DD was associated with better developmental outcomes later in life may be a result of adequate nutrient intake or status in early life.

In this cohort of Ghanaian children, 60-70% of children met the MDD criteria after 9 mo of age. Breast milk was the most commonly consumed food group, which aligns with the high prevalence of reported breastfeeding (99% at 6 mo and 74% at 18 mo) (Figure 1). The second most commonly consumed food group was grains, roots, and tubers, which includes foods such as porridge, banku, jollof, cassava, gari, yam, cocoyam, plantain, and fufu. Reported consumption of flesh foods (meat, poultry and fish) and other fruits and vegetables such as banana, paya, tomato, and onion was relatively consistent from 12 to 18 mo of age. The two least commonly consumed food groups were dairy products (animal milk, yogurt, cheese and infant formula) and pulses, nuts and seeds. Overall, the diet quality of the children in this cohort seemed nutritionally adequate based on the variety of foods consumed and the high proportion of children meeting the MDD criteria after 9 mo of age.

In addition to a diverse diet, consumption of certain nutrient-dense foods such as animal source foods (ASF) from 6-23 mo of age is especially important during this time of rapid growth and development. Previous longitudinal studies examining complementary feeding and development in LMICs have generally found that ASF consumption or protein and fat intake are positively associated with total development and cognition scores (41-44). Studies that investigated domain-specific developmental outcomes found mixed results. DD from 6-8 mo was positively associated with fine motor scores, but no other ASQ domain at 20-24 mo in Ugandan children (6). A study in Singapore examining relations of nutrient trajectories from 6-12 mo and development at 24 and 54 mo found both positive and negative associations with fine motor and language scores at 24 mo but no associations at 54 mo (45). Our finding that DD is associated

with language development at 18 mo and 4-6 y is consistent with previous observations that early DD and consumption of certain foods (proteins and fats) were associated with better language outcomes in toddlerhood in Haiti (46) and Kenya (47). Furthermore, a previous multi-cohort study examining factors associated with 18 mo language and motor development in Burkina Faso, Mali and Ghana (the Ghanaian cohort from this analysis) found that higher language scores were observed among children who had greater DD and higher quality stimulation from the home environment (48).

Our finding that early-life DD is associated with fewer hyperactivity/inattention problems in middle childhood is notable, as few longitudinal studies have investigated this relation. In a British cohort, consumption of ultra-processed foods and high sugar/high fat foods at 4 y of age was associated with increased hyperactivity at 7 y of age (49), but no association was found between this dietary pattern at age 7 y and behavioral problems over the following 16 months (50). A meta-analysis of studies examining dietary patterns and Attention Deficit Hyperactivity Disorder (ADHD) showed mixed results, possibly due to significant heterogeneity among the mostly cross-sectional studies (51). While the underlying biological mechanisms are not fully understood, one possible explanation is that diverse diets provide key micronutrients such as iron and zinc, which are essential for the production and regulation of dopamine (52). Dysfunction of the dopamine system may impair behavioral functioning and manifest as hyperactivity and inattention (53). Importantly, the timing of this nutritional exposure may matter, as the 6 to 18 mo period represents a sensitive window during which the neural circuits underlying attention and behavior regulation are being established (54).

In addition to providing essential nutrients for the growing child, complementary feeding entails regular caregiver-child interactions and thus, opportunities for developmental stimulation.

For example, infants may be exposed to different narratives and vocabulary during family mealtimes than they are elsewhere, which could support language development (55, 56). In our study, it is possible that a higher DD score was not only indicative of nutrient adequacy, but of responsive caregiving as well. Responsive feeding refers to a caregiver's attentive and reciprocal interactions in response to a child's hunger and satiety signals (57). It is also a form of responsive parenting, which stems from attachment and socialization theories of child development, and establishes a basis for healthy social-emotional development in children (58, 59). Parental responsivity (specifically parental verbal responsiveness) was associated with higher children's language scores in Ethiopia (60) and Kenya (61). Although our study has not measured responsive feeding practices, it may be correlated with DD (62) and thereby contributing to better language and social-emotional development in our cohort as well.

A major strength of this study was the dietary data collected at multiple time points from birth to 18 mo of age, which provided a unique opportunity to examine individual food intake patterns over time. While most studies use cross-sectional assessments of dietary intake often with a single day recall, we used dietary data from 5 days of recall over a period of 12 months allowing us to better capture the usual intake of food groups in this cohort. Similarly, because child development outcomes were measured at the end of the original iLiNS-DYAD-Ghana trial and each subsequent follow-up study, we were able to assess the prospective longitudinal relation between early-life DD and developmental outcomes at early, middle and late childhood. There was a high follow-up rate of the children from birth to preadolescence which reduces the likelihood of selection bias in our sample. We were also able to control for potentially confounding factors such as maternal education, household asset index and food insecurity. Finally, we developed a pre-specified statistical analysis plan before conducting this study.

However, our study also has important limitations. Although the 24-hr diet recalls were collected by in-person interviews, there is a possibility of recall bias by caregivers about children's food consumption. There may have been reporting bias from parents, children and teachers for the social-emotional outcomes. Furthermore, most children in our cohort were breastfed through 18 mo, which contributed to the cumulative DD score; therefore, our results may not be generalizable to populations with lower breastfeeding rates.

3.6 Conclusion

Our findings underscore the importance of DD in early life as a modifiable factor with potential long-term implications for language and social-emotional development. This study provides further evidence that both nutrient adequacy and caregiver responsiveness should be considered when developing programs or interventions aimed at improving infant and young child feeding practices and child development. Little is known about the impact and underlying mechanisms of responsive feeding on developmental outcomes so further investigation in this area is warranted.

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3.8 Tables and Figures

Table 3.1: Characteristics among participants included in the analysis (n=737)

	Mean (SD) or n (%)
Baseline characteristics	
Maternal age (y)	26.7 (5.4)
Maternal height (cm)	158.8 (5.9)
Pre-pregnancy BMI (kg/m ²)	24.4 (4.4)
Maternal education (y)	7.7 (3.5)
Primiparity	241 (33%)
Gestational age at delivery (wk)	39.3 (1.8)
Birthweight (kg)	3.0 (0.4)
Child male	355 (48%)
Married or co-habiting	690 (94%)
Household asset index at 6 mo	0.1 (0.9)
Food insecurity score at 6 mo	2.6 (4.3)
IYCF measurements	
Cumulative dietary diversity score (6-18 mo)	21.7 (3.9)
Dietary diversity score at 6 mo	2.3 (1.4)
Dietary diversity score at 9 mo	4.5 (1.5)
Dietary diversity score at 12 mo	4.9 (1.3)
Dietary diversity score at 15 mo	5.1 (1.3)
Dietary diversity score at 18 mo	5.0 (1.3)
Any BF at 6 mo	708 (99%)
Any BF at 18 mo	532 (74%)
Follow-up measurements	
Child age at 18 mo assessment (mo)	18.3 (0.3)
Child age at 4-6 y follow-up (y)	4.9 (0.6)
Child age at 9-11 y follow-up (y)	9.9 (0.5)
FCI score at 18 mo	8.2 (2.7)
EC-HOME score at 4-6 y follow-up	28.0 (4.5)
MC-HOME score at 9-11 y follow-up	35.2 (6.1)

BF: breastfeeding; EBF: exclusive breastfeeding; PBF: predominant breastfeeding; FCI: Family Care Indicators; EC-HOME: Early Childhood Home Observation for the Measurement of the Environment Inventory; IYCF: infant and young child feeding; MC-HOME: Middle Childhood Home Observation for the Measurement of the Environment Inventory.

Figure 3.1: Food group proportions of mean dietary diversity score across child age (6-18 mo)

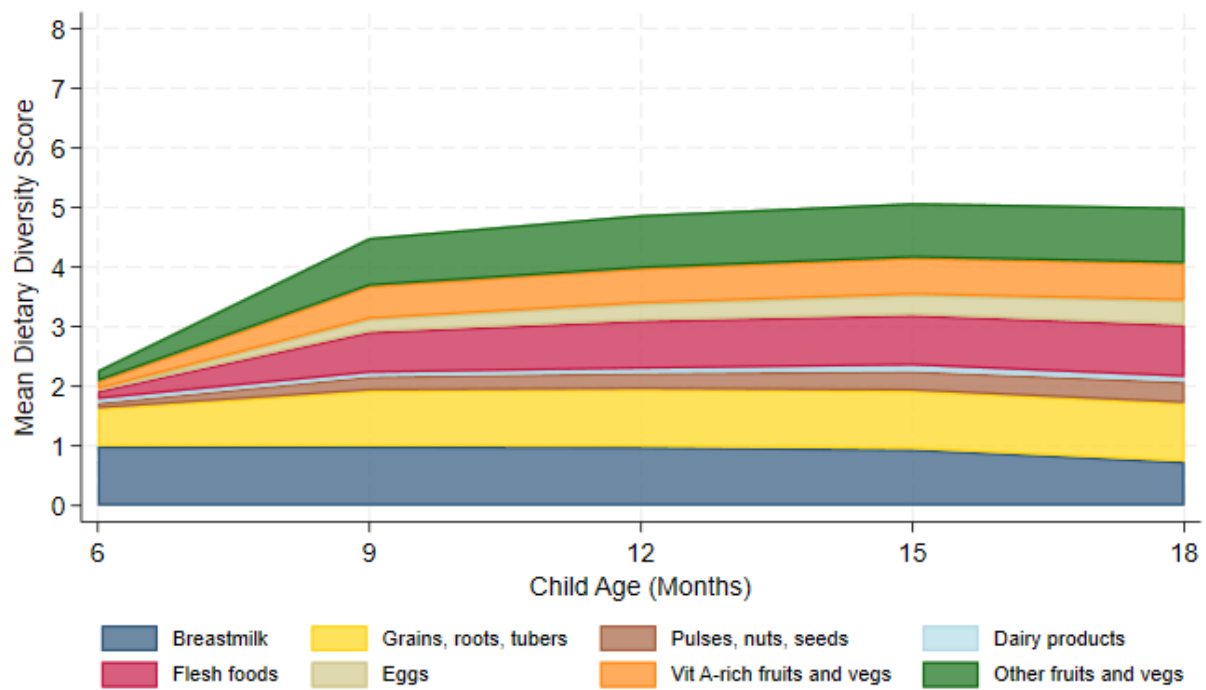


Figure 3.2: Associations of dietary diversity z-score from 6-18 mo with language z-scores at 18 mo and 4-6 y

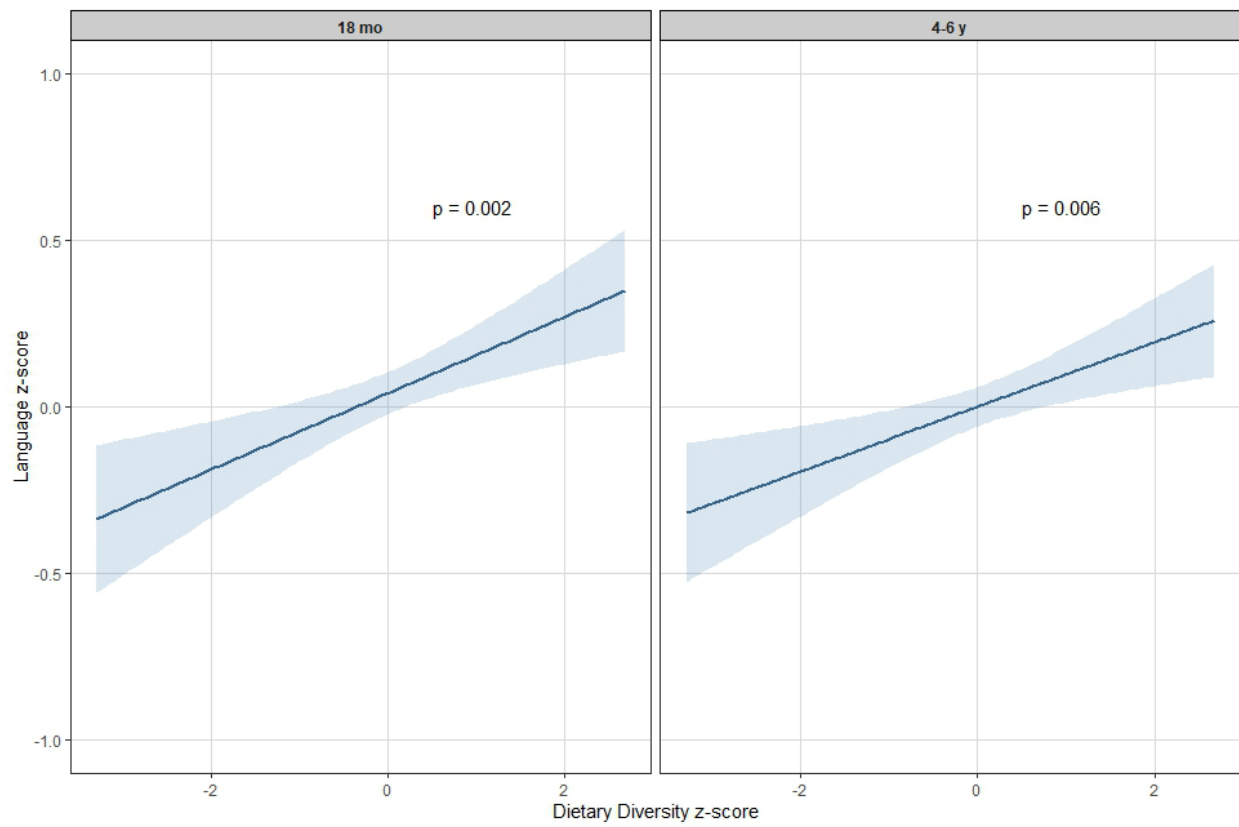
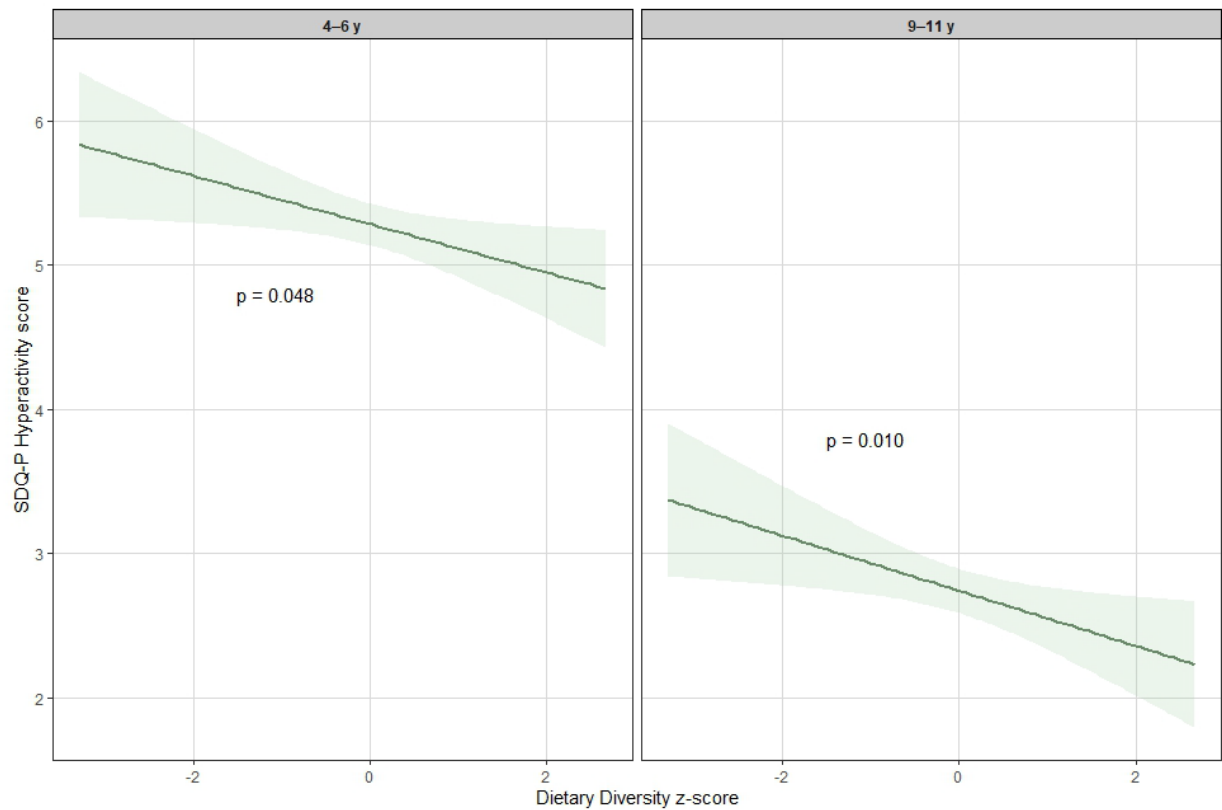


Figure 3.3: Associations of dietary diversity z-score from 6-18 mo with mean SDQ-P hyperactivity score* at 4-6 y and 9-11 y



*SDQ Hyperactivity subscale scores range from 0 to 10.

Table 3.2: Associations of dietary diversity z-score with developmental outcomes at 18 mo and 4-6 y

	Dietary Diversity (DD) score	
	Low vs. Adequate (n=457) vs. (n=151)	Cumulative z-score (per SD)
	β (95% CI)	
18 mo		
Motor z-score	0.09 (-0.06, 0.25)	0.04 (-0.02, 0.11)
Language z-score*	0.19 (0.03, 0.34) ^a	0.11 (0.04, 0.17) ^a
Executive function (A not B correct z-score)	0.09 (-0.09, 0.28)	0.01 (-0.07, 0.09)
Executive function (A not B perseverative errors z-score)	-0.12 (-0.32, 0.09)	-0.02 (-0.10, 0.07)
Social-emotional z-score*	0.12 (-0.02, 0.26)	-0.01 (-0.07, 0.05)
4-6 y		
Motor z-score	-0.08 (-0.25, 0.10)	0.03 (-0.05, 0.11)
Cognitive outcomes [†]		
Cognitive factor z-score	-0.02 (-0.19, 0.15)	0.03 (-0.04, 0.11)
Language	0.09 (-0.06, 0.23)	0.09 (0.02, 0.15) ^a
Visuospatial ability	-0.03 (-0.14, 0.07)	0.00 (-0.05, 0.04)
Declarative memory	-0.11 (-0.29, 0.07)	-0.02 (-0.10, 0.06)
Visual search test*	-0.03 (-0.14, 0.07)	-0.02 (-0.09, 0.06)
Head-to-toe inhibition*	-0.09 (-0.29, 0.10)	0.00 (-0.08, 0.08)
Pre-academic skills	0.05 (-0.13, 0.23)	0.05 (-0.03, 0.13)
Social-emotional outcomes (SDQ-P) [†]		
Total difficulties	-0.11 (-0.91, 0.70)	-0.20 (-0.55, 0.16)
Emotional symptoms	-0.09 (-0.43, 0.26)	-0.07 (-0.23, 0.08)
Conduct problems	0.08 (-0.24, 0.39)	0.02 (-0.12, 0.16)
Hyperactivity/ inattention	-0.25 (-0.60, 0.11)	-0.16 (-0.32, 0.00) ^a
Peer relationship problems	0.17 (-0.11, 0.44)	0.05 (-0.07, 0.17)
Prosocial behavior	-0.47 (-0.84, -0.10) ^a	-0.07 (-0.23, 0.09)

^ap -value < 0.05

*For visual search test and head-to-toe inhibition outcomes at 4-6 y: Low DD (cumulative DD score 11-24) (n=419), Adequate DD (cumulative DD score 25-40) (n=151).

[†]All cognitive outcomes are reported as differences in z-scores. All social-emotional outcomes are reported as differences in mean scores from a potential range of 0-40 for total difficulties and 0-10 for all 5 SDQ subscales. Values are β coefficients (95% CIs) from fully adjusted linear regression models with dietary diversity z-score as the exposure. Models adjusted for child age at assessment, child sex, outcome enumerator, maternal age, maternal education, maternal pre-pregnancy BMI, parity, gestational age, birth weight, intervention group, household asset index, household food insecurity.

SDQ-P: Strengths and Difficulties Questionnaire-Parent report.

Table 3.3: Associations of dietary diversity z-score with developmental outcomes at 9-11 y

	Dietary Diversity (DD) z-score	
	Low vs. Adequate (n=476) vs. (n=151)	Cumulative Score (per SD)
	β (95% CI)	
9-11 y		
Cognitive outcomes [†]		
Cognitive factor z-score	-0.10 (-0.25, 0.05)	-0.01 (-0.07, 0.06)
Intellect	-0.12 (-0.31, 0.07)	-0.03 (-0.11, 0.06)
Literacy	-0.05 (-0.22, 0.13)	0.02 (-0.06, 0.10)
Arithmetic	-0.03 (-0.22, 0.16)	0.01 (-0.08, 0.09)
Declarative memory*	-0.37 (-0.60, -0.13) ^a	-0.07 (-0.18, 0.03)
Procedural memory	-0.25 (-0.44, -0.05) ^a	-0.05 (-0.13, 0.04)
Executive function	-0.03 (-0.19, 0.15)	-0.04 (-0.11, 0.03)
Social-emotional outcomes (SDQ-P) [†]		
Total difficulties	-0.41 (-1.14, 0.33)	-0.29 (-0.61, 0.03)
Emotional symptoms	-0.17 (-0.41, 0.08)	-0.08 (-0.18, 0.03)
Conduct problems	-0.03 (-0.29, 0.24)	-0.05 (-0.16, 0.07)
Hyperactivity/ inattention	-0.33 (-0.69, 0.02)	-0.20 (-0.35, -0.05) ^a
Peer relationship problems	0.13 (-0.11, 0.37)	0.01 (-0.09, 0.12)
Prosocial behavior	0.31 (0.02, 0.61) ^a	0.13 (0.00, 0.26)
Social-emotional outcomes (SDQ-T) [†]		
Total difficulties	0.67 (-0.34, 1.69)	0.29 (-0.15, 0.73)
Emotional symptoms	0.43 (0.03, 0.83) ^a	0.11 (-0.06, 0.29)
Conduct problems	0.03 (-0.33, 0.38)	0.01 (-0.14, 0.16)
Hyperactivity/ inattention	0.31 (-0.13, 0.74)	0.16 (-0.02, 0.35)
Peer relationship problems	-0.03 (-0.33, 0.26)	-0.03 (-0.16, 0.10)
Prosocial behavior	-0.13 (-0.53, 0.27)	-0.05 (-0.22, 0.13)
Social-emotional outcomes (SDQ-C) [†]		
Total difficulties	-0.57 (-1.33, 0.20)	-0.29 (-0.63, 0.04)
Emotional symptoms	0.00 (-0.27, 0.28)	-0.05 (-0.17, 0.07)
Conduct problems	-0.16 (-0.42, 0.10)	-0.10 (-0.22, 0.01)
Hyperactivity/ inattention	-0.43 (-0.75, -0.10) ^a	-0.12 (-0.27, 0.02)
Peer relationship problems	-0.10 (-0.34, 0.14)	-0.04 (-0.14, 0.06)
Prosocial behavior	0.30 (0.03, 0.57) ^a	0.08 (-0.03, 0.20)

^ap -value < 0.05

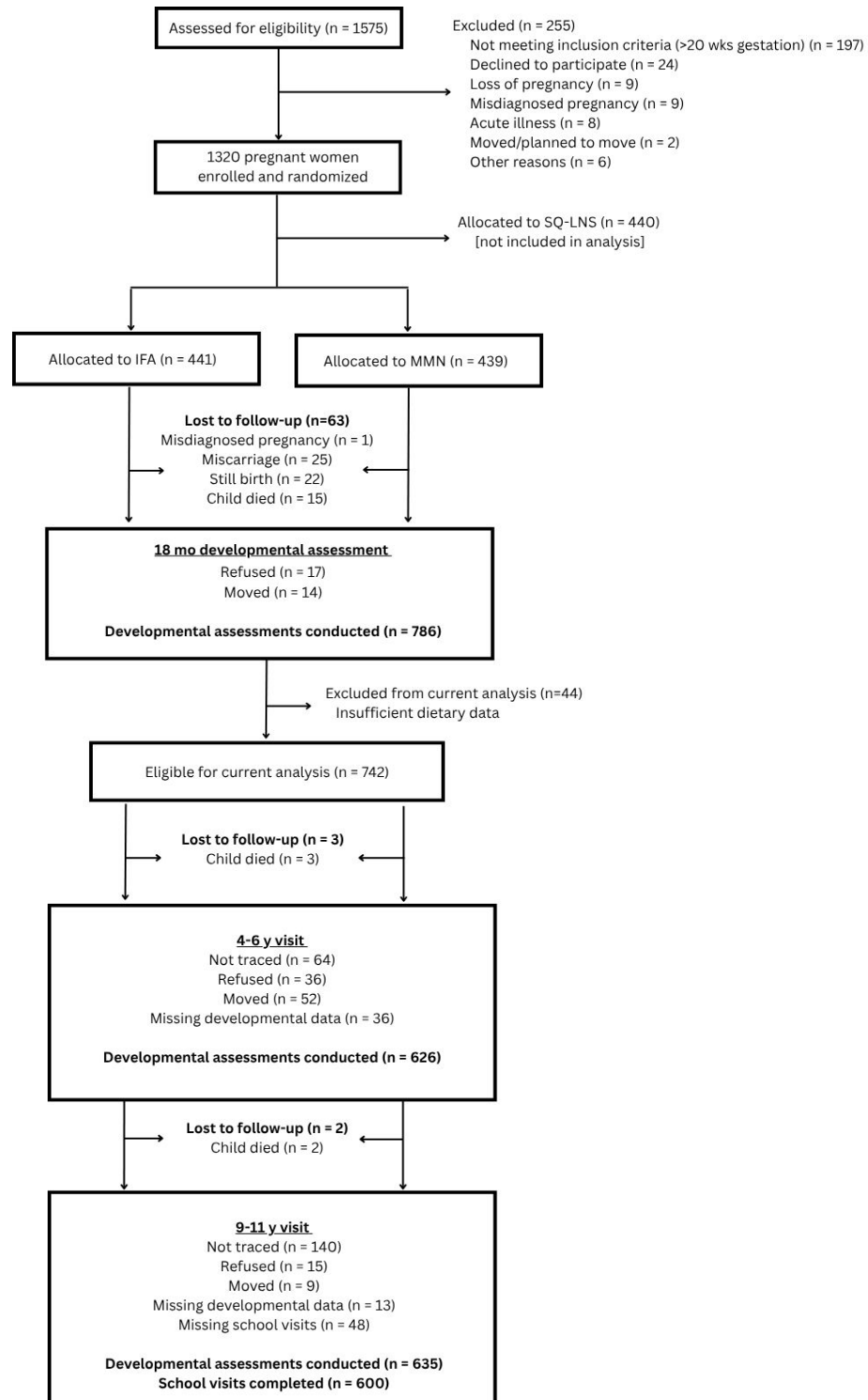
*For declarative memory outcome: Low DD (cumulative DD score 11-24) (n=360), Adequate DD (cumulative DD score 25-40) (n=104).

[†]All cognitive outcomes are reported as differences in z-scores. All social-emotional outcomes are reported as differences in mean scores from a potential range of 0-40 for total difficulties and 0-10 for all 5 SDQ subscales. Values are β coefficients (95% CIs) from fully adjusted linear regression models with dietary diversity z-score as the exposure. Models adjusted for child age at assessment, child sex, outcome enumerator, maternal age, maternal education, maternal pre-pregnancy BMI, parity, gestational age, birth weight, intervention group, household asset index, household food insecurity, and school quality.

SDQ-P: Strengths and Difficulties Questionnaire-Parent report; SDQ-T: Strengths and Difficulties Questionnaire-Teacher report; SDQ-C: Strengths and Difficulties Questionnaire-Child report.

3.9 Supplementary Materials

Figure S3.1: CONSORT diagram showing infants whose mothers were enrolled into the trial, and the reasons some infants were lost to follow-up



Chapter 4

Associations of early-life anemia and iron status with developmental outcomes

at 4-6 y and 9-11 y: a longitudinal study of Ghanaian preadolescents

4.1 Abstract

Background: Anemia and iron deficiency (ID) during the first 1000 days negatively affects child development. Infants in low- and middle-income countries, particularly males, are at high risk for anemia and ID.

Objective: We aimed to investigate the associations of early-life hemoglobin (Hb), zinc protoporphyrin (ZPP), and anemia/iron status with developmental outcomes at preschool age and preadolescence and whether these associations differed by child sex.

Methods: This is a secondary analysis of data from the iLiNS-DYAD-Ghana trial and its follow up studies. 1320 pregnant women were randomized to receive iron and folic acid (IFA), multiple micronutrient (MMN) or small-quantity lipid-based nutrient supplements (SQ-LNS) until delivery. Child Hb and ZPP concentrations were measured at 6 and 18 mo of age, and motor, cognitive and social-emotional development were assessed at 4-6 y and 9-11 y using a battery of tests (39 total outcomes). Multivariable regression analyses were restricted to the control group (i.e., IFA and MMN groups; n=657) because offspring of mothers in the SQ-LNS group also received SQ-LNS, containing 6 mg/d iron, from 6 to 18 mo of age.

Results: Mean $\pm$ SD Hb was 113.3 ± 9.9 g/L at 6 mo of age and 112.0 ± 10.7 g/L at 18 mo of age. Poorer iron status (higher ZPP) at 6 mo and lower Hb at 18 mo of age were associated with lower pre-academic skills at 4-6 y ($p < 0.035$). An inverse-U shaped relationship was found between Hb at 18 mo and intellect z-scores at 9-11 y ($p = 0.045$). Poorer iron status (i.e., lower Hb at 6 and 18 mo, higher ZPP at 6 mo) was associated with higher parent-reported conduct problems at 9-11 y ($p < 0.040$).

Conclusions: Overall, lower Hb and poorer iron status in early life were associated with worse cognitive and social-emotional outcomes at preschool age and preadolescence.

4.2 Introduction

Adequate nutrition during the first 1000 days of life is critical for infant survival and health. Iron-rich foods are particularly important during this time as infants' iron needs are very high yet their iron stores are usually depleted by 6 mo and after that they do not receive sufficient iron from breast milk alone (1). Infants with low iron intakes are at higher risk of developing iron-deficiency (ID) or iron-deficiency anemia (IDA), which could negatively affect motor, behavioral, and cognitive development, given the critical role iron plays in brain development (i.e., myelination, neurotransmitter synthesis and metabolism) (2-5). Furthermore, male infants are at higher risk of IDA than female infants possibly due to genetic or hormonal factors (6). Anemia, and particularly IDA, during infancy may have negative effects on children's long-term cognition and behavior yet few studies have examined this association specifically in geographical areas at highest risk of ID, such as in sub-Saharan Africa (7).

In 2017, about 35% of 6-59 mo old children in Ghana had anemia (using a hemoglobin (Hb) cutoff of <110 g/L), 22% had ID (serum ferritin <12 μ g/L), and 12% had IDA (Hb <110 g/L with serum ferritin <12 μ g/L) (8). The subgroup of 12-23 mo old children had the highest prevalences of anemia (46%), ID (34%) and IDA (22%) (8). Although anemia prevalence would likely be lower if the new recommended Hb cutoff of <105 g/L for children 6-23 months of age (9) is used, the fact that ID affected one in three children is cause for concern.

The International Lipid-based Nutrient Supplements (iLiNS) DYAD-Ghana trial provides a unique opportunity to investigate the association of infant anemia and iron status (indicated by

elevated ZPP concentration) with developmental outcomes in a cohort of Ghanaian children among whom about 12% had ID (zinc protoporphyrin (ZPP) >70 µmol/mol heme) at 6 mo of age (10). Previous research from this trial found that provision of small-quantity lipid-based nutrient supplements (SQ-LNS) to infants from 6 to 18 mo improved iron status at 18 mo of age (10) and behavioral function (e.g., lower social-emotional total difficulties scores, though not motor or cognitive scores) at 4-6 y (11). A prior study with this cohort also found that Hb concentration at 18 mo, but not Hb at 6 mo or at 4-6 y, was positively associated with cognitive (but not motor or social-emotional) development at 4-6 y (12). Since the associations of poorer 6 and 18 mo iron status with multiple developmental outcomes in early and middle childhood within this cohort have not been investigated, this analysis could help address questions about the longer-term impacts of iron in infancy.

The objective of this analysis was to investigate the associations of Hb concentration, ZPP concentration, and anemia/iron status at 6 and 18 mo of age with motor, social-emotional and cognitive outcomes at 4-6 y and 9-11 y of age. Our first hypothesis was that lower Hb and poorer iron status at 6 mo and 18 mo would be associated with worse developmental outcomes at 4-6 y and 9-11 y. Our second hypothesis was that poorer iron status would be more strongly associated with worse developmental outcomes among males than among females, given males' greater risk for ID.

4.3 Methods

Study design and participants

This is a secondary analysis study using data from the iLiNS DYAD-Ghana trial and its follow-up studies at 4-6 y and 9-11 y. The iLiNS DYAD-G trial was a randomized controlled

trial conducted between 2009 and 2014 in the semiurban Yilo and Lower Manya Krobo districts of the Eastern Region of Ghana. Details of the original trial design and methods (clinicaltrials.gov; NCT00970866) have been published previously (13, 14). In brief, 1320 women at least 18 y of age and no later than 20 wk pregnant were randomized into one of three intervention groups: 1) iron and folic acid (IFA), 2) multiple micronutrient (MMN), or 3) SQ-LNS. Women in the IFA group were assigned to receive a daily capsule containing 60 mg iron and 400 µg folic acid from enrollment until birth and then a daily placebo capsule containing 200 mg of calcium from birth to 6 mo postpartum. Women in the MMN group were assigned to receive a daily capsule containing 1-2 times the Recommended Daily Allowance (RDA) of 18 vitamins and minerals (including 20 mg iron and 400 µg folic acid) from enrollment to 6 mo postpartum. Women in the LNS group were assigned to receive daily 20 g SQ-LNS sachets, which contained 118 kilocalories, protein, essential fatty acids, the same vitamins and minerals as the MMN capsule, and 4 additional micronutrients (Ca, K, Mg, P), from enrollment to 6 mo postpartum. From 6 to 18 mo of age, children in the LNS group received daily 20 g SQ-LNS sachets, which provided 118 kilocalories, protein, essential fatty acids, and micronutrients (including 6 mg iron). Children in the IFA and MMN groups did not receive any supplementation. The current analysis was restricted to the control group (i.e., IFA and MMN groups) because offspring of mothers in the LNS group received SQ-LNS, containing 6 mg/d iron, from 6 to 18 mo of age. Ethical approval for the original trial and follow-up studies were obtained from the Institutional Review Board of the University of California Davis, the Ethics Committee for the College of Basic and Applied Sciences of the University of Ghana and the Ghana Health Service Ethical Review Committee. Follow-up visits were conducted in 2016, when children were 4-6 y old, and in 2021, when children were 9-11 y old.

Data collection

At enrollment into the original iLiNS-DYAD-Ghana trial, maternal and household information, including maternal age, education, parity, and household assets, was collected by trained field workers using a questionnaire. Maternal venous Hb concentration was measured by trained laboratory personnel at a clinic visit. Infant length and weight were measured within 48 hours of birth. Child Hb and ZPP were measured at 6 and 18 mo by trained laboratory personnel who collected blood by venipuncture (10). Within 2 minutes after the blood draw, Hb was measured using a digital Hemocue (HemoCue model 301). With a Hematofluorometer (Aviv Biomedical Co.), ZPP measurements were obtained using the original Aviv cover-slides and 3-level control material after red blood cells were washed three times with normal saline.

During the 4-6 y and 9-11 y follow-up studies, developmental outcome assessments were conducted by trained data collectors who were blinded to the treatment group, details of which have been published previously (11, 15). Fine motor function at 4-6 y was measured using the National Institutes of Health (NIH) Toolbox 9-hole pegboard test (16). To measure cognition at 4-6 y, a battery of assessments was administered. We assessed language ability using the Developmental Neuropsychological Assessment-II (NEPSY-II) body part naming and identification and comprehension of instructions subtests (17); visuospatial ability using the block design test (18, 19); declarative memory using the paired-associate task (20); executive function using the International Development and Early Learning Assessment (IDELA) head-toe subtest and visual search task (21); and pre-academic skills using the Parent's Evaluation of Developmental Status (PEDS) pre-academic subscale (22).

Cognition at 9-11 y was assessed using an additional battery of tools. We assessed general intellectual ability through a block design test based on the British Ability Scales II pattern construction subtest (18), the Wechsler Primary and Preschool Scale of Intelligence block design subtest (19) and the NEPSY-II (17); literacy with a letter, sentence, and word recognition test (20, 23); arithmetic ability through a test based on the Wechsler Intelligence Scale for Children (WISC) arithmetic task (24); and declarative memory, procedural memory, and executive function using the Rapid Assessment of Cognitive and Emotional Regulation (RACER) tasks (25). The RACER declarative memory task was developed based on the Paired-Associate Learning (PAL) paradigm. The RACER procedural memory task was developed based on the Serial Reaction Time (SRT) paradigm (26). The RACER inhibitory control task was based on the Simon Task, which is widely used to measure executive function (27).

Social-emotional skills were assessed using the parent-reported Strengths and Difficulties Questionnaire (SDQ-P) at 4-6 y and 9-11 y; teacher reports and child self-reports (SDQ-T, SDQ-C) were also measured at 9-11 y. The SDQ includes five subscales: 1) emotional symptoms, 2) conduct problems, 3) hyperactivity/inattention, 4) peer relationship problems, and 5) prosocial behavior; the scores from subscales 1-4 were summed to generate a “total difficulties score” with higher scores indicating greater problems (28, 29).

In addition, we assessed caregiving and stimulation available to children using the Home Observation for the Measurement of the Environment (HOME) Inventory (30). We used the Early Childhood version for the 4-6 y follow-up study and the Middle Childhood version for the 9-11 y follow-up study. Both versions were adapted to the local context in Ghana. Maternal depression was assessed using the Edinburgh Postnatal Depression Scale (EPDS) at 6 mo postpartum (31) and using the Center for Epidemiological Studies Depression Scale at the 9-11 y

follow-up (32). We assessed child pubertal development at 9-11 y of age using the youth self-reported Petersen Pubertal Development Scale (33).

Sample size and statistical analyses

A statistical analysis plan was posted to Open Science Framework (<https://osf.io/6dxs4>) before conducting analyses. Analyses were performed using Stata (version 18). For this analysis, we used data from children in the non-LNS groups (i.e., maternal IFA and maternal MMN groups) because children in these two groups did not directly receive iron supplementation during the trial, whereas infants in the LNS group received daily SQ-LNS containing 6 mg of iron from 6 to 18 mo of age. Furthermore, previous research from the iLiNS-DYAD-Ghana trial found that Hb concentrations did not differ between the IFA and MMN groups at 6 mo of age (10). Among the non-LNS groups with 4-6 y and 9-11 y outcome data, approximately 650 had Hb and ZPP measured at 6 mo and 18 mo of age. This sample size provided 80% power to detect associations between Hb or ZPP and continuous outcomes with a strength of correlation of 0.11 or larger at a significance level of $p < 0.05$.

Our primary exposure variables were child Hb concentration (g/L), ZPP ($\mu\text{mol/mol}$ heme) and anemia/iron status (categorical). Using the Hb and ZPP values, we created four categories for group comparisons: 1) anemia ($\text{Hb} < 105 \text{ g/L}$), 2) ID ($\text{ZPP} > 70 \mu\text{mol/mol heme}$), 3) IDA ($\text{Hb} < 105 \text{ g/L}$ plus $\text{ZPP} > 70 \mu\text{mol/mol heme}$), or 4) no anemia, no iron deficiency ($\text{Hb} \geq 105 \text{ g/L}$ and $\text{ZPP} \leq 70$), which was the reference group. These Hb values reflect the most recent WHO guideline on Hb cutoffs to define anemia in children 6-23 mo of age (9). The ZPP cutoff value of $70 \mu\text{mol/mol heme}$ is consistent with previous literature examining ID indicators in preschool-

aged children (10, 34). ZPP distributions were skewed and were therefore log-transformed before analysis.

Our primary outcomes for this analysis were motor score at 4-6 y, social-emotional SDQ-P total difficulties score at 4-6 y and 9-11 y, and cognitive factor score at 4-6 y and 9-11 y. The cognitive factor score at 4-6 y was calculated using the principal-axis factoring method including the following outcome measures: language, visuospatial ability, declarative memory, executive, pre-academic skills. The cognitive factor score at 9-11 y was also calculated using the principal-axis factoring method including general intellectual ability, literacy, arithmetic, declarative memory, procedural memory, and executive function. Secondary outcomes for this study included each of the 5 subscale scores of the SDQ-P (emotional symptoms, conduct problems, hyperactivity/inattention, peer relationship problems, prosocial behavior) at 4-6 y and 9-11 y and the SDQ-T and SDQ-C at 9-11 y, in addition to the cognitive scores at 4-6 y and 9-11 y listed above.

For all cognitive outcome scores, we calculated the age-adjusted z-score as the standardized residual of age predicting the score. This z-score represents the deviation of the child's score in units of SD from the score expected for the child's age. For social-emotional outcomes, we reported unadjusted mean scores since the SDQ is a commonly used screening tool for measuring psychosocial problems in children (28).

Multivariable linear regression models were used for all continuous outcomes. Because previous work has suggested a non-linear association between Hb and development (35), we included a squared term to allow for a potentially quadratic relationship when Hb was the exposure variable. If significant ($p < 0.1$), then this squared Hb term was retained in regression models. All models were minimally adjusted for child age at time of development assessment,

child sex, and outcome assessment enumerator. In fully adjusted models, we included covariates including maternal education, maternal depression, parity, household assets, household food insecurity, HOME scores, school quality and child pubertal development scores if they were associated with the outcome in bivariate testing ($p < 0.1$). For covariates that were highly correlated (e.g., $r > .50$) with each other and were associated with the outcome, we only included the covariate that was most strongly correlated with the outcome. For moderation testing, we included an additional interaction term of exposure with the pre-specified modifier (i.e., child sex) in both the minimally adjusted and fully adjusted models.

4.4 Results

Among children in the control group, we re-enrolled 662 and obtained developmental data from 626 children (77% of eligible) at 4-6 y and re-enrolled 648 and obtained developmental data from 635 children (78% of eligible) at 9-11 y (Supplementary figure 1); 564 were assessed at both time points and 698 were assessed at either time point. Of these 698 children, 570 had Hb or ZPP data at 6 mo and 600 had Hb or ZPP at 18 mo. The resulting analytic sample size was 657 with at least one exposure and outcome measurement combination.

Baseline characteristics of participants in this analytic sample are listed in Table 1. Similar to what has been reported in the larger sample previously (10), in the control group the mean $\pm$ SD Hb was 113 ± 9.9 g/L at 6 mo and 112 ± 10.7 g/L at 18 mo, and median (Q1, Q3) ZPP was 58 (36, 76) $\mu\text{mol/mol}$ heme at 6 mo and 56 (39, 81) $\mu\text{mol/mol}$ heme at 18 mo. At 6 mo, 7% of children in the control group had anemia (without ID), 23% had ID (without anemia), and

11% had IDA. At 18 mo, 11% had anemia (without ID), 20% had ID (without anemia), and 14% had IDA.

Cognitive outcomes

Hb at 6 mo was not associated with cognitive outcomes at 4-6 y (language, visuospatial ability, declarative memory, visual search test, head to toe inhibition, and pre-academic scores) or 9-11 y (intellect, literacy, arithmetic, declarative memory, procedural memory, executive function) (Supplementary figure 2). However, Hb at 18 mo was positively associated with pre-academic skills at 4-6 y ($p=0.013$) and negatively associated with procedural memory at 9-11 y ($p=0.014$) (Figure 1). We also found a non-linear association between Hb at 18 mo and intellect at 9-11 y, in which lower outcome scores were apparent at both low and high Hb concentrations ($p=0.045$) (Figure 1). No other associations were statistically significant. We did not find any associations of anemia/ID with cognitive outcomes at 4-6 y or 9-11 y.

In contrast to what was observed with Hb, we found associations between developmental outcomes and ZPP at 6 mo (Figure 2), but not 18 mo (Supplementary figure 3). Higher ZPP concentration, indicating poorer iron status, at 6 mo was associated with lower pre-academic skills at 4-6 y ($p=0.034$) as well as lower literacy scores at 9-11 y ($p=0.038$) (Figure 2). No other associations of ZPP or anemia/ID with cognitive outcomes at 4-6 y or 9-11 y were significant.

Child sex did not modify any associations of Hb at 6 or 18 mo with cognitive outcomes at 4-6 y or 9-11 y. While the main association between ZPP at 6 mo and cognitive factor score at 9-11 years was not significant ($p=0.120$), we found a significant interaction with child sex (p -interaction=0.027). In males, higher ZPP concentrations were associated with lower cognitive

factor scores ($p=0.026$), but this relation was not seen in females (Supplementary figure 4).

Although child sex modified associations between anemia/ID groups at 6 mo and language at 4-6 y (p -interaction= 0.046) as well as procedural memory at 9-11 y (p -interaction= 0.049), results were inconsistent (Supplementary table 1). Among females, those in the IDA group had higher language scores ($p=0.008$) compared to the reference group (no anemia, no ID) and there were no group differences for procedural memory (Supplementary table 1). Among males, compared to the reference group, those in the ID group had higher language scores ($p=0.019$), whereas those in the ID group ($p=0.049$) and IDA group ($p=0.031$) both had lower procedural memory scores (Supplementary table 1). There were no other significant interactions with child sex for associations between the categorical variable and cognitive outcomes.

Social-emotional outcomes

Hb at 6 and 18 mo were negatively associated with parent-reported (SDQ-P) conduct problems at 9-11 y ($p=0.015$ and $p=0.012$, respectively), but not the SDQ-P total difficulties score, emotional symptoms, hyperactivity/inattention, peer relationship problems, or prosocial behavior (Figure 3). In addition, higher ZPP at 6 mo (indicating poorer iron status) was associated with more conduct problems at 9-11 y ($p=0.040$), but not with other SDQ-P outcomes (Figure 4). Hb and ZPP at 6 mo were not significantly associated with any SDQ-P outcomes at 4-6 y (Supplementary figure 5) nor with any SDQ-T or SDQ-C outcomes at 9-11 y (Supplementary figures 6-7). Although anemia/ID at 18 mo was associated with SDQ-P and SDQ-T conduct problems at 9-11 y ($p=0.029$ and $p=0.021$, respectively), the directions were inconsistent (Supplementary table 2). Compared to the reference group, the anemia group had higher SDQ-P conduct problems ($p=0.003$), but lower SDQ-T conduct problems ($p=0.025$) (Supplementary

table 2). There were no differences when comparing the ID or IDA groups with the reference group for either outcome.

Hb at 18 mo was associated with teacher-reported (SDQ-T) peer relationship problems at 9-11 y ($p=0.026$) in a non-linear pattern such that higher problem scores were observed at lower and higher Hb concentrations (Figure 4). Anemia/ID at 18 mo was associated with SDQ-T prosocial behavior at 9-11 y ($p=0.009$) but again, the results were inconsistent (Supplementary table 2). Compared to the reference group, the anemia group had higher prosocial behavior scores ($p=0.026$) and the IDA group had lower prosocial behavior scores ($p=0.048$) (Supplementary table 2). The ID group did not differ from the reference group. There were no other statistically significant associations of Hb at 18 mo or anemia/ID with SDQ-T or SDQ-C outcomes at 9-11 y or SDQ-P outcomes at 4-6 y (Supplementary figure 8). Furthermore, ZPP at 18 mo was not associated with any social-emotional outcomes at 4-6 y or 9-11 y (Supplementary figures 9-10).

Effect modification by child sex was significant for associations of ZPP at 18 mo with SDQ-P total difficulties score (p for interaction= 0.024) and SDQ-P prosocial behavior (p for interaction= 0.006) at 9-11 y, but the results were unexpected. Among males, those with higher ZPP values at 18 mo had lower total difficulties scores ($p=0.017$) and higher prosocial behavior scores ($p=0.007$) at 9-11 y than those with lower ZPP (Supplementary figure 11). These associations were not significant in females. Child sex also modified the associations of anemia/ID at 18 mo with SDQ-P total difficulties score (p for interaction= 0.018) at 4-6 y and SDQ-P hyperactivity/inattention at 9-11 y (p for interaction= 0.030) (Supplementary table 1). Among females, those with anemia had higher SDQ-P total difficulties scores at 4-6 y ($p=0.030$) compared to the reference group while there were no differences with the ID or IDA groups. No

group differences were seen among males. Among males, those with ID at 18 mo had lower SDQ-P hyperactivity/inattention scores at 9-11 y ($p=0.044$) compared to the reference group. The anemic or ID groups did not differ from the reference group nor were there any group differences found among females. Finally, there was no evidence that child sex modified any associations of Hb or ZPP at 6 or 18 mo with SDQ-T or SDQ-C outcomes.

4.5 Discussion

In this cohort of Ghanaian children, we found that early-life Hb, ZPP and anemia/ID were associated with certain cognitive and social-emotional outcomes at 4-6 y and 9-11 y. For cognitive outcomes, poorer iron status at 6 mo (high ZPP) and low Hb at 18 mo were associated with lower pre-academic skills at 4-6 y. Similarly, higher ZPP concentration at 6 mo was associated with lower literacy scores at 9-11 y. However, we also found evidence of a non-linear relationship between Hb at 18 mo and intellect at 9-11 y, in which lower outcome scores were apparent at both low and high Hb concentrations. For social-emotional outcomes, lower Hb at 6 and 18 mo and poorer iron status at 6 mo were all associated with more conduct problems at 9-11 y, but not at 4-6 y.

Previously, we reported that Hb at 18 mo (but not at 6 mo or 4-6 y) was positively associated with an overall cognitive factor score at 4-6 y (12). Here, we extend this work by examining associations of Hb at 6 mo and 18 mo with specific cognitive assessments of language, visuospatial ability, declarative memory, executive function, and pre-academic skills at 4-6 y and find generally consistent results. Our findings are also consistent with other longitudinal studies (36, 37) showing that early-life anemia or IDA is associated with poorer

cognitive outcomes such as lower IQ scores in childhood (38), more frequent grade repetition in preadolescence (39), and impaired executive functioning and memory in early adulthood (40). In our previous (12) and current analyses, Hb at 6 mo was not associated with cognitive outcomes at 4-6 y or 9-11 y; this may be because Hb at 6 mo is strongly influenced by iron stores at birth, but after 6 mo, iron intake and absorption may be insufficient to meet infants' needs (41).

Anemia and IDA tend to be more prevalent in children 12-18 months (42), which may explain why we found associations of Hb at 18 mo, but not at 6 mo, with cognitive outcomes at 4-6 y and 9-11 y.

The inverse-U shaped relationship we found between Hb at 18 mo and intellect scores at 9-11 y is consistent with prior evidence indicating that associations of Hb with development outcomes may be nonlinear (35, 43-45). A study of healthy, term infants in the United Kingdom examining Hb at 8 mo of age and motor scores at 18 mo showed a similar inverse-U shaped association, in which lower motor scores were seen at low and high Hb concentrations (35). Similarly, in two-year-old Canadian children, researchers found a non-linear association between serum ferritin and cognitive function (as measured using the Early Learning Composite scores) (45). Few studies have examined potential negative consequences of high Hb concentrations in early-life and further investigation into the underlying mechanisms of this relation is warranted.

We previously reported no association between Hb at 6 or 18 mo and social-emotional development at 4-6 y, however we had only examined the parent-report SDQ total difficulties score, not the individual subscales (12). In the present analysis, we also did not find associations between early Hb and SDQ total difficulties scores at 4-6 y or 9-11 y, but Hb at 6 mo and 18 mo and ZPP at 6 mo were significantly associated with SDQ-P conduct problem subscale scores at 9-11 y. In all cases, higher conduct problems scores were seen with lower Hb concentrations and

higher ZPP concentrations (indicating poorer iron status). This finding is consistent with two studies of infants treated for ID that reported more behavioral problems such as anxiety/depression, social problems, attention problems and risk taking at 11 to 14 y of age compared to children who had not been iron deficient as infants (39, 46).

There are two potential mechanisms for this observed association. First, the “functional isolation hypothesis” posits that because children with ID are warier and less engaging with caregivers, caregivers in turn provide a less stimulating environment for the child, which can impair their development (47). Second, animal models of ID have documented alterations in the synthesis and function of several neurotransmitter systems, particularly dopamine, which plays a major role in behavioral activation and inhibition and positive affect (2). When comparing the relationships between iron status and the SDQ as reported by the parent, teacher or child, we found significant associations with conduct problems only with the parent report SDQ but not with the others. Previous research has suggested that generally parents report more problem behaviors in their children than teachers (48, 49), and so it is possible that they may have been more sensitive to observing difficulties than teachers.

We examined interactions with child sex for all of the associations in this analysis, given previous evidence that male infants may be at higher risk of ID compared to female infants (6). Of the 234 statistical tests we conducted with a child sex interaction, only seven were statistically significant (p for interaction < 0.05) and among those significant interactions, we did not find any consistent pattern. The associations of ZPP at 18 mo with total difficulties score and prosocial behavior at 9-11 y among males (but not females) were in the opposite direction than expected, in that better outcome scores were seen with poorer iron status. This leads us to believe that these observations are likely due to chance.

A major strength of this study is the extensive longitudinal data collected on this large cohort of mother-infant pairs in Ghana. This gave us the unique opportunity to examine how early-life anemia and iron status relates to motor, cognitive and social-emotional development outcomes at multiple time points (4-6 y and 9-11 y). We were also able to control for factors that could potentially confound these associations such as maternal education and depression, household food insecurity, HOME scores and school quality. There were several limitations to our study. We used Hb and ZPP measurements as indicators of iron status in the absence of other iron biomarker data such as serum ferritin and transferrin receptor measurements. Both Hb and ZPP may be influenced by inflammation but we were not able to adjust for this in the overall analysis because C-reactive protein (CRP) and alpha-1-acid glycoprotein (AGP) were measured only in a small subsample of children enrolled in the original trial. Another limitation is that our study sample had a lower prevalence of anemia, iron deficiency, and iron deficiency anemia compared to the national prevalences in Ghana (8) so our results may not be generalizable to the national level or other populations with different nutritional status.

4.6 Conclusion

Our findings contribute to the existing evidence that early-life Hb and iron status are associated with child development later in life. In general, lower Hb and poorer iron status were associated with worse cognitive and social-emotional outcomes, though the findings were domain-specific and some associations were non-linear. Further research is needed to investigate sensitive periods of exposure to anemia and ID during early-life and potential negative effects of high Hb concentrations on child development.

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4.8 Tables and Figures

Table 4.1: Characteristics among participants included in the analysis

	Control group (n=657)*
Baseline characteristics	
Maternal age, y	26.7 (5.4)
Pre-pregnancy BMI, kg/m ²	24.4 (4.5)
Maternal education, y	7.7 (3.4)
Maternal hemoglobin, g/L	111.3 (12.5)
Household asset index	0.1 (1)
Food insecurity score	2.6 (4.1)
Gestational age at delivery, wk	39.3 (1.8)
Child male, n (%)	312 (47)
6 mo and 18 mo measurements	
Child hemoglobin at 6 mo, g/L	113.3 (9.9)
Child hemoglobin at 18 mo, g/L	112.0 (10.7)
Child ZPP at 6 mo, µmol/mol heme	57.5 (44.0, 81.0)
Child ZPP at 18 mo, µmol/mol heme	56.0 (39.0, 81.0)
Prevalence of anemia (without ID) at 6 mo (%)	7
Prevalence of ID (without anemia) at 6 mo (%)	23
Prevalence of IDA at 6 mo (%)	11
Prevalence of anemia (without ID) at 18 mo (%)	11
Prevalence of ID (without anemia) at 18 mo (%)	20
Prevalence of IDA at 18 mo (%)	14
4-6 y and 9-11 y measurements	
Child age at 4-6 y follow-up, y	4.9 (0.6)
Child age at 9-11 y follow-up, y	9.9 (0.5)
EC-HOME score at 4-6 y follow-up	28.0 (4.5)
MC-HOME score at 9-11 y follow-up	35.2 (6.1)

BMI: body mass index; ZPP: zinc protoporphyrin; ID: iron deficiency; IDA: iron deficiency anemia; EC-HOME: Early Childhood Home Observation for the Measurement of the Environment Inventory; MC-HOME: Middle Childhood Home Observation for the Measurement of the Environment Inventory.

*Values are reported as mean (SD) or median (Q1, Q3) unless otherwise noted.

Figure 4.1: Associations of Hb at 18 mo with cognitive outcomes at 4-6 y and 9-11 y

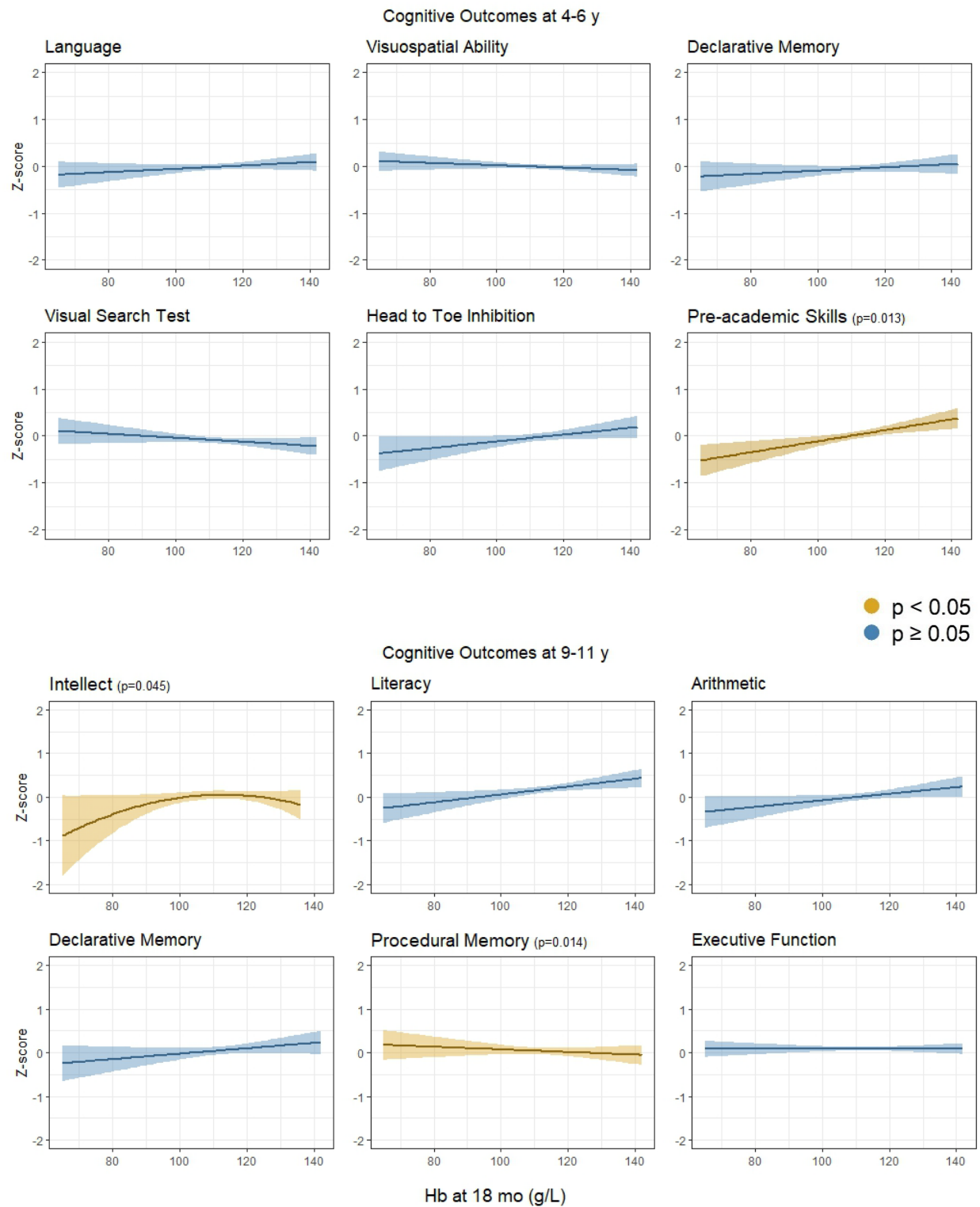


Figure 4.2: Associations of ZPP at 6 mo with cognitive outcomes at 4-6 y and 9-11 y

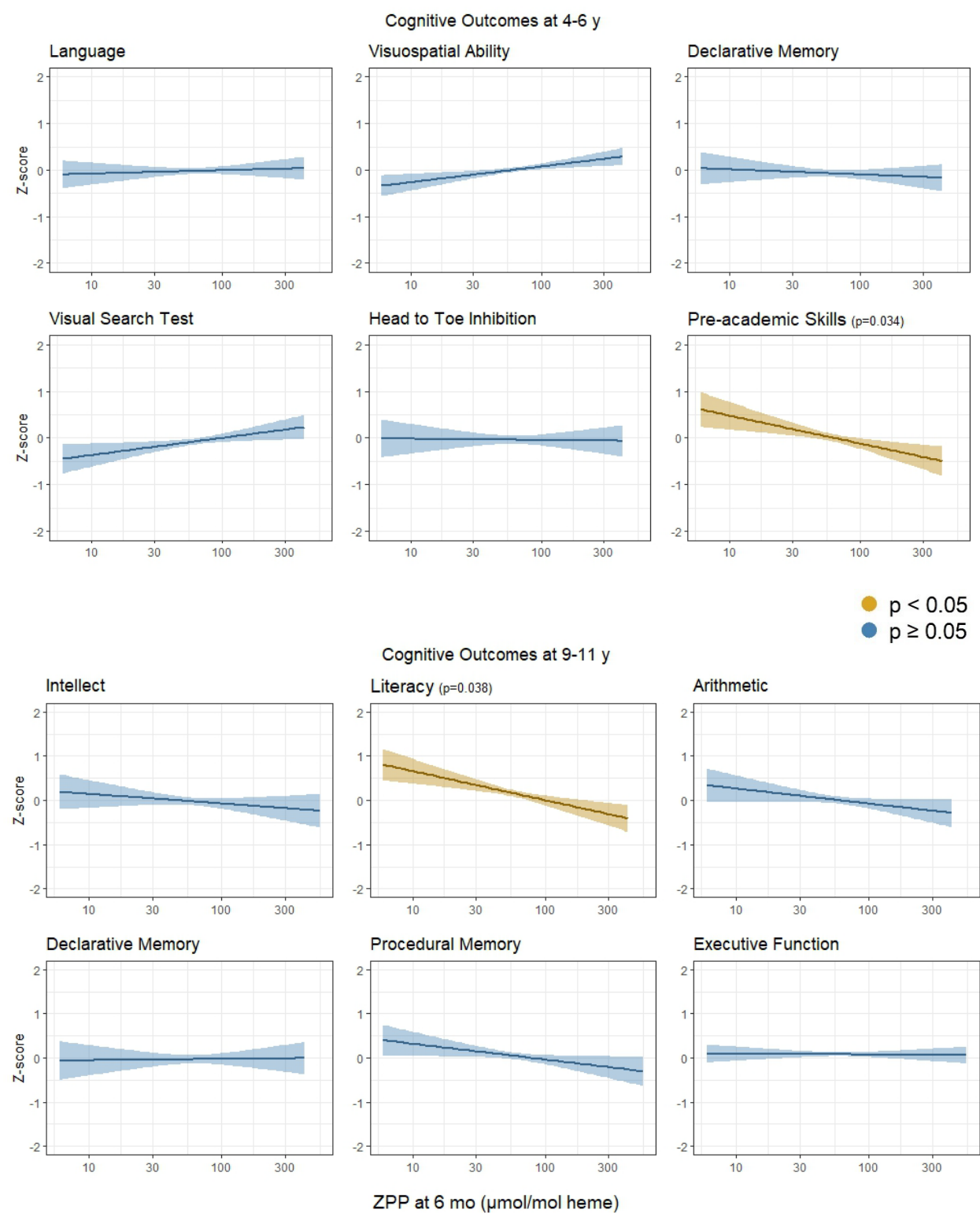


Figure 4.3: Associations of Hb at 6 and 18 mo with SDQ-P outcomes at 9-11 y

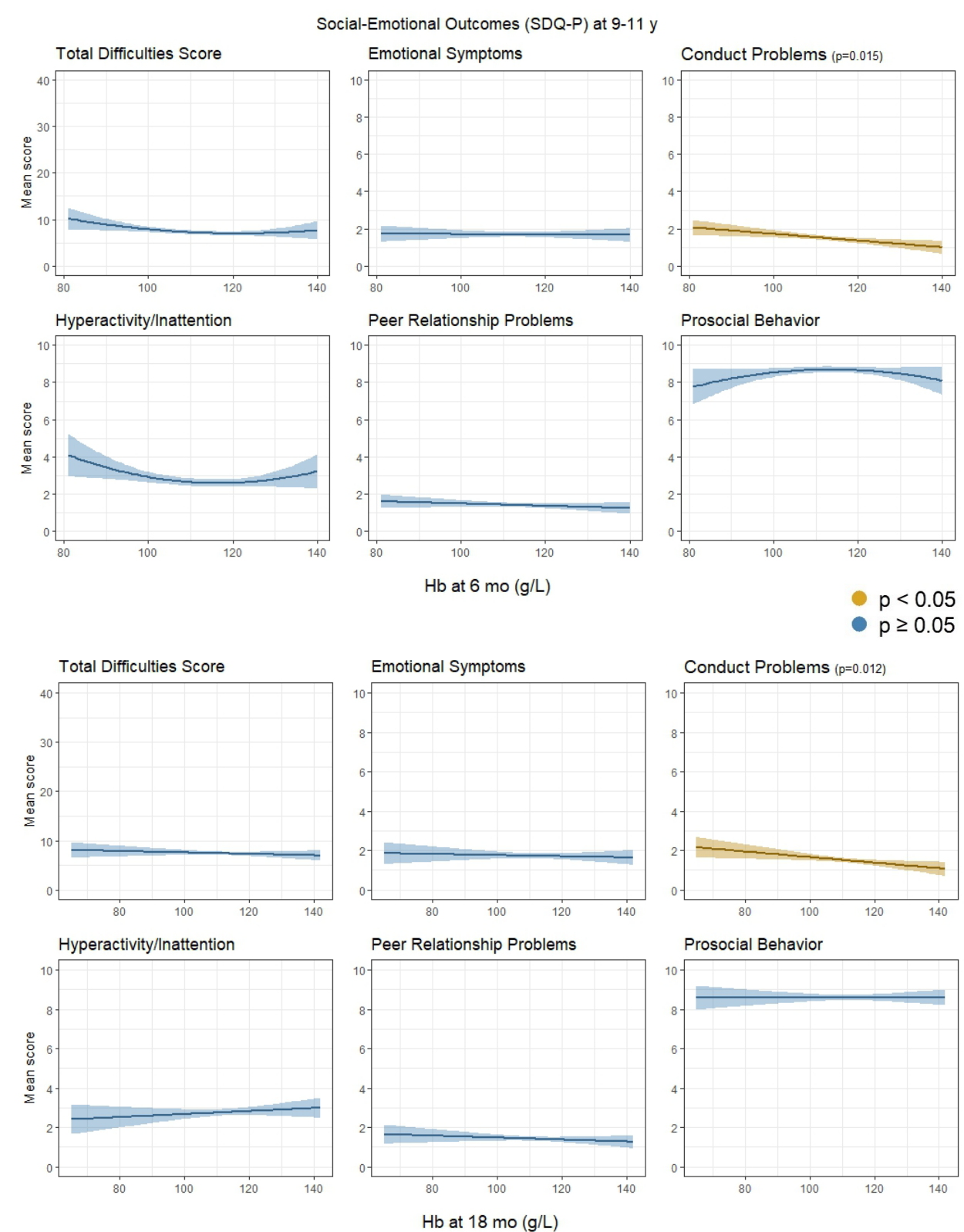
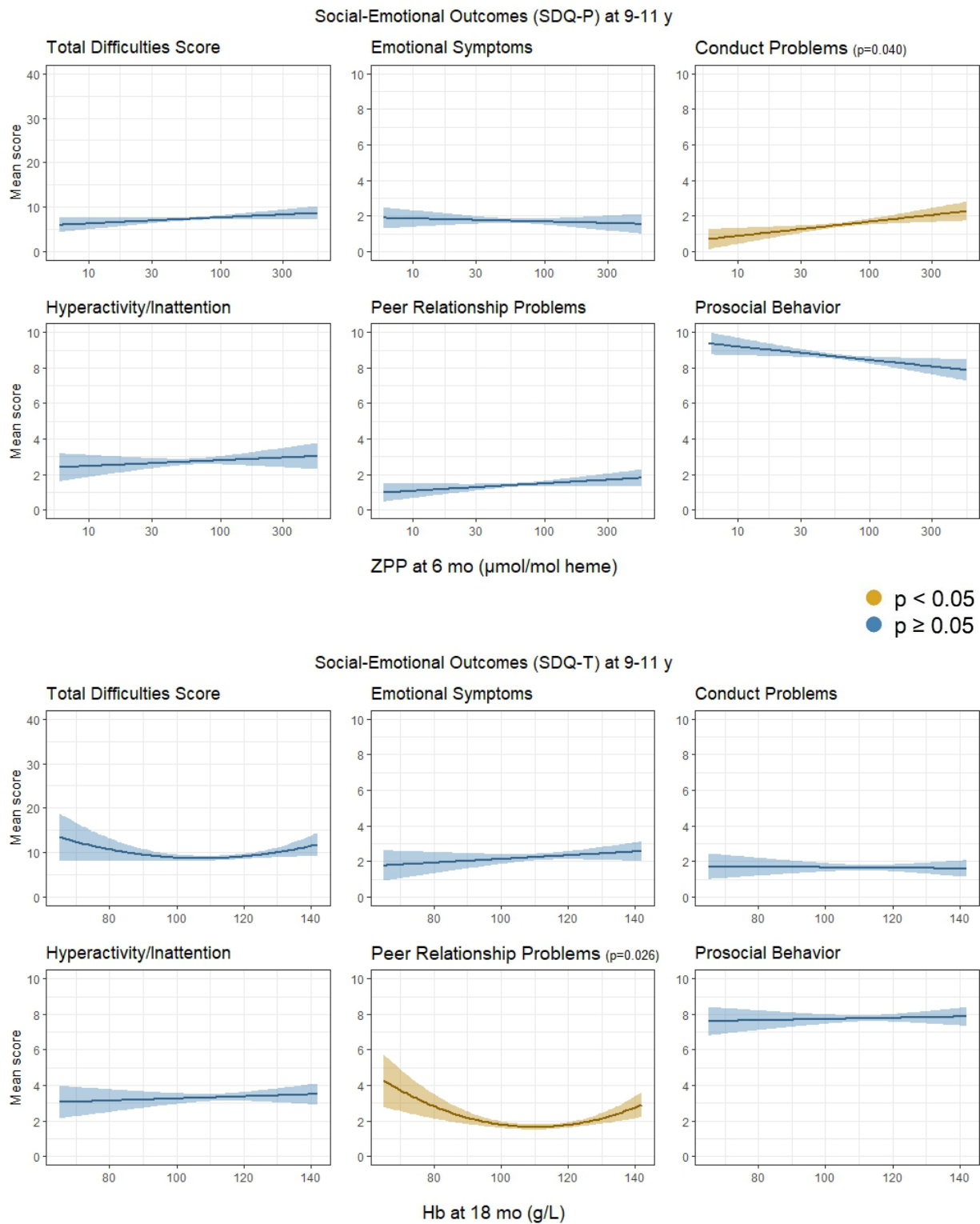


Figure 4.4: Associations of ZPP at 6 mo with SDQ-P outcomes and of Hb at 18 mo with SDQ-T outcomes at 9-11 y



4.9 Supplementary Materials

Figure S4.1: CONSORT diagram showing infants whose mothers were enrolled into the trial, and the reasons some infants were lost to follow-up

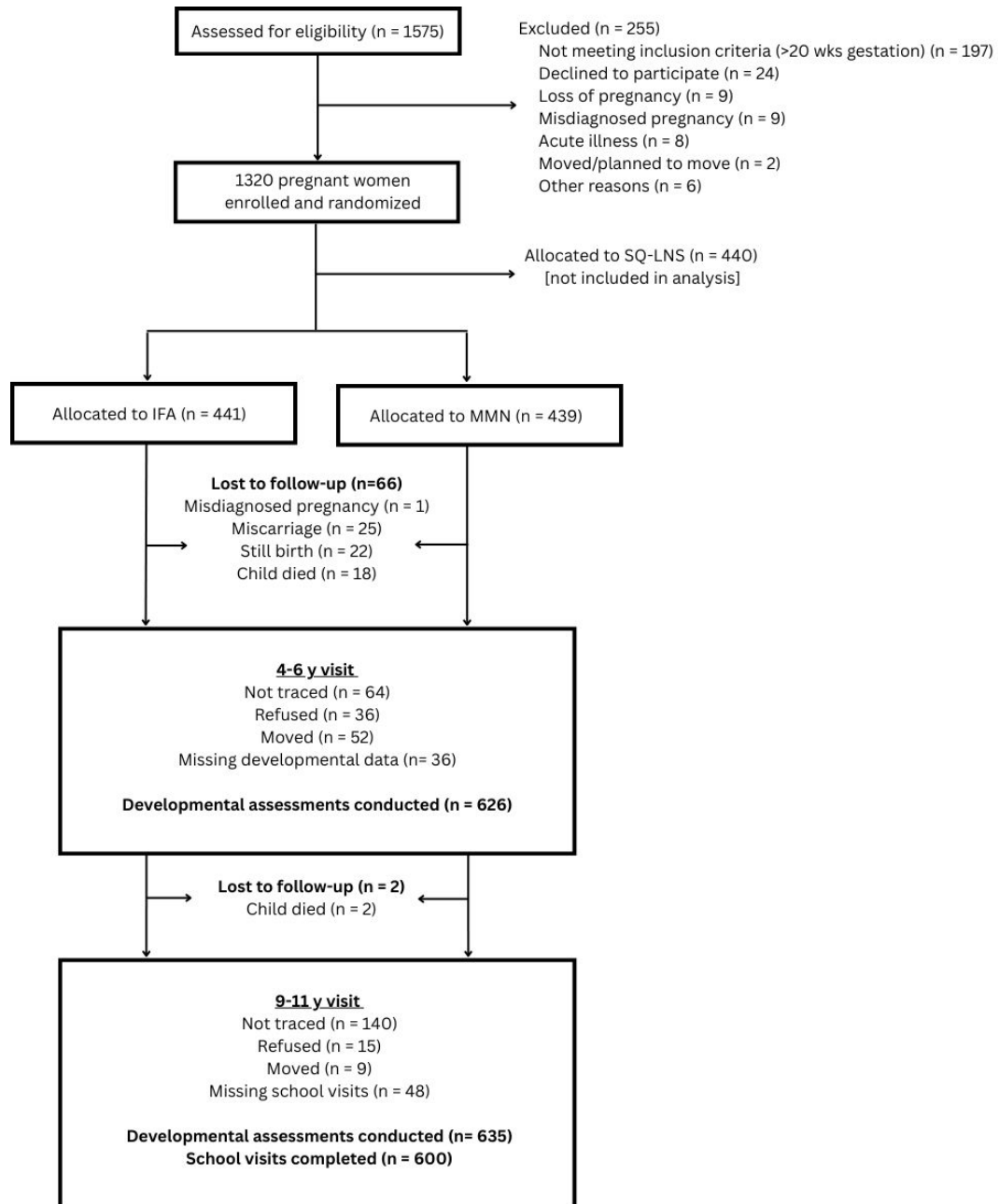


Figure S4.2: Associations of Hb at 6 mo with cognitive outcomes at 4-6 y and 9-11 y

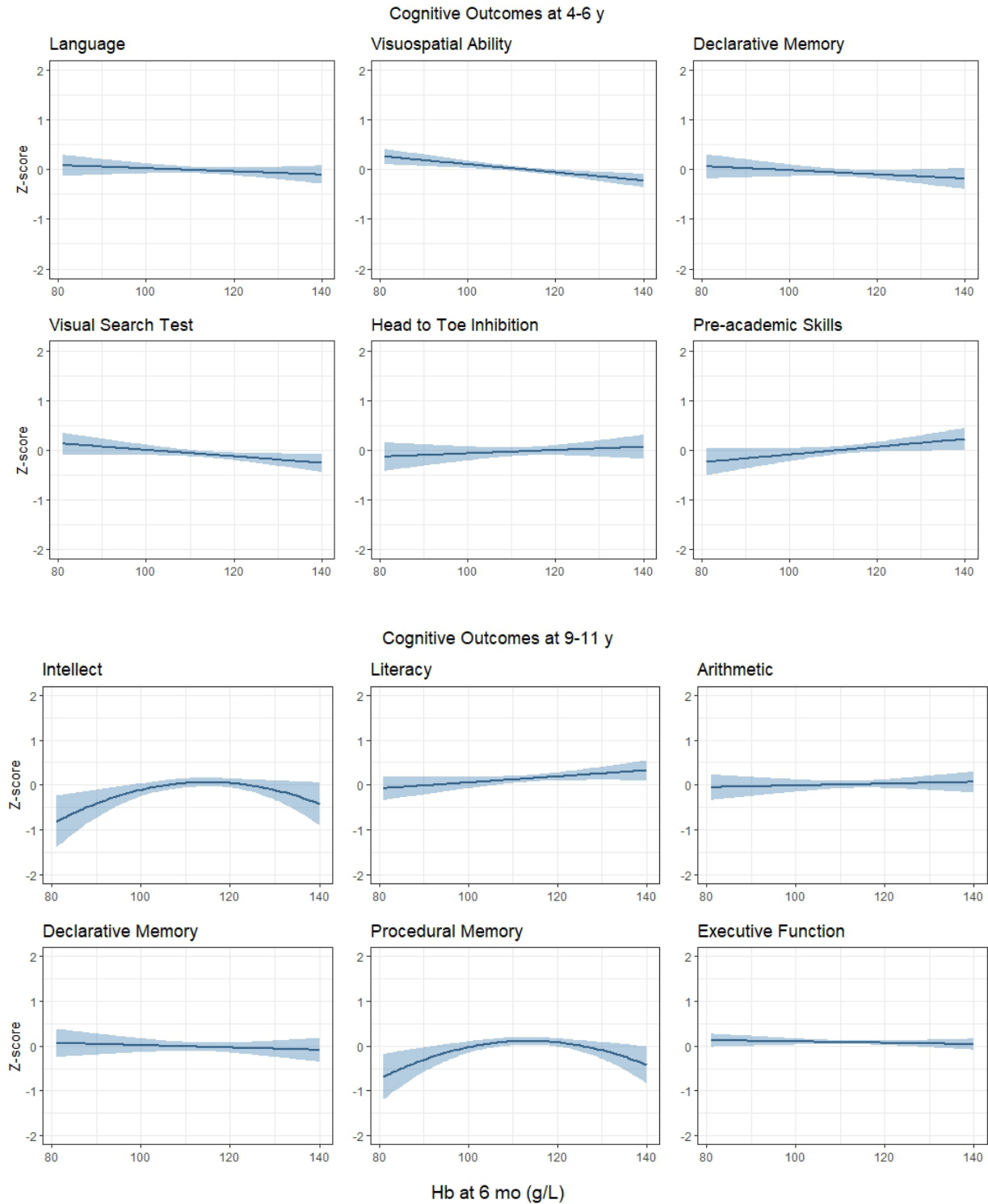


Figure S4.3: Associations of ZPP at 18 mo with cognitive outcomes at 4-6 y and 9-11 y

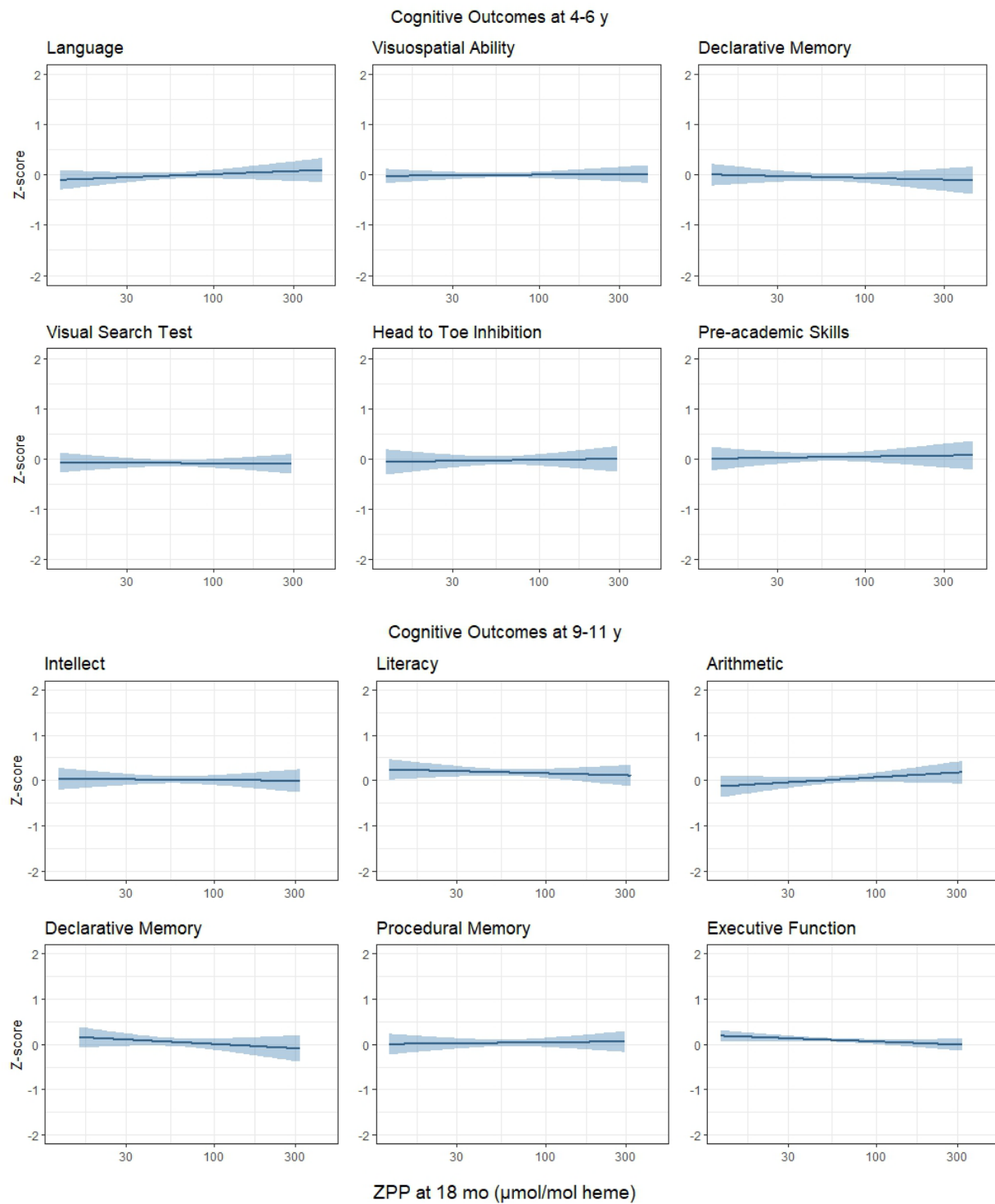


Figure S4.4: Child sex modified the association between ZPP at 6 mo and cognitive factor score at 9-11 y

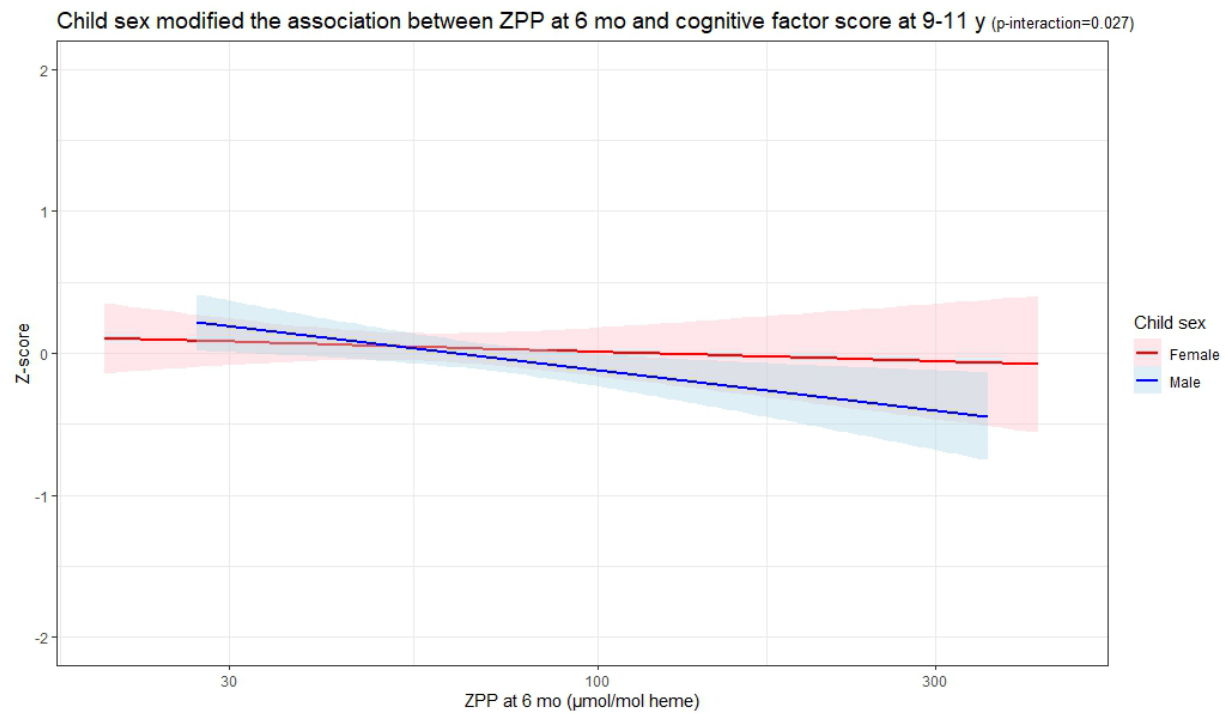


Table S4.1: Effect modification by child sex of the associations between anemia/ID groups at 6 mo and 18 mo and select development outcomes at 4-6 y and 9-11 y

	N	p-for- interaction (group x child sex)	Females β (95% CI)	p- value	Males β (95% CI)	p- value
Anemia/ID at 6 mo predicting:						
Language at 4-6 y		0.046				
No anemia, no ID (ref)	287		ref	—	ref	—
Anemia only	39		-0.20 (-0.50, 0.11)	0.207	0.11 (-0.26, 0.48)	0.542
Iron deficiency only	115		0.02 -0.19, 0.23)	0.851	0.27 (0.05, 0.50)	0.019 ^a
Iron deficiency anemia	60		0.49 (0.13, 0.85)	0.008 ^a	0.09 (-0.17, 0.36)	0.494
Procedural memory at 9-11 y		0.049				
No anemia, no ID (ref)	297		ref	—	ref	—
Anemia only	36		0.18 (-0.27, 0.64)	0.430	-0.30 (-0.79, 0.19)	0.225
Iron deficiency only	111		-0.23 (-0.54, 0.07)	0.137	-0.31 (-0.63, -0.00)	0.049 ^a
Iron deficiency anemia	51		0.41 (-0.13, 0.95)	0.134	-0.41 (-0.78, -0.04)	0.031 ^a
Anemia/ID at 18 mo predicting:						
SDQ-P total difficulties at 4-6 y		0.018				
No anemia, no ID (ref)	285		ref	—	ref	—
Anemia only	58		0.40 (0.04, 0.77)	0.030 ^a	0.08 (-0.32, 0.48)	0.704
Iron deficiency only	103		0.05 -0.23, 0.33)	0.739	-0.29 (-0.62, 0.05)	0.090
Iron deficiency anemia	74		-0.29 (-0.66, 0.08)	0.118	0.25 (-0.08, 0.57)	0.142
SDQ-P hyperactivity/inattention at 9-11 y		0.030				
No anemia, no ID (ref)	285		ref	—	ref	—
Anemia only	58		0.26 -0.51, 1.02)	0.507	-0.42 (-1.23, 0.38)	0.301
Iron deficiency only	111		0.30 (-0.26, 0.87)	0.289	-0.67 (-1.33, -0.02)	0.044 ^a
Iron deficiency anemia	75		-0.61 (-1.3, 0.12)	0.101	-0.12 (-0.81, 0.56)	0.727

^ap -value < 0.05

Values are β coefficients representing mean differences in outcome compared with the reference group. Estimates were obtained from linear regression models of anemia/ID at the indicated time point predicting indicated development outcome and adjusted for child age at assessment, child sex, outcome enumerator, maternal age, maternal education, baseline maternal Hb, maternal depression at 6 mo and 10 y, parity, gestational age, birth weight, intervention group, household asset index, household food insecurity, FCI score, HOME score at 5 y and 10 y, school quality, child pubertal score at 10 y.

Table S4.2: Associations between anemia/ID groups at 6 mo and 18 mo and select development outcomes at 9-11 y

	N	p-value	β (95% CI)	p-value
Anemia/ID at 18 mo predicting:				
SDQ-P conduct problems at 9-11 y		0.029		
No anemia, no ID (ref)	292		ref	—
Anemia only	58		0.58 (0.19, 0.97)	0.003 ^a
Iron deficiency only	112		0.01 (-0.28, 0.31)	0.924
Iron deficiency anemia	77		0.17 (-0.17, 0.51)	0.334
SDQ-T conduct problems at 9-11 y		0.021		
No anemia, no ID (ref)	278		ref	—
Anemia only	56		-0.59 (-1.11, -0.07)	0.025 ^a
Iron deficiency only	102		0.02 (-0.38, 0.43)	0.904
Iron deficiency anemia	68		0.41 (-0.06, 0.89)	0.089
SDQ-T prosocial behavior at 9-11 y		0.009		
No anemia, no ID (ref)	269		ref	—
Anemia only	56		0.63 (0.08, 1.19)	0.028 ^a
Iron deficiency only	100		0.14 (-0.30, 0.58)	0.536
Iron deficiency anemia	66		-0.52 (-1.04, -0.01)	0.048 ^a

^ap -value < 0.05

Values are β coefficients representing mean differences in outcome compared with the reference group. Estimates were obtained from linear regression models of anemia/ID at the indicated time point predicting indicated development outcome and adjusted for child age at assessment, child sex, outcome enumerator, maternal age, maternal education, baseline maternal Hb, maternal depression at 6 mo and 10 y, parity, gestational age, birth weight, intervention group, household asset index, household food insecurity, FCI score, HOME score at 5 y and 10 y, school quality, child pubertal score at 10 y.

Figure S4.5: Associations of Hb and ZPP at 6 mo with social-emotional outcomes (SDQ-P) at 4-6 y

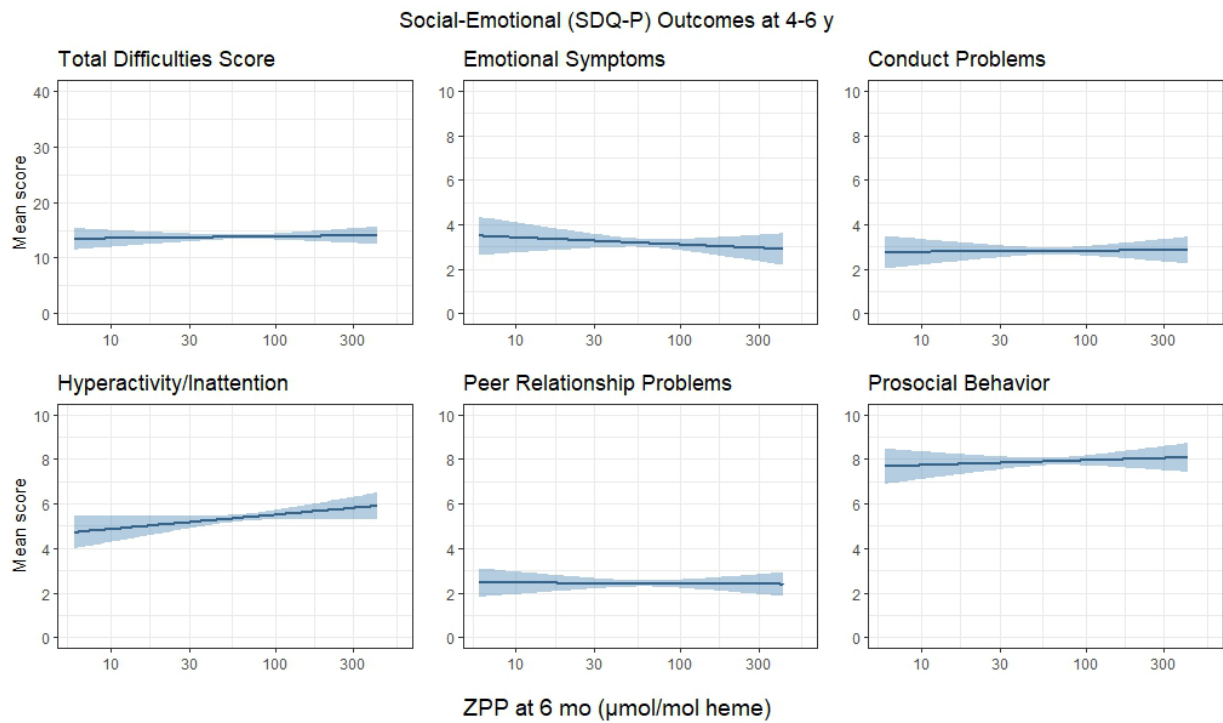
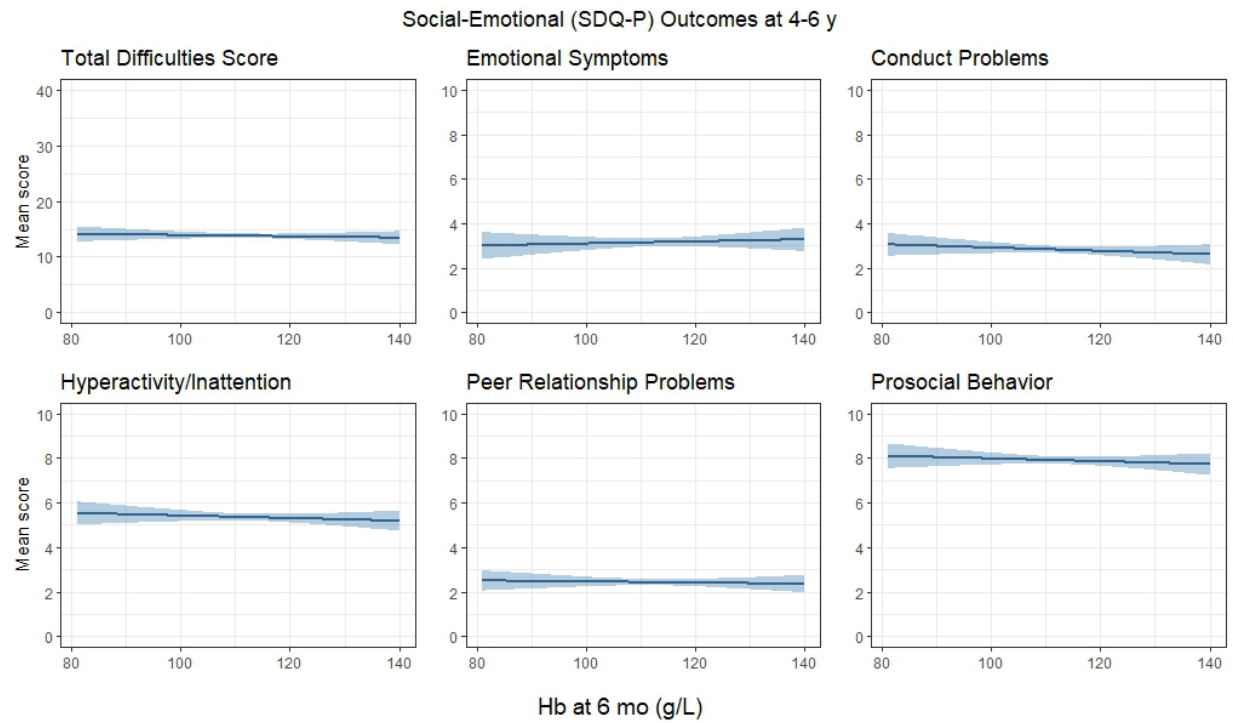


Figure S4.6: Associations of Hb at 6 mo with social-emotional outcomes (SDQ-T and SDQ-C) at 9-11 y

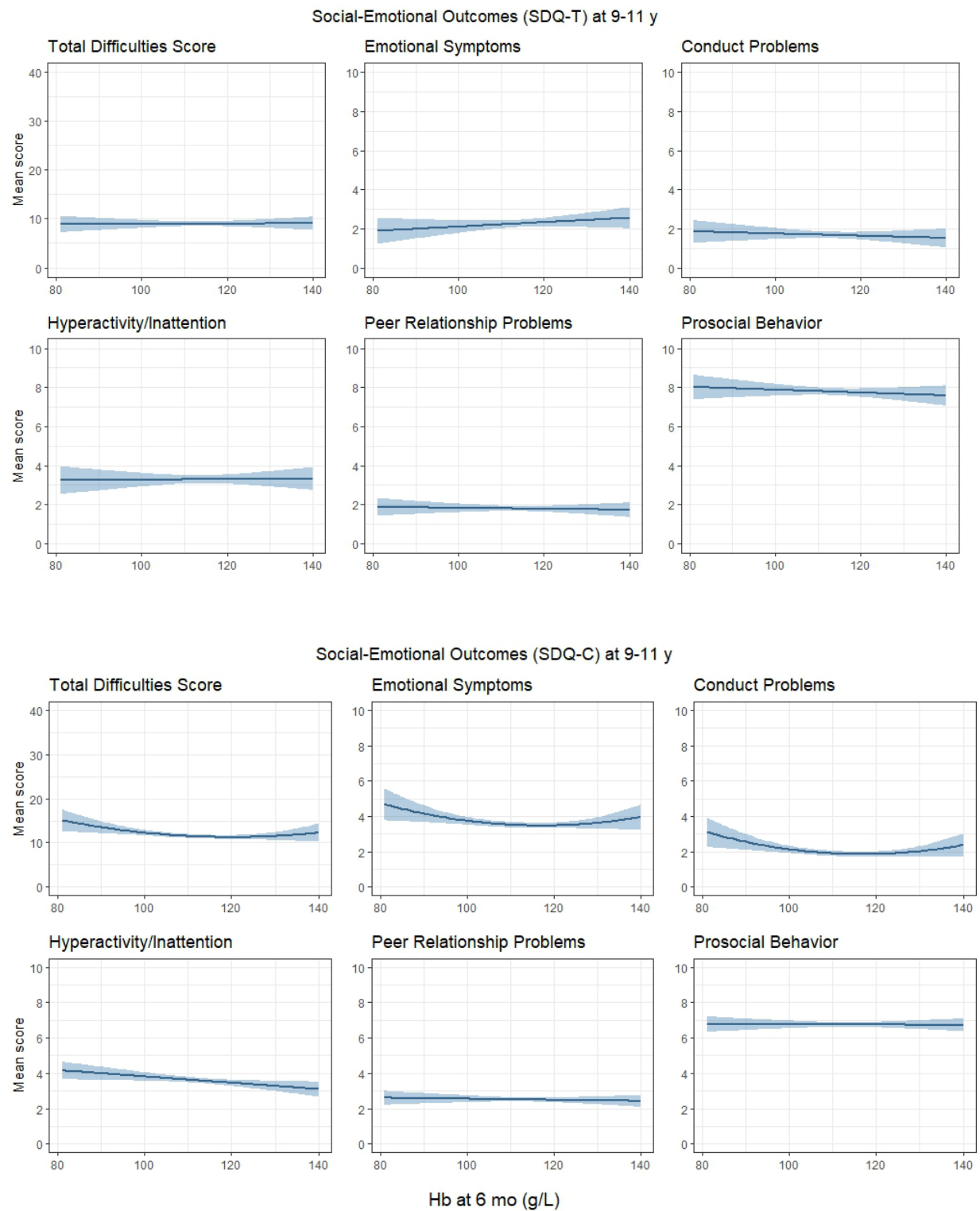


Figure S4.7: Associations of ZPP at 6 mo with social-emotional outcomes (SDQ-T and SDQ-C) at 9-11 y

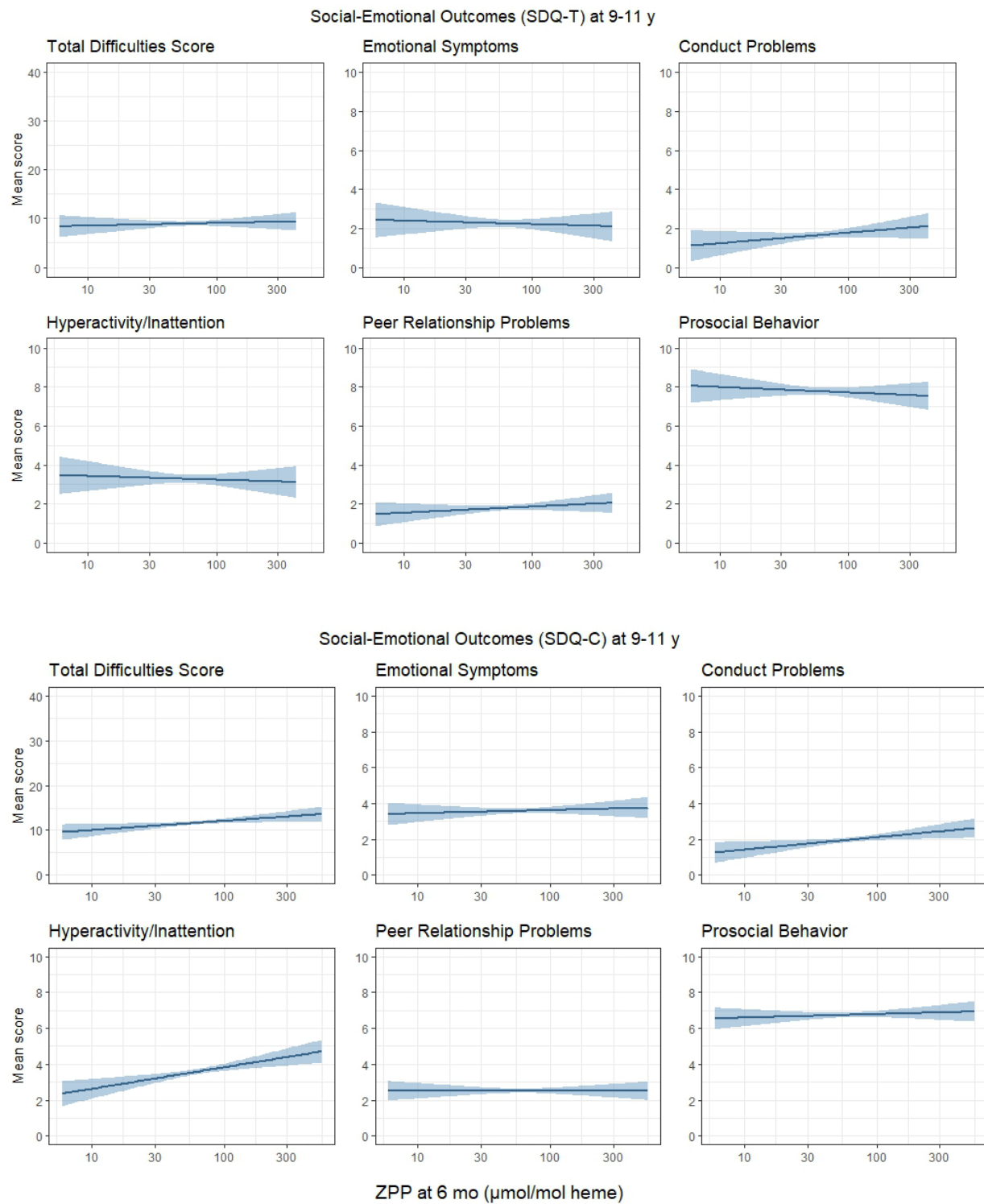


Figure S4.8: Associations of Hb at 18 mo with social-emotional outcomes (SDQ-P) at 4-6 y and (SDQ-C) at 9-11 y

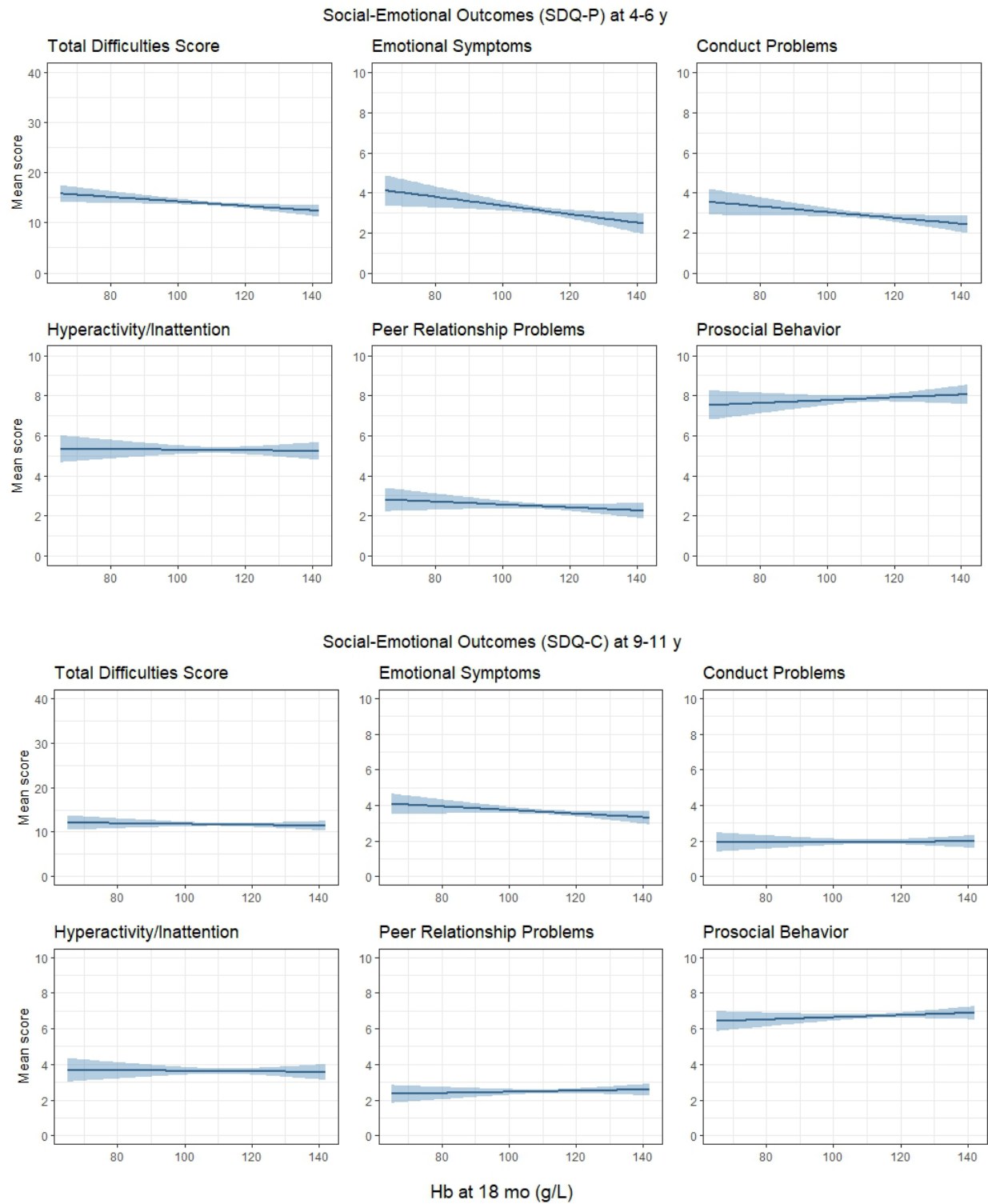


Figure S4.9: Associations of ZPP at 18 mo with social-emotional (SDQ-P) outcomes at 4-6 y and 9-11 y

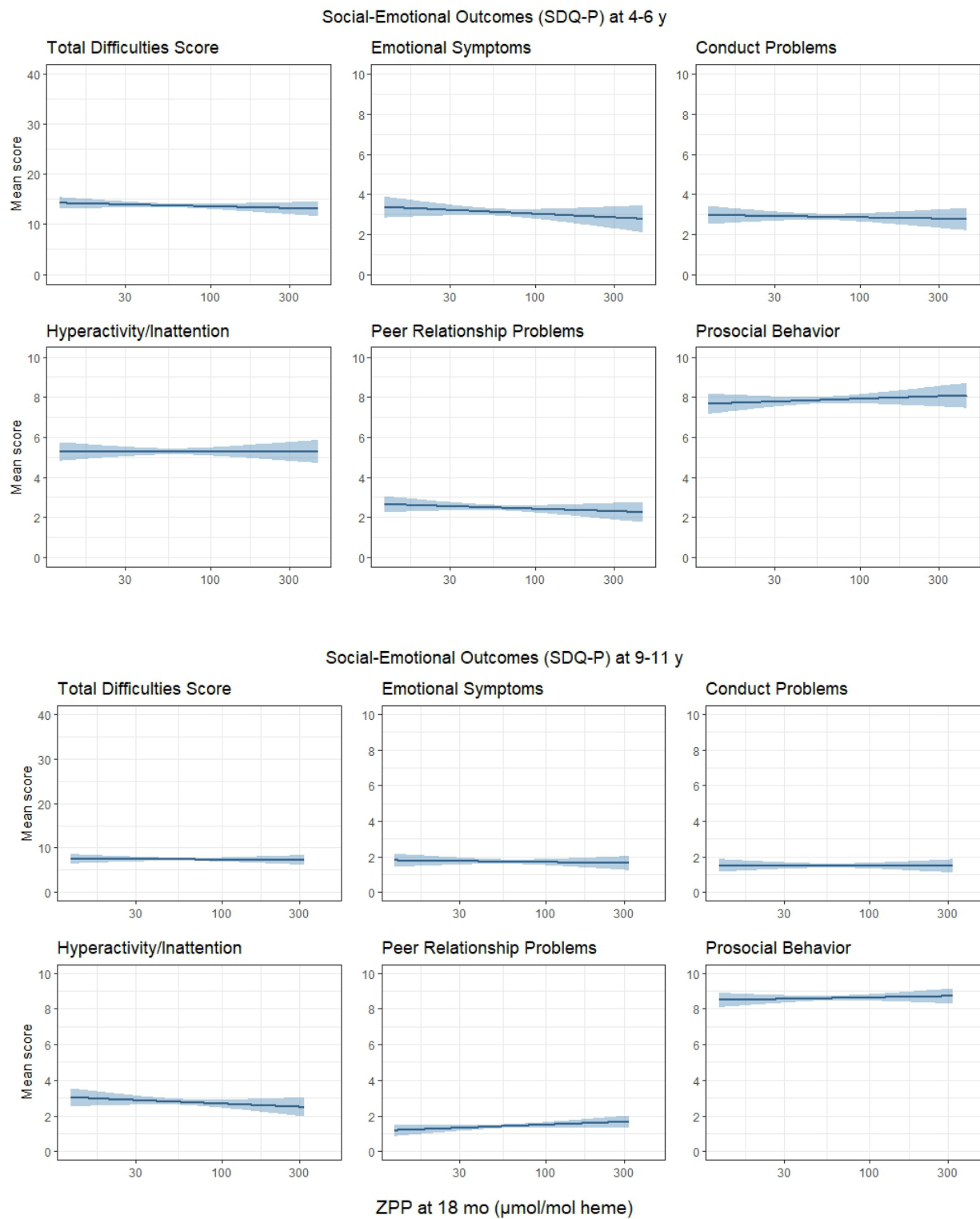


Figure S4.10: Associations of ZPP at 18 mo with social-emotional (SDQ-T and SDQ-C) outcomes at 9-11 y

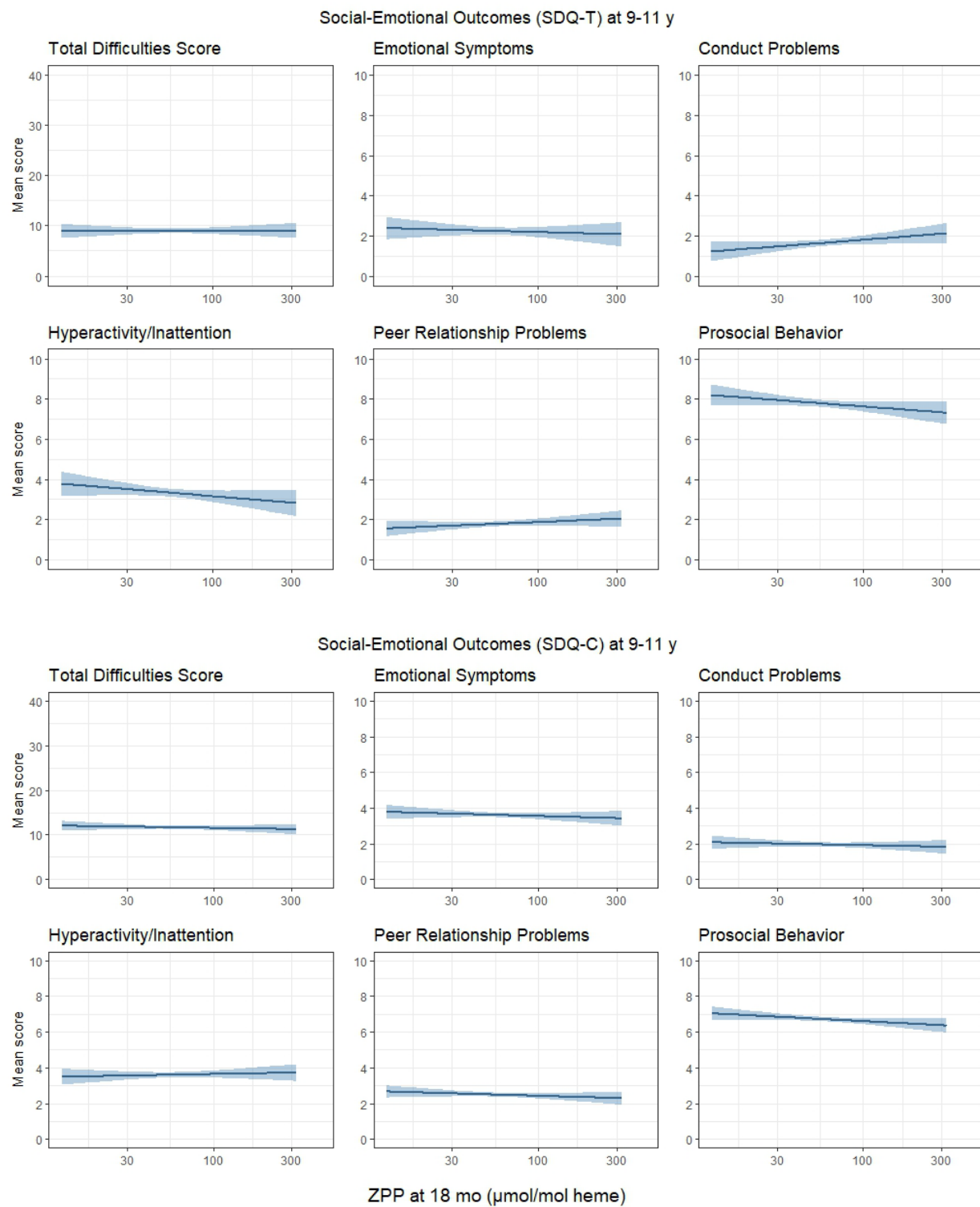


Figure S4.11: Child sex modified the associations between ZPP at 18 mo and SDQ-P total difficulties score and prosocial behavior at 9-11 y

