Linking Type, Timing, and Context Across Sensitive Windows
of Early-Life Nutrition and Cognitive Development

By

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ABSTRACT

Emerging evidence suggests that early-life nutrition exposures may influence specific cognitive domains, such as attention and memory. This dissertation proposes that these effects are not solely intrinsic properties of nutritional factors (*type*) but also reflect neurodevelopmental windows during which group differences become measurable (*timing*), as well as the *contexts* in which they are observed. I examine these relationships using eye-tracking-based tasks as objective measures of domain-specific effects of nutrition. In Chapter 1, I introduce the domain-specific effects of early-life nutrition and propose that linking type, timing, and context can improve our ability to evaluate nutrition-cognition relationships. In Chapter 2, I synthesize existing research through a scoping review of maternal and child nutrition on child cognitive development measured using eye-tracking assessments. I identify eye-tracking assessments as feasible and promising methods for evaluating the effects of early-life nutrition on attention and memory domains. In Chapter 3, I evaluate eye-tracking assessments of attention and memory within a longitudinal study of growth trajectories among infants and young children from cohorts in Malawi and suburban California, USA. Sex-specific growth trajectories were identified using repeated anthropometric measures collected between birth and 15 months through latent class trajectory modeling. Associations between these trajectories and cognitive outcomes varied across developmental time points and contexts. In Chapter 4, I examine associations of anemia and iron biomarkers at 6–9 months with attention, memory, and social engagement six months later among children in Malawi. Hemoglobin and iron biomarkers were associated with later social engagement and memory, respectively, although associations were modest in this high-burden settings. Thus, an overarching conclusion from this dissertation is that our ability to measure the effects of nutrition on development require specificity in alignment between the nutrient-specific biological mechanisms,

the developmental timing of both the effects of the nutritional exposure and the outcome assessment, and the contexts in which they occur.

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φιλόσοφος *(philósophos, "lover of wisdom")*

Completing this dissertation has been one of the most challenging, chaotic, transformative, and beautiful journeys of my life. It has been an intellectual journey, just as much an emotional one. I began this journey thinking that a successful Doctor of Philosophy meant papers, grants, and awards. It has surprised me, then, to arrive at the end not with pride in those achievements, but with a deeper love for science itself: for the ways it is beautiful, humbling, and transformative all at once. Science became my way to listen more wisely to the world, and to try, imperfectly but sincerely, to help people. This dissertation represents the people who carried me, challenged me, loved me, and believed in me along the way.

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Thank you, my PhD journey, for unraveling the beauty, chaos, and transformation of science; and for teaching me that, at its heart, science is a powerful way to help people.

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Chapter 1: General Introduction

Nutrition in early life and its role in brain development, including the concept of sensitive periods, are well established (1). The prenatal and early postnatal periods are characterized by extensive neurogenesis, synaptogenesis, myelination, and synaptic pruning, and thus represent a uniquely rapid and sensitive phase of brain development (2). During these periods, an adequate macro- and micronutrient supply is needed to support brain cell structure and function. A landmark study from the Guatemalan cohort by Pollitt et al. (1995) (3) found that macronutrient intake, specifically protein, during prenatal and early childhood periods was associated with long-term developmental trajectories. Another study showed that linear growth before 12 months, but not thereafter, and infant weight before four months predicted intelligence quotient at age 9 years (4). Together with other previous studies (5–7), these findings support the Developmental Origins of Health and Disease (DOHaD) theory, which proposes that early-life exposures program metabolic health, brain development, and influence long-term outcomes, including cognition.

Despite this early evidence, there has been substantial heterogeneity in the literature about the association between nutritional exposures and developmental outcomes (8,9). Yet, differences in the types of nutritional exposures assessed, tools used to measure impacts, and contexts in which they were measured present challenges with interpretation. A key question is *whether differential or lack of measured effects of nutrition in early life reflects true heterogeneity, or rather an inability to accurately measure the effect.* The central focus of this dissertation is to improve our understanding of domain-specific effects of nutritional exposures through improved conceptualization of the linkages between type, timing, and context of both the nutritional exposure and the outcome assessment.

1.1 Domain-specific effects of early-life nutrition on cognition

Emerging evidence suggests that different nutrients may influence specific cognitive domains, such as attention, memory, and language, referred to here as "domain-specific effects" (10,11). This terminology should not be confused with traditional uses of "domain specificity," which refers to specialized structures addressing specific cognitive tasks, as opposed to “domain generality” that subserves multiple tasks (12,13). Because nutrients act across multiple biological systems, the notion of domain-specific effects may initially seem counterintuitive. However, compelling evidence from both human and preclinical studies suggests that such domain-specific effects may emerge under certain biological conditions. One of the most extensively studied examples is the relationship between iron deficiency (ID) and memory, which will be used to illustrate the non-mutually exclusive mechanisms underlying domain-specific effects of early-life nutrition on cognition as follows.

First, specific nutrients support functions during periods of rapid growth in specific brain regions. For example, late gestation through 2–3 years of age represents a critical window of vulnerability to ID. This period coincides with rapid hippocampal maturation, which is crucial for memory development, alongside increased iron uptake and utilization in the brain (14). ID during this developmental stage may impair hippocampal metabolism via altered mitochondrial function and reduced cellular energy production (15,16). Additionally, iron is required for the activity of prolyl hydroxylase, an iron-dependent enzyme that regulates hypoxia-inducible factor 1α (HIF-1α), which further modulates neuronal function, including the expression of TfR-1 and Slc11a2 in the hippocampus (17). Altered expression of these pathways has been linked to deficits in learning and memory performance.

Second, some brain regions may differ intrinsically in their vulnerability to perturbations, independent of nutrient action. Neural systems vary in their rates of maturation and metabolic demand–differences that are endogenous to brain development itself (18,19). These intrinsic properties mean that even broad physiological or pathological influences can produce region-specific outcomes. For example, in a study testing the effect of acute global and region-specific cerebral blood flow (CBF) responses to moderate-intensity aerobic exercise, decreased CBF was found across all brain regions but was attenuated in the hippocampus (20). The hippocampus also showed a rebound effect that was most robust than in other regions assessed. This hippocampus-specific CBF effect in response to this broader exposure, as compared to specific micronutrients, suggests an additional pathway underlying domain-specific effects of nutrition.

1.2 Challenges in evaluating domain-specific effects of early-life nutrition

Despite compelling evidence linking early nutrition to overall brain development, challenges remain in evaluating the domain-specific effects of nutrition. In a meta-analysis of randomized controlled trials conducted during pregnancy or between 0–5 years of age, which included 75 studies with 122 comparisons between intervention and control groups and outcomes for 72,275 children, pooled effect sizes of nutrition interventions on cognitive, language, and motor scores were small (0.05–0.08 standard deviation) (8). Among studies measuring cognitive outcomes, around 70% used a global developmental test of the Bayley Scales of Infant and Toddler Development. Similarly, in a systematic review of nutrition interventions on cognitive development among preschool-age children, the majority of studies used global developmental tests, such as the Bayley Scales of Infant and Toddler Development and the Wechsler Preschool and Primary Scale of Intelligence (9). These global developmental tests are based on normative

developmental milestones and assume the presence of a single underlying factor that accounts for all individual differences in cognitive status. Thus, they are typically quantified as single overall composite scores and may obscure subtle differences in specific domains affected by nutrition (10,21).

Because children live in varied ecologies across countries, another gap relates to the reporting of reliability and validity of developmental assessments in the local context. Most tests have not been locally adapted; in the previously mentioned meta-analysis, among the 61 studies conducted in low- and middle-income countries, 60 used tests originally developed and validated in a different country (8). This validation process is important because assessment methods developed in high-income countries with Western cultures may result in tasks in which children are unfamiliar with the testing materials and procedures, potentially leading to erroneous results (22). For example, adaptation of the Denver Developmental Screening Test II in Sri Lanka required changing the picture used for the "name pictures" item from a horse to a cow, as cows are more common than horses in Sri Lanka, and changing playing card/board games to interactive games because board games are uncommon (23).

1.3 Evaluating domain-specific effects through interaction between type, timing, and context across sensitive windows of development

This dissertation proposes that the apparent domain-specific effects of nutrition reflect the interaction between the type of nutritional exposure, cognitive domain, developmental timing, and context. Understanding relationships between early-life nutrition and cognition requires moving beyond the question of which nutrient affects which cognitive domain. Instead, it requires

considering how the type of nutritional exposure, the cognitive domain assessed, the timing of exposure, the timing of cognitive assessment, and context together shape observable outcomes.

The link between type, timing, and context across sensitive windows of development can be illustrated through the analogy of building a house. A house is constructed progressively, with foundational structures, framing, bricklaying, wiring, and finishing completed at different stages. The same underlying materials contribute to the entire structure, yet what is *affected*, *visible*, and therefore *measurable* depends on what is being built at a given time and when the inspection occurs. Early in construction, two houses may appear similar in their framing yet differ in their bricklaying; later, differences may be observed in their interior finishing while the underlying structure remains comparable. Similarly, because different cognitive domains mature along different developmental timelines (19), group differences in nutritional exposures may be most observable when specific cognitive domains are actively maturing. At other time points, these differences may not be detectable, even when assessed using identical tasks. Hence, domain-specific effects are not solely properties of nutrition exposures but also reflect specific developmental windows during which group differences in specific cognitive domains are most likely to be measurable.

This framework therefore emphasizes that both exposure timing and assessment timing matter. The previous example of iron and the hippocampus highlights the importance of the type of nutritional exposure, the specific cognitive domain, and the timing of nutritional exposure. However, examples from developmental science also support the importance of the timing of cognitive outcome measurement. Ross-Sheehy et al. (2017) (24) found differences in spatial attention between premature and full-term infants at 5 months, but these differences were less apparent by 10 months. Similarly, Shinskey and Munakata (2010) (25) showed that infants' visual

preferences changed with age: 7-month-old infants showed a preference for familiar stimuli, whereas 11-month-old infants showed a preference for novelty. This shift was attributed to improvements in processing speed, which allowed older infants to form representations of familiar objects more rapidly and then orient toward novelty (25,26). Together, these examples illustrate why the timing of cognitive assessment is critical and must be selected to capture periods when group differences are detectable. Moreover, when a specific period is hypothesized to best predict an outcome, it is necessary to assess whether the strength of the association between exposure and cognitive domain differs depending on when the outcome is measured, or remains stable across assessment timing (27). Thus, rather than assuming a single, generalized sensitive period across the first 1,000 days of life, periods of heightened sensitivity may vary across cognitive domains, depending on when each domain is undergoing rapid development and when it is measured.

Context further shapes how differences in cognition associated with early-life nutrition exposures are expressed and interpreted, especially for experience-dependent processes (as compared to experience-expectant processes) (28–30). Experience-dependent development refers to processes shaped by environmental information that varies across individuals, such as the particular language a child hears or the caregiving context in which development occurs. A cross-cultural study by DeBolt et al. (2025) (31) identified population-specific differences in spatial attention, measured by an eye-tracking-based task, between cohorts from rural Malawi and a suburban area in California, United States. Children in Malawi demonstrated slower reaction times but greater accuracy, while children in the United States showed the opposite, suggesting that different behavioral strategies may underlie task performance across settings. Returning to the house analogy, this is similar to the way two houses may be wired differently while still supporting the same overall function. Likewise, children may arrive at adaptive developmental outcomes

through somewhat different pathways, some of which depend on contextual exposures (32,33). Therefore, developmental context shapes the interpretation of domain-specific effects of nutrition on cognition and is incorporated across all chapters of this dissertation.

1.4 Integrating interaction between type, timing, and context in nutrition studies using eye-tracking assessments

In **Chapter 2**, I synthesize existing research on early-life nutrition and child cognitive development that utilize eye-tracking assessments, and evaluate their feasibility across settings. Eye-tracking-based tasks provide objective (34) and domain-specific measures of cognition by capturing children's eye fixations on stimuli, providing insights into how long they look, where they look, and how they visually scan stimuli (35). These methods enable the assessment of early differences in various cognitive domains, including attention, memory, language, social cognition, executive function, and predictive processing (36–40), which may not yet be observable through global developmental assessments. Importantly, eye-tracking-based tasks reduce the necessity to have observers manually measure looking time. Stimuli presented during the tasks can also be adapted to local contexts, enabling cross-cultural applications (41). Because eye-tracking assessments do not rely on caregiver report or children's subjective responses, the adaptation process may require less extensive effort, time, and money (42).

In **Chapter 3**, I evaluate eye-tracking assessments of visual attention and memory in a longitudinal study of growth trajectories among infants and young children across cohorts in Malawi and suburban California, USA (43–45). Unlike static anthropometric indicators, growth trajectories derived from repeated measures provide a life-course perspective that captures cumulative nutritional and health influences during sensitive periods of brain development (46).

Deviations from expected growth, such as accelerated or decelerated weight gain, may signal increased risk for suboptimal neurodevelopment through inadequate supply of nutrients, altered metabolic programming, and inflammatory processes (47–50). A few previous studies have identified distinct growth trajectory groups in children and demonstrated associations between these groups and later outcomes related to body size, stature, schooling, cognitive and socioemotional outcomes (51–54). Using unsupervised learning of latent class trajectory modeling, I identify subgroups of children with distinct growth patterns and examine their associations with cognitive outcomes. Furthermore, growth trajectories are conceptualized as a general indicator rather than a domain-specific nutritional exposure. Hence, by comparing associations of growth trajectories with attention and memory outcomes across multiple time points (e.g., 6–9 months vs. 12–15 months), this chapter evaluates whether apparent domain-specific effects vary as a function of timing of cognitive assessments.

In **Chapter 4**, I focus on iron deficiency (ID) and anemia as exposures with both domain-specific and general pathways of influence. ID influences early neurodevelopment in a brain-region-dependent manner, resulting in effects on specific cognitive domains, with strong evidence for hippocampal involvement supporting memory development (55,56). Iron supports the transcriptome, metabolome, intracellular signaling pathways, and electrophysiological response properties of the developing hippocampus for recognition learning and memory (57). Beyond memory outcomes, there is growing interest in associations of iron status with attention and early social engagement (58,59); however, evidence for both is more limited than for memory. In contrast, anemia–with or without iron deficiency (60)–may lead to broader effects by impairing oxygen delivery to the brain (61) and could lead to lethargy, thereby reducing social engagement between young children and their caregivers and limiting opportunities to explore the environment.

This contrast provides an opportunity to explore whether biologically specific and general mechanisms lead to different cognitive outcomes when measured at the same developmental time point. In a longitudinal study of children aged 6–9 months at baseline, I evaluate whether biomarkers of anemia and ID are associated with learning and memory performance on a series of eye-tracking assessments six months later.

This dissertation incorporates contextual factors across all chapters. In **Chapter 2**, I assess the feasibility of eye-tracking assessments across diverse settings, where logistical constraints may influence implementation despite the method not requiring highly specialized personnel. In **Chapter 3**, I compare growth trajectories and their associations with attention and memory across settings. Previous studies across Malawi and the USA suggest broadly similar patterns of engagement and attentional behavior (31,41), yet contextual factors, such as health burden and caregiving environments, may buffer or amplify associations between nutrition and cognition (62). Extending this framework, **Chapter 4** examines iron deficiency within a high-burden setting, where co-occurring stressors such as infection and inflammation may influence the interpretation of these associations.

Ultimately, this dissertation informs our understanding of the specificity of nutritional effects on child development, and highlights the importance of improving measurement methods. To understand and accurately observe a true signal through the noise of measurement error, there must be alignment between nutrient-specific biological mechanisms, the developmental timing of both the effects of the nutritional exposures and outcome assessment, and the contexts in which they occur.

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Chapter 2:

Eyes on development: Exploring the impact of early life nutrition exposures on child cognition through eye-tracking assessment[1]

[1] This paper has been previously published (Rianda D, Mjaaseth UN, DeBolt MC, Agustina R, Oakes LM, Stewart CP. Eyes on development: Exploring the impact of early life nutrition exposures on child cognition through eye-tracking assessment. Nutr Rev 2025; nuaf235). Reprinted with permission.

2.1 Abstract

The effects of certain nutrients on cognition in early life are hypothesized to be domain-specific. Previous studies have examined the associations between child nutrition and cognitive, motor or socio-emotional development, but the assessment methods are often insensitive to the specific functional domains affected by nutrition. Our scoping review has two objectives: 1) to synthesize evidence on the impact of early life nutrition exposures on child cognition evaluated using eye-tracking assessment and 2) to describe the use of eye trackers for child cognitive assessment and to provide recommendations for eye-tracking implementation in future research. A systematic search was performed using PubMed, Embase, and Food Science and Technology Abstracts, with time restricted until April 2024. Additionally, we hand-searched reference lists of eligible studies to identify additional relevant publications. We included studies of any nutrition exposures (intervention or assessment) involving pregnant or lactating mothers, or children aged 0–5 years, with child cognitive outcomes assessed using eye-tracking assessments. The review was conducted following the PRISMA-ScR checklist. A total of 1,140 article titles and abstracts were screened, with 9 articles included in this review (3 randomized controlled trials, 5 prospective cohort studies, and 1 cross-sectional and prospective cohort study nested in a trial). The majority of the studies were conducted in low- and middle-income countries. Despite the limited number of nutrition studies using eye-tracking assessments, 5 of the 9 studies demonstrated significant relationships between nutrition exposures and attention and memory outcomes. Eye-tracking assessments were also found to be feasible across multiple settings. Eye-tracking assessment is a feasible and promising method for evaluating early life nutrition exposures on attention and memory outcomes across settings. However, the selection of eye-tracking-based tasks should be tailored to the type

of nutrition exposures, desired long-term outcomes, and the feasibility of proper training for local staff.

2.2 Introduction

Cognitive development in early life lays the foundation for life-long capabilities, with the period from birth to three years of age considered the most critical.[1–3] During this period, infants and young children experience rapid development of their cognitive abilities (e.g., attention and memory), many of which are important for later academic performance.[4,5] Nutrition in early life, encompassing both maternal and child nutrition, is a pivotal biomedical factor impacting cognition.[6,7]

Adequate nutrient intake is essential for infants and young children to support the structural development of their brains and enable effective engagement with their environment.[8] Moreover, maternal nutrition during pregnancy represents an additional sensitive period during which nutrition interventions could impact fetal neurodevelopment, when neurogenesis and synaptogenesis occur.[9] Several nutrients have been linked with brain development in children[8], including omega-3 fatty acids[10], iron[11], zinc[12], folic acid and choline[13], vitamin D[14], vitamin B6 and vitamin B12[15].

Notably, the effects of certain nutrition exposures on cognitive development are hypothesized to be domain-specific.[16] Previous studies have examined the associations between child nutrition and cognitive, motor or socio-emotional development. However, the assessment methods are often insensitive to the specific functional domains affected by malnutrition. In a systematic review of nutrition intervention on cognitive development among preschool-age children, the majority of the studies used global developmental tests [e.g., Wechsler Pre-school

and Primary Scale of Intelligence (WPPSI), Bayley Scales of Infant and Toddler Development 3rd edition (Bayley-III)].[17] These tests are based on normative developmental milestones and assume the presence of a single underlying factor that accounts for all individual differences in cognitive status. Thus, they are typically quantified as a single, overall composite score. Rather than affecting global outcomes, the effects of a given nutrition intervention might be specific to certain developmental domains influenced by the nutrients' role in specific biological pathways. Therefore, more precise results may be obtained using specific cognitive tasks. For instance, Cao et al.[18] found that docosahexaenoic acid (DHA) omega-3 is primarily accumulated in the hippocampus of rats and the brain's parietal lobe, associated with visual attention and working memory. In humans, a study by Rodrigue et al.[19] demonstrated that iron also accumulates in the hippocampus and correlates with memory performance. This finding is consistent with another study showing lower recognition memory among neonates with iron deficiency.[20] Thus, the effect of such nutrients on development may be more clearly observed if the assessment targets those cognitive systems.

There has been growing interest in utilizing eye-tracking technology in nutrition research to measure specific areas of cognitive development. This technology enables the automatic measurement of children's eye fixation on stimuli, providing insights into how long they look, where they look, and how they visually scan the stimuli.[21] These looking behaviors can then be used to evaluate specific domains of child cognition, including attention, memory, language, social cognition, executive function, and predictive processing.[22–26] Importantly, eye-tracking-based tasks reduce the necessity to have observers manually measure looking time. Stimuli presented during the tasks can also be adapted to local contexts, enabling cross-cultural applications.[27]

This scoping review aims to synthesize existing research assessing the relation between maternal and child nutrition exposures in early life and child cognitive development as measured by eye tracking assessments. We begin with a literature review describing the history and application of eye-tracking technology in assessing cognitive development in children, including its biological rationale and relevant developmental domains. We then present findings from the scoping review of nutrition studies on child cognition utilizing eye-tracking methods. Finally, we provide recommendations for future research implementing eye-tracking assessments to evaluate the impact of nutrition on child cognitive development.

History of eye-tracking assessment

Vision is one of the first senses to develop and is crucial for allowing infants to begin gathering information about the world around them. Even as newborns, humans selectively direct their gaze toward particular objects and events. Before language and advanced motor skills emerge, measuring looking behavior provides an indirect way to assess brain development, including cognition. Variations in looking behavior among children may reflect differences in their cognitive growth. For example, variations in visual attention, as reflected in how children direct their gaze, can indicate differences in their ability to focus on the meaningful parts of a visual stimulus.

Pioneering work using children's looking behavior to make inferences on their cognitive function emerged around the 1960s and 1970s.[28,29] This early work revealed important characteristics of visual attention in infants. For example, infants look longer at novel or more complex stimuli than at familiar or less complex stimuli.[30–33] Moreover, infants' preferences for visual novelty indicates early visual recognition memory and has been associated with later intelligence quotients (IQ).[34] Because the development of visual attention milestones varies among

individuals, visual attention serve as a marker for later outcomes, including mathematics and literacy skills, as well as the potential for an ASD diagnosis.[35,36]

Historically, a child's looking behavior was recorded via observations by a human coder through a peephole or from offline coding of films or videotapes; these methods are still widely used. Coders are trained to assess the direction of an infant's gaze when it falls within regions of a stimulus field. However, this method has challenges. First, it is more difficult to assess the vertical gaze position compared to the horizontal position.[37] As a result, this type of observation is primarily suitable for measuring visual attention in paradigms that do not require precise measurement of vertical gaze, such as single-stimulus habituation paradigms or two-stimulus preferential-looking paradigm (when the items are presented side-by-side). More complex attention patterns, such as selectively attending to the eyes of a face, are challenging to evaluate. Additionally, assessing quantitative characteristics of eye movements, such as the timing of saccades and smooth pursuit, is not feasible using human observers. Lastly, although trained coders achieve high reliability ratings, and video recordings allow more detailed temporal resolution, determining eye position through these recordings and analyzing the data are often extremely time-consuming.

Objective evaluation of eye movements emerged when two automated eye-tracking techniques were developed.[37] Electro-oculography (EOG) is a technique based on the gradient of metabolic activity between the front and back of the eyeball. However, a fundamental problem came with this technique: it requires a pair of electrodes to be placed near the eyes, which is often not feasible for infants. Metabolic activity also varies over time and may cause the EOG signal to drift without any gaze change. Another technique, corneal-reflection photography, was developed based on the occurrence of corneal reflection (CR) when a small point of light is directed to the

eye. Although this technique can provide a detailed measure of changes in gaze, it requires the child's head to be fixed in position. CR photography uses a video camera with a close-up lens that fills the video frame with the eye, focusing on the pupil. Any movement that causes the pupil to move outside the video frame results in a loss of usable data. To resolve this issue, eye-tracking systems require position-sensing to monitor head movements. Early systems used a magnetic head tracker (MHT) receiver to be placed in the infant's head and connected to an MHT control unit. More modern systems involve video solutions to monitor head movements. The development of the CR eye-tracking technique allowed children to move freely while maintaining high spatial and temporal accuracy of eye position recording.[38] This recent technique is promising for use in nutrition studies across various settings, including remote or resource-constrained areas, when measuring cognition as an outcome.

Cognitive domains evaluated using eye-tracking assessment

Eye-tracking assessments capture eye gaze, reflecting visual processing in the brain. The mechanistic pathway of visual processing assessed by an eye tracker is described in **Figure 2.1**. The child's retina receives stimuli displayed on the screen. Two visual processing pathways–subcortical and cortical–are activated from the retina. The subcortical pathway transmits visual input to the superior colliculus in the midbrain.[45] The information is relayed to the basal ganglia. This allows saccadic eye movements–quick, simultaneous shifts of both eyes that change the fixation point. These eye movements are important for a wide range of visual abilities, including tasks involving attentional cues.

The cortical pathways of visual processing pass visual input to the cortical areas via the primary visual cortex in the occipital lobe.[40] From there, the input is transmitted via two pathways,

the ventral visual pathway through the temporal lobe and the dorsal visual pathway through the parietal lobe.[40,41] Visual information is processed in distinct ways – object recognition and identification occur via the ventral pathway while spatial awareness is processed via the dorsal pathway. These functions help children identify stimuli and then focus their attention on selective information when planning saccades. Some stimuli trigger the recognition and recall of memories, leading to eye movements that differentiate between novel and familiar stimuli. Finally, voluntary eye movements are initiated as visual processing passes through several areas in frontal lobe responsible for voluntary eye movement, including the frontal eye field, the supplementary eye field, and the dorsolateral prefrontal cortex.[45,47] Thus, a child's looking behavior in response to varying stimuli results from visual processing that reflects attention and interactions with other cognitive and perceptual systems, such as memory and learning. Eye-tracking procedures allow for the evaluation of these cognitive domains, including attention and memory.

Attention is a fundamental cognitive process involving the selection of relevant information and inhibition of irrelevant information. This allows children to orient toward, shift between, and maintain focus on targets.[43] Visual attention promotes information gathering and selection, which determine the targets for subsequent learning and influence developmental trajectories across various cognitive domains. Multiple functions are constructed within attention. Four attentional functions are considered crucial for the learning process. These can be assessed using eye-tracking methods: orienting toward and away from objects at various spatial locations, selective filtering of visual inputs, processing visual inputs, and maintaining focus on a target.[44] However, these attentional functions are often closely intertwined in everyday life. Therefore, a task designed to evaluate one function may also engage other attentional functions and/or cognitive domains. For instance, tasks measuring the duration of attention to screen-based stimuli primarily

assess the ability to maintain focus on a target. Yet, they may also involve attentional function of processing visual inputs, executive attention, and motivational factors (i.e., child's preference for specific stimuli).

Another cognitive domain that can be assessed using eye-tracking methods is memory. Memory is a product of encoding, storing, and retrieving information, and therefore is tightly linked with learning.[43] When a child looks at an object, they begin to form a memory representation; whether that memory is fleeting and stored in visual short term memory or becomes consolidated as a long-term memory depends on several factors including how long the child looks at the object. However, it is worth noting that fidelity of memory is also affected by attentional selectivity, which redirects infants to relevant objects, and perceptual discrimination, required for children to discriminate objects as different entities. Most studies assessing visual memory among infants and young children are based on habituation/novelty preference, which first allows children to become familiar with, or form a memory of, an image and then compares their responses to novel and familiar images. As children's memory for an image grows, they will exhibit increased looking behavior at a novel stimulus. Therefore, the appearance of a "novelty preference" indicates memory of a familiar image. Moreover, certain tasks might be able to assess functions related to specific brain areas. For example, a relational binding task evaluates an infant's ability to bind features (i.e., identify objects by features and bind them to locations, learning both "what" and "where", for example learning that one's toys are in the family room). This ability is related to the further development of medial temporal lobe structures, including the parahippocampal cortex.[45]

General procedure of eye-tracking assessment

Eye-tracking systems estimate a point of gaze and calculate aspects of eye movements from corneal and pupil reflections. When a light source reflects off the cornea, the reflection remains relatively stationary as the eye moves. This reflection is called the first Purkinje image. The gaze location can then be calculated by comparing the first Purkinje image's position to the pupil's center.[38] To link the angular rotations of the eye to known spatial positions on the screen, a short (~20 s) calibration procedure is performed at the start of the eye-tracking assessment.[46]

When selecting the type of eye tracker, an important consideration is the sampling rate. Modern eye trackers have sampling rates ranging from 30 Hz to 1200 Hz; earlier models had rates ranging from 60 to 120 Hz. A trade-off may occur between sampling rate and data quality (e.g., lost samples, accuracy, precision). A lower sampling rate decreases temporal precision (e.g., 16 ms at 60 Hz vs. 8 ms at 120 Hz), but it eases the calibration process, making it easier for non-experts to track infants, and therefore, minimizes data loss from the analysis.[46]

Figure 2.2 illustrates the general setup for an eye-tracking assessment**.** The assessment should be conducted in a room or curtained booth with minimal distractions and a quiet environment. The testing area should have no windows because daylight contains some frequencies in the infrared spectrum. Artificial lighting in the room should be diffused to prevent reflections on the pupil, which could interfere with the eye tracker's algorithm.

During the assessment, the child is positioned on their mother's lap, in an infant carrier, or seated independently, facing the screen. The eye tracker is attached to the monitor and placed approximately 60 cm from the child's eyes (the specific distance will depend on the make and model of the eye tracker). A monitor mount is often used to help adjust this distance. Additionally, a webcam records the child's behavior, allowing the assessor to note any significant activities, such as when the infant becomes fussy.

A plain-coloured divider is placed between the assessor and the mother and child. The assessor may either face the mother and child or sit beside them, as long as the position allows them to intervene when needed. For instance, if the child loses focus, the assessor can shake a rattle behind the monitor to redirect the child's attention back to the screen. Therefore, adequate space behind the monitor is necessary during the assessment.

Assessing child cognition using eye-tracking methods in nutrition studies

Utilizing eye-tracking methods is a promising approach with significant implications for understanding child development and nutrition. When evaluating the influences of nutrition exposures on child cognition, selecting appropriate eye-tracking-based tasks requires several considerations. Certain tasks have been validated and demonstrated stronger associations with long-term outcomes. For example, although both assess memory, the visual paired comparison (VPC) task has shown larger associations with later intelligence quotient (IQ) than the habituation task.[47,48]

Importantly, assessments of infant looking behavior have shown large individual and developmental differences, suggesting that they might be more suitable for assessing group-level differences rather than individual changes.[47] A study conducted among infants in Malawi demonstrated low test-retest correlations for eye-tracking results, even over a period as short as two months (i.e., between 7 and 9 months of age).[49] In general, looking measures in infancy that are separated at points close in time (a few days or weeks) tend to give moderate reliability (0.40–0.60), while those separated by a month or more showed lower estimates (0–0.20).[50] This variation can be attributed to the different rates at which children develop, leading to increased within-

person differences over time. Due to this high variability in eye-tracking results, ensuring a sufficient sample size is also crucial.

Assessing general cognitive development with eye-tracking methods is more sensitive in detecting subtler deficits or benefits from a nutrition intervention than using broader assessments of early neurodevelopment, such as Bayley Scales of Infant and Toddler Development.[16] Notably, some eye-tracking-based tasks are associated with specific brain regions, which may be advantageous for evaluating nutrition interventions targeting particular cognitive areas. For instance, a nutrition intervention hypothesized to affect the parahippocampal cortex could benefit from using a relational binding task.[51] Tasks involving preferential tracking of faces can be used to evaluate interventions that are hypothesized to affect the posterior temporal cortex.[52] However, although eye-tracking-based tasks are designed to assess specific cognitive domains, most of them may also engage other domains. For instance, habituation/novelty preference tasks primarily assess memory but also require attentional engagement. Moreover, some tasks may involve activation across multiple brain regions. For example, tasks targeting spatial attention, such as the Infant Orienting with Attention (IOWA) task, engage frontal, prefrontal, and parietal cortical areas.[53,54] Thus, these tasks are suitable even when the cognitive domains or brain regions influenced by specific nutrients are not yet identified.

One potential challenge for the use of eye-tracking methods is data loss. Enrolled children may not complete eye-tracking assessment due to loss to follow-up, refusal to participate, or failure to achieve successful calibration. Even among those who are assessed, data loss can still occur if children move excessively or turn their heads away from the screen (e.g., due to inconsolable crying, too tired, or not focused on the screen) or if the eye tracker fails to detect the pupil in the camera image.[55,56] These issues can result in children having insufficient usable data for analysis.

To the best of the authors' knowledge, the typical rate of data loss in eye-tracking studies among infants and young children has not been systematically evaluated. However, one study examining eye-tracking data quality in infants aged 5 and 10 months found that data loss was predicted by both participant age and assessor.[56] Data loss was higher among 5-month-old infants, with a peak around 15%, and showed greater variability across assessors compared to 10-month-olds. In nutrition studies, data loss is particularly an issue if exclusions are not random but systematically related to the child's nutritional status. For example, a nutrition intervention that reduces fussiness alongside improving attention and memory might result in more available data in the intervention group compared to control.

2.3 Methods

We used the Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) Checklist to guide our scoping review process. This review's protocol was registered with the Open Science Framework in April, 2024 (https://osf.io/fkab2/).

Search strategy

We performed a literature search using PubMed, Embase, and Food Science and Technology Abstracts, with time restricted to April 22, 2024, when our protocol was registered. We used the search terms in **Supplementary Table 2.1**, adapted for each bibliographic database and developed under the guidance of an experienced librarian at the University of California, Davis. To identify any additional relevant publications, we individually searched reference lists from eligible studies. Specific nutrients of interest defined as search terms were selected based on reviews by Nyaradi et al.[57], Prado and Dewey[8], and Cusick and Georgieff[58]. 5/21/26 11:18:00 AMOur search strategy used the following structure: *Population* AND *Intervention/Comparison*

AND *Outcome*. All studies found based on the combined search across all databases were uploaded into Covidence (www.covidence.org) to remove duplicates and screen the titles and abstracts of the papers.

After removing duplicates, two authors (D.R., U.N.M.) independently performed title and abstract screening. We included studies of any nutrition exposures (intervention or assessment) involving mothers and/or children with outcomes on child cognition assessed using any eye-tracking methodology. We searched for any human observational studies or trials in pregnant or lactating mothers, infants (< 1 year old), or young children (1–5 years old), where eye-tracking methods were used to assess child cognition at any point after supplementation. We included any types of nutrition exposure or indicator of status (e.g., serum ferritin concentration) and nutrition interventions, including foods, supplements, or dietary recommendations to consume nutrient-rich foods. Systematic reviews of nutrition intervention on child development were included to identify potential additional studies. Unpublished studies were not included, and studies were restricted to those published in English only.

Full-text review and data extraction

To determine the eligibility of the identified studies, one author (D.R.) reviewed all full-text versions of the potentially relevant studies, and 33.3% (n=62 studies) were randomly selected for a double review by another author (U.N.M.). Any uncertainties during this full-text eligibility assessment were discussed with other authors. We contacted the corresponding authors of studies with undisclosed data, requiring clarification, or published as study protocols to determine whether outcome data had since been published.

We charted data from the included studies using a pre-designed form. One author (D.R.) performed data charting for all studies, with 33.3% (n=3 studies) of randomly selected studies doubled-charted by another author (U.N.M.). These data included (1) first author and year, (2) study design, (3) study population and sample size, (4) study location, (5) type and duration of nutrition exposure, (6) type of eye-tracking-based task as the outcome, and (7) findings. We considered critical appraisal of the studies unnecessary for this scoping review. A narrative synthesis and overview tables were provided to summarize all included studies.[59] We created figures using BioRender (www.biorender.com) for the mechanistic pathway of visual processing and SketchUp (www.sketchup.com) for the general setup of eye-tracking assessment.

2.4 Results

We screened a total of 1,140 articles' titles and abstracts, and 186 full-text articles were retrieved and assessed for eligibility, resulting in 9 articles included in this review (**Figure 2.3**). The primary reason for excluding empirical articles was the absence of an eye-tracking assessment to measure cognition (n=60 articles), and reviews (n=102 articles) were excluded when they did not provide additional relevant nutrition studies that used eye-tracking assessment. Of the 9 articles included, 3 were controlled trials[60–62], 5 were prospective cohort studies[49,55,63–65], and 1 was a cross-sectional and prospective cohort study nested in a trial[66]. Only 2 studies were conducted in high-income countries[63,64] (i.e., Singapore and England); the remaining 7 studies were conducted in low- and middle-income countries[49,55,60–62,65,66] [i.e., Malawi (n=4), Sierra Leone (n=1), South Africa (n=1), and Gambia (n=1)].

All nine included studies involved varied nutrition exposures, with two reporting on the same study sample (**Table 2.1**)[49,55,60–66]. Eye-tracking procedures used in the included studies are

described in **Table 2.2**[49,55,60–66]. These procedures focus on evaluating two cognitive domains: attention and memory. Across 11 different eye-tracking-based tasks, only 3 were used in more than one study: saccadic reaction time (SRT), VPC, and IOWA tasks. Randomized controlled trials included in this review tested various nutrition interventions, consisting of eggs, ready-to-use therapeutic food (RUTF) formulation for moderate acute malnutrition (MAM), and home visit interventions that included child nutrition. Other nutrition exposures were assessed through prospective cohort and cross-sectional studies, which examined breastfeeding, length-for-age (LAZ) and weight-for-age z-scores (WAZ), prenatal docosahexaenoic acid (DHA) intake, supplementary feeding for MAM, plasma choline and trimethylamine N-oxide (TMAO), and iron status. Our literature search revealed that the earliest study on nutrition utilizing eye-tracking assessment was published in 2015.[63] Of the 9 studies included, significant associations between maternal and child nutrition exposures and eye-tracking outcomes were demonstrated in 5 studies with varied types of exposures and eye-tracking-based tasks, as summarized in **Figure 2.4**[49,55,60–66].

Findings from randomized controlled trials

Three studies evaluated nutrition exposures in children through randomized controlled trials. Prado et al.[62] evaluated the intervention of providing one egg per day to infants, using the VPC task to assess infant memory and the IOWA task to assess attention. This study involved the same sample assessed by Bragg et al.[66] The trial enrolled 660 children aged 6–9 months old in Malawi, with 10% loss to follow-up rate. About 2/3 of the children were assessed using eye-tracking methods at the end of the intervention period when they were between 12 and 15 months. No significant effects of the intervention on the eye-tracking-based tasks were observed. Similarly, the

intervention did not significantly affect other developmental outcomes assessed by non-eye-tracking methods, such as the Malawi Developmental Assessment Tool (MDAT) and elicited imitation.

Similarly, another randomized controlled trial in Malawi by Stephenson et al.[61] used the same eye-tracking-based tasks (i.e., VPC and IOWA tasks) and found no effects of three different RUTF formulations [RUTF made with high-oleic (HO) peanuts and palm oil with added DHA (DHA-HO-RUTF) vs. without added DHA (HO-RUTF) vs. standard RUTF] among infants with MAM. Nonetheless, significant effects were observed on gross motor and social domain z-scores assessed using the MDAT. It is worth noting that the intervention period lasted only until a clinical outcome was reached, or for a maximum of six bi-weekly follow-up visits. The study enrolled 2,758 infants aged 6–59 months old. However, for the eye-tracking outcomes, a subset of participants was systematically selected for testing based on age at the time of assessment. Among those selected, around 60-70% completed eye-tracking assessments and were included in the analysis (n=380 in DHA-HO-RUTF vs. n=350 in HO-RUTF vs. n=402 in S-RUTF group). Notably, this study did not include a group without intervention or healthy controls, nor did it perform eye-tracking assessments at baseline.

The most recent randomized controlled trial involving children that utilized eye trackers, conducted by Rockers et al.[60], evaluated a home visit intervention in South Africa aimed to improve child attention assessed using the SRT task. The intervention, which included nutrition counselling, began at birth and continued until the children reached 24 months of age, with additional follow-up until 36 months. Assessments were conducted at multiple time points (7, 17, and 36 months old), with the percentage of children assessed and analyzed varying across these time points (55.7%, 86.7%, and 82.6%, respectively). The home-visit intervention resulted in

lower SRT at 7 and 17 months of age, indicating faster attention shifting, but these differences were no longer present at 36 months old. This study did not observe any significant effects on developmental scores assessed using MDAT.

Findings from prospective cohort and cross-sectional studies

We identified 5 prospective cohort studies and 1 cross-sectional and prospective cohort study that utilized eye-tracking methods, with only 1 study assessing a prenatal exposure. Rees et al.[64] conducted a prospective cohort study evaluating the effects of prenatal DHA intake during 2nd and 3rd trimester of pregnancy on infant memory, assessed using a habituation task, and attention, assessed using a visual attention task. Data on DHA intake was collected using the Diet History Questionnaire (DHQ, National Institute for Cancer, NIC: US), which assesses diet over three months and includes an evaluation of dietary supplements. This study followed mothers in England from pregnancy until their children reached 9 months of age, with a relatively high proportion of children assessed (94 out of 107) despite the small sample size. However, no significant differences were observed on either task based on prenatal DHA intake during the 2nd and 3rd trimesters.

Cai et al.[63] examined the influence of breastfeeding on the relational binding task, which indicates infant memory, and visual expectation task depicting attention. This prospective cohort study followed pregnant women in Singapore until their children were 24 months old. Of the 1,247 pregnant women enrolled, around 15% of children were assessed and included in the analysis at 6, 18, and 24 months old. Infants with high breastfeeding exposure –defined as those who were exclusively or predominantly breastfed until 4 months and continued with at least partial breastfeeding until the age of 6 months or beyond–showed better memory at 6 months on the

relational binding task compared to those who were weaned and exclusively formula-fed before 3 months. However, no other significant associations were identified in this study.

In a prospective cohort study in Malawi, anthropometric indicators of infant nutritional status, including LAZ and WAZ, were evaluated by Pyykkö et al.[49] Infants were followed from the neonatal period until they were 9 months old. Eye-tracking-based tasks focusing on attention–comprising visual search, anticipatory attention shifts, and attention to faces–were performed at 9 months, with 86% of children assessed and analyzed out of 444 enrolled. However, neither LAZ nor WAZ at birth, nor 9 months, was associated with any attention markers at 9 months.

MAM status has been shown to influence eye-tracking results. Leppänen et al.[55] compared infants undergoing treatment for MAM in Sierra Leone compared to infants without MAM using the SRT task. This prospective cohort study enrolled 138 infants with MAM, defined by mid-upper arm circumference ≥ 11.5 cm and < 12.5 cm without bipedal edema, and 60 aged-matched controls aged 7–11 months old. Around 70% of the infants completed the SRT task. Infants with MAM demonstrated higher adjusted SRT at baseline, indicating slower attention shifting, but a significant improvement in SRT was observed after four weeks of treatment compared to controls.

Infant plasma choline, TMAO, and iron status were also significantly associated with eye-tracking-based tasks related to infant attention and memory. Bragg et al.[66] conducted cross-sectional and cohort analyses of the randomized controlled trial in Malawi conducted by Prado et al.[62] The data from 400 infants was examined at baseline, when they were 6 to 9 months old and at endline, when the infants were 12 to 15 months old. Higher plasma choline level was associated with slower IOWA response time, indicating slower attention shifting, but faster processing speed on the VPC task, indicating faster learning. Meanwhile, higher plasma TMAO level at baseline was associated with slower processing speed on the VPC task six months later. Iron status at 5

months old was evaluated by McCann et al.[65] in Gambia. This cohort study assessed visual disengagement at multiple time points (5 months, 8 months, 12 months, 18 months, 24 months, and 3–5 years), with the percentage of children assessed and analyzed ranging from 41% to 65% of the 258 children enrolled. Significant differences in visual disengagement trajectories were found between infants in the highest and lowest terciles of soluble transferrin receptor levels at 5 months. However, no differences were observed based on ferritin and hemoglobin levels.

Cross-cultural feasibility

Out of the 9 studies, only 2 were conducted in high-income countries. There was considerable variability in how studies reported the number of children assessed and included in the analysis for eye-tracking assessments. All studies conducted in low- and middle-income countries reported the number of children included in the analysis. In contrast, although both studies from high-income countries reported the number of children who attended the visit or were assessed, they either partially reported the number analyzed (i.e., only for 1 out of 3 time points)[63] or did not specify it at all (i.e., for any time point)[64]. The proportion of enrolled children who were assessed and had sufficient usable data for at least one eye-tracking-based task ranged from 41% to 87%. An exception was one study in Singapore, which captured only 15% of enrolled participants.[63] Among children assessed, data loss (i.e., proportion without sufficient usable data for analysis) ranged from 2% to 44% across studies and time points. The criteria used to define usable data varied depending on the eye-tracking task (e.g., children with trials showing at least 70% fixation on the central stimulus prior to attention shift[49], children with at least 10 valid SRTs per visit[55,60]). Moreover, some of the included studies made adaptions to the stimuli, such as using local faces, highlighting the potential for cross-cultural applicability.[49,55,62] One study reported initial negative

perceptions in a low-income community.[62] However, a similar challenge was found in a high-income country, where participants expressed a lack of interest in having their child undergo neurocognitive assessments.[63] Overall, these suggest that eye-tracking assessments in nutrition studies are feasible across diverse settings, although data quality and reporting practices remain inconsistent.

2.5 Discussion

Despite the limited number of nutrition studies utilizing eye-tracking assessments, 5 of the 9 studies reviewed demonstrated significant relationships between maternal and child nutrition exposures and child cognitive outcomes, particularly in attention and memory domains. However, there was a heterogeneity in both the types of nutrition exposures and eye-tracking assessments used, making it difficult to draw generalizable conclusions about early life nutrition exposures on child cognitive outcomes measured using eye trackers. Nutrition exposures associated with significant results included breastfeeding, supplementary feeding for MAM, home visit interventions, infant plasma choline, TMAO, and iron status.[55,60,63,65,66]

Eye-tracking methods allow for detecting subtle cognitive deficits or improvements when evaluating certain nutrition exposures. In contrast, global developmental assessments, which require more complex integration across domains, may underestimate these effects.[16] However, one of the included studies observed impacts on gross motor and social domains when using global assessments (e.g., MDAT), while no effects were found in the eye-tracking results.[61] This suggested that eye-tracking-based tasks complement global developmental assessments, particularly for evaluating attention and memory domains.

Furthermore, our scoping review showed that eye-tracking assessments are feasible across different settings. This finding aligns with a previous study comparing caregiver acceptance of the method in high-income and low-income countries, demonstrating high acceptance in both settings with comparable completion rates to traditional observational tests.[67] A prior study comparing novelty preference scores using the VPC task among infants in the US and rural Malawi also observed no significant differences, suggesting comparable performance across settings.[27] However, more studies are needed to compare performance on other eye-tracking-based tasks across settings, because some cultural contexts may influence the results. For instance, infants in rural areas might be less familiar with looking at a computer screen, which could affect eye-tracking outcomes.

However, we noted several challenges in using eye-tracking assessments to evaluate impacts. Negative perceptions and lack of interest may occur among caregivers[62,63], which should be addressed through proactive community engagement and communication to describe the nature and purpose of the assessment from the researchers or field teams. Because eye-tracking assessments may be relatively new in some areas, adequate training for local staff and piloting the assessments are crucial to ensure high-quality data.[46,56] This approach can also help in detecting technical issues early on. Nonetheless, certain adjustments can be made to make the procedure easier for assessors, such as reducing the sampling rate to ease calibration.[46] Researchers must also be cautious when transporting eye-tracking tools to remote areas with inadequate transportation infrastructure. One study conducted in a rural setting reported a software error[62], although the specific cause of the error was not further described.

We also identified considerable within-person variation in eye-tracking results over time. Hence, evaluating changes or improvements of the scores at the individual level may be less

suitable than group-level evaluations (e.g., intervention vs. control groups) measured over time. For group-level comparisons, however, obtaining baseline assessments and including healthy infants as controls are crucial to confirm whether a nutrition intervention helps children catch up. For example, in one of the included studies comparing infants treated for MAM with healthy controls, the MAM-treated group had higher baseline SRT, but it was shortened after 4 weeks compared to controls, suggesting that the treatment may have helped to catch up in reaction time.[55] In our review, we also observed heterogeneity in the timing of assessments, which may influence the ability to detect group-based differences. Of note, the group-level evaluations are best performed during periods when differences are still potentially detectable using the same tasks. Otherwise, modified or alternative tasks that are more sensitive to differences in older children might be needed. For instance, preterm infants showed slower IOWA response time at 5 months compared to full-term infants, but these differences were no longer detectable at 10 months, suggesting a "catch-up".[68] Nonetheless, at ten months, preterm infants exhibited attentional deficits specifically in tasks involving saccade cancelling, shifting, and/or inhibition.[69] Additionally, a minimum number of successful attempts may be required during assessments, which can lead to potential data loss. Inconsolable crying and lack of focus among children were identified as key challenges contributing to data loss, highlighting the need for an assessor experienced with children to address these issues.

We noted a few limitations of this scoping review. First, we limited our review to peer-reviewed, published studies to ensure methodological transparency and completeness of reported data; however, this approach may have introduced publication bias by excluding unpublished studies, conference abstracts, or registered clinical trials without published outcomes. The data extracted from the studies only allowed qualitative interpretation of the evidence due to the lack

of uniformity across studies in nutrition exposures and types of eye-tracking-based tasks. Hence, this review was only able to summarize the current state of the field, and more nutrition studies utilizing eye-tracking assessments are warranted to identify which tasks are more sensitive to certain early life nutrition exposures. Furthermore, when selecting tasks, careful consideration should be given to those that are reliable and associated with long-term outcomes. Because nutrition exposures may interact with environmental factors in influencing cognition[8], future studies conducted in varied contexts and populations are also warranted. Lastly, although our scoping review aimed to include studies evaluating any cognitive domain using eye trackers, only attention and memory were represented among the included studies. Prior research that did not include nutrition exposures have used eye-tracking assessments to measure other cognitive domains, including language, social cognition, executive function, and predictive processing.[22–25] These domains should be explored in future nutrition studies, particularly as our understanding of early cognitive precursors continues to grow and more eye-tracking tasks become available for evaluating development in early life.

Overall, our scoping review suggests that eye-tracking assessment is a promising method for evaluating child attention and memory in nutrition studies, with applicability across various settings. The strengths of this review included a comprehensive literature research, and double title-abstract screening, full-text eligibility assessment, and data extraction. Although eye-tracking assessment is a feasible method for use in studies focusing on early-life nutrition, it is crucial to select appropriate eye-tracking-based tasks for a more sensitive evaluation of nutrition interventions.

2.6 Conclusion

Eye-tracking assessment is a feasible and promising method for use in nutrition studies across settings. It enables the detection of subtle deficits or benefits of certain nutrition interventions, a challenge often faced with global developmental tests. However, the selection of eye-tracking-based tasks should be carefully tailored to the type of nutrition exposures, desired long-term outcomes, and the feasibility of proper training for local staff. Future studies are warranted to draw more generalizable conclusions about the impact of early life nutrition exposures on child cognitive outcomes measured using eye trackers.

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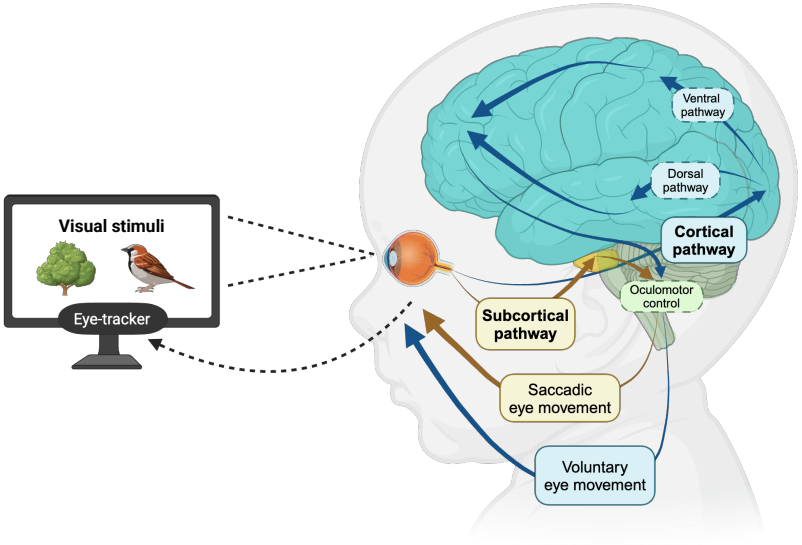

Figure 2.1. Mechanistic pathway of visual processing captured by eye-tracking assessment

(a) (b)

Figure 2.2. General setup for eye-tracking assessment: (a) Assessor facing the mother and infant, or (b) Assessor sitting next to the mother and infant

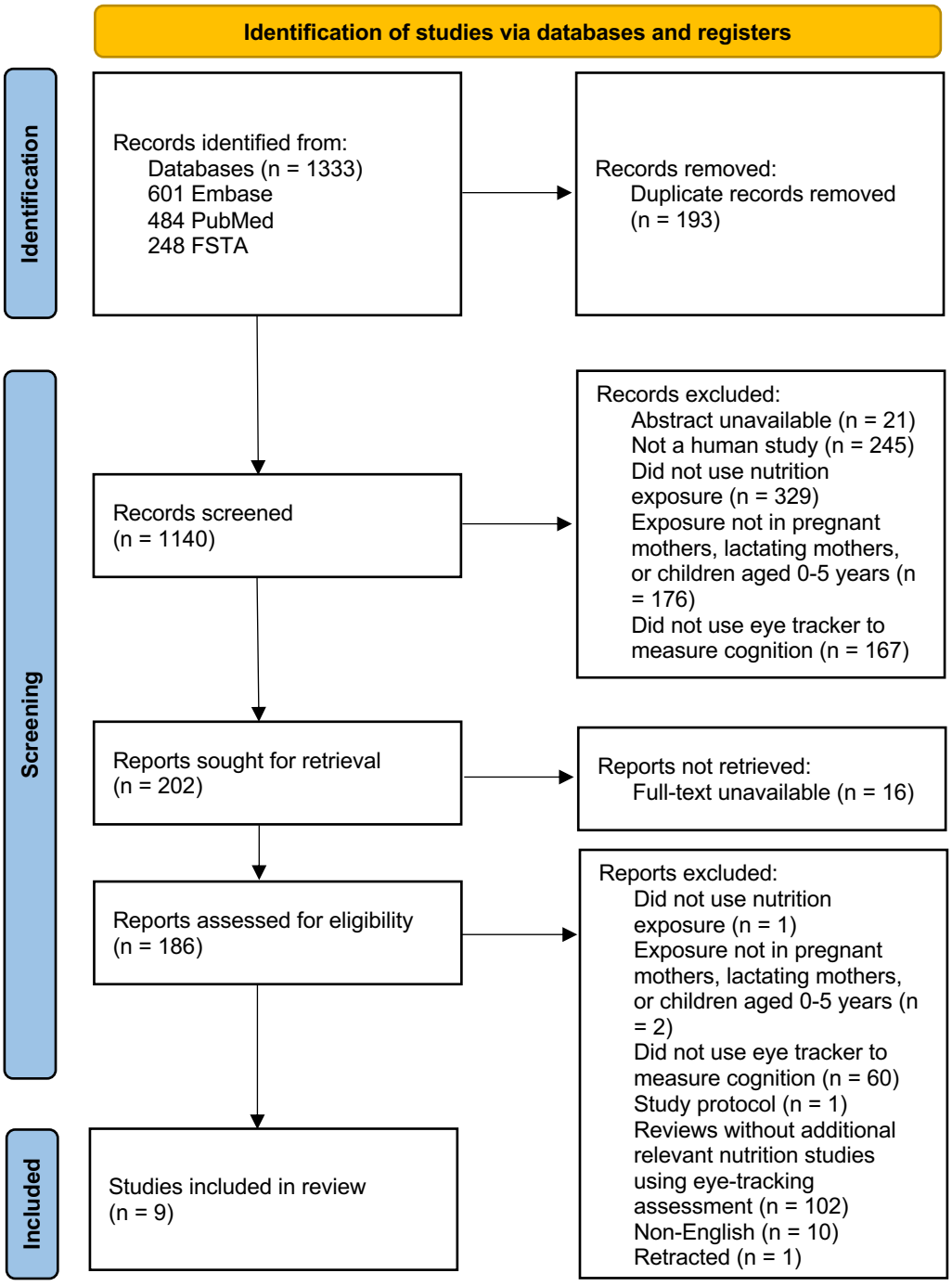

Figure 2.3. PRISMA flow diagram

Table 2.1. Description of included studies

Reference	Study design	Country	Study population for eye-tracking assessment[a]	Nutrition exposure	Duration	Eye-tracking-based tasks	Outcome
Cai et al. (2015)[63]	Prospective cohort study	Singapore	N pregnant women enrolled = 1,247 N children born and enrolled = 1,247	Breastfeeding (duration and exclusivity)	From pregnancy until 24 mo	Memory - Relational binding Attention - Visual expectation	- Better memory at 6 mo, indicated by greater preferential looking toward correctly matched items during early portions of a

			N at 1st visit (6 mo) = 473 (attending); 185 (analyzed) N at 2nd visit (18 mo) = 431 (attending); number of children included in the analysis not reported N at 3rd visit (24 mo) = 514				relational binding task, among higher breastfeeding exposure - No significant association between breastfeeding and visual expectation

			(attending); number of children included in the analysis not reported				
Pyykkö et al. (2019)[49]	Prospective cohort study	Malawi	N infants enrolled = 444 N at 9 mo = 397 (assessed); 384 (analyzed)	LAZ and WAZ at enrolment (birth–28 days old) and 9 mo	From birth until 9 mo	Attention - Visual search - Anticipatory attention shifts - Attention to faces	No significant correlations between any eye tracking scores and LAZ or WAZ

Rees et al. (2019)[64]	Prospective cohort study	England	N pregnant women enrolled = 125 N children born and enrolled = 107 N at 1st visit (4.5 mo) = 101 (attending); number of children included in the analysis not reported	Prenatal DHA intake (maternal intake at 2nd and 3rd trimester of pregnancy)	From pregnancy until 9 mo	Memory - Habituation Attention - Visual attention	No significant differences on habituation and visual attention tasks based on prenatal DHA intake

			N at 2nd visit (9 mo) = 94 (attending); number of children included in the analysis not reported				
Prado et al. (2020)[62]	Randomized controlled trial	Malawi	N infants enrolled = 331 intervention vs. 329 control N = 282 intervention	Egg (1 per day)	6 months	Memory - VPC Attention - IOWA	No significant effects of the intervention on VPC and IOWA tasks

			vs. 290 control (assessed); 236 intervention vs. 239 control (analyzed) Age: 6–9 mo				
Leppänen et al. (2022)[55]	Prospective cohort study nested in a cluster-randomized trial	Sierra Leone	N infants enrolled = 138 with MAM vs. 60 age-matched control	Supplementary feeding for infants in MAM group: - corn-soy blend plus with oil - corn-soy-whey blend with oil	Follow-up until 4 weeks after intervention started	Attention - SRT	Higher adjusted SRT at baseline and improvement in SRT 4 weeks after intervention among MAM infants compared to controls

			N = 135 MAM vs. 56 control (assessed); 96 MAM vs. 46 control (analyzed) Age: 7–11 mo	- super cereal plus with amylase, or - ready-to-use supplementary food			
Stephenson et al. (2022)[61]	Randomized controlled trial	Malawi	N infants enrolled = 864 DHA-HO-RUTF vs. 930 HO-RUTF vs. 964 S-RUTF	Three different RUTF formulations for MAM: - DHA-HO-RUTF - HO-RUTF - S-RUTF	Until reaching a clinical outcome or for a maximum of 6 bi-weekly	Memory - VPC Attention - IOWA	No significant effects of the DHA-HO-RUTF or HO-RUTF, as compared with S-RUTF, on VPC and IOWA tasks

			N subset of infants invited for eye-tracking assessment = 380 DHA-HO-RUTF vs. 350 HO-RUTF vs. 402 S-RUTF N = 248 DHA-HO-RUTF vs. 244 HO-RUTF vs. 297 S-RUTF (analyzed)		follow-up visits		

			Age: 6–59 mo with MAM				
Rockers et al. (2023)[60]	Cluster-randomized controlled trial	South Africa	N infants enrolled = 182 intervention vs. 134 control N at 1st visit (7 mo) = 182 intervention vs. 134 control (assessed); 103 intervention	Home visit intervention integrated to community health workers operations, which included child nutrition	Intervention until children reached the age of 24 mo; follow-up until 36 mo	Attention - SRT	Lower SRT at 7 mo and 17 mo among intervention group

			vs. 73 control (analyzed) N at 2nd visit (17 mo) = 184 intervention vs. 132 control (assessed); 161 intervention vs. 113 control (analyzed) N at 3rd visit (36 mo) = 164 intervention				

			vs. 120 control (assessed); 148 intervention vs. 113 control (analyzed)				
Bragg et al. (2023)[66]	Cross-sectional and prospective cohort studies nested in a randomized controlled	Malawi	N = 400 (analyzed) Age: 6–9 mo	Infant plasma choline and TMAO	6 months follow-up for longitudinal assessment	Memory - VPC Attention - IOWA	- Significant association between higher plasma choline and slower IOWA response time - Significant association between higher

	trial (Prado et al.[62])						plasma choline and faster processing speed on VPC task - Significant association between higher plasma TMAO at baseline and slower processing speed on VPC task at follow-up

McCann et al. (2023)[65]	Prospective cohort study	Gambia	N infants enrolled = 258 N at 1st visit (5 mo) = 195 (assessed); 150 (analyzed) N at 2nd visit (8 mo) = 188 (assessed); 143 (analyzed) N at 3rd visit (12 mo) = 187	Infant iron status at the age of 5 mo	From 5 mo to 5 yo	Attention - Visual disengagement time	- Significant differences on the trajectory of visual disengagement time between infants in highest and lowest terciles of sTfR - No significant differences on the trajectory of visual disengagement time based on

			(assessed); 134 (analyzed) N at 4th visit (18 mo) = 177 (assessed); 134 (analyzed) N at 5th visit (24 mo) = 164 (assessed); 106 (analyzed) N at 6th visit (3–5 yo) = 171				ferritin and Hb status

			(assessed); 167 (analyzed)				

Table 2.2. Overview of eye-tracking-based tasks in the included studies: Assessed domain and function, procedures, and outcome indicators

Cognitive domain evaluated	Function assessed	Eye-tracking-based tasks	Procedures	Indicators	Reference citation
Memory	Binding in memory	Relational binding	Infant watched blocks of trials involving scenes and picture of toys. Each block consisted of study trial and test trial. 1) Study trial: Infant watched 3 audiovisual scenes with a picture of a toy superimposed on each scene. 2) Test trial: Infant watched 1 of the 3 audiovisual scenes, with all 3 pictures of toys viewed	- Proportion of time the child spent looking at each picture to evaluate relational memory (child's preference to look at the correct match)	Cai et al. (2015)[63]

			previously superimposed on the scene.		
	Memory formation	Habituation	Infant watched repeated paired presentations of a familiar and novel stimulus. The side presentation of the novel or familiar stimulus was random. - Familiar stimuli: 4 yellow dots arranged in a shape of a square - Novel stimuli: Patterns of 2, 3, 4, or 5 dots in either blue or red Trial was stopped if infant did not look at the screen for two consecutive trials.	- Look duration to the familiar stimulus - Number of trials required to be habituated (i.e., a decrease to half of the average looking time compared to the initial looking time to the familiar stimulus)	Rees et al. (2019)[64]

	Recognition memory	Visual paired comparison	The task consisted of 4 trials for 4 different pairs of local faces (i.e., adult male-adult female, adult male-child male, adult female-child female, child male-child female). Each trial was composed of a familiarization followed by a recognition memory period. 1) Familiarization period: Two identical faces were presented on the left and right side of the screen. 2) Recognition memory period: A new pair of faces were presented, consisting of the same face shown in	- Novelty preference score, or the proportion of time child spent looking at the novel face compared to the previously seen face - Peak look length - Mean familiarization fixation	Prado et al. (2020)[62], Stephenson. et al (2022)[61], Bragg. et al (2023)[66]

			familiarization period (familiar stimulus) in one side and a novel face in the other side. These stimuli were side-reversed after the first 5 seconds.		
Attention	Planning and learning patterns from visual inputs	Visual expectation	1) Baseline phase: Infant watched 18 movie clips presented in random locations on a screen 2) Experimental phase: Infant watched similar clips presented in locations on the screen that varied according specific pattern (e.g., left-left-right or right-right-left)	- Saccadic reaction time - Reaction time - Average proportion of time spent looking at stimuli - Proportion of trials when infants correctly anticipated - Average duration of anticipation	Cai et al. (2015)[63]

				- Average proportion of time spent looking at the correct location in advance of the stimulus appearing on the screen	
	Selective filtering of visual inputs	Visual search	1) Infant watched a presentation of an image of red apple with an *oh* sound on the center of the screen. 2) A blank screen appeared, followed by the reappearance of the apple in a randomly chosen location. Depending on the experimental condition, the apple was presented alone (*one-object condition*), among	- Number of successful search in *one-object* - Number of successful search in *multiple-objects* - Number of successful search in *conjunction condition*	Pyykkö et al. (2019)[49]

			four or eight distractors of one kind (*multiple-objects condition*), or among distractors of two kinds (*conjunction condition*). 3) If the infant fixated the apple within 4 s from the start of the trial, a reward sound was played while the apple spun. Infant received a total of 8 trials per condition in 2 sessions. Successful search was determined when the gaze hit the area of interest (apple) within predefined time limit.		

	Planning and learning patterns from visual inputs	Anticipatory attention shifts	1) Infant were presented with a fixation stimulus (a pink pig face) in the center of the screen. 2) After infant looked at the fixation stimulus, an auditory cue and two visual placeholders (empty rectangles) were presented to the left and right side of the screen. The furthest edge of the rectangles bordered the edge of the screen. 3) If the infant made a correct anticipatory saccade to the placeholder where a salient	- Percentage of correct anticipation in pre-switch - Percentage of correct anticipation in post-switch	Pyykkö et al. (2019)[49]

			visual stimulus was to be presented, or after 1,000 ms had passed, an audiovisual reward appeared within the rectangle. The reward appeared on one and the same side (left or right) during the first 8 trials (*pre-switch*), then the side was switched on the last 8 trials (*post-switch*). Infant received a total of 16 pre-switch and 16 post-switch trials in 2 sessions.		

	Maintaining focus on a target	Attention to faces	1) Infant watched a dynamic attention grabber on the center of the screen. 2) After the infant fixated on the stimulus, two stimuli were presented with a 1,000 ms onset asynchrony. The first stimulus was a picture of a non-face pattern, or a face displaying a happy or fearful expression, presented on the center of the screen. The second stimulus was a geometric shape, superimposed by a still picture showing the first frame of a	- Percentage of occurred shifts to non-face control stimuli - Percentage of occurred shifts to lateral happy stimulus - Percentage of occurred shifts to lateral fearful stimulus - Dwell time index on non-face control stimuli - Dwell time index on faces	Pyykkö et al. (2019)[49]

			child-friendly cartoon animation. This stimulus was displayed laterally on the left or right side, bordered the edge of the screen. 3) If the infant shifted gaze to the second stimulus, the still picture turned into a dynamic cartoon. Infants received a total of 8 non-face trials and 8 face trials (4 happy, 4 fearful) in a random order in 2 sessions.		

	Maintaining focus on a target	Visual attention	Infant watched four consecutive trials presenting a stimulus, which was a circular, yellow, smiley face rotated around the monitor on a circular path. On each trial, there was a randomly selected critical position (at 0^o, 90^o, 180^o, or 270^o). If the infant were able to continually track the stimulus for the 10 degrees preceding the critical position, then the second stimulus, a 3D blue box, would appear in the center of the screen. The original smiley face stimulus	- Number of box (i.e., second stimulus) appearances - Number of rotations preceding box - Switch response time from the first to second stimulus	Rees et al. (2019)[64]

			continued to rotate on its path despite the appearance of the second stimulus. If infant remained fixated on the first stimulus (i.e., smiley face), both stimuli remained on the screen for a further four rotations of the first stimulus, then both disappeared, followed by a new trial. If the infant did not make the box appear after 9 rotations of the first stimulus, the next trial begin.		
	Orienting toward and away from	Infant orienting with attention	1) A central fixation appeared as a bright yellow dynamic smiley face, accompanied with	- Response time - Cue facilitation score - Cue interference score	Prado et al. (2020)[62], Stephenson

	objects at various spatial locations		classical music. When the infant's gaze was fixed on the central fixation, the eye-tracking assessor pressed a key to advance to the trial. 2) Infant saw a 100 ms spatial precue depending on the trial type. The task consisted of 3 experimental conditions (valid, invalid, and double cues) and 1 control condition (no cue). A black dot serves as a spatial precue preceding a randomly presented target image. The number and location of the		et al. (2022)[61], Bragg et al. (2023)[66]

			black dot presented depend on the types of cue conditions. 3) A 100 ms blank screen. 4) Infant was displayed a target image for 1000 ms, The target images consisted of 96 colorful everyday objects, some familiar and others unfamiliar to the children, such as fruits, balls, and baskets. In the valid cue condition, both the cue and target image appeared on the same side, while in the invalid cue condition, the target		

			image was presented on the opposite side of the cue. The double cue condition involved cues on both sides, with the target image presented at the spatial location of 1 of the 2 cued locations. In the baseline condition, no cue was presented, but the target image still appeared on either the left or right side of the screen. Infants received up to 24 trials per trial type.		
	Orienting toward and	Saccadic reaction time	After calibration, in each session, infant was presented (1) a long	Saccadic reaction time, calculated as time interval	Leppänen et al.

	away from objects at various spatial locations		social scene, followed by (2) five saccade targets, (3) a short social scene, (4) five saccade targets, (5) a long social scene, (6) five saccade targets, (7) short social scene, and (8) five saccade targets. Infants received 2 sessions (8 blocks of 5 saccade target, 4 long and 4 short social scenes. - Saccade targets: Targets were colorful cartoon animations of common objects (e.g., fish, soccer ball). Targets were presented one at a time in the	from the onset of the saccade target to the first entry of the gaze into the area of the saccade target. Analysis of the eye-tracking data during social scene viewing had not been reported.	(2022)[55], Rockers et al. (2023)[60]

			center of the screen, and each subsequent target in a new randomly chosen on-screen location 10^{o} away. After appearing on screen the animations remained paused at the first frame and the accompanying audio silenced until the infant looked at the animation or a 1-s wait period elapsed. The animation was subsequently played for 2500 ms with an accompanying sound.		

			- Social scenes: Stimuli were 5-15s video recordings of female models. In the short social scene 5 s video, the model looked directly at the camera and greeted the infant in infant's natural language. In the long social scene 9.5-15 s video, the model looked at the camera while enacting a short story or a song appropriate for infant's age range. The videos were taken in various environments (e.g., living room, kitchen).		

	Orienting toward and away from objects at various spatial locations	Visual disengagement time	1) Infant watched a dynamic attention grabber on the center of the screen. 2) After the infant fixated on the stimulus, the stimulus started to spin to maintain infant attention. After 600-700 ms, a peripheral stimulus was presented to the left or right side of the screen in one of three conditions: *baseline, gap,* and *overlap.* - Baseline condition: Peripheral stimulus appeared and central	Disengagement time, calculated as the difference in saccadic reaction time between the baseline and overlap conditions.	McCann et al. (2023)[65]

			stimulus disappeared simultaneously. - Gap condition: Central stimulus disappeared 200 ms prior to the appearance of the peripheral stimulus, reducing competition for attention. - Overlap condition: Central stimulus remained on the screen, but stopped rotating when the peripheral appeared, increasing competition for attention		

			3) The peripheral stimulus remained on the screen until gaze fixation was detected, or until 2000 ms had passed. It became animated, and engaging sounds were played. Infants received fifteen trials in each condition.		

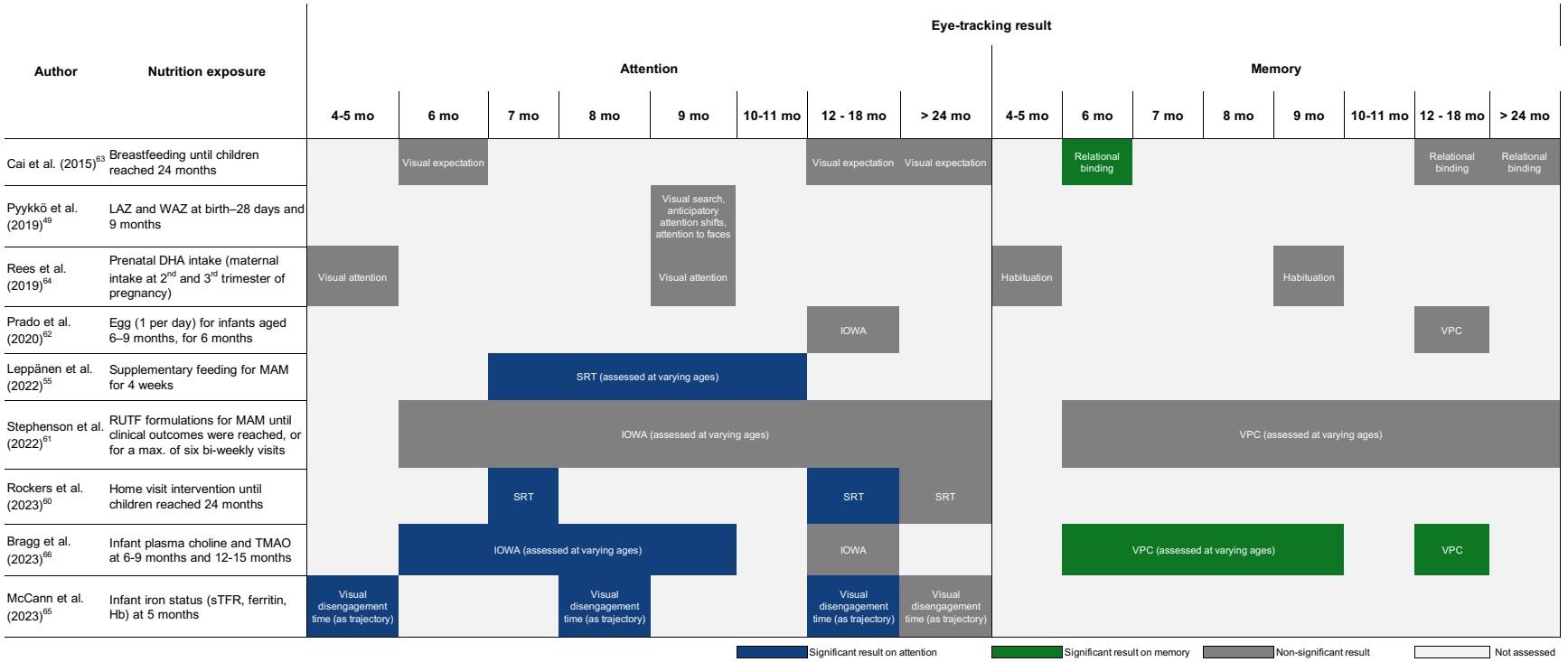

Figure 2.4. Summary of relationships between maternal and child nutrition exposures and eye-tracking outcomes in the included studies. DHA, docosahexanoic acid; Hb, hemoglobin; IOWA, Infant Orienting with Attention; LAZ, Length-for-age z-score; MAM, moderate acute malnutrition; SRT, saccadic reaction time; sTfR, soluble transferrin receptor; TMAO, trimethylamine N-oxide; VPC, visual paired comparison; WAZ, Weight-for-age z-score.

2.8 Supplementary materials

Supplementary Table 2.1. Search terms used for the scoping review

Database	Population	Intervention/Comparison	Outcome
Pubmed	child OR infant OR toddler OR infancy OR pregnan* OR lactati* OR breastfe*	"Diet, Food, and Nutrition"[Mesh] OR "Nutrition Disorders"[Mesh] OR nutrition*[tiab] OR supplement*[tiab] OR food*[tiab] OR diet*[tiab] OR meal*[tiab] OR macronutrient*[tiab] OR micronutrient*[tiab] OR protein*[tiab] OR lipid*[tiab] OR omega*[tiab] OR polyunsaturated fatty acids[tiab] OR PUFA[tiab] OR "Fatty Acids, Omega-3"[Mesh] OR iron[tiab] OR zinc[tiab] OR iodine[tiab] OR copper[tiab] OR	"Eye Movement Measurements"[Mesh:NoExp] OR "Eye-Tracking Technology"[Mesh] OR ("Visual Perception"[MeSH] AND (Cognition[Mesh] OR Attention[Mesh] OR Memory[Mesh])) OR “eye track*”[tiab] OR "eye-track*"[tiab] OR eyetrack*[tiab] OR “gaze track*”[tiab] OR "Gaze-Track*"[tiab] OR Gazetrack*[tiab] OR "eye movement track*"[tiab] OR “looking time”[tiab] OR “looking behavi*”[tiab] OR Fagan[tiab] OR habituation*[tiab]

		vitamin*[tiab] OR "Carotenoids"[Mesh] OR "Vitamin B 6"[Mesh] OR "Folic Acid"[Mesh] OR "Vitamin B 12"[Mesh] OR "Ascorbic Acid"[Mesh] OR "Vitamin D"[Mesh] OR choline[tiab] OR probiotic*[tiab] OR prebiotic*[tiab] OR protein*[tiab] OR selenium[tiab]	
Embase	child* OR infant* OR toddler* OR infancy OR pregnan* OR lactati* OR breastfe*	'nutrition'/exp OR 'fatty acid'/exp OR 'nutritional disorder'/exp OR nutrition*:ab,ti,kw OR supplement*:ab,ti,kw OR food*:ab,ti,kw OR diet*:ab,ti,kw OR meal*:ab,ti,kw OR macronutrient*:ab,ti,kw OR micronutrient*:ab,ti,kw	'eye movement monitor'/exp OR 'eye-tracking technology'/exp OR 'eye tracking'/exp OR ('vision'/exp AND 'cognition'/exp) OR "eye track*":ab,ti,kw OR "eye-track*":ab,ti,kw OR eyetrack*:ab,ti,kw OR "gaze track*":ab,ti,kw OR "Gaze-Track*":ab,ti,kw OR Gazetrack*:ab,ti,kw OR "eye

		OR protein*:ab,ti,kw OR lipid*:ab,ti,kw OR omega*:ab,ti,kw OR polyunsaturated fatty acids:ab,ti,kw OR PUFA:ab,ti,kw OR iron:ab,ti,kw OR zinc:ab,ti,kw OR iodine:ab,ti,kw OR copper:ab,ti,kw OR vitamin*:ab,ti,kw OR 'carotenoid'/exp OR 'pyridoxine derivative'/exp OR 'folic acid derivative'/exp OR folate OR 'cobalamin derivative'/exp OR 'ascorbic acid'/exp OR 'vitamin D'/exp OR choline:ab,ti,kw OR probiotic*:ab,ti,kw OR prebiotic*:ab,ti,kw OR	movement track*":ab,ti,kw OR “looking time”:ab,ti,kw OR “looking behavi*”:ab,ti,kw OR Fagan:ab,ti,kw OR habituation*:ab,ti,kw

		protein*:ab,ti,kw OR selenium:ab,ti,kw	
Food Science and Technology Abstracts	(child* or infant* or toddler* or infancy or pregnan* or lactati* or breastfe*).mp.	nutrition/ or malnutrition/ or omega-3 fatty acids/ or retinoids/ or vitamin b6/ or folates/ or folic acid/ or cobalamins/ or ascorbic acid/ or vitamin d/ or (nutrition* or supplement* or food* or diet* or meal* or macronutrient* or micronutrient* or protein* or lipid* or omega* or "polyunsaturated fatty acids" or pufa or iron or zinc or iodine or copper or vitamin* or choline or probiotic* or prebiotic* or protein or selenium).ab,ti.	(visual.mp. and (cogniti* or attenti* or memory or language or perception).ab,ti.) or ("eye movement" or "eye track*" or "eye-track*" or eyetrack* or "gaze track*" or "Gaze-Track*" or Gazetrack* or "eye movement track*" or "looking time" or "looking behavi*" or Fagan or habituation*).ab,ti.

Chapter 3:

Growth trajectories and cognitive outcomes in childhood:

A comparative study of Malawi and the USA

3.1 Abstract

Variations in early-life growth patterns may reflect differences in metabolic programming influencing child attention and memory. We identified growth trajectories and their associations with attention and memory among infants and young children across multiple time points in diverse contexts. Anthropometric data were collected from cohorts in Malawi (n=600) and suburban California, USA (n=101) at birth and at 6–9, 8–12, and 11–15 months. Latent class trajectory modeling identified sex-specific growth pattern classes separately for weight and weight-for-age z-score in both cohorts, and for length, length-for-age, and weight-for-length z-scores in Malawi. In a subgroup (n=458 in Malawi; n=92 in the USA), associations between growth trajectories and cognitive outcomes were assessed with eye-tracking-based tasks between 6–15 months. The Infant Orienting with Attention task measured visual attention (reaction time, task error); the Visual Paired Comparison task measured memory (novelty preference, peak look duration, shift rate; only in Malawi). Mixed effects models were used to evaluate associations, adjusting for child, parental, and household characteristics. Growth trajectory classes varied by cohort, some aligning with WHO standards and others showing either acceleration or deceleration. In Malawi, boys who started with the lowest intercept at birth and ended with the highest weight by 12 months demonstrated poorer memory (via novelty preference) at later ages (12–15 months) compared with those who had a higher starting point at birth but end the lowest ($\beta \pm$ SE: -0.13 $\pm$ 0.05; p=0.026) and average (-0.14 $\pm$ 0.05; p=0.016). Associations with reaction time and shift rate were identified in Malawi at earlier ages (6–9 months). No significant associations were observed for USA cohort. Early growth patterns are linked to emerging attention and memory, highlighting the importance of longitudinal evaluation. Further studies are warranted to understand pathways linking nutrition, growth, and development prenatally through six months of life.

3.2 Introduction

Globally, over 250 million children under the age of five are estimated to be at risk of not reaching their developmental potential, particularly in low- and middle-income countries (1). This is of concern because early childhood is a critical period for brain development, including the emergence of core cognitive domains such as attention and memory. These abilities form the foundation for learning, and early deficits can lead to long-term consequences (2–5). To better mitigate these risks, it is crucial to assess early-life adversities that shape cognitive outcomes during this critical window. Among these adversities, nutrition is a key modifiable factor that provides essential nutrients for brain development and supports children's ability to engage effectively with their environment (6,7).

However, capturing how nutrition influences cognitive development is complex. Both nutrition and cognition are dynamic processes that evolve over time, especially during the first years of life. Traditional approaches that rely on single time-point measurements often fail to reflect these changing patterns. Therefore, longitudinal methods may better capture the evolving nature of these relationships. One such approach is evaluating child growth trajectories rather than static anthropometric indicators. Growth trajectories, derived from repeated measures of weight and length over time (8), provide a life-course analysis that reflect the cumulative impact of nutrition and health during the sensitive period of brain development. Deviations from expected growth, such as decelerated weight gain or linear growth faltering, may signal increased risk for suboptimal neurodevelopment through inadequate supply of nutrients, altered metabolic programming, and inflammatory processes (9–12). A few previous studies have identified distinct growth trajectory groups in children and demonstrated associations between these groups and later outcomes related to body size, stature, schooling, cognitive and socioemotional outcomes (13–16).

Alongside longitudinal growth assessment, cognitive evaluations in early life must also account for developmental timing and effects on specific cognitive domains. Many global developmental tests produce composite scores that may obscure subtle differences in specific domains affected by nutrition (17,18). In contrast, eye-tracking technology offers scalable assessments that can detect differences in certain cognitive domains (19). Decades of research provide robust evidence that gaze behavior systematically reflects underlying cognitive processes (20–23). Eye-tracking-based tasks such as Infant Orienting with Attention (IOWA) and Visual Paired Comparison (VPC) assess aspects of visual attention and recognition memory, respectively, based on children's eye movements (24,25). These processes are particularly sensitive to early-life perturbations and are strongly associated with later academic and cognitive outcomes (26,27). The IOWA task measures a child's ability to shift attention and program and execute eye movements to a rapidly appearing target, while the VPC task evaluates novelty preference, a behavioral marker of recognition memory.

Our study evaluated the associations between child growth trajectories and visual attention and memory among infants and young children across different cohorts in Malawi and the suburban California, United States (USA). We applied an unsupervised learning approach of latent class trajectory modeling to classify growth patterns based on repeated anthropometric measures. Cognitive outcomes were assessed using eye-tracking-based IOWA and VPC tasks at multiple time points. Previous studies have compared these two tasks in Malawi and the USA, in which both study populations showed similar levels of engagement and patterns of shifting attention and novelty preference (28,29). By integrating longitudinal assessments of both growth and cognition, this study aimed to provide a clearer understanding of how early growth patterns are associated with specific cognitive systems across diverse settings.

3.3 Methods

Study cohorts

We used cohort data from randomized trials conducted in Malawi (Mazira study) and the USA (IMiND study). The Mazira study was a randomized controlled trial evaluating the impact of daily consumption of eggs for 6 months conducted from 2018 to 2019 in rural Lungwena and Malindi, Mangochi District, Malawi, involving 660 children (egg: n=331; no egg: n=329) aged 6–9 months at baseline (30,31). The Infant Microbiome, Nutrition, and Development (IMiND) study was a randomized crossover trial carried out from 2017 to 2020 in Davis, California, involving 101 exclusively breastfed infants aged 6 months at baseline (32). Two cohorts were used for this study. In the first cohort, infants entered a 7-day lead-in period of exclusive breastfeeding, followed by 7 days of either pear first (n=31) or sweet potato first (n=33) as a complementary food with continued breastfeeding, a 4-day washout period of exclusive breastfeeding, 7 days of the alternate study food with continued breastfeeding, and a final 4-day follow-up period of exclusive breastfeeding. Amidst the study, a published report found high concentrations of heavy metals in baby food containing sweet potatoes. In response to these findings, the IMiND study dropped the sweet potato arm and in a second cohort only used pears (n = 38) as the study food. For this cohort, infants entered a 7-day lead-in period of exclusive breastfeeding, followed by 7 days of pear as a complementary food with continued breastfeeding, and a 4-day washout period of exclusive breastfeeding. For the present analysis, we collapsed all intervention arms for both the Mazira and IMiND studies and included children with eye-tracking data at any time point and anthropometric data at least two postnatal time points.

Anthropometric assessments

Repeated anthropometric measurements were performed within each study to identify the growth trajectories. In the Malawi cohort (n=600), anthropometric assessments were performed at baseline (6–9 mo), midline (9–12 mo), and endline (12–15 mo). Available birth weight data extracted from child health passport were included (n=152); birth weight data recalled by mothers (n=226) were excluded. In the USA cohort (n=101), children were measured by study personnel at repeated timepoints (3 to 6 timepoints) between baseline (6–7 mo), midline (8–10 mo), and endline (11–12 mo), with all birth weight recalled by the mothers. As part of data cleaning, we excluded extreme birthweight values below 500 g and above 5000 g in both cohorts (Malawi: 135 final birth weight observations available out of 600; USA: 101 out of 101) (33). The timing and methods of anthropometric assessments across cohorts are summarized in **Supplementary Table 3.1**.

Growth trajectories identification

We used latent class trajectory modeling (LCTM) to determine child growth trajectories, adapted from the modeling framework developed by Lennon et al. (2015) (8,34). Growth trajectories were identified for weight, weight-for-age z-score (WAZ), length, length-for-age z-score (LAZ), and weight-for-length z-score (WLZ), stratified by sex and analyzed separately for each cohort. LCTM was performed using '*hlme*' function from '*lcmm*' package in R software environment and SAS Proc Traj in SAS software (8,35). This approach identifies latent trajectory classes based on repeated anthropometric measurements as a function of child age and assigns individuals to the class for which they have the highest posterior probability. Model adequacy of LCTM was evaluated using three criteria: average posterior probability assignment (APPA) greater than 70% for all classes, odds of correct classification (OCC) values greater than 5.0, and relative entropy

values (E_K) greater than 0.5. We assessed class separation using Degree of Separation (DoS_k), where a DoS_k values of zero indicates no distinction between mean trajectories.

Figure 3.1 summarizes the modeling steps. We first determined the initial fixed- and random-effect structure by examining the linearity of the shape of residual plots of each trajectory group in a model without random effects. We then tested models with varying numbers of classes (1 to 7) and selected the model with the lowest Bayesian Information Criteria (BIC). For each favored number of classes, we compared six model variants under different assumptions about random effects and residual variances (e.g., random intercept, random slope, random quadratic with common variance structure across classes), which underwent further refinement to achieve the most parsimonious model while fulfilling the model adequacy criteria (8,36).

If the tested models failed to meet adequacy criteria, we refined them through several steps. We first added natural splines while retaining the number of classes. If this did not resolve the issue, we tested a different number of classes with the next lowest BIC, reintroducing splines if necessary. If criteria were still unmet, we evaluated alternative model models (i.e., linear vs quadratic model) using the previously selected number of classes. This iterative process was repeated until the adequacy criteria were fulfilled. If multiple models met all criteria, the most parsimonious model was chosen. When only two trajectory groups were identified and one comprised less than 5% of the sample (37,38), we excluded individuals in the small group and repeated LCTM to assess whether group proportions improved. If three or more trajectory groups were identified and one remained too small (<5%), we excluded that group from associational analyses with development outcomes.

Visual attention and memory assessments

We assessed visual attention and memory using eye-tracking-based IOWA and VPC tasks. IOWA tasks were performed in both cohorts, while the VPC task was administered only in the Malawi cohort (**Supplementary Table 3.2**). In both cohorts, tasks were performed longitudinally. In Malawi, assessments were conducted when children were 6–9 months and 12–15 months (28,29,31,39). In the USA, assessments occured at 6, 8–9, and 11–12 months (32).

All tasks were conducted in quiet, minimally distracting settings. Children sat facing a monitor approximately 60 cm away, either on their mother's lap, in an infant carrier, or seated independently. Tobii Pro X2 eye tracker (60 Hz sampling rate) was used in Malawi, and the SMI-Red-M system (120 Hz sampling rate) was used in the USA cohort. Eye fixation coordinates (x, y) were recorded and processed using default fixation filters (i.e., Tobii I-VT Fixation Filter and the SMI's BeGaze software default fixation parameters for low-speed eye tracking data).

The IOWA task assessed visual orienting speed and accuracy under varying levels of visual competition (24). Each trial followed a structured sequence: (1) a central fixation stimulus (a bright yellow, dynamic smiley face) was presented at the center of the display to attract the infant's attention; (2) once the infant's gaze was fixed on this central stimulus, the eye-tracking assessor initiated the trial by pressing a key in the Malawi cohort, or a trigger area-of-interest (AOI) process automatically initiated the trial in the USA cohort; prompting (3) the brief (100 ms) appearance of a spatial precue (black dot), whose number and location varied depending on the cue type; (4) this was followed by a brief (100 ms) blank screen and then (5) presentation of the target image. There were four cue conditions that each presented a different amount of visual competition. The *no cue* condition involved no competition; no spatial cue was provided, and the target was randomly shown on either the left or right side of the display. In the valid cue condition, the cue and target

image appeared on the same side of the screen, and thus drew attention to the same location. In the invalid cue condition, the target appeared on the opposite side of the cue, providing competition for attention to two locations. In the double cue condition, cues appeared on both sides, and the target appeared at one of the two cued locations, providing competition for attention during the cue period. Each child was exposed to up to 24 trials per cue condition. Target images varied across trials and were drawn from a set of 96 colorful, everyday objects (e.g., fruits, balls, baskets), with some images likely to be familiar and others unfamiliar to the child. In all studies, IOWA task trials were intermixed with a completely different task, which helped to maintain infants' interest.

In IOWA task, we calculated the reaction time, defined as the duration from target onset to the first fixation within the AOI for the target. Only correct trials, defined as those in which the first fixation occurred within the target AOI, were used for reaction time calculation. Trials were excluded if central fixation was <200 ms (indicating potential lack of engagement), or if latencies to the AOI were <100 ms or >1000 ms (24,31). We also calculated task error as an measure of accuracy. Task error was computed from the proportion of correct invalid and double cue trials, the two trial types that provided a competition for attention; we used a binary indicator (task error = 0 vs. >0), with zero error reflecting better accuracy.

The VPC tasks assessed visual recognition memory and has been previously used in diverse contexts with varying stimuli (40–44). In the Malawi study included here, each trial included a familiarization phase in which a single (culturally appropriate) face was presented, followed by a recognition test phase in which that now-familiar face was paired with a novel (culturally appropriate) face. The side of the novel face was reversed after five seconds. The four trials included adult male-adult female, adult male-child male, adult female-child female, and child

male-child female pairs of faces. Trials with less than one second of total looking time during either phase were excluded.

Three look-defined outcomes were derived from the VPC task using the same methods described in Beckner et al. (2023) (28). The novelty preference score was calculated as the proportion of time the child spent looking at the novel face AOI, with higher scores reflecting better recognition memory. Peak look duration, measured during the familiarization phase, represented the duration of the longest look children executed during learning. Shift rate, calculated as the number of gaze shifts between AOIs per second of total looking time across both familiarization and recognition test phases, captured the child's visual exploratory behavior. Shorter peak look duration and faster shift rate have been associated with higher novelty preference, reflecting more efficient processing (45,46).

Statistical analysis

A detailed statistical analysis plan was preregistered on the Open Science Framework (https://osf.io/z8quh/). We analyzed associations between child growth trajectories and visual attention and memory outcomes separately by cohort, anthropometric indicator, and child sex. The child's identified growth trajectory class was used as the predictor variable, with the class most closely resembling the World Health Organization growth standard median trajectory (47) used as the reference. For outcomes with trial-level data (i.e., multiple observations per participant), we evaluated associations with growth trajectories using a robust linear mixed-effects model (RLMM) (48) for reaction time and linear mixed-effects model (LMM) for novelty preference score, peak look duration, and shift rate; all models included random intercepts for participant and stimulus. RLMM was specifically chosen for reaction time because latency-based outcomes are commonly

non-normally distributed, leading to a non-normal distribution of the error variance. RLMM accounts for this by down-weighting extreme values, allowing more reliable parameter estimation (49). Task error, the only outcome with participant-level data (i.e., single observation per participant), was evaluated using logistic regression due to its binary classification (i.e., 0% task error vs. non-zero task error).

All outcomes assessments included minimally adjusted and fully adjusted models. Minimally adjusted models included fixed effects of growth trajectory group, child age at assessment, and cue type (for reaction time). Fully adjusted models incorporated additional covariates related to child characteristics (e.g., birth order, prematurity) and household and parental characteristics (e.g., maternal and paternal age, maternal height, and parental education) selected through bivariable screening for association with the outcome ($p < 0.1$).

We used a stepwise approach for each minimally and fully adjusted models. For reaction time outcomes, we first assessed whether to include an interaction term between growth trajectories and cue type by comparing the model fit of full and reduced models using analysis of variance (ANOVA). Interactions with $p < 0.1$ were retained, and subsequent analyses were stratified by cue type. For all outcomes, we evaluated the main effect of growth trajectories by comparing the fit of full and reduced models using ANOVA. A threshold of $p < 0.1$ was used to determine whether to proceed with pairwise comparisons. When applicable, pairwise comparisons between growth trajectory groups were conducted using a two-sided superiority framework with 95% confidence intervals. We did not apply corrections for multiple comparisons because the outcome indicators (e.g., reaction time, task error, novelty preference) represent conceptually related and interdependent aspects of visual attention and memory (50).

Ethics approvals

This secondary analysis used de-identified data from previously approved randomized trials. The Mazira study was approved by the institutional review boards at the University of California, Davis and the College of Medicine in Malawi. The IMiND study was approved by the institutional review board at the University of California, Davis.

3.4 Results

In this cross-country comparative study, we included children with eye-tracking data available at any time point of assessment. To identify growth trajectory groups, we used the full sample from each study with at least two postnatal assessments, excluding birth, regardless of the availability of eye-tracking data (n=600 from Malawi and n=101 from the USA). Some eye-tracking results were missing, collected using different eye-tracking protocol (for Malawi cohort at baseline), or classified as 'unsuccessful' and were therefore excluded from the analysis due to various reasons (e.g., children were fussy, not focused on the screen, or had reaction times outside the acceptable range). Final usable data for analysis included 245 children at baseline and 458 at endline in the Malawi cohort (**Supplementary Figure 3.1**), and 87 at baseline, 92 at midline, and 80 at endline in the USA cohort (**Supplementary Figure 3.2**).

Comparisons of child, household, and parental characteristics of the Malawi and USA cohorts are presented in **Supplementary Table 3.3**. Both cohorts were comparable in child sex distribution (50.0% girls) and paternal age but differed in other characteristics. The Malawi cohort had a lower proportion of parents who were married or living together (57.6%) compared to the USA (96.7%). Mothers in the USA cohort were generally older (32.4 ± 3.7 years vs. 26.1 ± 6.6 in Malawi), taller (165.0 ± 6.9 cm vs. 157.0 ± 5.3 in Malawi), and had higher body mass index (24.2

± 3.1 kg/m^2 vs. 21.9 ± 3.1 in Malawi). The USA cohort also had the highest proportions of both maternal and paternal education at the bachelor's level (45.6% and 38.0%, respectively) and at the graduate/professional level (31.5% and 29.4%, respectively). Paternal occupations varied widely across both cohorts.

Growth trajectories across cohorts

Growth trajectories identified in the Malawi and USA cohorts are summarized in **Supplementary Table 3.4** and **Supplementary Table 3.5.** The number of trajectory classes ranged from two (e.g., weight and WAZ among boys in the USA) to five (e.g., WAZ among girls in Malawi). The Malawi cohort showed a greater number of trajectory classes for weight and WAZ than the USA, except WAZ among boys. All models met adequacy criteria, with DoS_k ranging from 0.13 (WLZ among boys in Malawi) to 127.64 (length among girls in Malawi).

After excluding trajectory classes representing fewer than ~5% of participants, we described ponderal (i.e., weight, WAZ, and WLZ) and linear (i.e., length and LAZ) growth trajectories from 0 to 12 months for each cohort in **Figure 3.2** and **Figure 3.3**, respectively, based on time points specific to each cohort. Growth trajectory patterns varied across cohorts. Both cohorts had at least one class that aligned with the World Health Organization (WHO) growth standards, while other classes showed either acceleration or deceleration. Within each cohort, weight trajectory classes showed similar intercepts at birth but they diverged notably by 6 months. From this point onward, WAZ, LAZ, and WLZ trajectories exhibited varying intercepts among classes, followed by mostly flat or negative trends through 12 months. Girls in both Malawi and the USA cohorts generally demonstrated more heterogeneous ponderal growth trajectories from 6 to 12 months, with more classes and greater variability in intercepts than boys. In Malawi, ponderal

growth trajectories tended to result in more classes than linear growth trajectories. Additionally, girls overall revealed more linear growth trajectory classes than boys, indicating greater variability.

Growth trajectories associations with attention and memory in early life

Assessment time points of the attention and memory outcomes varied across cohorts. In the Malawi cohort, the mean age at assessment was 7.2 ± 1.3 months (baseline) and 13.3 ± 1.2 months (endline). In the USA cohort, children were assessed at 6.0 ± 0.5 months (baseline), 8.0 ± 0.7 months (midline), and 11.9 ± 0.5 months (endline). Associations between growth trajectories and early-life visual attention and memory, as evaluated using fully adjusted models, are summarized in the heatmap in **Figure 3.4** and **Supplementary Table 3.6**[2]. Measures of attention and memory were associated with growth trajectories in the Malawi cohort, but not in the USA cohort. Recall that the VPC task of memory was administered only in the Malawi cohort.

Novelty preference, an indicator of memory, was significantly associated with growth trajectories in the Malawi cohort; these associations were found only for weight gain among males. Boys in the 'end high' group, who started with the lowest intercept at birth and ended with the highest weight by 12 months, had lower novelty preference at 12–15 months compared to the 'end low' group (estimated mean difference ± SE: -0.13 ± 0.05; p=0.026) and 'end average' group (-0.14 ± 0.05; p=0.016). Shift rate, another indicator of memory, was associated with linear growth trajectories in Malawi. Boys in the 'start low–end low' LAZ trajectory group showed lower shift rate at 6–9 months compared to the 'start average–end average' group (-0.06 shifts/s ± 0.03; p=0.040).

[2] Supplemental Excel table is available on Open Science Framework at https://osf.io/rva6p/.

Analysis of the IOWA task, the measure of attention administered to both cohorts, revealed that reaction time was associated with linear growth trajectories only in the Malawi cohort. Linear growth trajectories between 6 and 12 months were significantly associated with reaction time at 6–9 months in both boys and girls (**Figure 3.5**). Girls in the 'start lowest–end lowest' LAZ and length trajectory group showed slower reaction time in invalid cue trials compared to the 'start average–end average' trajectory group (90.55 ± 31.10 ms; p=0.019 for LAZ and 77.2 ± 28.51 ms; p=0.019 for length). Boys in the 'start low–end low' LAZ and length trajectory group showed faster reaction time in valid cue trials (-54.57 ± 18.34 ms; p=0.003 for both LAZ and length) and double cue trials (-26.29 ± 11.89 ms; p=0.027 for both LAZ and length), as compared to the 'start average–end average' group. No significant associations were identified between growth trajectory groups and attention outcomes in the USA cohort.

3.5 Discussion

Our cross-cultural analyses in Malawi and the USA revealed variations in early life growth trajectories across cohorts, with some aligning with WHO growth standards and others showing acceleration or deceleration. These trajectories were primarily distinguished by their intercepts at 6 months, suggesting that unmeasured prenatal or early postnatal factors likely influenced subsequent growth. Growth trajectory groups were associated with cognitive outcomes, and these associations varied across time points, sexes, and contexts. For boys in Malawi, the memory measure of novelty preference was significantly associated with ponderal growth trajectories at later ages (12–15 months) with between-group differences ranging from 0.13-0.14 (score range: 0–1), and shift rate was linked to linear growth trajectories at earlier ages (6–9 month), with between-group difference of 0.06 shifts/s. Reaction time, a measure of visual orienting speed, was

also associated with linear growth in Malawi at similar early ages, although directionality differed by sex: girls in linear growth trajectory groups with lower intercepts at 6 months showed slower orienting speed by 77.2–90.55 ms, while boys showed faster speed by 26.29–54.57 ms. No significant associations were observed between growth trajectories and attention outcomes in the USA cohort at any time point; memory outcome were not assessed in this cohort.

Growth trajectories from 6 to 12 months were primarily differentiated by their intercepts at 6 months, suggesting they reflect a continuation of earlier, unmeasured growth patterns originating prenatally or within the first 6 months of life. Similar findings were reported by Ramírez-Luzuriaga et al. (2021), who documented that length trajectories from birth to 84 months were differentiated primarily by intercepts at birth (16). The underlying mechanisms are thought to involve a combination of genetic factors and intrauterine environmental exposures (e.g., delivery of nutrients) that contribute to fetal programming (51). Additionally, in both cohorts, we found that some–if not most–children aligned closely with the WHO standard median trajectory.

In our study, children with lower starting point–either at birth (for ponderal growth) or at six months (for linear growth)–generally demonstrated poorer cognitive outcomes. Differences between these trajectory groups were apparent by 6 months and often persisted through 12 months, hinting at either the influence of unmeasured prenatal and early postnatal growth patterns during the first six months or other shared antecedents of both growth and memory outcomes in early life. This was evident as early as 6 months in the Malawi cohort, as seen in the associations between linear growth trajectories (6–12 months) and shift rate (6–9 months) among boys. At later ages (i.e., 12–15 months), boys in Malawi who belonged to the weight trajectory group with lowest intercept at birth but reaching the highest weight by 6 months and persisted though 12 months exhibited poorer recognition memory compared to other groups that began with higher weight at

birth. Findings on reaction time were less consistent. In linear trajectory groups with lower intercepts at 6 months and persisted through 12 months, girls showed slower orienting speed while boys showed faster orienting speed at 6–9 months.

Our longitudinal approach allowed us to capture these growth dynamics compared to cross-sectional assessments (e.g., stunting status or single-point LAZ measures), providing a more comprehensive reflection of overall health in early life. Infants with higher intercepts by six months may have benefited from more optimal intrauterine conditions, as well as early nurturing environments, in which their nutritional, physical, and psychosocial needs were met when environmental exposures and experiences exert strong influences on development (52). Healthy children are also more likely to engage actively with their surroundings, consistently receiving stimulation that promotes brain development. In contrast, children with poorer nutritional status may receive less stimulation due to caregivers' lower expectations of their developmental potential (53). Previous studies have also linked lower-than-expected growth trajectories with lower cognitive abilities in adulthood (16) and poorer schooling outcomes (14), especially when growth faltering occurs early in life.

Although our longitudinal analyses provide a novel perspective on the associations between growth and developmental outcomes, these links may not represent true causality (54,55). For instance, impaired growth reflecting inadequate nutrient intake has been associated with disrupted brain functional connectivity (10). However, both growth and brain development may be influenced by shared underlying determinants, such as inflammation and repeated infections, which may independently affect both domains, further complicating causal interpretations (54,56,57). Additionally, optimal growth and brain development may rely on different key nutrients, potentially leading to different outcomes. Linear growth mainly involves bone

development and is influenced by nutrients such as protein, zinc, vitamin D, and calcium (58), while brain development relies more directly on nutrients like iron, fatty acids, and choline (7). Hence, rather than establishing causality, our findings provide insight for future studies to move beyond static anthropometric indicators and instead consider deviations from expected trajectories as a more sensitive marker of environmental inadequacy. Longitudinal growth studies with repeated outcome measurements are needed to clarify the temporal relationships between growth patterns and cognitive development, including different influences of linear vs. ponderal growth, as well as potential sex-specific mechanisms. These sex differences remain unclear in our study; however, prior evidence has identified sex-specific responses to adversity in brain development (59), fetal programming of metabolism (60), and differences in energy requirements during rapid growth periods (61).

Importantly, our study found that associations between growth trajectories and attention and memory outcomes varied across time points and contexts. Associations with reaction time and shift rate were identified at earlier ages, while those with novelty preference tended to emerge later. Early delays in attention and memory indicators–particularly lower-level and stimulus-driven visual processes (62), such as slower reaction time at 6–9 months–may resolve by the next assessment (i.e., 12–15 months) due to physical maturation and postnatal experiences, making group differences harder to detect over time. Ross-Sheehy et al. (2017) reported similar findings, in which preterm infants exhibited slower reaction time than full term infants at 5 months but not at 10 months (63). In contrast, novelty preference reflects more goal-directed processes that continue developing throughout late infancy (64). Another potential explanation lies in behavioral discontinuity (i.e., series of distinct stages) across development (65), highlighting the importance of age-appropriate tasks. For example, Shinskey and Munakata (2010) found that 7-month-old

infants showed a preference for familiar stimuli, but by 11 months, they demonstrated a preference for novelty (66). This transition was attributed to improvements in processing speed, which enabled infants to form representations of familiar objects more rapidly, allowing a shift toward novelty preference. Additionally, our findings suggest that associations may vary across context. Significant associations with reaction time were observed in Malawi but not in the USA cohort, despite similar ages at assessment. These differences could be attributed to higher socioeconomic status, including parental education levels, and lower heterogeneity within the USA cohort, where all infants were exclusively breastfed, in contrast to Malawi cohort. Such factors may buffer the negative effects of malnutrition on development (7). Moreover, the prevalence of malnutrition in the US was generally lower than in Malawi (67), which may have limited our ability to detect associations between growth trajectories and child development. Notably, the USA cohort had a smaller sample size than Malawi cohort, which may have contributed to the lack of statistically significant findings.

Our study has important strengths. We utilized robust longitudinal data analyses benchmarked against WHO growth standards, providing a life-course analysis of early-life nutritional status relative to a universal standard. The use of eye-tracking-based tasks enabled assessments of specific cognitive domains and allowed for the detection of subtle differences using trial-level data, including interactions with cue types in the IOWA task for orienting attention that theorized to reflect activation of different brain regions (24). Assessing attention and memory outcomes at multiple time points also allowed us to explore the role of assessment timing–an important consideration given the limited application of these tasks across developmental stages in nutrition studies. Furthermore, in evaluating associations, we adopted a stepwise modeling approach to determine model structure more robustly and improve model parsimony.

However, this study also has some limitations. Although the inclusion of datasets from two different contexts in our study offered opportunities for cross-site replication, differences in data availability and protocols limited interpretability. Anthropometric data from 0 to 6 months were limited in the Malawi and USA cohorts, restricting analysis of early postnatal growth patterns. Additionally, birth weight was not directly measured in either cohort (extracted from birth records in Malawi and recalled by parents in the USA) and no information on history of prematurity was available, though extreme birth weight values were excluded in our analysis. Moreover, although we excluded trajectory classes representing fewer than 5% of participants, the relatively small sample sizes in the USA resulted in some retained classes with small absolute numbers of children (e.g., n=3 in the 'end high' group in the USA for WAZ trajectories among boys). These small group sizes, as expected in an exploratory analysis, could limit the statistical power to detect differences. Finally, although we adjusted for potential important confounders, some unmeasured shared antecedents of growth and cognitive outcomes, such as child morbidity, may have influenced the associations observed.

Growth trajectories in early life are linked to the development of attention and memory, highlighting the importance of longitudinal growth monitoring. These associations varied across developmental time points and contexts. Importantly, our findings reinforce the need to better understand the biological pathways linking nutrition, growth, and development, particularly during the prenatal period through the first six months of life.

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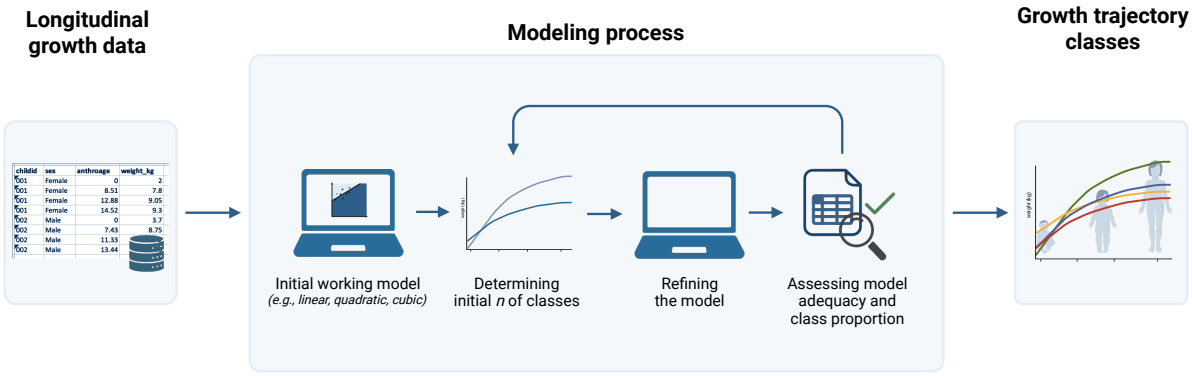


Figure 3.1. Modeling process to identify growth trajectories using latent class trajectory modeling (LCTM), adapted from the framework developed by Lennon et al (2015). Longitudinal growth data were used to classify individuals into trajectory groups based on the highest posterior probability of class membership. Model adequacy was assessed using three criteria: average posterior probability assignment (APPA) greater than 70% for all classes, odds of correct classification (OCC) values greater than 5.0, and relative entropy values (E_K) greater than 0.5.

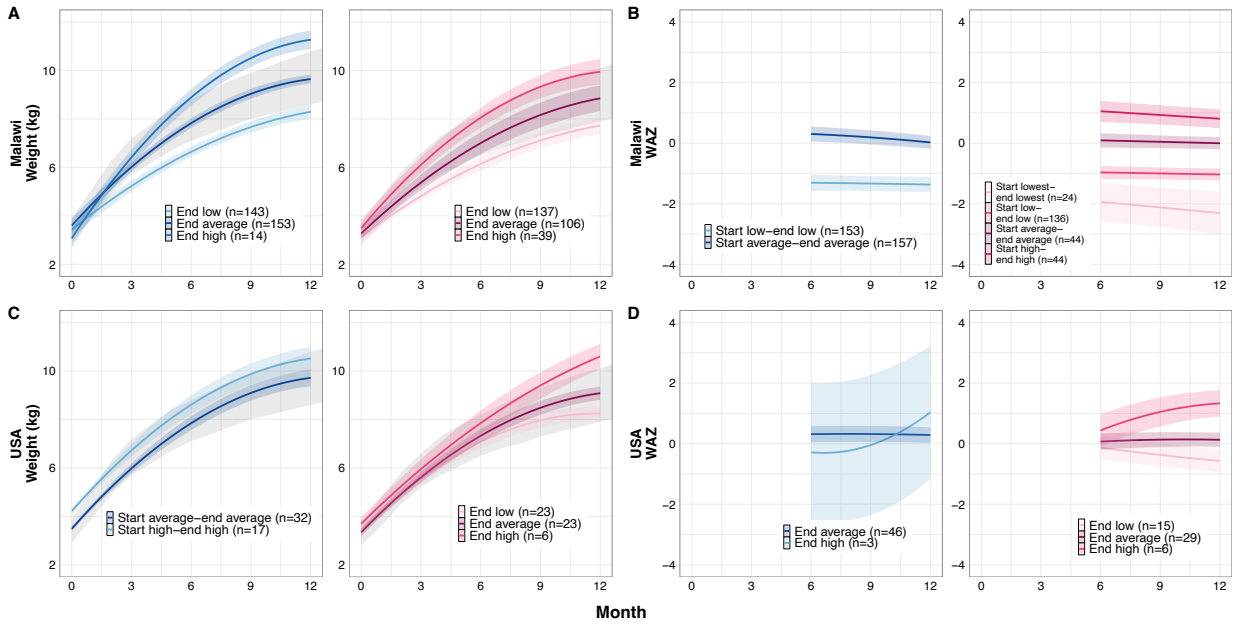


Figure 3.2. Comparison of ponderal growth trajectories among children in Malawi (A–B) and the USA (C–D) for weight (A, C) and weight-for-age z-score (WAZ; B, D). Blue lines represent boys and pink lines represent girls. Gray shaded areas indicate the -1 to +1 standard deviation range of the World Health Organization growth standard; within each sex, darker lines represent trajectory classes more closely aligned with these standards. Trajectory classes with a proportion below ~5% were excluded. Abbreviations: USA, The United States of America; WAZ, weight-for-age z-score.

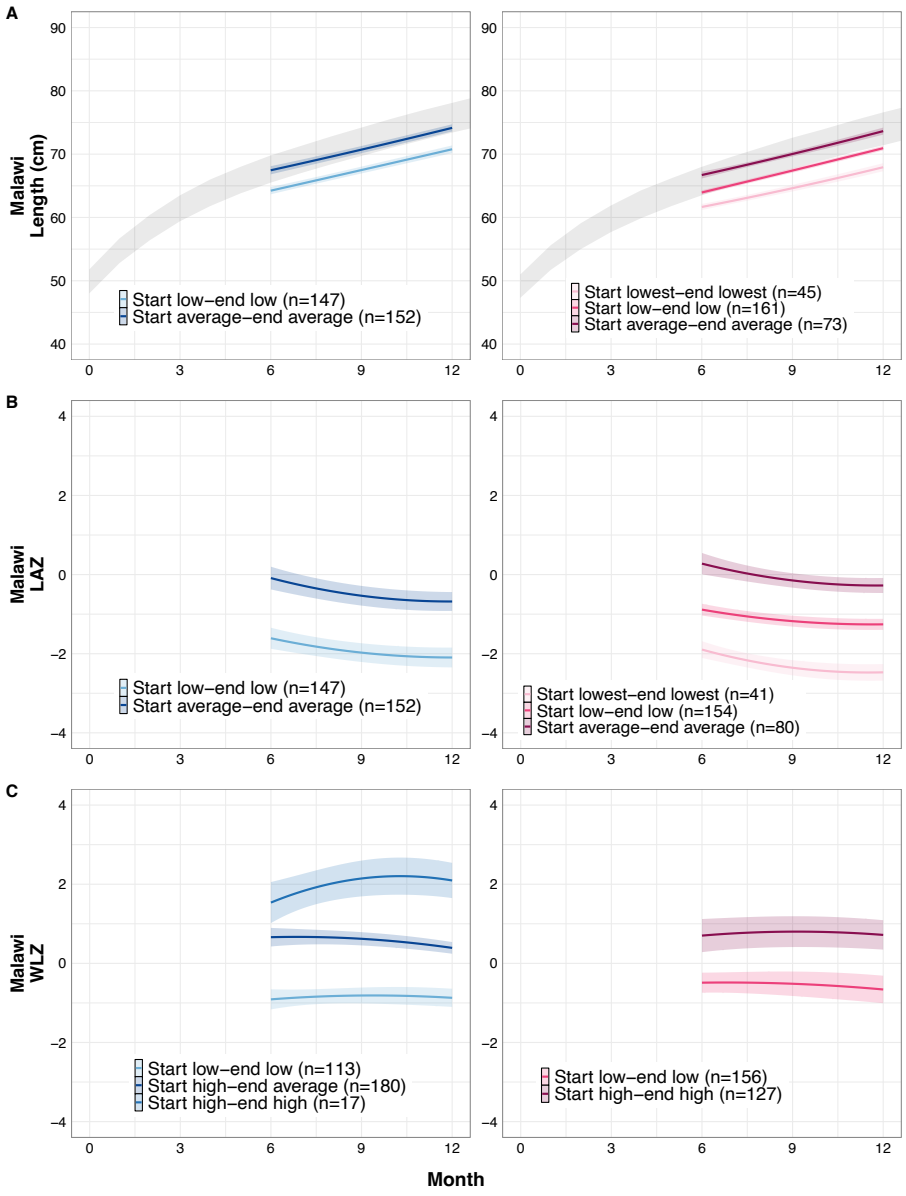


Figure 3.3. Comparison of linear growth trajectories among children in Malawi for length (A), length-for-age z-score (LAZ; B), and weight-for-length z-score (WLZ; C). Blue lines represent boys and pink lines represent girls. Gray shaded areas indicate the -1 to +1 standard deviation range of the World Health Organization growth standard; within each sex, darker lines represent trajectory classes more closely aligned with these standards. Trajectory classes with a proportion below ~5% were excluded. Abbreviations: LAZ, length-for-age z-score; WLZ, weight-for-length z-score.

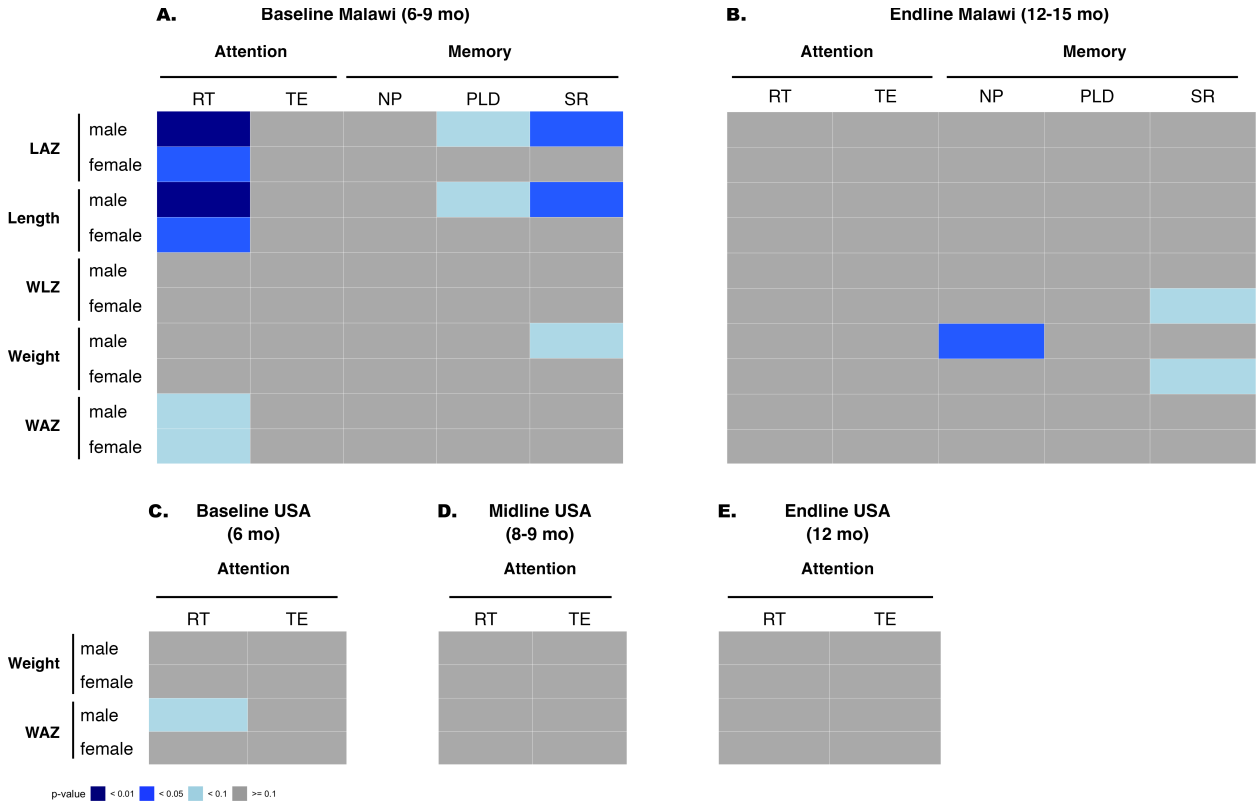


Figure 3.4. Heatmap summarizing the associations between early-life growth trajectories and attention and memory among infants and young children in Malawi (A–B) and the USA (C–E), assessed using fully adjusted models. P-values represent the lowest value observed in pairwise comparisons between trajectory groups for each outcome indicator. Abbreviations: LAZ, length-for-age z-score; NP, novelty preference; PLD, peak look duration; RT, reaction time; SR, shift rate; TE, task error; USA, The United States of America; WAZ, weight-for-age z-score; WLZ, weight-for-length z-score.

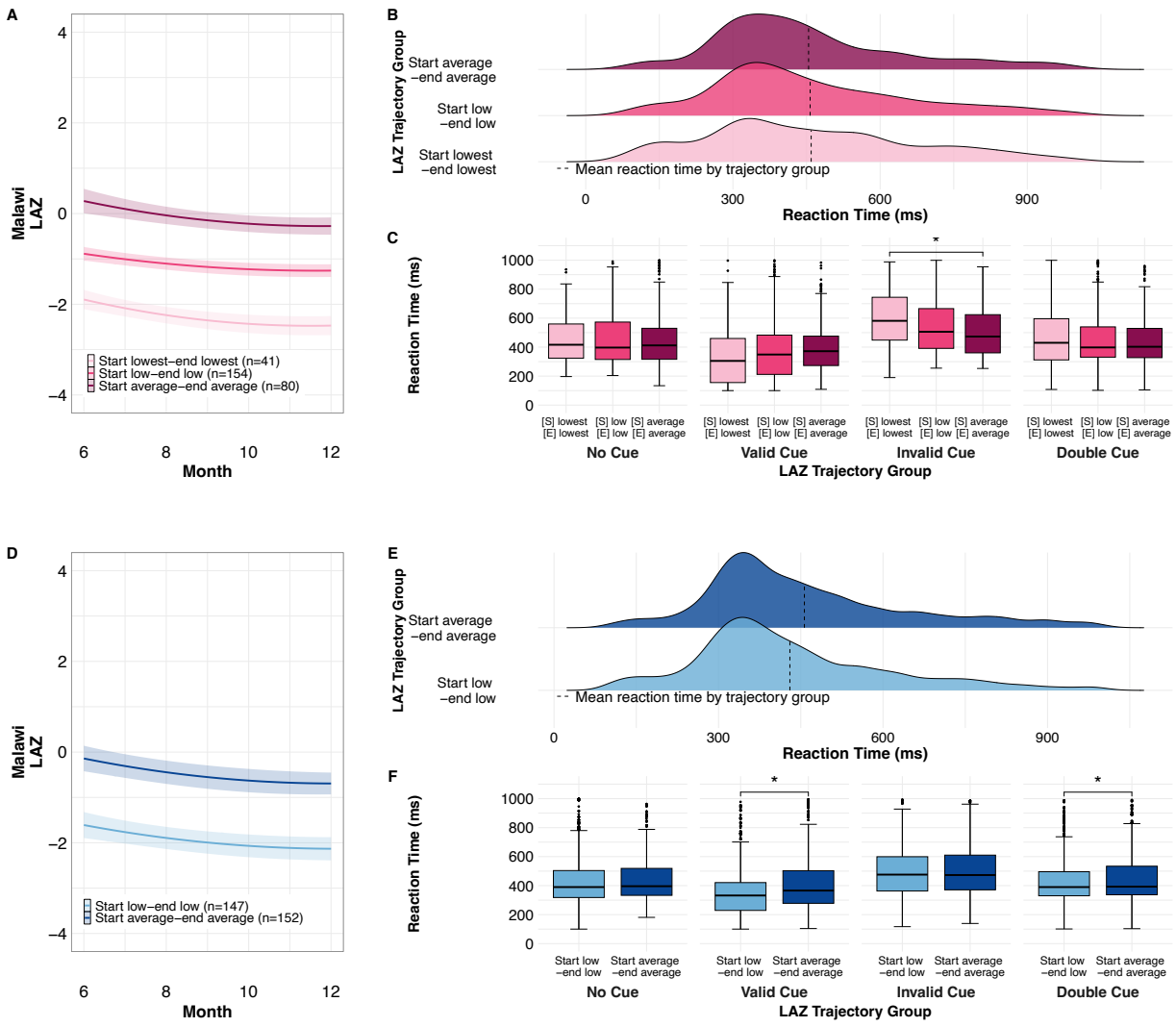


Figure 3.5. Associations between length-for-age Z-score (LAZ) trajectories from 6 to 12 months and reaction time at 6–9 months among girls (A–C) and boys (D–F) in Malawi. Panels A and D show LAZ trajectories; B and E show the distribution of reaction time collapsed across cue types; C and F display pairwise differences in reaction time by cue type between trajectory groups. Pairwise comparisons using robust linear mixed-effects models showed significantly slower reaction time in the 'lowest' LAZ trajectory group compared to the 'average' group among girls in invalid cue trials (estimated mean difference ± SE: 90.55 ± 31.1 ms; p=0.0189), and significantly faster reaction time in the 'below average' group compared to the 'average' group among boys in

valid cue trials (-54.57 ± 18.34 ms; p=0.0029) and double cue trials (-26.29 ± 11.89 ms; p=0.027).

* Indicates significant difference between trajectory groups within cue type, $p < 0.05$. Abbreviations: [E], end; LAZ, length-for-age z-score; [S], start.

3.7 Supplementary materials

Supplementary Table 3.1. Anthropometric assessments in Malawi and the USA

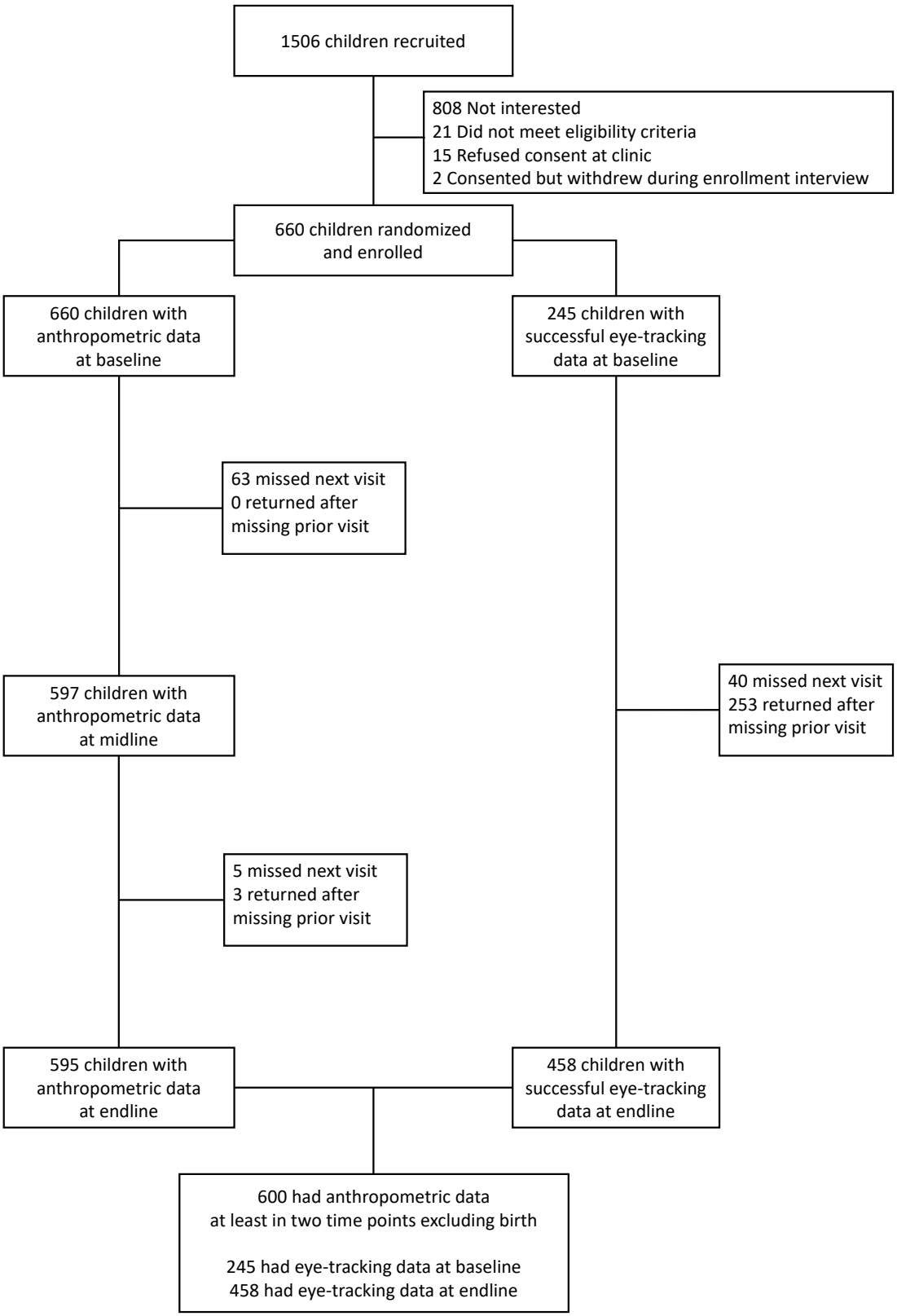
Study	Anthropometric indicator for growth trajectory	Timepoint	Weight	Length	Data collection methods	
					Weight	Length
Mazira (Malawi)	• Weight (0–15 mo) • WAZ (6–15 mo) • Length (6–15 mo) • LAZ (6–15 mo) • WLZ (6–15 mo)	Birth	✓		Child health card	–
		Baseline (6–9 mo)	✓	✓	Measured in the caregiver's arms using the tare function of the Seca 874 digital scale	Measured using Holtain length board
		Midline (9–12 mo)	✓	✓		
		Endline (12–15 mo)	✓	✓		
IMiND (USA)	• Weight (0–12 mo) • WAZ (6–12 mo)	Birth	✓		Self-reported by the caregiver	–
		Baseline (6–7 mo)	✓		Measured during home or lab visit using a Tanita BD 815U digital scale	
		Midline (8–10 mo)	✓			
		Endline (11–12 mo)	✓			

– = not assessed. Abbreviations: LAZ, length-for-age z-score; USA, The United States of America; WAZ, weight-for-age z-score; WLZ, weight-for-length z-score.

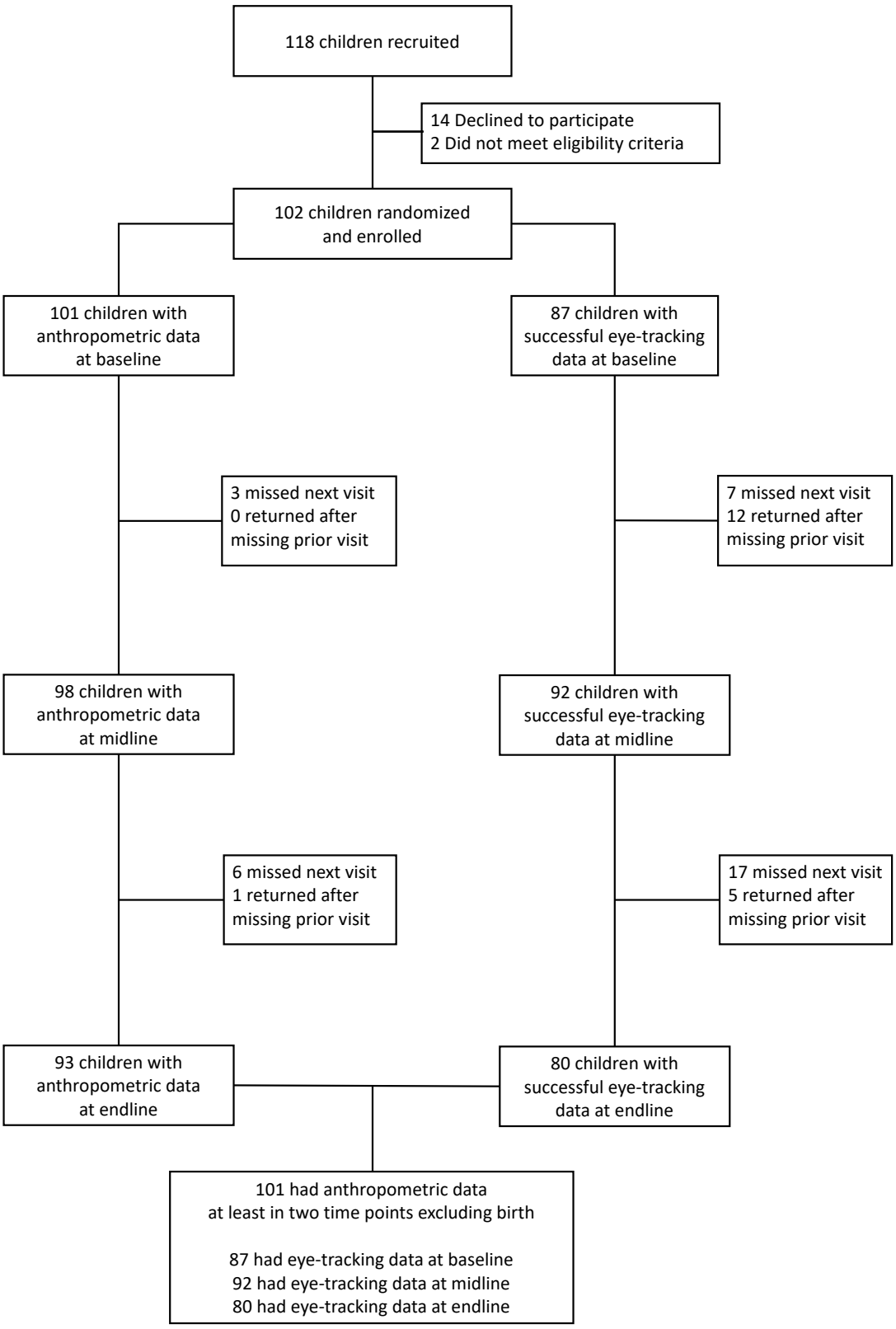
Supplementary Table 3.2. Eye-tracking assessment settings in Malawi and the USA

Study	Attention and memory outcome	Time point	Room settings	Mother-infant positioning	Eye-tracking system
Mazira (Malawi)	IOWA task (attention): • Mean latency • Task error VPC task (memory) • Novelty preference score • Peak look duration • Shift rates	6–9 mo 12–15 mo	A room with black curtain covering the computer/assessor	Child was placed in an infant carrier worn by the caregiver. Caregiver was asked to look away from the monitor or close their eyes.	Tobii Pro X2-60
IMiND (USA)	IOWA task (attention): • Mean latency • Task error	6 mo 8–9 mo 12 mo	A room with white curtain covering the computer/assessor	Child sat in a high chair, and their caregiver sat to their right. Caregiver wore felt-covered glasses or turned around.	SMI-Red-M 120 Hz

Abbreviations: IOWA, Infant Orienting With Attention; USA, The United States of America; VPC, Visual Paired Comparison.



Supplementary Figure 3.1. CONSORT flow diagram of the Malawi cohort



Supplementary Figure 3.2. CONSORT flow diagram of the USA cohort

Supplementary Table 3.3. Comparison of participant characteristics between Malawi and the USA

Characteristic		Malawi (n=458)[a]	USA (n=92)
Child characteristics			
Female		229 (50.0)	46 (50.0)
First born		124 (27.1)	21 (22.8)
Group allocation			
	Egg	226 (49.3)	–
	No egg	232 (50.7)	–
	Pear First	–	27 (29.4)
	Sweet Potato First	–	30 (32.6)
	Only Pears	–	35 (38.0)
Household and parental characteristics			
Maternal age, years		26.1 ± 6.6	32.4 ± 3.7
Paternal age, years		32.3 ± 8.6	34.2 ± 4.8
Maternal height, cm		157 ± 5.3	165 ± 6.9
Maternal BMI, kg/m²		21.9 ± 3.1	24.2 ± 3.1
Marital status			
	Married/living together	264 (57.6)	89 (96.7)
	Married, polygamous	91 (19.9)	–
	Unmarried/divorced, separated	103 (22.5)	3 (3.3)
Maternal education			

	No formal education	68 (14.9)	–
	Incomplete primary	289 (63.1)	–
	Completed primary or greater	101 (22.1)	–
	Completed vocational, incomplete bachelor, or unknown	–	22 (23.9)
	Completed bachelor	–	41 (45.6)
	Graduate/professional degree	–	29 (31.5)
Paternal education			
	Unknown	153 (34.6)	–
	No formal education	42 (9.5)	–
	Incomplete primary	120 (27.2)	–
	Completed primary or greater	127 (28.7)	–
	Completed vocational, incomplete bachelor, or unknown	–	30 (32.6)
	Completed bachelor	–	35 (38.0)
	Graduate/professional degree	–	27 (29.4)
Maternal occupation as a housewife		165 (36.3)	29 (31.5)

Paternal occupation			
	Farming/fishing	178 (48.2)	–
	Service	191 (51.8)	–
	Non-office work	–	61 (66.3)
Having >1 children <5 yrs old		261 (57.0)	49 (53.3)
Household members >4		291 (63.5)	29 (31.5)
HOME Inventory		24.2 ± 3.5	–

– = not assessed/not applicable. Abbreviations: BMI, body mass index; HOME Inventory, The Home Observation for Measurement of the Environment Inventory.

[a] Missing 107 data on paternal age and 89 data on paternal occupation.

Supplementary Table 3.4. Model adequacy assessments of latent class trajectory models among children in Malawi

Parameter	Sex	K	n	Proportion per class %	Average posterior probability assignment (APPA)	Odds of correct classification (OCC)	Relative entropy (E_K)	Degree of separation (DoS_k)	Kappa	Accuracy	Model	n excluded during modeling
Weight	Male	3	310	46.13:49.35:4.52	0.884:0.853:0.916	8.746:6.349:185.529	0.720	2.009	-0.010	0.205 (0.161 - 0.254)	Model E (3 groups)	0
	Female	4	290	47.2:36.55:13.45:2.76	0.837:0.731:0.805:0.906	6.2:5.138:25.789:142.782	0.618	1.559	-0.011	0.209 (0.164 - 0.261)	Model F (4 groups)	0

WAZ	Male	2	310	49.35:51.65	0.857:0.846	5.989:5.530	0.509	0.544	0.269	0.636 (0.580 - 0.690)	Model E (2 groups)	0
	Female	5	290	8.28:46.90: 27.59: 15.17: 2.07	0.856:0.881: 0.775:0.867: 0.848	56.50:8.79 :10.10:34. 90:149.95	0.735	0.562	-0.065	0.150 (0.111 - 0.197)	Model F linear (5 groups)	0
Length	Male	3	300	0.33:49: 50.7	0.996:0.833: 0.850	60458.7:5. 303:5.399	0.675	120.7	0.459	0.663 (0.607 – 0.717)	Model E (3 groups)	0
	Female	3	279	16.13:57. 71: 26.16	0.884:0.873: 0.851	35.77:5.35 :15.92	0.71	127.6	0.524	0.699 (0.641	Model E (3 groups)	11

										-0.753)		
LAZ	Male	3	300	0.33:49:50.7	0.995:0.840:0.857	50253.21:5.47:5.37	0.68	0.514	0.449	0.657 (0.600 – 0.710)	Model E (3 groups)	0
	Female	4	288	14.58:54.51:28.12:2.78	0.915:0.886:0.845:0.884	57.95:6.87:14.06:224.79	0.784	0.822	0.581	0.730 (0.674 - 0.781)	Model E (4 groups)	2
WLZ	Male	3	310	36.45:58.06:5.48	0.88:0.885:0.846	12.26:5.899:86.953	0.733	0.129	-0.083	0.094 (0.064 - 0.132)	Model E (3 groups)	0

	Female	3	290	53.79:43.79:2.41	0.853:0.801:0.834	5.324:5.679:74.317	0.594	0.148	-0.088	0.045 (0.024 – 0.076)	Model F (3 groups)	0

Supplementary Table 3.5. Model adequacy assessments of latent class trajectory models among children in the USA

Para-meter	Sex	K	n	Proportion per class %	Average posterior probability assignment (APPA)	Odds of correct classification (OCC)	Relative entropy (E_K)	Degree of separation (DoS_k)	Kappa	Accuracy	Model	n excluded during modeling
Weight	Male	2	49	65.31: 34.69	0.963: 0.924	13.760: 23.003	0.812	16.304	0.301	0.630 (0.476 - 0.768)	Model E (2 groups)	0
	Female	3	52	44.23: 44.23: 11.54	0.906: 0.879: 0.930	12.354: 9.507: 90.672	0.801	4.943	0.085	0.467 (0.317 - 0.621)	Model E (3 groups)	0

WAZ	Male	2	49	93.88: 6.12	0.996: 1.000	16.03: 29368.73	0.973	0.515	0.013	0.413 (0.270 - 0.568)	Model F (2 groups)	0
	Female	4	52	3.85: 28.85: 55.77: 11.54	0.862: 0.879: 0.866: 0.919	150.70: 15.491: 5.938: 82.044	0.792	4.041	-0.106	0.067 (0.014 - 0.183)	Model E (4 groups)	0

Chapter 4:

Longitudinal associations of anemia and iron status on early learning and memory: Evidence from eye-tracking assessments

4.1 Abstract

Anemia and iron deficiency (ID) remain prevalent among young children and may influence development in specific cognitive domains. We examined associations of anemia and iron status at 6–9 months with spatial attention, recognition memory, and social engagement six months later using eye-tracking assessments. We analyzed cohort data from a randomized egg provision trial in rural Mangochi District, Malawi (n=660), with no effects on anemia, iron status, or development. In a subgroup (n=406), baseline anemia and iron biomarkers were measured, including hemoglobin, plasma ferritin, soluble transferrin receptor (sTfR), and body iron index (BII). Children were categorized as anemic (hemoglobin <10.5 g/dL), ID (ferritin <12 μg/dL, sTfR >8.3 mg/L, or BII <0 mg/kg), and iron deficient anemic (IDA). At 12–15 months, attention was assessed using eye-tracking-based tasks of Infant Orienting with Attention, while memory and social engagement were assessed using Visual Paired Comparison. Associations were evaluated using mixed-effect models adjusted for child, parental, and household characteristics. Among 406 children, 185 (45.6%) had anemia at baseline. Of 366 with iron data, 308 (84.2%) had ID, including 150 with IDA. Hemoglobin was associated with social engagement, and iron biomarkers with memory. Per interquartile range (IQR) increase in hemoglobin, eyes dwell time decreased by 4% (95%CI 1%, 6%), mouth dwell time increased by 2% (95%CI 0%, 6%), and eye-mouth index decreased by 0.04 (95%CI 0.00, 0.08). IQR increases in ferritin, sTfR, and BII were associated with higher novelty preference (0.02; 95%CI 0.00, 0.02), lower peak look duration (-9.5%; 95%CI -14.8%, -2.9%), and lower shift rate (-0.02 shifts/s; 95%CI 0.00, -0.02), respectively. Categorical anemia, ID, and IDA were not associated with outcomes. Hemoglobin at 6–9 months was associated with social engagement, and iron biomarkers with memory. Effect sizes were small, indicating modest predictive associations in this high-burden setting.

4.2 Introduction

Iron deficiency (ID) remains one of the most prevalent micronutrient deficiencies globally and is a leading cause of anemia in early childhood (1), a period marked by rapid brain growth. In Malawi, the prevalence of ID, functional ID [i.e., defined by plasma soluble transferrin receptor (sTfR) cutoff], iron deficiency anemia (IDA), and all-cause anemia among children aged 6–59 months in 2015–2016 was 19.6%, 50.3%, 4.7%, and 29.5%, respectively, with younger children (<36 months) at significantly greater risk (2). However, these estimates may be underestimated; the most recent Demographic and Health Survey in 2024 reported anemia prevalence among children aged 6–59 months as high as 56% (3).

In young children, a combination of diet and infection contributes to anemia and poor iron status. Typical diets are high in phytates with non-heme iron sources that have low iron bioavailability, such as maize-based porridge along with legumes and dark leafy greens (4,5). Infectious diseases can further compromise iron status through multiple pathways. Malaria can cause ID through hepcidin-mediated blockage of iron absorption (6), and can cause anemia through hemolysis and disrupted erythropoiesis (7). Enteric infections may impair iron absorption through intestinal inflammation and barrier disruption (8), while certain parasitic infections, such as schistosomiasis and hookworm infection, can increase the risk of anemia through intestinal or urinary blood loss (9–11). Together, these factors result in widespread anemia and ID during the sensitive period of brain development. ID in early life has been associated with long-term impacts on cognitive function (12,13), even following iron repletion (14).

ID influences early neurodevelopment in a brain-region-dependent manner, resulting in effects on specific cognitive domains, with particularly strong evidence for hippocampal neurons that support memory development (14,15). Iron supports the transcriptome, metabolome,

intracellular signaling pathways, and electrophysiological response properties of the developing hippocampus for recognition learning and memory (16). Beyond memory outcomes, there is growing interest in associations between iron status with attention outcomes, such as sluggish cognitive tempo, and early social engagement (17,18); however, evidence for both is more limited than for memory. In contrast, anemia–with or without ID (19)–may lead to broader effects on neurodevelopment. Anemia impairs oxygen delivery to the brain (20) and could lead to lethargy, thereby reducing social engagement between young children and their caregivers and limiting opportunities to explore environment. Associations between anemia and ID with neurodevelopment may also be developmentally dynamic (14), such that iron status during sensitive windows has disproportionate downstream consequences. Previous evidence has shown that ID in infancy is associated with lower performance on executive function and recognition memory tasks, suggesting long-term impacts on the dopamine system and hippocampus (21). Additionally, concurrent anemia may influence child behavior at the time of assessment (22) and thus might bias observed relationships in cross-sectional evaluations. These considerations motivated analyses that evaluate predictive associations from earlier iron status to later developmental outcomes.

Eye-tracking assessments provide a means of measuring specific developmental outcomes, including visual attention, learning, and memory in infants and young children (23,24). These developmental domains, particularly memory, may be sensitive to ID and were assessed during a period expected to be sensitive for detecting group differences, as they are still maturing (25,26). Thus, these assessments present an opportunity to evaluate the impact of anemia and ID that may be more specific than general global developmental tests (24,27). In a longitudinal study of 6–9

months old children at baseline, we evaluated the associations between biomarkers of anemia and ID with performance on a series of eye-tracking assessments 6 months later.

4.3 Methods

Study cohorts

We used cohort data from the Mazira study, a randomized trial conducted in rural Lungwena and Malindi, Mangochi District, Malawi, from 2018 to 2019 to evaluate the impact of daily consumption of eggs for 6 months. This study involved 660 children (egg: n=331; no egg: n=329) aged 6–9 months (28,29) who were assessed at the start of the trial (baseline) and at follow-up points 3 months (midline) and 6 months (endline) later. At the baseline and endline visits, venous blood samples were collected by trained nurses to measure anemia and iron status biomarkers, and developmental assessments were performed using eye-tracking-based tasks.

Inclusion criteria for the parent trial were singleton infants aged 6–9.9 months, and exclusion criteria consisted of mid-upper arm circumference <12.5 cm or the presence of bipedal edema, severe anemia (<5 g/dL), acute illness or injury warranting hospital referral, history of egg allergy or allergic reaction to egg during test feeding, history of anaphylaxis or serious allergic reaction requiring emergency medical care, congenital defects or chronic morbidity associated with growth or developmental impairments or which may impact feeding, or plans to leave the study area within the next 6 months. For the purposes of this analysis, we included children with laboratory data on anemia or iron status who underwent eye-tracking assessment at 12–15 months. Detailed descriptions of the study have been published previously (28,29). The study reported no effect of the intervention on anemia, iron status, or development (29,30).

Anemia and iron status assessments

Venous blood samples were collected from all participating children at baseline and endline. Hemoglobin concentration was measured by trained nurses at the study clinic using portable HemoCue Hb 201 analyzers (HemoCue, Inc.). Plasma samples were frozen and stored at -80 C before shipping on dry ice to the VitMin Lab for analysis where plasma ferritin, soluble transferrin receptor (sTfR), c-reactive protein (CRP), and alpha-1 acid glycoprotein (AGP) concentrations were measured using a combined sandwich enzyme-linked immunosorbent assay (ELISA) method. All analytes were measured from a single well containing 50–75 μL of plasma, and a 10% subset of samples was reanalyzed for quality assurance. Coefficients of variation (CV) for each iron biomarker were calculated from replicates of pooled plasma samples run with each assay tray. The CVs were 2.3% for ferritin, 3.6% for sTfR, 5.8% for CRP, and 8.1% for AGP (30). Both plasma ferritin and sTfR levels were corrected for subclinical inflammation [i.e., elevated C-reactive protein (CRP) and α-1-acid glycoprotein (AGP)] using the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) regression approach (31,32). Using measured plasma ferritin and sTfR, the body iron index (BII) was calculated using Cook's formula (33) by applying constants to the ratio of the inflammation-adjusted sTfR:ferritin. Finally, children were categorized as having anemia if they had hemoglobin <10.5 g/dL (34) and as having ID if they had plasma ferritin <12 μg/dL, sTfR >8.3mg/L, or BII <0 mg/kg (35,36). IDA was defined as the presence of both anemia and ID.

Early learning and memory assessments

Learning and memory outcomes were assessed using eye-tracking-based tasks; detailed protocols have been described previously (29,37,38). These tasks were performed in a room with a black

curtain separating the computer and assessor from the child. The child was placed in an infant carrier worn by the caregiver, and the caregiver was asked to look away from the monitor or close their eyes. A monitor was positioned approximately 60 cm from the child's face. Caregiver and child activities were monitored using a webcam throughout the procedure. The Tobii Pro X2–60 eye-tracking system was used to detect the child's eye fixations while stimuli were presented on the monitor. The eye-tracking system recorded the coordinates (x, y) of the focal point of the infant's gaze at a sampling rate of 60 Hz. The Tobii I-VT Fixation Filter was used to identify fixations from the continuous datastream of eye gaze positions.

Spatial attention outcomes were measured using the Infant Orienting with Attention (IOWA) task (38,39). This task assesses how fast children shift attention and make fast and accurate eye movements to a randomly presented target images on the screen, consisting of colorful everyday objects, such as fruits, balls, and baskets on a gray background. Prior to presenting the target images, children were exposed to a variety of cue conditions, consisting of 3 experimental conditions (valid, invalid, and double cues) and 1 control condition (no cue). A black dot served as a spatial precue preceding the target image. The number and location of the presented black dot(s) depended on the type of cue condition. In the valid cue condition, both the cue and target image appeared on the same side, while in the invalid cue condition, the target image was presented on the opposite side of the cue. The double cue condition involved cues on both sides, with the target image presented at the spatial location of one of the two cued locations. In the baseline condition, no cue was presented, but the target image still appeared on either the left or right side of the screen. Each child was exposed to up to 24 trials per cue condition, with an equal proportion on each side of the screen. Two attention outcomes were computed: reaction time and task error. Reaction time measures the duration from the beginning of target image presentation to

the first eye fixation within the area-of-interest (AOI) of the target image; shorter reaction time across all cue types reflects faster response to the target. Task error is the proportion of incorrect responses in invalid and double cue trials, in which high task error reflects inaccuracy. Based on previous age-related findings reported by Ross-Sheehy et al. (39), we assumed that more advanced visual spatial attention is indicated by lower mean latency and lower odds of task error.

Memory outcomes were assessed using the Visual Paired Comparison (VPC) task (37). This task specifically assesses recognition memory by comparing the proportion of time the child spends looking at a novel face compared to a previously seen face (i.e., novelty preference). Children exhibit a robust preference for novelty by looking longer at novel face stimuli than at familiar ones (40). Children were first exposed to a familiarization period where two images of a culturally appropriate face were presented on the left and right sides of the screen. During a recognition memory period, a new pair of faces was presented, consisting of the same face shown in familiarization period (familiar stimulus) on one side and a novel face on the other side. These stimuli were side reversed after the first 5 seconds. Each child was exposed up to 4 trials each including a different pair of faces (i.e., adult male-adult female, adult male-child male, adult female-child female, and child male-child female). Three look-defined outcomes were calculated (37): novelty preference, peak look duration, and shift rate. The novelty preference score was computed by measuring the child's total time looking at the novel face relative to the total time looking at both faces. Peak look duration was determined by assessing the longest individual look on each trial during the familiarization phase. Shift rate was calculated by dividing the total number of shifts during the familiarization and test phase by the total look duration; the values were collapsed across both familiarization and recognition memory periods. Based on previous findings

reported by Beckner et al. (2023) (37), we assumed that more advanced recognition memory was indicated by higher novelty preference, lower peak look duration, and higher shift rate.

Visual social engagement outcomes were measured using face stimuli from the VPC task by evaluating engagement with eyes vs. mouth areas. Previous studies have used the proportion of looking time to the eye and mouth areas as indicators of social engagement (26,41). Moreover, infants' attention to mouth may predict language development (42). We created AOIs of the eyes and mouth using MediaPipe Face Mesh (**Supplementary Figure 4.1**) by segmenting the eyes and mouth of the stimuli and extracting the area coordinates (43). To accommodate calibration drift, the areas were overdrawn by 1.82 visual degrees (third quartile of mean accuracy error). The outcomes derived from this task followed Souter (2020) (26) to include the eye-mouth index (EMI), eyes percent dwell time (PDT), and mouth PDT. EMI was computed based on looking time to the eyes AOI divided by looking time to the eyes and mouth AOIs within each stimulus frame during the familiarization and recognition memory periods. Eyes and mouth PDT were calculated based on the proportion of looking time that fell within the eyes AOI and mouth AOI, respectively, within each stimulus frame during the familiarization and recognition memory periods.

Statistical analysis

We evaluated anemia and iron status at 6–9 months as predictors of visual attention, memory, and social engagement outcomes at 12–15 months. Each indicator of anemia and iron status (hemoglobin, plasma ferritin, plasma sTfR, BII, anemia status, ID status, IDA status) was examined separately with each outcome. Before analysis, peak look duration was transformed because of a skewed distribution, and task error was dichotomized as 0 errors vs. >0 errors. A

detailed statistical analysis plan was preregistered on the Open Science Framework (https://osf.io/vfrg7/).

For learning and memory outcomes with trial-level data within participants, we used a robust linear mixed-effects model based on Koller (2016) (44) for reaction time, and linear mixed-effects models for novelty preference score, peak look duration, shift rates, EMI, eyes PDT, and mouth PDT. Task error, which was measured at the participant level, was analyzed using logistic regression. For all models, we included fixed effects of child age at outcome assessment, trial group assignment (e.g., intervention vs. control), and covariates related to blood sample collection and eye-tracking assessment (e.g., time of last meal before blood draw, child activity level and interaction with the assessor during the eye-tracking-based task). Coefficient estimates for continuous biomarkers were scaled to represent an interquartile range (IQR) increase (i.e., an increase in biomarkers level from the 25th percentile to the 75th percentile), to improve interpretability given the skewness of the biomarkers distribution.

For each learning and memory outcome, we also conducted fully adjusted analyses that included malaria status at baseline, along with child, household, and parental characteristics. We first examined bivariate associations of each child, household, and parental covariate on the outcome using likelihood ratio tests; covariates with $p < 0.10$ were included in the fully adjusted models. To address missing covariate data, we imputed the mean for continuous covariates with ≤10% missingness; no continuous covariate had more than 10% missing data. For categorical covariates with ≤10% missingness, we imputed the most frequent category. For categorical covariates with >10% missingness, we created a separate "missing" category.

As a sensitivity analysis, we evaluated associations between sustained anemia, ID, and IDA over time (baseline and endline) with learning and memory outcomes. Children were classified

into four groups: (a) anemia/ID/IDA at both baseline and endline (sustained status), (b) anemia/ID/IDA at baseline only, (c) anemia/ID/IDA at endline only, and (d) no anemia/ID/IDA at either time point as the reference group. We also compared children with moderate to severe anemia with children who were not iron deficient or anemic at baseline. Finally, because plasma ferritin and plasma sTfR reflect different biological processes related to ID, we additionally examined associations between ID and learning and memory outcomes using each biomarker separately: low plasma ferritin only (ferritin <12 μg/dL) and high plasma sTfR only (sTfR >8.3 mg/L).

Ethics approvals

This secondary analysis used de-identified data from the Mazira study (Clinical Trial Registry number: NCT03385252) and was approved by the institutional review boards at the University of California, Davis and the College of Medicine in Malawi.

4.4 Results

Of 660 children randomized in the parent trial, a total of 406 children with baseline anemia and iron assessments, along with endline eye-tracking data were included (**Figure 4.1**). Children were excluded due to a variety of reasons: 78 children did not provide blood sample at baseline (e.g., veins could not be located, caregivers refused), 157 had missing eye-tracking data at endline, and 19 were excluded based on eye-tracking analysis criteria. The mean age at assessment for anemia and iron status was 7.36 ± 1.20 months, and the sex distribution was balanced between females and males (**Table 4.1**). Of the 406 included subjects, 185 (45.6%) had anemia at baseline. Among 366 children with available iron biomarkers data, 308 (84.2%) had ID; out of 308, IDA was found

in 150 children. Among the children with data on anemia and iron status at both baseline and endline (n=357), only 14 children did not have ID or anemia at both time points. Most children had ID and/or IDA at least once across the two time points (**Figure 4.2A**). Importantly, when using the plasma ferritin cutoff to define ID (**Figure 4.2B**), fewer children were classified as iron deficient compared to using the plasma sTfR cutoff (**Figure 4.2C**). Of the 406 children included in the analysis, 67 were categorized as having ID at baseline using the plasma ferritin cutoff (<12 µg/dL), whereas 302 were classified as having ID using the plasma sTfR cutoff (>8.3 mg/L).

The associations between baseline anemia and iron status with early learning and memory outcomes 6 months later are presented in **Supplementary Table 4.1** for the fully adjusted models, including baseline malaria as a covariate (complete results are provided in **Supplementary Table 4.2**[3]). Our analysis revealed that hemoglobin levels were associated with visual social engagement. Each IQR increase in baseline hemoglobin concentration was associated with a 4% (95%CI: 0.6%, 6%; p=0.020) lower percent dwell time on the eyes, 2% (95%CI: 0.2%, 6%; p=0.033) higher percent dwell time on the mouth, and 0.04 point (95%CI: 0.004, 0.08; p=0.028) lower eye-mouth index at endline (**Figure 4.3**). No associations were observed between categorical anemia status and any outcomes (**Figure 4.4**).

All iron biomarkers were associated with memory outcomes, although the patterns were not consistent (**Figure 4.3**). Each IQR increase in plasma ferritin, indicating better iron status, was associated with better memory via higher novelty preference by 0.02 point (95%CI: 0, 0.02; p=0.039). Conversely, each IQR increase in plasma sTfR, suggesting poorer iron status, was associated with better memory, reflected by a 9.5% decrease in peak look duration (95% CI: 2.9%, 14.8%; p=0.004). Lastly, each IQR increase in BII, suggesting better iron status, was associated

[3] Supplemental Excel table is available on Open Science Framework at https://osf.io/rva6p/.

with poorer memory, as shown by a decrease in shift rates by 0.02 shifts/s (95%CI: 0, 0.04; p=0.041). Despite significant associations observed for continuous iron biomarker levels, no significant associations were observed for categorical ID or IDA status (**Figure 4.4**).

In a sensitivity analysis, we further explored whether sustained anemia, ID, and IDA status were associated with early learning and memory outcomes (**Supplementary Table 4.3**), with children without these conditions at both time points as the reference. Children who had ID at endline only demonstrated faster reaction time (β: -36.13 ms; 95% CI: -71.57, -0.68; p=0.046) and lower odds of task error (adjusted OR: 0.18; 95% CI: 0.03, 0.98; p=0.048), compared with those without ID at both time points. Similarly, children who had ID at baseline only showed lower odds of task error (adjusted OR: 0.14; 95% CI: 0.02, 0.92; p=0.04), compared with those without ID at both time points. Children who had IDA at outcome assessment only had a lower percentage of dwell time on the eyes (β: -9%; 95% CI: -2%, -16%; p=0.012), compared with those without IDA at both time points. No significant associations were observed when comparing children with anemia, ID, or IDA at both time points (i.e., sustained anemia, ID, or IDA) with those without these conditions at both time points. Notably, we identified a trend toward slower reaction time and more task errors among children with sustained ID compared with those with ID at baseline or endline only (**Supplementary Figure 4.2**). However, we did not pursue further analyses of this pattern in order to adhere to the pre-specified reference group.

In additional post hoc analyses, baseline moderate/severe anemia (compared with no ID/anemia) was also not associated with any outcomes. In analyses of baseline ID using the ferritin cutoff, children with ID showed faster reaction time (β: -18.02 ms; 95% CI: -35.95, -0.10; p=0.049) and higher shift rates (β: 0.04 shifts/s; 95% CI: 0.003, 0.08; p=0.036) compared to non-ID. No significant associations were observed when ID was defined using the sTfR cutoff alone.

4.5 Discussion

In this study of 6–15-month-old children with a high prevalence of ID, hemoglobin concentrations were associated with visual social engagement, and iron status biomarkers were associated with recognition memory 6 months later. However, all observed associations were small in magnitude, and no associations were identified with baseline anemia, ID, or IDA. Given the small effect sizes, our findings suggest modest predictive associations of anemia and iron biomarkers on later developmental outcomes, as assessed using eye-tracking methods. Previously, we reported that 77% of children had ID and 52% had IDA (30). Using a longitudinal approach in the present analysis, we found that overall 93.6% of children experienced ID, with or without anemia, at least once between 6–9 and 12–15 months of age. This suggests that the overall burden of ID among young children may be underestimated when using only cross-sectional analyses.

In our study, higher hemoglobin was associated with lower eye-mouth index, reduced dwell time on the eyes, and increased dwell time on the mouth. Increased attention to the mouth at 12–15 months might reflect developmental shifts in visual social engagement related to emerging language development (41). For example, a previous study demonstrated that attention to the mouth at 12 months predicted vocabulary size at 18 and 24 months (45). Additionally, lower hemoglobin in early infancy may be associated with reduced overall behavioral engagement, consistent with prior studies linking anemia with lethargy and reduced social interaction (46,47).

We also observed associations between iron biomarkers and recognition memory indicators. In our study, higher plasma ferritin, indicative of greater iron stores, was associated with greater novelty preference, suggesting better recognition memory. Our results corroborate previous findings of postnatal ID during infancy on recognition memory (48,49). Late gestation through 2–3 years of age represents a critical window of vulnerability to ID, corresponding to a

period of rapid hippocampal maturation, which is crucial for memory development, and increased iron uptake and utilization in the brain (16). Deficiency during this developmental stage may impair hippocampal metabolism via altered mitochondrial function and regionally distributed losses in cellular energy production (50,51). Additionally, iron is required for the activity of prolyl hydroxylase, an iron-dependent enzyme for regulating hypoxia-inducible factor 1α (HIF-1α), which further modulates neuronal function, including the expression of TfR-1 and Slc11a2 in the hippocampus (52). Altered expression of these genes has been linked to deficits in learning and memory performance.

However, associations for other iron biomarkers (plasma sTfR and BII) were in unexpected directions and were inconsistent with plasma ferritin results. Higher plasma sTfR, indicating ID, was associated with shorter peak look duration, which may suggest a shorter, potentially more efficient, learning period. We also found that higher BII, indicating better iron status, was associated with lower shift rates, reflecting less shifting of attention between simultaneously presented stimuli during learning and recollection. Notably, novelty preference represents a behavioral endpoint, whereas peak look duration and shift rates reflect intermediate attention-related processing strategies that may support subsequent memory performance, including novelty preference (53,54). The lack of consistent associations across memory indicators therefore underscores the need for careful interpretation of process-based measures of infant development. For instance, prior work from this cohort in Malawi suggested population-specific differences in IOWA task performance compared with a cohort from a suburban area in California, United States (38). Children in Malawi demonstrated slower reaction times but greater accuracy, suggesting that different behavioral strategies may underlie task performance across settings. If so, process measures such as peak look duration and shift rates may not map directly onto developmental

function consistently across populations. This may partially explain the inconsistent directionality observed for peak look duration and shift rates in the present study. Finally, the observed associations with recognition memory and the absence of associations with spatial attention indicators in our main analysis are consistent with developmental expectations. Goal-directed processes assessed by VPC task are still maturing at 12–15 months, whereas stimulus-driven attention assessed in the IOWA task may be less sensitive to variation in iron status at this age (25,55).

The different patterns of association observed for ferritin and sTfR with memory outcomes likely reflect their distinct biological roles. Ferritin primarily reflects iron stores, whereas sTfR reflects cellular iron demand and erythropoiesis. These biomarkers therefore capture related but non-identical aspects of iron biology, and discordant associations are not unexpected (12). Although both biomarkers were adjusted for inflammation using BRINDA methods, residual confounding may remain, particularly in settings with a high burden of infection. In addition, plasma sTfR levels may be affected by rapid growth during infancy, although a previous study reported similar reference intervals for sTfR from birth to 12 months in a low-risk population for ID (56). We also examined BII as a composite measure (33,57). However, this index was developed in adults and has not been extensively validated in infants, particularly in high-inflammation settings. More importantly, because ID was extremely common in our study context, shifts in plasma ferritin and sTfR levels may not have been large enough to detect differences. Together, these factors may contribute to the lack of consistent directionality in associations across biomarkers.

An important finding in our study was the absence of associations when iron status and anemia were analyzed categorically. This contrasts with some prior studies that reported dose–

response trends across iron-sufficient, ID, and IDA groups (58), although we noted different criteria for defining ID. Moreover, categorical classifications may be less informative in this context due to the high prevalence of ID and anemia. In our study population, children without ID may still have non-ID anemia, and comparison groups for IDA may include children with ID alone, non-ID anemia, or neither condition. If anemia independent of ID also influences development, these mixed categories would reduce contrast between groups and bias estimates toward the null. This interpretation is consistent with previous evidence showing limited differences between non-IDA and IDA in some neurodevelopmental domains (59). The number of children without ID or anemia at baseline was small, and even fewer remained in this group at outcome assessment, reducing the ability to detect differences. Thus, results could be different if we examine these same measures in a population with lower prevalence of ID. We further explored alternative contrasts of anemia and iron status in sensitivity analyses (e.g., sustained anemia and iron status, moderate/severe anemia). However, due to the imbalance in group sizes, these results should be interpreted with caution.

Strengths of this study include its prospective design, multiple iron biomarkers with adjustment for inflammation, and the use of eye-tracking-based tasks to detect subtle differences in learning and memory outcomes. Importantly, eye-tracking-based tasks provide objective measures of cognitive outcomes in infancy (60), unlike measures that rely on parental report. The use of multiple iron biomarkers, along with varied learning and memory indicators, allowed for a more robust evaluation of how anemia and iron status are linked to early neurodevelopment. However, our observational design precludes causal interpretation. Confounding factors, such as other micronutrient deficiencies (e.g., zinc, vitamin A), which commonly coexist in settings with a high burden of ID (61), may distort the associations. Additionally, the interpretation of process-

based developmental measures (e.g., peak look duration, shift rates) remains challenging, particularly across diverse cultural contexts. Determining iron status categories in infancy also warrants greater caution in settings with a high burden of inflammation, infection, and multiple etiologies of anemia.

In conclusion, we found that early anemia and iron biomarkers levels were associated with recognition memory and social engagement outcomes, although these associations were modest and varied by biomarker and developmental domain. The absence of associations using categorical definitions of anemia and iron status highlights the need for cautious interpretation in settings with a high prevalence of ID and anemia. Although our study provides an objective measurement of developmental outcomes, careful selection of developmental indicators and interpretation of the associations are warranted, particularly in settings where multiple risk factors for anemia, ID, and developmental vulnerability coexist.

4.6 References

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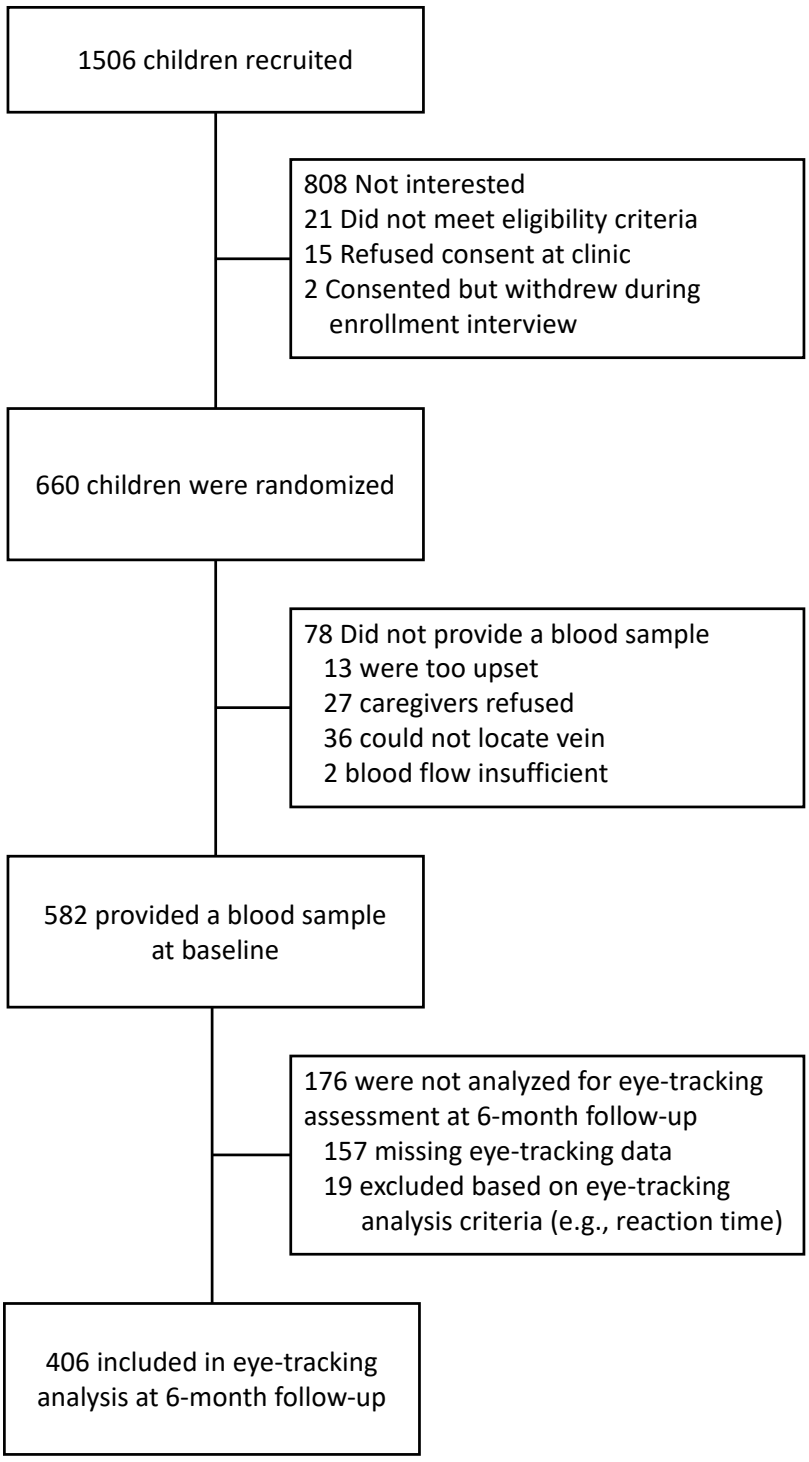


Figure 4.1. Study flow diagram

Table 4.1. Participant characteristics (n=406)

Characteristic		n (%) or Mean ± SD[1]
Child characteristics		
Child age at baseline anemia and iron status assessment, mos		7.4 ± 1.2
Female		199 (49)
First born		114 (28.1)
Group allocation		
	Egg	200 (49.3)
	No egg	206 (50.7)
Positive malaria test at baseline		58 (14.3)
Positive malaria test at outcome assessment		19 (4.7)
Length-for-age z-score		-0.87 ± 1.05
Weight-for-age z-score		-0.54 ± 1.08
Weight-for-length z-score		0.04 ± 1.02
Household and parental characteristics		
Maternal age, y		26.0 ± 6.7
Paternal age, y		32.3 ± 8.6
Maternal height, cm		157.1 ± 5.2
Maternal BMI, kg/m^2		21.8 ± 3.0
Marital status		
	Monogamous	235 (57.9)
	Polygamous	76 (18.7)

	Unmarried	95 (23.4)
Maternal education		
	No formal education	62 (15.3)
	Incomplete primary	254 (62.6)
	Completed primary or greater	90 (22.2)
Paternal education		
	Unknown	156 (38.4)
	No formal education	37 (9.1)
	Incomplete primary	102 (65.4)
	Completed primary or greater	111 (27.3)
Maternal occupation as a housewife		150 (37.2)
Paternal occupation		
	Farming/fishing	149 (45.6)
	Service	178 (54.4)
Having >1 children <5 yo		226 (56.6)
Household members >4		257 (63.6)
Village location		
	Malindi	212 (52.2)
	Lungwena	194 (47.8)
Household asset index		0.05 ± 1.04
Moderate/severe food insecurity		315 (77.6)
HOME Inventory		24.1 ± 3.5

Abbreviations: BII, body iron index; BMI, body mass index; HOME Inventory, The Home Observation for Measurement of the Environment Inventory. [1] Missing 89 data on paternal age and 79 data on paternal occupation

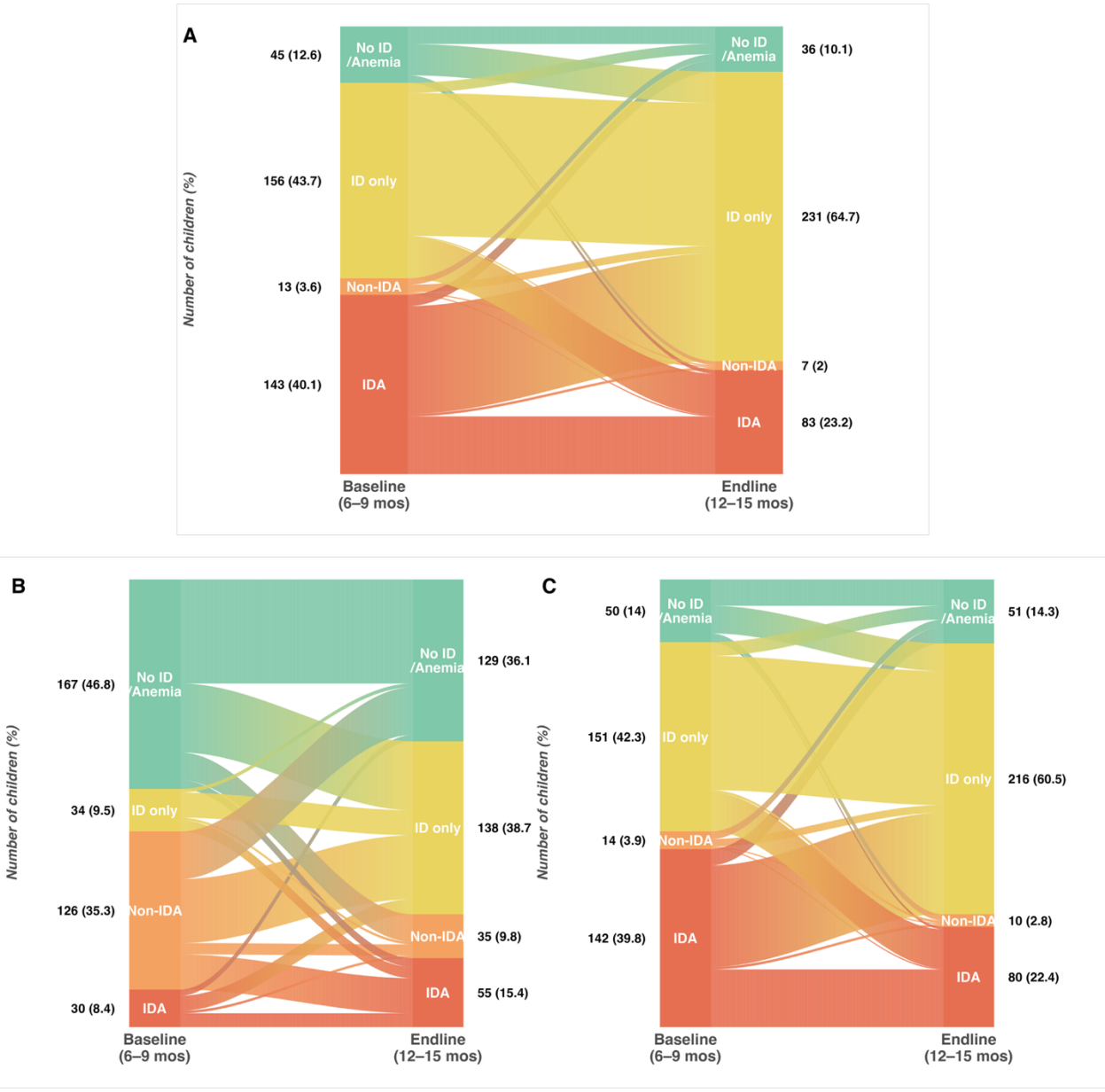


Figure 4.2. Proportion of children with iron deficiency (ID, yellow), non-iron deficiency anemia (non-IDA, orange), iron deficiency anemia (IDA, red), and without iron deficiency or anemia (green) over time. Panel A: iron deficiency was defined using at least one of the following criteria: plasma ferritin < 12 µg/dL, plasma soluble transferrin receptor (sTfR) > 8.3 mg/L, or body iron index < 0 mg/kg. Panel B: iron deficiency defined using the plasma ferritin cutoff only. Panel C: iron deficiency defined using the plasma sTfR cutoff only.

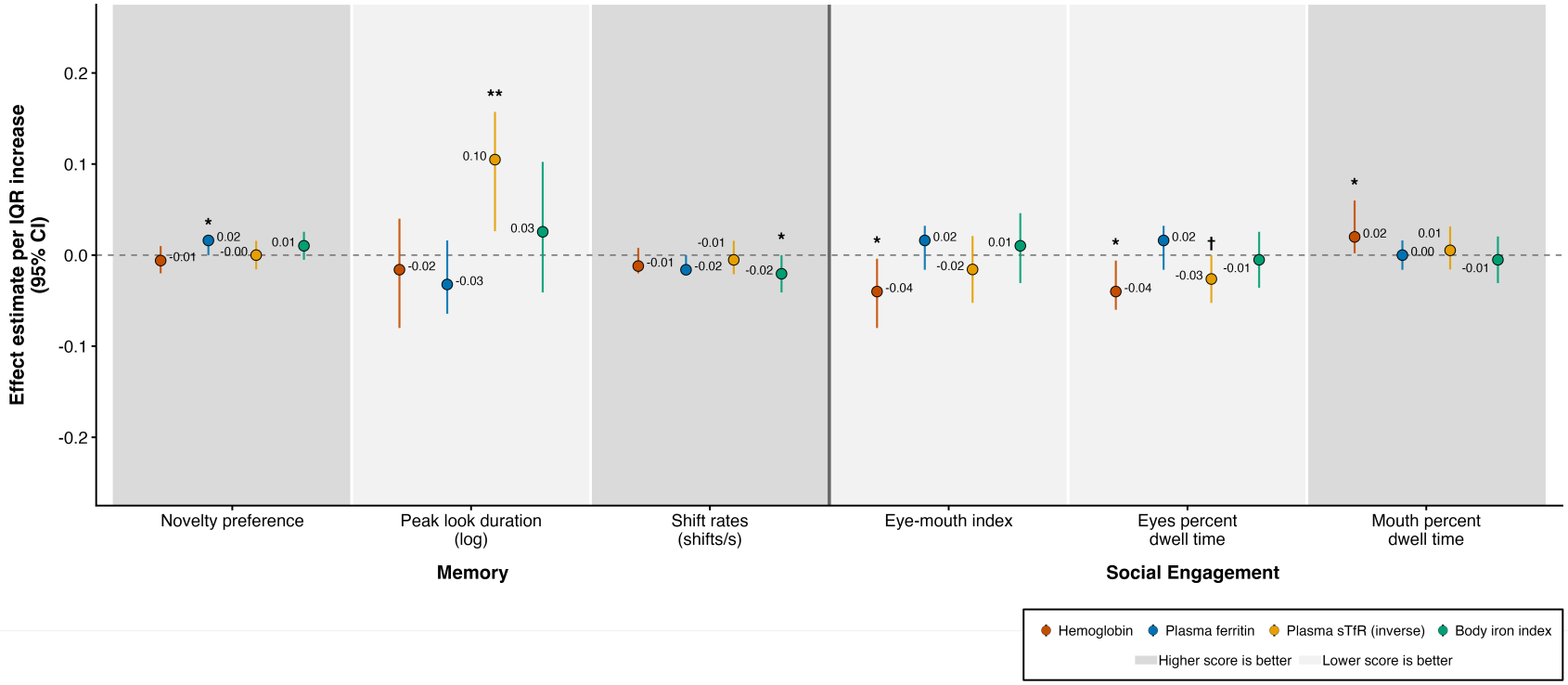


Figure 4.3. Associations of anemia and iron status biomarkers with memory and social engagement outcomes measured 6 months later using the Visual Paired Comparison task. Memory indicators included novelty preference, peak look duration, and shift rates. Social engagement indicators included eye-mouth index, eyes percent dwell time, and mouth percent dwell time. Estimates are from fully adjusted models and represent effects per interquartile range increase in each biomarker. Plasma ferritin and sTfR were adjusted for inflammation using the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) regression approach.

Plasma sTfR was inverted to align directionality with other biomarkers (i.e., higher biomarker levels reflect better anemia or iron status). Outcomes with a dark background are those for which higher scores indicate better performance, whereas outcomes with a light background are those for which lower scores indicate better performance. Abbreviations: IQR, interquartile range; sTfR, soluble transferrin receptor. * $p<0.05$; ** $p<0.01$; † $p<0.1$.

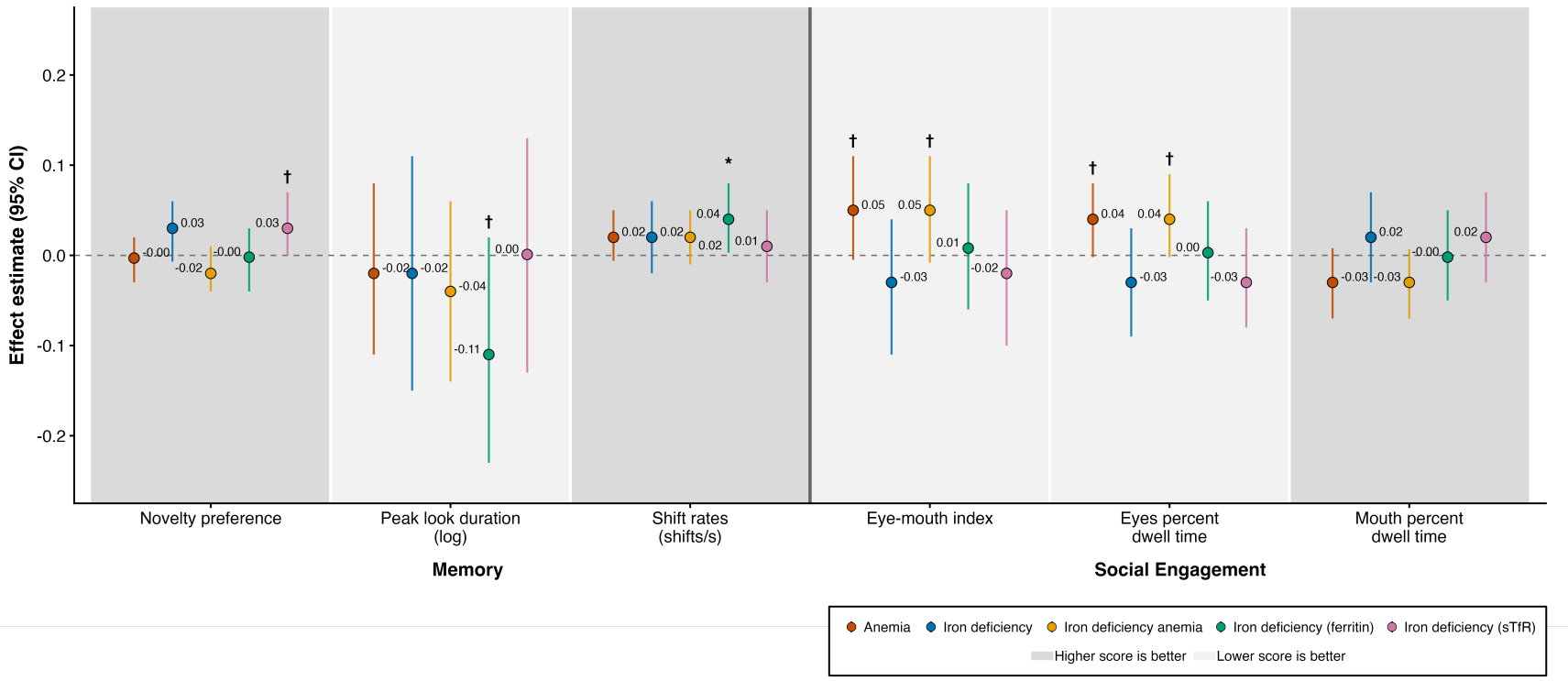
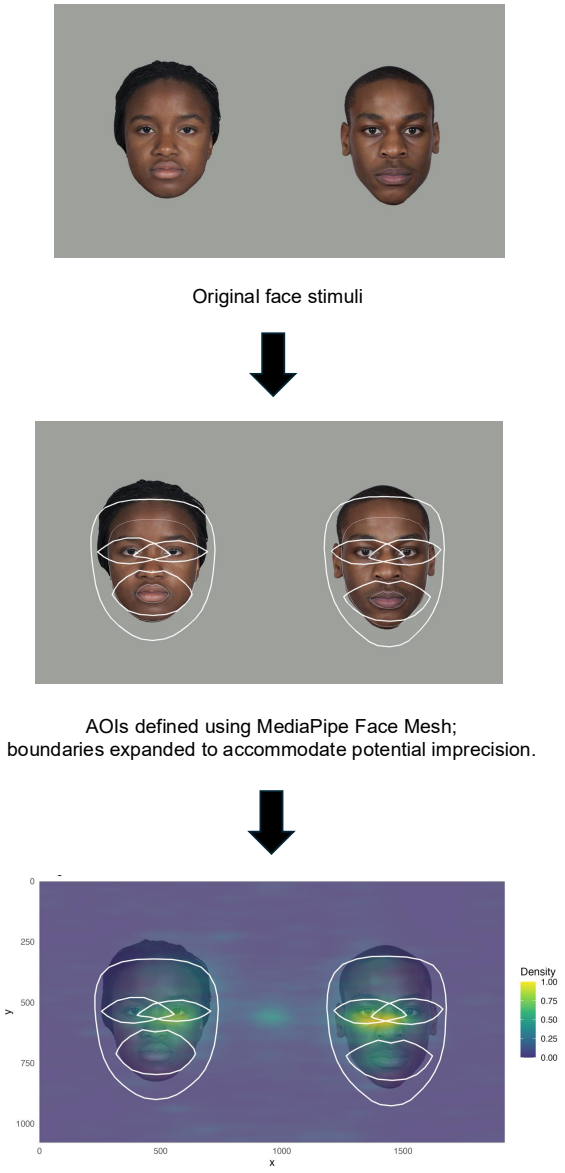


Figure 4.4. Associations of anemia and iron status categories with memory and social engagement outcomes measured 6 months later using the Visual Paired Comparison task. Memory indicators included novelty preference, peak look duration, and shift rates. Social engagement indicators included eye-mouth index, eyes percent dwell time, and mouth percent dwell time. Estimates are from fully adjusted models. Iron deficiency status included: (1) iron deficiency defined using at least one of the following criteria–plasma ferritin < 12 µg/L, plasma soluble transferrin receptor (sTfR) > 8.3 mg/L, or body iron index < 0 mg/kg; (2) iron deficiency defined using the plasma ferritin cutoff only; and (3) iron deficiency defined using the plasma sTfR cutoff only. Outcomes with a dark background are

those for which higher scores indicate better performance, whereas outcomes with a light background are those for which lower scores indicate better performance. * $p<0.05$; † $p<0.1$.

4.7 Supplementary materials



Supplementary Figure 4.1. Development of the eye-tracking-based visual social engagement assessment, with an example fixation heatmap. Abbreviations: AOI, area of interest.

Supplementary Table 4.1. Associations between baseline anemia and iron status with early learning and memory outcomes 6 months later.

	IQR (P25–P75)	β (95% CI)							
		Attention[1,2]		Memory[1,3]			Social Engagement[1,4]		
		Reaction time, ms	Task error[5]	Novelty preference[6]	Peak look duration (log)	Shift rates, shifts/s	Eye-mouth index	Eyes percent dwell time	Mouth percent dwell time
Hemoglobin (per IQR increase)	2.00 (9.50–11.50)	-3.24 (-12.50, 6.04)	1.72 (1.40, 2.12)	-0.01 (-0.02, 0.01)	-0.02 (-0.08, 0.04)	-0.01 (-0.02, 0.01)	-0.04 (-0.08, -0.004)*	-4.0% (-6.0, -0.6)*	2.0% (0.2, 6.0)*

Plasma ferritin (per IQR increase)	16.10 (7.04–23.14)	0.16 (-5.80, 6.28)	16.26 (15.94, 16.42)	0.02 (0.00, 0.02)*	-0.03 (-0.06, 0.02)	-0.02 (-0.02, 0.00)	0.02 (-0.02, 0.03)	1.6% (-1.6, 3.2)	0.0% (-1.6, 1.6)
Plasma sTfR (per IQR increase)	5.24 (7.86–13.10)	8.07 (-0.21, 16.36)†	5.50 (5.14, 5.92)	0.00 (-0.02, 0.02)	-0.10 (-0.16, -0.03)**	0.01 (-0.02, 0.02)	0.02 (-0.02, 0.05)	2.6% (0.0, 5.2)†	-0.5% (-3.1, 1.6)
Body iron index (per IQR increase)	5.12 (-3.05–2.07)	-1.13 (-10.95, 8.70)	5.07 (4.66, 5.48)	0.01 (-0.01, 0.03)	0.03 (-0.04, 0.10)	-0.02 (-0.04, 0.00)*	0.01 (-0.03, 0.05)	-0.5% (-3.6, 2.6)	-0.5% (-3.1, 2.0)
Anemia	–	0.98 (-13.03, 15.00)	1.49 (0.78, 2.83)	-0.003 (-0.03, 0.02)	-0.02 (-0.11, 0.08)	0.02 (-0.006, 0.05)	0.05 (-0.005, 0.11)†	4.0% (-0.2, 8.0)†	-3.0% (-7.0, 0.8)

ID	–	7.69 (-11.57, 26.95)	0.74 (0.31, 1.76)	0.03 (-0.007, 0.06)	-0.02 (-0.15, 0.11)	0.02 (-0.02, 0.06)	-0.03 (-0.11, 0.04)	-3.0% (-9.0, 3.0)	2.0% (-3.0, 7.0)
IDA	–	5.90 (-8.68, 20.48)	1.28 (0.65, 2.54)	-0.02 (-0.04, 0.01)	-0.04 (-0.14, 0.06)	0.02 (-0.01, 0.05)	0.05 (-0.008, 0.11)†	4.0% (-0.2, 9.0)†	-3.0% (-7.0, 0.7)

Abbreviations: ID, iron deficiency; IDA, iron deficiency anemia; sTfR, soluble transferrin receptor.

[1] Covariates included baseline malaria status; variables related to blood sample collection and eye-tracking assessment; and child, household, and parental characteristics. The latter were selected based on bivariate associations with the outcome using likelihood ratio tests ($p < 0.10$). All final models were adjusted for child age at outcome assessment, trial group assignment, time of last meal before blood draw, calendar month of blood draw, child mood, activity level and interaction with assessor during the developmental tasks, developmental data assessor, eye-tracking station, and baseline malaria.

[2] Additionally adjusted for HOME Inventory score, village location, and paternal education.

[3]Additionally adjusted for child sex, household food insecurity access scale, number of children under 5 yo, and endline malaria

[4] Additionally adjusted for first born status, maternal age, and endline malaria.

[5] Odds ratio for having > 0 task error.

[6] Novelty preference ranges from 0 to 1, with values closer to 1 indicating greater preference for novel stimuli.

* $p<0.05$; ** $p<0.01$; † $p<0.1$.

Supplementary Table 4.3. Associations between sustained anemia and iron status with early learning and memory outcomes.

	N	β (95% CI)							
		Attention[1,2]		**Memory[1,3]**			**Social Engagement[1,4]**		
		Reaction time, ms	**Task error[5]**	**Novelty preference[6]**	**Peak look duration (log)**	**Shift rates, shifts/s**	**Eye-mouth index**	**Eyes percent dwell time**	**Mouth percent dwell time**
Anemia									
Sustained anemia	60	3.11 (-17.57, 23.78)	2.11 (0.83, 5.35)	0.004 (-0.03, 0.04)	-0.04 (-0.18, 0.10)	-0.007 (-0.05, 0.03)	0.03 (-0.05, 0.11)	3.0% (-3.0, 9.0)	-0.5% (-6.0, 5.0)
Anemic at baseline assessment only	122	0.46 (-16.27, 17.19)	1.48 (0.69, 3.18)	0.000 (-0.03, 0.03)	0.02 (-0.09, 0.13)	0.03 (-0.003, 0.06)†	0.04 (-0.03, 0.10)	2.0% (-3.0, 7.0)	-3.0% (-7.0, 2.0)

Anemic at outcome assessment only	45	-6.36 (-28.42, 15.71)	1.79 (0.67, 4.80)	0.01 (-0.02, 0.05)	0.05 (-0.10, 0.20)	0.001 (-0.04, 0.04)	-0.05 (-0.13, 0.04)	-6.0% (-12.0, 0.4)†	3.0% (-3.0, 9.0)
Not anemic	174	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
ID									
Sustained ID	279	-15.81 (-45.28, 13.66)	0.26 (0.06, 1.12)†	-0.007 (-0.06, 0.05)	0.03 (-0.18, 0.23)	0.02 (-0.05, 0.08)	-0.06 (-0.18, 0.06)	-5.0% (-15.0, 4.0)	3.0% (-5.0, 11.0)
ID at baseline assessment only	20	-30.95 (-71.97, 10.06)	0.14 (0.02, 0.92)*	-0.010 (-0.08, 0.06)	0.11 (-0.16, 0.39)	-0.01 (-0.10, 0.07)	0.04 (-0.13, 0.20)	-4.0% (-16.0, 9.0)	-2.0% (-13.0, 9.0)
ID at outcome assessment only	35	-36.13 (-71.57, -0.68)*	0.18 (0.03, 0.98)*	-0.05 (-0.12, 0.01)	0.09 (-0.16, 0.34)	0.001 (-0.08, 0.08)	-0.03 (-0.17, 0.11)	-3.0% (-15.0, 8.0)	0.6% (-9.0, 10.0)
Not ID	23	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref

IDA									
Sustained IDA	46	14.81 (-7.24, 36.86)	1.57 (0.56, 4.43)	-0.01 (-0.05, 0.03)	-0.06 (-0.21, 0.09)	-0.02 (-0.06, 0.03)	0.007 (-0.08, 0.09)	2.0% (-5.0, 9.0)	0.7% (-5.0, 7.0)
IDA at baseline assessment only	97	0.88 (-16.07, 17.82)	1.26 (0.57, 2.79)	-0.01 (-0.05, 0.02)	0.000 (-0.12, 0.12)	0.03 (-0.005, 0.07)†	0.05 (-0.02, 0.12)	2.0% (-3.0, 8.0)	-4.0% (-8.0, 1.0)
IDA at outcome assessment only	37	-3.51 (-26.70, 19.67)	1.66 (0.57, 4.81)	0.02 (-0.02, 0.07)	0.08 (-0.08, 0.23)	0.007 (-0.04, 0.06)	-0.07 (-0.16, 0.02)	-9.0% (-16.0, -2.0)*	5.0% (-2.0, 11.0)
Not IDA	177	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref

Abbreviation: ID, iron deficiency; IDA, iron deficiency anemia.

1 Covariates included baseline malaria status; variables related to blood sample collection and eye-tracking assessment; and child, household, and parental characteristics. The latter were selected based on bivariate associations with the outcome using likelihood ratio tests ($p < 0.10$). All final models were adjusted for child age at outcome assessment, trial group assignment, time of last meal

before blood draw, calendar month of blood draw, child mood, activity level and interaction with assessor during the developmental tasks, developmental data assessor, eye-tracking station, and baseline malaria.

[2] Additionally adjusted for HOME Inventory score, village location, and paternal education.

[3] Additionally adjusted for child sex, household food insecurity access scale, number of children under 5 yo, and endline malaria

[4] Additionally adjusted for first born status, maternal age, and endline malaria.

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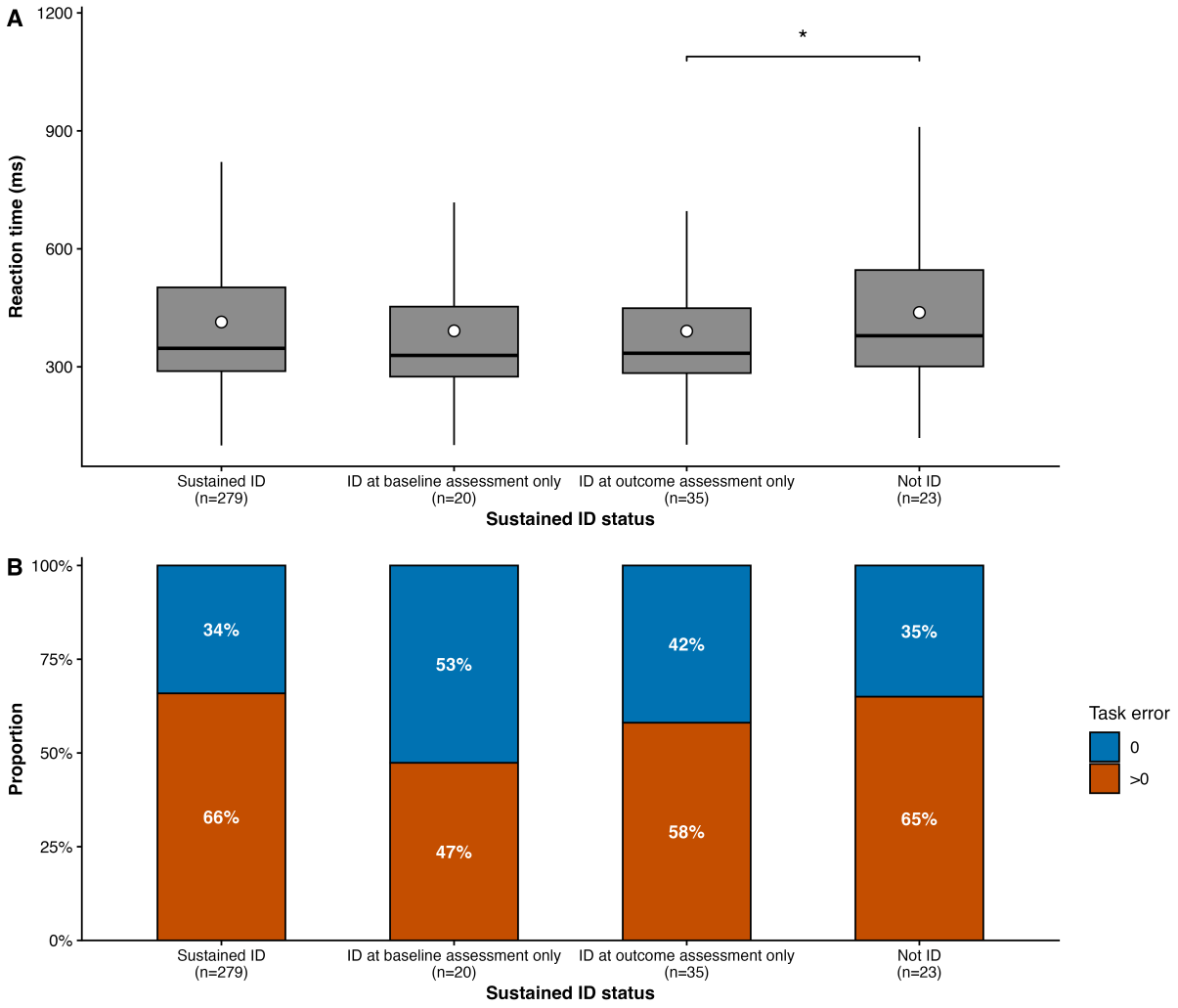
[6] Novelty preference ranges from 0 to 1, with values closer to 1 indicating greater preference for novel stimuli.

* $p<0.05$; † $p<0.1$.



Supplementary Figure 4.2. Associations of sustained iron deficiency (ID) status with reaction time (Panel A) and task error (Panel B), with the "not ID" category as the reference group. For task error, significant associations were observed for the "ID at baseline assessment only" and "ID at outcome assessment only" groups versus the reference group, although these are not marked in the figure. * $p<0.05$