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EFFECTS OF IRON AND ZINC SUPPLEMENTATION DURING THE PRESCHOOL YEARS ON SCHOOL-AGE CHILD BEHAVIOR

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Abstract

Data on the long-term effects of preschool micronutrient supplementation on behavior are sparse. We examined behavior in seven to nine year old Nepali children (n=694) who received micronutrient supplements from 12 to 35 months of age in a double-masked randomized controlled trial. Children were randomized to daily iron plus folic acid (IFA), zinc (Zn), iron plus folic acid and zinc (IFAZn) or control. Behavior was assessed on six dimensions (positive mood, negative mood, lively/active, sociability, attention and demandingness) during two home visits. Differences in behavioral rating scores were assessed using logistic regression, comparing the control group to each of the three supplementation groups. Additionally, groups who received any iron plus folic acid (IFA or IFAZn) or any zinc (Zn or IFAZn) were compared to groups who received no iron plus folic acid or no zinc, respectively.

Overall, those who received IFA were 2.90 times more likely to receive higher ratings of demandingness than controls (p=0.005) and those who received IFAZn were 1.43 times more likely to receive higher ratings of sociability than controls (p=0.048). After adjusting for confounders, those who received IFA were 3.13 times more likely to receive higher ratings of demandingness than controls (p=0.007); the greater likelihood of children supplemented with IFAZn to receive higher ratings of sociability than controls lost significance.

Additionally, those who received any iron plus folic acid were 1.81 times more likely to receive higher ratings of demandingness than those who received none (p=0.03) and those who received any zinc were 1.58 times more likely to receive higher ratings of sociability than those who received none (p=0.0005). After adjusting for confounders, those who received any iron plus folic acid were 2.10 times more likely to receive higher ratings of demandingness than those who received none (p=0.01) and those who received any zinc were 1.49 times more likely to receive higher ratings of sociability than those who received none (p=0.004).

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Chapter 1

Literature Review

I. Iron

a. Diet and Deficiency

Iron, an essential micronutrient, is found in amounts ranging from two to four grams in the human body, or approximately 38 mg iron/kg body weight for women and 50 mg iron/kg body weight for men (1). Most iron in the body (65%) is found as a component of hemoglobin, while the remaining iron is found in myoglobin, enzymes, or storage (2). While iron is one of the most abundant elements on the earth's surface, dietary intakes are often inadequate, a function of the amount of iron in an individual's diet, the bioavailability of the iron in the diet and the individual's iron losses (3). It is estimated that two billion people suffer from iron deficiency worldwide (4). Developing nations, especially those in south Asia and sub-Saharan Africa, bear a significant amount of the burden (4–6).

As noted, iron status is directly affected by the iron content of the food consumed by an individual. Good sources of heme iron (iron contained within a porphyrin ring and derived from hemoglobin and myoglobin) include various animal products including red meat, oysters, fish and poultry (7). Good sources of nonheme iron include nuts, legumes, seeds, green leafy vegetables and dried fruits (7). The distinction between heme and nonheme iron is important since heme iron is more bioavailable than nonheme iron. While heme iron has a rate of absorption ranging from 5% to 35%, nonheme iron only has a rate of absorption from 2% to 20%

(3). So, while heme iron makes up only 10% of iron in the diet, up to one third of absorbed iron is of the heme variety (8).

Other elements of the diet can affect the absorption of iron. Dietary enhancers of nonheme iron absorption include fructose, sorbitol, meat, fish, poultry, mucin and acids, including ascorbic, citric, lactic and tartaric (1). Of these acids, ascorbic is the most efficient enhancer (9). These factors help to improve absorption through reduction and chelation. For example, ascorbic acid reduces ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}), the state nonheme iron must be in to be absorbed in the small intestine. Without dietary enhancers, nonheme iron absorption is typically only 2% to 3% but with 75 units of these factors, nonheme iron absorption can be increased anywhere from 8% to 20%, depending on the iron status of the individual (10).

Dietary inhibitors of iron, both heme and nonheme, include polyphenols in tea and coffee, oxalic acid, phytates, phosvitin, calcium phosphate salts and divalent minerals such as calcium, zinc, manganese and nickel (1). Of these, phytate is of note since it is a particularly potent inhibitor of iron and is a common component of plant food like grains and legumes, the staple foods of the developing world (11). These factors diminish iron absorption through complex formation and competitive inhibition. For example, divalent minerals compete for absorption across the apical membrane with iron since they can all be transported via divalent metal transporter 1 (DMT1).

As noted earlier, iron deficiency is a common nutrition problem worldwide. In South-East Asia, 45.7 percent of non-pregnant women and 65.5 percent of preschool-age children are estimated to have anemia; approximately half of these cases are attributable to iron deficiency (12,13). Individuals who are at the highest risk for developing iron deficiency include infants and young children, due to the low iron content of breast milk, rapid growth rate and low body

reserves, adolescents during their growth spurt, women of childbearing age, due to menstruation, and pregnant women, due to expanding blood volume, the demands of the fetus and the child birthing process. Early-life iron deficiency is of particular concern since evidence of long-term effects in humans is beginning to accumulate (14–16). The consequences of iron deficiency include weakness, tiredness, behavioral alterations, decreased cognitive ability, attention span and brain function, and impaired immune system functioning (17–21).

b. Metabolism

The digestion of iron begins in the stomach where hydrochloric acid (HCl) secretions denature proteins, helping to release protein-bound iron, and reduce iron from the ferric state (Fe^{3+}) to the more soluble ferrous state (Fe^{2+}) (3). Iron absorption does not occur, however, until the micronutrient enters the small intestine. The absorption of iron is a tightly regulated process since there are no efficient mechanisms that can maintain iron homeostasis through excretion (22).

Nonheme iron, released from food components in the stomach and small intestine, must be in the ferrous state to be absorbed across the apical membrane of the enterocyte. However, most nonheme iron is present in the ferric state after its release from food components. In the relatively basic environment of the small intestine, this ferric iron can complex to form the insoluble compound ferric hydroxide ($Fe(OH)_3$), reducing absorption and thereby decreasing the bioavailability of nonheme iron. As stated earlier, some nonheme iron is reduced to the ferrous state by HCl in the stomach. Additionally, enhancers of nonheme iron absorption such as ascorbic acid reduce iron to the ferrous state, allowing iron to be transported directly through DMT1 (1). The rest of the nonheme iron that will eventually be taken into the enterocyte is reduced from its ferric state at the brush border by the ferrireductase duodenal cytochrome B

(DcytB); the ferrous iron can then be absorbed by the enterocyte via DMT1. DMT1 is one point of homeostatic control since synthesis of the transporter is upregulated when iron stores are low.

Heme iron, the more bioavailable form of the micronutrient, must first be hydrolyzed from the globin portion of hemoglobin before intestinal absorption may occur. This process occurs in both the stomach and small intestine by the action of proteases. The heme molecule can then be absorbed across the apical membrane by the enterocyte via heme carrier protein-1 (hcp-1) (22). Once inside the enterocyte, the heme iron is further hydrolyzed by heme oxygenase, releasing ferrous iron from the protoporphyrin ring (2). At this point, the metabolisms of heme and nonheme iron converge, following the same pathways throughout the body.

Following absorption, iron can either be transported through the cell for transport across the basolateral membrane and into circulation or remain in the enterocyte, either stored as ferritin or for functional use, depending on systemic iron needs (22). For export out of the enterocyte, ferrous iron must bind to the membrane transport protein ferroportin (Fp). As Fp moves out of the cell, the copper-containing protein hephaestin oxidizes the ferrous iron back to its ferric state, allowing it to bind to apotransferrin. Apotransferrin, or transferrin (Tf) once iron is bound, is the main iron transport protein and can carry up to two iron atoms throughout the body to tissues.

For iron uptake into cells, Tf must bind to transferrin receptor (TfR), a transmembrane protein consisting of two subunits that can bind one Tf molecule each (2). Following binding, the TfR-Tf complex is endocytosed into the cytosol of the cell, forming an endosome (1). Once inside the cell, the pH of the endosome is lowered, decreasing the affinity of Tf for iron and thereby initiating the release of ferric iron (7). The ferric iron can then be reduced to the ferrous state and used within the cell or stored in ferritin when it is not needed in a functional capacity.

The process of absorption is regulated by hepcidin, a protein which is released from the liver when systemic iron status is adequate or high. Upon release from the liver, hepcidin binds to Fp, leading to the internalization and degradation of the transport protein (22). This subsequent loss of Fp prevents iron export and leads to storage in apoferritin. When the intestinal cells are sloughed off, the iron contained in ferritin is excreted as well.

Intracellular iron homeostasis is regulated posttranscriptionally by iron response elements (IREs) and IRE binding proteins (IRE-BPs) (7). By interacting with IREs, RNA stem-loop motifs on 3' or 5' untranslated regions (UTRs), IRE-BPs can enhance the translation of TfR and repress the translation of ferritin in response to low intracellular iron, respectively (2). In times of high intracellular iron, the IRE-BP exists as a 4Fe-4S cluster and cannot function as a binding protein (23). However, when there is low intracellular iron, the IRE-BP exists as a 3Fe-4S cluster and can function as a binding protein (23). In the case of the TfR mRNA, binding of the IRE-BP to the 3'-UTR stabilizes the mRNA and enhances the translation of TfR (1). In contrast, when IRE-BPs bind to the 5'-UTR of the ferritin mRNA, translation of the protein is inhibited (1). Both mRNA bindings have the effect of increasing intracellular iron content, one by upregulating iron uptake, the other by downregulating iron storage.

c. Brain Metabolism

Key to optimal functioning, iron is the most abundant mineral in the brain (24). While much brain iron is accumulated before the blood-brain barrier (BBB) fully matures, iron continues to enter the brain via this structure throughout the lifespan (25). Paracellular transport is precluded by the presence of endothelial cell tight junctions (26). For entry into the brain, iron as a component of Tf binds to TfR at the luminal membrane of the blood capillary endothelial cells (BCECs) of the BBB and is endocytosed (27). As the endosome becomes acidic, iron is

released from Tf, reduced to the ferrous state and transported across the endosomal membrane, possibly by DMT1 (28). Another method of iron transport into the brain has been suggested in which BCECs interact with astrocytic end-feet, without the involvement of DMT1 (27). The Tf-TfR complex would still be endocytosed and transported to the abluminal side of the BCEC but the complex would remain intact until it is exposed to brain extracellular fluid near the BCEC-astrocyte end-foot junction, high in hydrogen ions, ATP and citrate, allowing for the release of iron from Tf (27).

Iron is transported throughout the brain primarily bound to Tf synthesized and secreted by oligodendrocytes (26). However, in the brain the concentration of iron has been shown to exceed that of the binding capacity of Tf, indicating that iron is also transported bound to other substances, such as citrate (28). Neuronal cell uptake of iron is proposed to involve a variety of transporters and proteins including ceruloplasmin (Cp), a ferroxidase, and DMT1. In one pathway, after binding of diferric Tf to TfR, iron is brought into the neuronal cell with the creation of an endosome, and iron is transported into the cytosol via DMT1 at the endosomal membrane (27). This is thought to be the main source of iron for neuronal cells because of the high expression of both TfR and DMT1 in neuronal cells (25). In another pathway, Cp attached to the astrocytic end-feet by a glycophosphoinositide (GPI) linkage oxidizes ferrous iron to ferric iron, allowing for entrance into the neuronal cell bound to citrate or ATP (27,28). In iron efflux, Fp is believed to transport ferrous iron from the neuronal cell, which is then neutralized by ascorbic acid in the interstitial fluid (27).

While iron is found in all areas of the brain, the highest levels are found in the globus pallidus of the basal ganglia, substantia nigra and deep cerebellar nuclei (29–31). Iron deficiency can have a profound effect on the brain, especially during neural development, due to its

involvement in numerous CNS processes. In utero and early postnatal iron deficiency may result in impaired neurogenesis and differentiation of specific brain cells, such as those in the hippocampus and striatum, causing decreased arborization of dendrites and a change in functioning of oligodendrocytes, cells responsible for myelin formation (27,32). As a result, iron deficiency can interfere with proper myelination of neurons (33). As demonstrated by studies in rats, alterations in the composition and amount of myelin due to early iron deficiency may be irreversible if they occur during a sensitive period of brain development either prenatally or in early childhood (27,34,35). Iron deficiency also affects the synthesis of neurotransmitters including dopamine, serotonin, GABA and norepinephrine due to its essentiality to the enzymes involved (30,32).

d. Functions in the Body

In the body, iron primarily functions as a component of many proteins, including acting as a cofactor for several enzymes. While iron can be found in clusters with sulfur, as a single atom, or as part of a bridge with oxygen, iron is most commonly present as a component of heme in body proteins (1). Proteins which contain heme include hemoglobin, myoglobin, cytochromes, monooxygenases, dioxygenases and oxidases.

Hemoglobin and myoglobin are essential to oxygen transport throughout the body. While hemoglobin transports the vast majority of oxygen in the blood, myoglobin is found in the cytosol of muscle cells and helps to enable the diffusion of dioxygen from capillary red blood cells to the cytosol and mitochondria of muscle cells (3). In both cases, the iron atom at the center of the heme molecule in the protein binds loosely to oxygen, allowing for rapid transfer to tissues.

Cytochromes represent another significant iron containing protein, playing an essential role in the electron transport chain. The transfer of electrons from cytochrome to cytochrome is facilitated by oxidation state changes in iron, located at the active site of the protein (3). When a cytochrome receives an electron, iron is reduced to the ferrous state; when the electron is transferred to the next cytochrome, the iron atom is oxidized back to the ferric state (7).

Important iron containing enzymes participate in a variety of reactions throughout the body. Monooxygenases insert one oxygen atom into substrates such as phenylalanine, tyrosine and tryptophan, while dioxygenases insert two oxygen atoms into substrates including tryptophan, homogentisate and trimethyl lysine (1). Other iron containing enzymes, including catalase and myeloperoxidase are required to protect the body from cellular damage (7).

e. Assessment of Status

There are numerous biomarkers that allow for the reliable assessment of iron status and can discriminate between the different levels of iron deficiency – depleted iron stores, early functional iron deficiency and iron-deficiency anemia. Depleted iron stores are assessed using serum ferritin, with levels less than 12 µg/L indicative of iron deficiency (36). Despite the sensitivity to depleted iron stores, serum ferritin varies with gender and is elevated during inflammation or infection since it is an acute-phase protein (37). In situations where inflammation or infection is present, the cut off of 30 µg/L is commonly used to indicate iron depletion (38). Another, and increasingly popular, method of accounting for inflammation or infection is to apply a correction factor to the ferritin value itself; one such correction includes the method developed by David Thurnham (39). In this method, individuals in a sample are stratified into categories based on C-reactive protein (CRP) and α_1-acid glycoprotein (AGP) levels (apparently healthy reference group, incubation, early convalescence and late

convalescence) and then individual ferritin values are adjusted by the application of the correction factor, the ratio of the median ferritin value of the apparently healthy reference group to the median of the individual's inflammation group (39,40).

Serum transferrin receptor concentration (sTfR) is a sensitive and specific indicator that can be used to detect the progression of iron depletion to functional iron deficiency. During iron deficiency, the expression of sTfR is upregulated by the IRE/IRE-BP system to levels greater than normal values which range from approximately 5 to 8 mg/L (41). Levels greater than 8.5 mg/L are believed to be indicative of iron deficiency (1). While sTfR is not as affected by inflammation or infection as ferritin, sTfR has been shown to be elevated in cases of malaria (42,43). Another measure of iron deficiency is total iron-binding capacity (TIBC) which represents the amount of iron that transferrin can bind to in the plasma. While normal levels range from 250 to 400 µg/dL, a TIBC greater than 400 µg/dL is suggestive of iron deficiency (1). Additional biomarkers that are used to assess iron deficiency include serum iron and erythrocyte protoporphyrin, in which levels less than 60 µg/dL and greater than 70 µg/dL indicate inadequate iron, respectively (7). Calculated from serum iron and TIBC, serum transferrin saturation may also be used to assess iron deficiency (1). While normal levels range from approximately 30% to 35%, serum transferrin saturation below 15% is indicative of iron deficiency (36).

If iron deficiency continues to progress, anemia is induced and can be detected by a variety of indices. The most commonly used measure to assess anemia is hemoglobin, in which levels below 12 g/dL and 13 g/dL are indicative of anemia in women and men, respectively (44). However, hemoglobin is not specific to iron deficiency anemia and decreased levels could be due to deficiencies in folate or vitamin B12 since both are also crucial to erythropoiesis (45).

Other causes of anemia include, but are not limited to, chronic infections, renal failure, leukemia, lymphoma, thalassemia, sickle cell, trauma, peptic ulcers, menorrhagia and blood donation (46). Additionally, adjustments to the cutoffs for anemia must be considered for children, pregnant women, smokers and people who live at altitude (44). Hematocrit may also be used to assess anemia, with concentrations less than 36% suggestive of anemia (7). Much like hemoglobin, hematocrit is not specific to iron deficiency anemia and can be affected by a variety of factors. Iron deficiency anemia may also be determined by examination of the red blood cells themselves. In anemia caused by iron deficiency, red blood cells are hypochromic and microcytic (36).

II. Zinc

a. Diet and Deficiency

Zinc (Zn^{2+}), an essential micronutrient, is found in amounts ranging from 1.5 to 2.5 g in the human body. Most zinc is found in the muscle and bone, but, the micronutrient is present in all organs, tissues and body fluids (47). While hard to characterize, due to regulation in the body and the lack of an easily interpretable biomarker, it is believed that 17% of the world's population is at risk of zinc deficiency (48). While individuals in high income countries are at a relatively low risk of zinc deficiency (7.5%), persons from south Asia and sub-Saharan Africa are at a particularly high risk (29.6% and 25.6%, respectively), with individuals from developing nations in these regions experiencing the highest rates of zinc deficiency (48–50).

The best sources of zinc are animal products, including poultry, pork and dairy, but especially red meats and seafood. Plant sources that provide the most zinc include whole grains and vegetables, but absorption can be limited due to the presence of phytates in those foods (51).

Zinc absorption has been estimated to range from 10% to 59%, depending upon intake, zinc status and dietary factors (1).

In addition to increased absorption due to low zinc status, zinc absorption can be enhanced by several dietary factors. Higher amounts of protein, especially animal proteins, have been correlated with increased zinc absorption (51). Ligands serve as an important enhancer of zinc absorption because of their ability to maintain zinc solubility in the intestine. These ligands include organic acids, such as citric and picolinic acid, and amino acids, such as histidine and cysteine (1). However, the absorption of zinc is inhibited by many components of the diet. Chiefly, phytates, common in plant foods, form large, insoluble complexes with zinc and consequently decrease absorption of the mineral (47). Other substances that form complexes with zinc and decrease absorption include oxalate, found in tea, spinach, chocolate and berries, and polyphenols, found in tea, vegetables, fruits and whole grains (1). Additionally, zinc absorption is negatively impacted by other divalent cations, including iron, calcium and copper (51). Decreased absorption is caused by competition for ligands and transporters such as DMT1 in the intestine (52).

As noted, zinc deficiency is believed to be a common nutritional issue worldwide. Early and mild zinc deficiency is characterized by slowed growth and decreased appetite, activity and attention (18,47). Other symptoms may include higher susceptibility to infection, increased rates of diarrhea and decreased neuropsychological performance (53). Prolonged and severe zinc deficiency results in a variety of symptoms affecting several organ systems including the epidermal, gastrointestinal, central nervous, immune, skeletal and reproductive systems (54). Symptoms may include severe growth retardation and delayed sexual maturation in children, skeletal abnormalities, immune system dysfunction, dermatitis and sexual dysfunction (47).

b. Metabolism

Much like iron, zinc must be released from food components before it can be absorbed in the small intestine. It is believed that zinc is hydrolyzed from protein and nucleic acids by proteases, nucleases, and HCl in the stomach and small intestine (1). Once released, zinc is absorbed across the brush border membrane of the enterocyte via the transporter Zrt- and Irt-like protein 4 (ZIP4) (55). It is thought that ZIP4 does not require ATP, but the mechanism is still not completely characterized.

Once inside the enterocyte, zinc may be used or stored in the cell, or moved across the basolateral membrane for use by other tissues. Initially, zinc will bind to cysteine-rich intestinal protein (CRIP) and be transported transmucosally to the basolateral membrane for export (56). As levels of zinc increase in the enterocyte, however, zinc will bind to thionein for storage. Metallothionein (MT), as thionein is called once bound to zinc, captures the zinc and is most often sloughed off with intestinal cells. For zinc that does not get bound by MT, the CRIP transported zinc is moved across the basolateral membrane by zinc transporter 1 (ZnT1) and most often binds loosely to the protein albumin for transport in the blood to the liver (1).

After transport to the liver, where zinc is initially concentrated, zinc can be transported to other tissues in the body, primarily bound to albumin (approximately 60%) but also to transferrin, α-2 macroglobulin and immunoglobulin G (1,47). Cellular zinc influx is facilitated by a variety of ZIP carriers (1, 2, 4, 6, 7, 8 and 14) and exhibits some tissue specificity (55). Cellular efflux and intracellular sequestration of zinc is mediated by numerous ZnTs including ZnT1, expressed ubiquitously, ZnT3, expressed in the brain, ZnT4, expressed in mammary glands, and ZnT8, expressed in the pancreatic β-cells (57). As in enterocytes, when zinc is not needed for functional uses in the cell, zinc is stored in MT. Expression of thionein appears to be

affected in part by zinc. Metal regulatory elements (MREs) located on the promoter region of the gene for thionein bind to metal transcription factors (MTFs) to induce thionein synthesis in a process dependent on zinc (58). Low cellular zinc disrupts the binding of the MTF to the MRE and therefore decreases synthesis of the protein.

c. Brain Metabolism

Found in relatively high concentrations in the cerebral cortex and hippocampus, Zinc is a critically important mineral in the brain, second in abundance only to iron (59,60). To maintain a constant concentration in the brain, zinc must be transported across the BBB and the blood-CSF barrier (61). Transported to the BBB bound to histidine or potentially albumin, zinc is shuttled through the BBB via a transporter, such as DMT1, ZIP2 or peptide/histidine transporter (PHT1), all potential candidates for zinc transport (61,62). The mechanism for how zinc moves from the brain capillary endothelial cells into the extracellular fluid of the brain is currently unknown (62). Additionally, the exact mechanism of uptake of zinc from the brain extracellular fluid and into the neural and glial cells has yet to be characterized completely (61,63). However, it is believed that DMT1, present in large quantities on cells such as the hippocampal pyramidal and granule cells, is involved in zinc uptake into neural cells and that PHT1 may be involved in uptake in neural and glial cells as well (61,62). Additionally, a number of membrane channels and the Na^{+}/Zn^{2+} exchanger is believed to facilitate this complex process (63).

While 90% of brain zinc is incorporated and bound in proteins, the remaining free zinc is found primarily in presynaptic vesicles, with especially high concentrations in the hippocampal mossy fiber terminals (61,64). Maintaining homeostasis between the protein-bound and free zinc is important for preserving normal brain function because of zinc's roles in gene expression, enzymatic activity, cell signaling and the activity of neurotransmitters (59). Critical to brain zinc

homeostasis are ZnTs which mediate the movement of zinc from the cytosol to organelles or out of the cell (65). While ten have been identified in the brain, ZnTs of particular importance include ZnT1, expressed on the plasma membrane of neural and glial cells, and ZnT3, expressed primarily on the synaptic glutamatergic vesicles in the hippocampus, cortex and olfactory bulb (65). ZIPs are also present in the brain, mediating the influx of zinc into the cytosol of the neural and glial cells (65). Additionally, MT plays an important role in maintaining the homeostasis of zinc in the brain and protecting the neural and glial cells from oxidative stress, releasing zinc in times of reactive oxygen species production (59).

The importance of brain zinc is demonstrated by a variety of processes. During neurodevelopment in utero, zinc is essential to the function of proteins, enzymes, hormones and growth factors that guide stem cell proliferation and differentiation (59). Additionally, zinc plays a key role during periods of developmental pruning and plasticity of neural cells, due to its involvement in apoptosis, although high levels of zinc may induce detrimental cell death (59,64). Finally, zinc is important in neurotransmission, modulating several postsynaptic receptors via glutamate, including those for NMDA and GABA (59,65,66).

Zinc deficiency, most common in periods of rapid growth, can cause alterations in brain function. Studies in rats have shown that there can be up to a 30% reduction in zinc concentration in the mossy fibers of the hippocampus, a region of the brain associated with learning, memory, emotional control and mood (59,67). Additionally, low brain zinc will activate expression of an isoform of MT, MT-III, which results in cell growth retardation (68). Behavioral alterations have been observed in zinc deficiency during childhood and adulthood. Rodent and primate studies have indicated that these alterations in behavior may not respond to zinc repletion if the zinc deficiency occurs during a sensitive period (18). In states of zinc

deficiency, reduced activity and responsiveness is often noted in developing children, while decreased learning ability and visual attention has been observed in adult animal studies (62).

d. Functions in the Body

Zinc has numerous biochemical roles in the body, due in part to the fact that the mineral is a component of more than 300 metalloenzymes (69). In these enzymes, zinc can function in a catalytic, cocatalytic or structural capacity by participating directly in the reaction by interacting with substrates, either singularly (catalytic) or with multiples zinc atoms (cocatalytic), or by providing structural stability to the enzyme (structural) (47,69). Examples of zinc-dependent enzymes include carbonic anhydrase, which aids in the disposal of carbon dioxide, Δ-aminolevulinic acid dehydratase, which is involved in heme synthesis, and superoxide dismutase, which catalyzes the removal of superoxide radicals (1). In addition to its role in enzymes, zinc is important in cell growth and replication, bone formation, membrane stability, transcription and gene expression, and cell- and antibody-mediated immunity (1,70).

e. Assessment of Status

The accurate assessment of zinc status in humans is difficult due to tight homeostatic control and the lack of a readily mobilized storage form of the micronutrient in the body when intake is inadequate (71). Additionally, the search for adequate zinc biomarkers has largely been foiled by the extensive involvement of zinc in numerous bodily processes and the small decreases in body zinc that are related to morbidity and altered development (36). Despite these complications, there are a few biomarkers that are regularly used to assess zinc status.

The most commonly used biomarker is plasma zinc, with fasting concentrations below 70 µg/dL suggestive of deficiency (72). However, plasma zinc has several limitations including poor sensitivity, imperfect specificity and a variety of influencing factors such as meals, time of

day, stress, infection and medication (1,36). In addition, plasma zinc is a fairly poor measure of marginal zinc deficiency (73). Other biomarkers that have been shown to respond to changes in zinc status include urinary zinc excretion and hair zinc concentration (72). But, much like plasma zinc, there are a variety of limitations associated with these biomarkers and they are not always reliable (1). The use of red blood cell metallothionein has been proposed as a potentially useful marker of zinc deficiency, including at the marginal level, but further research is needed before more conclusive statements can be made (73).

Zinc status can also be assessed at the population level. In addition to the use of plasma zinc as an indicator of a population at high risk for zinc deficiency (greater than 20% of a population below the designated cutoff), measures such as dietary zinc intake and the functional indicators of height- or length-for-age are commonly used to assess the zinc status of the population (74). In the case of dietary intake, risk of zinc deficiency is considered to be of a public health concern when the prevalence of low intake is greater than 25% (75). In the case of functional indicators, risk of zinc deficiency is considered to be of a public health concern when the prevalence of low height- or length-for-age (-2.0 SD below the age-specific median) is greater than 20% (76).

III. Child Development

Child development, especially brain development, is a long and complicated process, taking place over the course of years and is affected by a variety of factors including early experiences and diet. Brain development begins in utero and can continue through an individual's twenties in some areas, involving the basic processes of neural induction, neural and glial proliferation, cell migration, programmed cell death, cell differentiation, synaptogenesis and

synapse pruning (77,78). Of these processes, cell differentiation, synaptogenesis and synapse pruning continue to occur postnatally in normal development (78). In fact, approximately 83% of an individual's total dendritic growth occurs postnatally, with the brain producing two times as many synapses in early childhood as it will need in adulthood (77). Because of the intensity and rapidity of this "brain growth spurt", the first two years of life have been characterized as a critical period of development, in which deficits and deprivations could have a negative long lasting or irreversible effect on the function of the brain (79,80).

Coinciding with the periods of cell differentiation, synaptogenesis and synapse pruning is the important process of myelination (77). Myelin, a lipid based sheath produced by glial cells, insulates axons, allowing for the fast transmission of electrical signals, making it critically important to proper information flow and the conduction of action potentials (81). Therefore, the disruption of myelination, as is caused by iron deficiency, can create alterations in the functioning of the brain such as decreased conduction speed and increased refractory periods following synaptic firing (81,82). Myelination occurs slowly, beginning in the midbrain and cerebellum just after birth, continuing in subcortical areas of the forebrain during the first and second year of life and ending in the cerebral cortex, an area in which maturation and myelination continues to occur through the second decade of life (77). Of note, the limbic system, which is important to the emotional and social development of children, myelinates slowly, especially axons within the limbic cortex and axons which transmit information to and from the hippocampus (77).

As noted earlier, child development can be affected by one's diet and experiences. In fact, the failure of children to reach their developmental potential is a common worldwide issue; over 200 million children fail to reach their full capabilities due to poverty, illness, malnutrition

and insufficient care (5). One area which appears to play an important role in child development is cognitive stimulation and experience. Several studies have shown that early cognitive stimulation can improve cognitive functioning, social behavior, self-confidence and positive affect (83). Experience during the sensitive or critical period of brain development can shape and guide the connectivity of neural circuits; if such experience is deficient or lacking, behavior and cognition may be irreversibly altered (80).

Nutrition represents another area which has a significant impact on child development. Firstly, micronutrient deficiencies can change the structure and functioning of the brain. In addition to altering myelination, iron deficiency has been associated with modified brain and neurotransmitter metabolism (82,83). The outward manifestations of these alterations in the developing brain include diminished affective response, delays in motor development, lower cognitive test scores and decreased social-emotional functioning (79,82,83). Zinc deficiency is also thought to directly alter the brain, resulting in altered motor development, cognitive development and behavior, although studies have not been conclusive (84,85). Secondly, malnutrition can manifest itself in a variety of other bodily systems, negatively affecting child development. Examples of these effects include stunting and decreased immunity in zinc deficiency, reduced energy in iron deficiency and poor growth and activity in protein energy malnutrition (50,83,85).

IV. Nepal

Described as “a yam between two rocks”, Nepal is a landlocked nation in southern Asia, located between its powerful neighbors, India and China (**Figure 1**) (86). Roughly the size of Arkansas with an area of 56,827 square miles, this Hindu nation is home to over 30 million

Nepali (87). While the capital Kathmandu is fairly urbanized, the vast majority of Nepal is rural, with 88% of the population residing outside of cities (86). Unsurprisingly, agriculture is the main component of the Nepali economy and is what sustains the livelihood of approximately 75% of the population (87). Nepal can be separated into three distinct geographic regions, the Parbat (mountains), the Pahar (hills) and the Tarai (plains) (86). While the Pahar contains the fertile Kathmandu Valley and is the traditional political and cultural heart of Nepal, the hot and humid Tarai contains 57% of Nepal's cultivatable land and 48% of its population (86). Despite the economic importance of the Tarai, it has historically had lower sociopolitical standing due to overpowering by the elite of Kathmandu (86).

Figure 1. Map of Nepal (87)

There are three broad ethnic groups in Nepal; the Indo-Nepali living primarily in the Tarai and the lower Pahar, the Tibeto-Nepali living primarily in the Parbat and the indigenous living throughout the Tarai and Kathmandu Valley (86). The Indo-Nepali ethnic group can be further categorized into two ethnic groups, the Pahadi and the Madhesi (86). Although both

groups relocated from India, the Pahadi are generally high caste and represent the elite landowners of society while the Madhesi are generally low caste and represent the peasantry (86). Furthermore, the Pahadi minority dominates the political, social and economic realms of Nepal (88). In addition to the stratification of society by ethnicity, the Hindu caste system continues to play a major role in Nepali life. A person's caste, either Brahmin (priests and scholars), Chhetri (rulers and warriors), Vishya (merchants and traders) or Shudra (artisans and laborers), is unchanging and is a primary determiner of one's status in society (86). In combination with one's ethnicity, a Nepali's caste can be a significant barrier to economic mobility, political participation and social inclusion (89).

Nepal is one of the poorest and least developed countries in the world, receiving a Human Development Index (HDI) score of 0.463 (ranking 157 out of 186 countries) and a Multidimensional Poverty Index (MPI) score of 0.217, one of the highest in south Asia (90). In line with these measures of poverty and development, Nepal has high rates of infant mortality (41.76 deaths/1,000 live births), low life expectancy (66.86 years), and low rates of literacy (73% for men; 48.3% for women) (87). Strikingly, these statistics represent vast improvements in development since the first end of the Nepali monarch system in 1951, due in part to increased access to education, vaccination and improved knowledge of sanitation and basic health care (91).

The majority of Nepali prescribe to a conservative social and family structure in which women are subordinate to men (91). Using the Gender Gap Index, a measure which assesses economic participation and opportunity, educational attainment, health and survival and political empowerment, Nepal has one of the highest gender gaps in the world, scoring better than only Iran and Pakistan in the region of Asia and the Pacific (92). This disadvantage for Nepali women

begins at birth with the traditional preference for sons, with daughters often receiving less food and care than their brothers, especially during times of economic hardship (86,91). While the constitution guarantees the educational rights of women, economic and cultural factors, as well as high dropout rates, limit the enhancement of the status of women (86).

V. Previous Research

Research on the effects of iron and zinc supplementation on child behavior is scarce and use different measures, but the few studies that have been conducted point towards an association between supplementation and positive outcomes. Berglund et al. found that in a sample of low birth weight (LBW) Swedish infants, iron supplemented children were less emotionally reactive and had fewer attention problems at 3.5 years of age (93). Furthermore, Lozoff et al. found that Chilean infants supplemented with iron were more likely to show positive affect, interact socially and check caregivers' reactions (94). Additionally, in a study of Chinese 4 year olds, it was found that children who had chronic iron deficiency anemia (IDA) in infancy had less positive affect and frustration tolerance and more passive behavior, unengaged affect and physical self-soothing than children who were nonanemic during infancy or children who had IDA that was corrected before 24 months of age (95). Finally, in a study of French children with attention deficit hyperactive disorder (ADHD) aged 5 to 8 years, those supplemented with iron had greater decreases in the severity of ADHD symptoms, especially inattention, compared to controls (96).

In a study of LBW Brazilian infants, Ashworth et al. found that infants supplemented with zinc for eight weeks were rated higher than controls on responsiveness, emotional tone, activity level, cooperation and vocalization which, when taken in aggregate, indicated a

significant difference between groups (97). Additionally, Sazawal et al. found that 12 to 23 month old Indian children supplemented with zinc had significantly higher ratings of activity (98). Finally, in a study of Turkish children with ADHD aged 6 to 14 years, those supplemented with zinc had greater reductions in hyperactive, impulsive and impaired socialization symptoms compared to controls (99).

However, not all studies have shown effects of iron or zinc supplementation. In one of the few studies examining the long term effects of iron deficiency on behavior, Lozoff et al. found that Costa Rican children who were severely and chronically iron-deficient in infancy were significantly more likely to have internalizing problem scores than their good-iron-status counterparts at 11 to 14 years of age, despite correction of the iron deficiency in infancy (100). Additionally, Hamadani et al. found no difference in behavior ratings of approach, activity, cooperation, emotional tone or vocalization at both 7 and 13 months between Bangladeshi infants supplemented with zinc versus control (101).

The effects of iron and zinc supplementation in combination on behavior have yet to be explored in depth. Of the studies that examine the effects of iron and zinc supplementation in combination during infancy, most focus on the development of cognitive or motor skills, rather than behavior, and have produced mixed results (102–106). In the only study found that directly examined the effect of iron and zinc supplementation in combination on child behavior, Kordas et al. found that supplementation with iron, zinc or iron and zinc did not decrease parent or teacher ratings of oppositional, hyperactive or cognitive problems in comparison to control in first grade Mexican children exposed to lead (107).

Chapter 2

Methods

I. Study Design

Ethical approval for the study was obtained from institutional review boards at the Johns Hopkins Bloomberg School of Public Health, The Pennsylvania State University, and The Institute of Medicine, Tribhuvan University, Kathmandu, Nepal.

From June 2007 to April 2009, a follow-up study of 7- to 9-year old children who received supplementation with different combinations of micronutrients in utero and during the preschool years (12-35 months of age) was conducted in a Tarai district in Sarlahi, Nepal. The study area was chosen based on location within the district and consisted of 30 Village Development Committees (VDCs). This analysis focuses on the effects of supplementation during the preschool years on behavior in middle childhood. As such, only children born to mothers who received the placebo in the prenatal supplementation trial were eligible for this analysis.

Supplementation during the preschool years was part of a 4-group, double-masked, cluster-randomized, controlled trial (108,109). The groups were placebo, iron (12.5 mg) plus folic acid (50 µg), zinc (10 mg) and iron plus folic acid and zinc, with supplementation given in the form of a pill. Additionally, as part of a national program, all children were eligible to receive a 200,000 IU dose of Vitamin A once every 6 months. Adherence to supplementation was high (75% [interquartile range, 65% - 90%] of all possible doses) but varied by supplementation group.

II. Data Collection

Households with eligible children were invited to participate in the follow-up study. The purpose of the study was explained and oral parental consent and child assent were obtained. At enrollment, demographics, socioeconomic status, morbidity symptoms of both mother and child during the previous 7 and 30 days, dietary intake, iodine content of household salt and history of child's enrollment in school was recorded during an interview at the child's home. An index of socioeconomic status was created by establishing an "asset score", which ranged from 0-11 and consisted of any household ownership of goats, cattle, cart, bicycle, motorcycle, electricity, radio, television, telephone, mobile phone or watches.

At a clinic visit, anthropometry and hemoglobin of the child was assessed. Field workers trained in anthropometry measured weight, height and middle upper arm circumference (MUAC) in triplicate. Weight was measured to the nearest 0.1 kg with a SECA floor scale while height and MUAC were measured to the nearest 0.1 cm using a right-angle head board and insertation tape respectively. From these measurements, weight-for-age, height-for-age and weight-for-height z scores were calculated using international reference standards (110). Hemoglobin was assessed using a fingerstick with a B-Hemoglobin Analyzer (HemoCue, Lake Forest, California). Observed and maternally reported abnormalities in the child's vision, hearing, motor function and behavior were recorded. Additionally, the middle childhood Home Observation for the Measurement of the Environment (111) (HOME) (assessed at the home) and Raven's Colored Progressive Matrices (112) (administered at the clinic) were used to evaluate the quality and quantity of stimulation available to the child in his or her home environment and the mother's reasoning ability, respectively. Additional psychometric testing, including the Universal

Nonverbal Intelligence Test (UNIT) (113) and the Movement Assessment Battery for Children (MABC) (114), were collected and reported in a previous paper (115).

III. Behavior Ratings

Ratings of child behavior were assessed during two home visits using the child ratings portion of the Parent-Child Interaction Rating Scale (PCIRS) (116). Ratings were made on a scale of 1 (low amount of trait) to 5 (high amount of trait) and included 6 dimensions: positive mood (extent to which a child is satisfied, content and pleased with the situation overall), negative mood (extent to which the child cries, fusses, tenses body while crying, throws temper tantrums and otherwise expresses his or her discontent), lively/active (extent to which the child is motorically active), sociability (extent to which the child initiates social interactions with the parent or any other person and responds to their social initiations), attention (extent of the child's sustained involvement with the physical world and objects) and demandingness (extent to which the child makes or appears to make excessive persistent and/or negative bids for attention as characterized by demanding attention above and beyond when basic needs have already been met or initial appropriate requests have already been addressed by the parent). A more in depth explanation of the PCIRS can be found in the Appendix.

Field workers were trained to make appropriate behavior ratings using video examples and a thorough explanation of the scale, and were then certified to collect data when they reached the desired level of proficiency. At each home visit, the trained field worker (BR worker) observed the child in a naturalistic setting for 10 minutes, making notes and ratings on each dimension for an additional 5 minutes. This procedure was completed 6 consecutive times

at each home visit (12 ratings per dimension per child). The average time between visits was 2.12 days.

IV. Statistical Analysis

The four preschool supplementation groups were compared to identify any significant differences on potentially confounding variables using analysis of variance (ANOVA) for continuous variables and Chi square calculations for categorical variables. Variables significantly different between supplementation groups or variables known to be associated with outcomes were controlled for in the adjusted analyses. The 12 behavioral ratings for each child were averaged within each dimension to establish a single score per dimension.
Two-sided testing was employed and data were analyzed as intent to treat.

Since behavior ratings were made using a Likert-type scale, logistic regression was employed to examine the associations between behavior rating scores and child supplementation group. Each of the three non-placebo supplementation groups were compared to the placebo group. Additionally, the supplementation courses containing any iron plus folic acid or any zinc were compared to the supplementation courses containing no iron plus folic acid or no zinc, respectively.

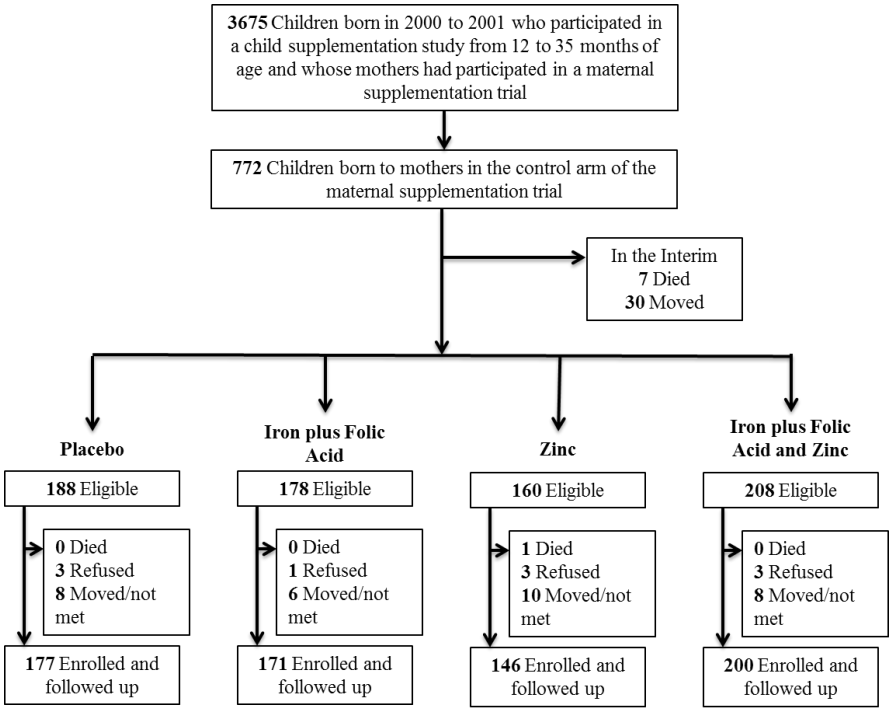
Adjusted analyses controlled for child age, adherence to supplementation, dark green leafy vegetable intake, tea intake, hemoglobin, maternal literacy and HOME score since differences between groups were found for these variables. Additionally, adjusted analyses controlled for child sex, VDC and BR worker because of potential associations between sex and behavior and potential design effects. Data were analyzed using SAS software, version 9.3 (SAS Institute Inc, Cary, North Carolina).

Chapter 3

Demographics and Sample Characteristics

Children in this study were drawn from the group of children (n=3675) whose mothers took part in a micronutrient supplementation trial during pregnancy. Children who were eligible for this analysis had mothers who were in the control group during in utero supplementation (n=772). After accounting for those children who died (n=7) or moved out of the study area (n=30) during the preschool supplementation trial, 735 children were eligible for the preschool supplementation analyses. Between the conclusion of the preschool supplementation trial and the beginning of the follow-up study at 7 to 9 years, 1 child died, 33 children could not be located and 7 children refused to participate, bringing final total enrollment to 694 children. The number of participants by treatment group ranged from 146 to 200. The loss to follow-up rate was approximately 5% (**Figure 2**).

Figure 2. Study participation and follow-up by treatment group



At follow-up, the mean (SD) age of children was 8.4 (0.7) years and differed (p=0.0003) between treatment groups (**Table 1**). Females comprised 53% of the sample and the majority of children (76%) had started school. Diet in the past 7 days showed that the majority of children consumed milk and dairy products (73%) and dark green leafy vegetables (71%). In contrast, a minority of children consumed citrus fruits (40%) and yellow fruits and vegetables (39%). At the time of follow-up, morbidity across treatment groups was very low; 1% reported lower respiratory tract infections while 4% reported diarrhea or dysentery. Children in the study were wasted and stunted as evidenced by their average weight for age z-score (SD) of -2.06 (0.91) and height for age z-score (SD) of -1.91 (0.89), respectively. The prevalence of anemia in the sample (35%) was lower than the prevalence of anemia in developing countries (12). Additionally, there

were very low levels of reported abnormalities in vision (0.2%), hearing (0.7%), motor function (2%) and behavior (5%).

Literacy was extremely low among the mothers (16%) and differed (p=0.004) between treatment groups (**Table 1**). Additionally, the vast majority of mothers (81%) had no formal education. Markers of socioeconomic status indicated a low level of development in the sample; 23% of the households lived in a home with stone or cement walls while only 5% of households had a cement roof. Furthermore, the mean asset score (SD) was only 4.5 (2.3) out of a possible 11.

Statistically significant differences between treatment groups were observed with respect to child age, supplementation adherence, intakes of dark green leafy vegetables and tea in the past 7 days, hemoglobin levels, maternal literacy and HOME inventory score.

Table 1. Baseline characteristics of the enrolled children, their mothers, and households by child supplementation group in Sarlahi, Nepal (2007-2009)

Characteristic	Control (n=177)	IFA (n=171)	Zn (n=146)	IFAZn (n=200)	P Value
Child Characteristics					
Age, mean (SD), y	8.3 (0.65)	8.3 (0.76)	8.4 (0.63)	8.5 (0.61)	0.0003
Male, n (%)	83 (46.9)	88 (51.5)	65 (44.5)	90 (45.0)	0.56
Primary caretaker mother, n (%)	170 (96.1)	167 (97.7)	142 (97.3)	189 (94.5)	0.38
Ever sent to school, n (%)	131 (74.0)	124 (72.5)	117 (80.1)	155 (77.5)	0.37
Adherence, mean (SD), %	75.2 (20.6)	73.4 (23.3)	79.7 (18.0)	72.0 (21.5)	0.008
Diet in the past 7 d (any intake), n (%)					
Milk and dairy products	138 (78.0)	128 (74.9)	110 (75.3)	133 (66.5)	0.07
Meat, chicken, or fish	107 (60.5)	97 (56.7)	80 (54.8)	116 (58.0)	0.77
Dark green leafy vegetables	113 (63.8)	119 (69.6)	103 (70.6)	157 (78.5)	0.02
Citrus fruits	64 (36.2)	78 (45.6)	58 (39.7)	75 (37.5)	0.28
Yellow fruits and vegetables	75 (42.4)	60 (35.1)	51 (34.9)	88 (44.0)	0.17
Tea	61 (34.5)	84 (49.1)	65 (44.5)	89 (44.5)	0.04
Morbidity in the past 7 d, n (%)					
Lower respiratory tract infection	1 (0.6)	3 (1.8)	1 (0.7)	3 (1.5)	0.67
Diarrhea/dysentery	2 (1.1)	10 (5.9)	8 (5.5)	7 (3.5)	0.09
Child anthropometry and anemia					
Weight for age z score, mean (SD)	-2.09 (0.91)	-2.07 (0.93)	-1.99 (0.87)	-2.08 (0.94)	0.8
Height for age z score, mean (SD)	-1.89 (0.90)	-1.85 (0.91)	-1.97 (0.84)	-1.93 (0.87)	0.65
BMI z score, mean (SD)	-1.25 (0.86)	-1.26 (0.84)	-1.03 (0.77)	-1.20 (0.86)	0.06
MUAC, mean (SD), cm	15.6 (1.2)	15.7 (1.4)	15.8 (1.4)	15.8 (1.3)	0.40
Hemoglobin, mean (SD), g/dL	12.2 (1.0)	12.2 (1.1)	12.6 (1.0)	12.5 (1.0)	0.0003
Anemia (hemoglobin) <12 g/dL, n %	72 (41.4)	62 (36.9)	40 (28.0)	66 (33.3)	0.08
Any reported abnormality, n (%)					
Vision	0	0	0	2 (1.0)	0.18
Hearing	1 (0.6)	1 (0.6)	1 (0.7)	2 (1.0)	0.96
Motor function	2 (1.1)	4 (2.4)	3 (2.1)	2 (1.0)	0.67
Behavior	9 (5.1)	7 (4.2)	6 (4.2)	14 (7.0)	0.57
Maternal characteristics					
Age at enrollment, mean (SD), y	30.7 (5.4)	31.8 (6.0)	31.8 (6.1)	32.0 (6.1)	0.14
Literacy, n (%)	21 (12.0)	43 (25.8)	38 (26.2)	42 (21.0)	0.004
Raven score, mean (SD)	15.7 (4.6)	16.4 (5.2)	16.9 (5.5)	16.9 (4.9)	0.10
Maternal edu level, yrs of schooling, n (%)					0.02
None	156 (89.1)	128 (76.7)	109 (75.2)	166 (83.0)	
1-5	9 (5.1)	12 (7.2)	11 (7.6)	12 (6.0)	
>6	10 (5.7)	27 (16.2)	25 (17.2)	22 (11.0)	
Adult education in past 3 y, n (%)	14 (8.1)	13 (7.7)	15 (10.6)	15 (7.9)	0.78
Household characteristics					
Household salt iodine level >= 15 ppm, n (%)	115 (65.3)	110 (65.5)	106 (72.6)	145 (72.9)	0.23
Walls made with stone or cement, n (%)	50 (28.3)	33 (19.3)	29 (19.9)	45 (22.5)	0.18
Cement Roof, n (%)	8 (4.5)	10 (5.9)	3 (2.1)	11 (5.5)	0.37
Asset score, mean (SD)	4.5 (2.3)	4.5 (2.2)	4.8 (2.2)	4.4 (2.3)	0.30
HOME score, mean (SD)	23.0 (5.7)	25.6 (6.2)	24.6 (5.7)	24.2 (6.3)	0.001

Chapter 4

Results

Mean behavioral ratings by treatment group are presented in **Table 2**. Logistic regression revealed that children supplemented with iron plus folic acid were 2.90 times more likely to receive higher ratings of demandingness than controls (p=0.005) while children supplemented with iron plus folic acid and zinc were 1.43 times more likely to receive higher ratings of sociability than controls (p=0.048) (**Table 3**). Additionally, results that approached but did not reach significance included higher ratings of attention (1.39 times) in children supplemented with iron plus folic acid versus controls (p=0.08), higher ratings of sociability (1.42 times) in children supplemented with zinc versus controls (p=0.07) and higher ratings of positive mood (1.38 times) in children supplemented with iron plus folic acid and zinc versus controls (p=0.07) (**Table 3**). All other odds ratios between supplementation groups and controls were found to be statistically insignificant.

Table 2. Mean (SD) behavior ratings by child supplementation group assessed among children aged 7 to 9 years in Sarlahi, Nepal (2007-2009)

Dimension	PL (n=177)	IFA (n=171)	Zn (n=146)	IFAZn (n=200)
Positive Mood	2.58 (0.41)	2.59 (0.40)	2.62 (0.39)	2.66 (0.39)
Negative Mood	1.05 (0.12)	1.06 (0.11)	1.06 (0.09)	1.06 (0.09)
Lively/Active	2.61 (0.35)	2.59 (0.35)	2.63 (0.34)	2.63 (0.36)
Sociability	2.60 (0.35)	2.54 (0.45)	2.66 (0.41)	2.68 (0.42)
Attention	2.62 (0.44)	2.71 (0.36)	2.67 (0.40)	2.68 (0.31)
Demandingness	1.01 (0.06)	1.03 (0.07)	1.02 (0.14)	1.01 (0.04)

Abbreviations: PL, placebo; IFA, iron plus folic acid; Zn, zinc; IFAZn, iron plus folic acid and zinc.

Table 3. Odds of supplementation groups receiving higher behavior ratings than controls among children aged 7 to 9 years in Sarlahi, Nepal (2007-2009)

	IFA (n=171)		Zn (n=146)		IFAZn (n=200)	
Dimension	**Odds Ratio (95% CI)**	**P Value**	**Odds Ratio (95% CI)**	**P Value**	**Odds Ratio (95% CI)**	**P Value**
Positive Mood	1.00 (0.70 to 1.44)	0.99	1.12 (0.76 to 1.64)	0.57	1.38 (0.97 to 1.97)	0.07
Negative Mood	1.16 (0.74 to 1.82)	0.51	0.96 (0.60 to 1.55)	0.87	1.25 (0.82 to 1.92)	0.30
Lively/Active	0.94 (0.65 to 1.35)	0.72	1.10 (0.75 to 1.62)	0.61	1.06 (0.75 to 1.51)	0.73
Sociability	0.77 (0.54 to 1.12)	0.17	1.42 (0.97 to 2.08)	0.07	1.43 (1.004 to 2.04)	0.048
Attention	1.39 (0.96 to 2.01)	0.08	1.20 (0.82 to 1.76)	0.34	1.32 (0.93 to 1.88)	0.12
Demandingness	2.90 (1.39 to 6.05)	0.005	1.36 (0.58 to 3.18)	0.48	1.50 (0.69 to 3.26)	0.31

Abbreviations: IFA, iron plus folic acid; Zn, zinc; IFAZn, iron plus folic acid and zinc; CI, confidence interval.

After adjustment for confounders, children supplemented with iron plus folic acid remained more likely to receive higher ratings of demandingness than controls (p=0.007) while the finding of higher ratings of sociability in those supplemented with iron plus folic acid and zinc was no longer significant (**Table 4**). All other odds ratios between supplementation groups and controls were found to be statistically insignificant.

Table 4. Odds of supplementation groups receiving higher behavior ratings than controls, adjusted for confounders*, among children aged 7 to 9 years in Sarlahi, Nepal (2007-2009)

	IFA (n=162)		Zn (n=142)		IFAZn (n=198)	
Dimension	**Odds Ratio (95% CI)**	**P Value**	**Odds Ratio (95% CI)**	**P Value**	**Odds Ratio (95% CI)**	**P Value**
Positive Mood	1.01 (0.68 to 1.50)	0.95	0.91 (0.60 to 1.37)	0.64	1.26 (0.87 to 1.84)	0.22
Negative Mood	1.30 (0.79 to 2.14)	0.30	1.07 (0.63 to 1.81)	0.80	1.48 (0.93 to 2.37)	0.10
Lively/Active	0.92 (0.62 to 1.37)	0.69	0.95 (0.63 to 1.44)	0.81	0.98 (0.67 to 1.42)	0.91
Sociability	0.81 (0.55 to 1.20)	0.30	1.34 (0.89 to 2.04)	0.16	1.29 (0.88 to 1.88)	0.19
Attention	1.30 (0.88 to 1.92)	0.19	1.13 (0.75 to 1.71)	0.56	1.13 (0.78 to 1.65)	0.51
Demandingness	3.13 (1.37 to 7.13)	0.007	1.53 (0.57 to 4.11)	0.39	1.98 (0.82 to 4.80)	0.13

Abbreviations: IFA, iron plus folic acid; Zn, zinc; IFAZn, iron plus folic acid and zinc; CI, confidence interval.

*Confounders adjusted for: child age, child sex, adherence to supplementation, dark green leafy vegetable intake, tea intake, hemoglobin, maternal literacy, HOME score, VDC, BR field worker.

Additionally, logistic regression of those receiving any iron plus folic acid versus not and those receiving any zinc versus not showed that those receiving any iron plus folic acid were 1.81 times more likely to receive higher ratings of demandingness than those receiving none (p=0.03) and those receiving any zinc were 1.58 times more likely to receive higher ratings of sociability than those receiving none (p=0.0005) (**Table 5**). Also, results that approached but did not reach significance included those supplemented with any iron plus folic acid were 1.25 times more likely to receive higher ratings of attention than controls (p=0.09), while those supplemented with any zinc were 1.26 times more likely to receive higher ratings of positive mood than controls (p=0.08) (**Table 5**). All other odds ratios between iron plus folic acid or zinc and no iron plus folic acid or zinc were found to be statistically insignificant.

Table 5. Odds of any iron plus folic acid or zinc supplementation receiving higher behavior ratings than no iron plus folic acid or zinc supplementation among children aged 7 to 9 years in Sarlahi, Nepal (2007-2009)

	Any IFA (n=371) vs No IFA (n=323)		Any Zn (n=346) vs No Zn (n=348)	
Dimension	**Odds Ratio (95% CI)**	**P Value**	**Odds Ratio (95% CI)**	**P Value**
Positive Mood	1.14 (0.88 to 1.48)	0.32	1.26 (0.98 to 1.64)	0.08
Negative Mood	1.23 (0.90 to 1.69)	0.19	1.04 (0.76 to 1.43)	0.80
Lively/Active	0.96 (0.74 to 1.24)	0.74	1.12 (0.87 to 1.46)	0.38
Sociability	0.93 (0.72 to 1.21)	0.60	1.58 (1.22 to 2.06)	0.0005
Attention	1.25 (0.96 to 1.62)	0.09	1.06 (0.82 to 1.37)	0.66
Demandingness	1.81 (1.07 to 3.07)	0.03	0.77 (0.46 to 1.27)	0.30

Abbreviations: IFA, iron plus folic acid; Zn, zinc; IFAZn, iron plus folic acid and zinc; CI, confidence interval

When the model was adjusted for confounders, those receiving any iron plus folic acid were 2.10 times more likely to receive higher ratings of demandingness than those receiving none (p=0.01) and those receiving any zinc were 1.49 times more likely to receive higher ratings

of sociability than those receiving none (p=0.004) (**Table 6**). While all other odds ratios were determined to be statistically insignificant, one approached significance: those supplemented with any iron plus folic acid were 1.34 times more likely to receive higher ratings of negative mood than those receiving none (p=0.09) (**Table 6**).

Table 6. Odds of any iron plus folic acid or zinc supplementation receiving higher behavior ratings than no iron plus folic acid or no zinc supplementation, adjusted for confounders*, among children aged 7 to 9 years in Sarlahi, Nepal (2007-2009)

	Any IFA (n=360) vs No IFA (n=312)		Any Zn (n=340) vs No Zn (n=332)	
Dimension	**Odds Ratio (95% CI)**	**P Value**	**Odds Ratio (95% CI)**	**P Value**
Positive Mood	1.10 (0.84 to 1.44)	0.48	1.16 (0.89 to 1.53)	0.28
Negative Mood	1.34 (0.96 to 1.86)	0.09	1.09 (0.78 to 1.53)	0.61
Lively/Active	0.92 (0.71 to 1.21)	0.55	1.07 (0.81 to 1.40)	0.64
Sociability	0.86 (0.66 to 1.12)	0.26	1.49 (1.13 to 1.96)	0.004
Attention	1.15 (0.88 to 1.50)	0.31	0.95 (0.73 to 1.25)	0.73
Demandingness	2.10 (1.19 to 3.71)	0.01	0.76 (0.44 to 1.31)	0.32

Abbreviations: IFA, iron plus folic acid; Zn, zinc; IFAZn, iron plus folic acid and zinc; CI, confidence interval.

*Confounders adjusted for: child age, child sex, adherence to supplementation, dark green leafy vegetable intake, tea intake, hemoglobin, maternal literacy, HOME score, VDC, BR field worker.

All analyses were rerun without children with any reported abnormalities in vision, hearing, motor function and behavior (n=47) and all adjusted results remained the same (data not shown).

Chapter 5

Discussion

I. Effects of Supplementation

In this sample of 7 to 9 year old Nepali children, children supplemented with iron plus folic acid were 2.90 times more likely to receive higher demandingness scores than controls and children supplemented with iron plus folic acid and zinc were 1.43 times more likely to receive higher sociability scores than controls. Additionally, children who received any iron plus folic acid supplementation were 1.81 times more likely to receive higher demandingness scores than children who received none and children who received any zinc supplementation were 1.58 times more likely to receive higher sociability scores than children who received none.

After controlling for confounders, children supplemented with iron plus folic acid were 3.13 times more likely to receive higher demandingness scores than controls. Additionally, children who received any iron plus folic acid supplementation were 2.10 times more likely to receive higher demandingness scores than children who received none and children who received any zinc supplementation were 1.49 times more likely to receive higher sociability scores than children who received none. Other aspects of behavior did not differ significantly between supplementation groups and controls, and no other odds ratios were significant.

The finding of increased sociability with zinc supplementation is in accordance with a previous study (1). In this study, Bilici et al. found that in Turkish children with ADHD aged 6 to 14 years, those supplemented with zinc had greater reductions in impaired socialization symptoms compared to controls, as measured by the Attention Deficit Hyperactivity Disorder

Scale (ADHDS) (1). Significant differences in improvement between groups on the specific ADHDS Impaired Socialization Subscale were observed after the 12th week of supplementation (1). Additionally, rates of recovery, as determined by subscale cutoff values, were significantly higher in the zinc treated group than the placebo group (1). While there is an overlap in age at testing between the Bilici et al. study and our own, age at supplementation differed greatly with our sample being supplemented during the preschool years (12 – 35 months) and Bilici et al.'s being supplemented for only 12 weeks prior to assessment.

Due to the unique and specific nature of the study dimension "demandingness", there appears to be no literature that is specific to the link between demandingness and iron supplementation.

II. Connecting Sociability and Zinc to Neurobiology

While the exact mechanism relating zinc to sociability has yet to be determined, neurobiology provides many possible routes of impact. One way in which zinc could affect sociability is through serotonin, as was suggested by Bilici et al. (1). In this mechanism, zinc affects the production of serotonin through its role in a substrate for pyridoxal kinase, the enzyme responsible for converting vitamin B6 to its active form pyridoxal-5'-phosphate (PLP) (2). PLP in turn is a necessary cofactor for the conversion of tryptophan to serotonin (3). Another way in which zinc could affect sociability is through its connection to oxytocin. Oxytocin, a neuropeptide which has been linked consistently with sociability, is the central component of a neuroendocrine system which coordinates social behaviors, decreases withdrawal characteristic and increases stress tolerance (4,5). The action of oxytocin is transduced by its receptor (Oxtr), which is found throughout the brain (5). Zinc facilitates the binding of oxytocin to Oxtr through

extensive conformational changes induced by zinc, thereby influencing the expression of oxytocin throughout the brain (6). If an individual were to experience zinc deficiency their sociability may then be negatively impacted. However, these mechanisms of action would be more plausible if behavior were measured and differences were detected immediately after the supplementation course had finished, not years after as was seen in this study.

Finally, one mechanism of action that could relate early zinc deficiency to later deficits in sociability is through the zinc deficiency induced dysfunction of extracellular signal-regulated kinases (ERK 1/2) (7). Even at a marginal level, zinc deficiency has been shown to impair activation of ERK 1/2, whose signaling is nearly ubiquitous in neural development, through hypophosphorylation (7). A study of ERK2 conditional knock-out (CKO) mice showed that normal social behavior was impaired in the ERK2 CKO mice compared to controls (8). Additionally, similar deficits in social behavior that continue into adulthood have been observed in other animals exposed to zinc deficiency (9). Therefore it is possible that zinc deficiency in early childhood could cause irreversible alterations in ERK2 function causing reduced sociability in later childhood. Although this is plausible, we do not have data on zinc status in early childhood and, therefore, cannot test this hypothesis.

III. Impact of Sociability

The benefits of sociability have been demonstrated across a variety of domains. Firstly, sociability has been shown to be an important factor related to school success. In a study of Chinese children, sociability-competence was significantly, positively correlated with academic achievement in both concurrent and cross lagged analyses (10). Additionally, in a study of third and fourth grade American children, social skills were found to be positively predictive of

concurrent and future academic functioning (11). Finally, in a study of American children in the third grade, academic achievement was positively correlated with both peer acceptance and engagement in positive interactions with peers (12).

Additionally, child sociability is related to several other positive outcomes, both during childhood and early adulthood. In a study of Chinese children, sociability in sixth grade was positively correlated with peer preference, social standing, and self-perceptions of competence and self-worth and negatively correlated with loneliness and depression two years later (13). A later follow up of these same children found that sociability in sixth grade was positively correlated with family interaction, peer integration and self-perceptions of competence and self-worth and negatively correlated with loneliness and internalizing problems seven years later (14). Both studies demonstrate the significant relationship between sociability and socioemotional adjustment.

Conversely, social withdrawal, or the tendency to isolate oneself from peers, in children has been shown to relate to a variety of negative consequences (15). For example, in a study of Australian children, social withdrawal at age 5 was predictive of social impairment at age 15, which in turn was predictive of depression at age 20 (16). Additionally, studies have demonstrated that social withdrawal during adolescence is associated with anxiety, depression and low self-worth (17,18).

However, because of the lack of empirical studies on the relationship between sociability and school success and adjustment in developing and South Asian countries it is difficult to extrapolate these findings to Sarlahi, Nepal. Studies in Nepal which examine sociability and assess school achievement, such as performance on examinations or culturally appropriate

standardized tests, and markers of success and adjustment at multiple time points through a child's life would help determine the nature or existence of this relationship in Nepal.

IV. Demandingness

Unlike zinc and sociability, it is more challenging to identify biologically plausible mechanisms linking demandingness to iron and neurotransmitters due to the difficulty of identifying a standard definition of demandingness. Therefore, the finding that children supplemented with iron plus folic acid were more likely to be scored higher on the dimension of demandingness compared to controls could be interpreted in several ways. Higher demandingness could be indicative of a child's increased energy from iron supplementation since iron deficiency and iron deficiency anemia are associated with decreased energy (19). If a child has more energy they may be able to more easily demand attention. Additionally, studies have shown that iron deficient anemic infants are more wary and hesitant than infants with normal iron status (20). Perhaps decreases in these types of behavior were interpreted as demandingness, leading to the greater likelihood of higher demandingness scores in children supplemented with iron plus folic acid. However, it is also possible that the increased demandingness seen is simply indicative that the children are "fussier", engaging in whining and complaining, and more intrusive. In both cases, demandingness, as it is defined in the rating criteria, could be interpreted as disruptive by caretakers and could engender negative feelings toward the child.

Despite the significance of the finding, the levels of demandingness across all supplementation groups were low. A potential explanation of the low levels of demandingness seen across supplementation groups could be that in the presence of an authority figure (field worker) the children felt like they could not "behave badly". Nepali culture could also explain

why very low levels of demandingness were seen in this sample. Firstly, Nepal is a collectivist society in which the group is generally valued more than the individual and ideals such as group harmony, conformity and deference to authority are emphasized (21,22). Furthermore, collectivism is especially emphasized in rural, agrarian communities in which group harmony and interdependence are critically important in sustaining the family unit's success and livelihood (23). Therefore, it is feasible that by the age of eight, rural Nepali children would have internalized these values and learned that being "demanding" would disrupt harmony within the household and would be extremely socially inappropriate. For example, in a cross-cultural study of Nepali and United States children, Nepali children are significantly more likely to report that they accept negative and difficult situations than their United States counterparts (22). In light of this information, higher scores of demandingness seen in those children supplemented with iron plus folic acid could be because of decreased social inhibition.

V. Strengths and Weaknesses

One strength of this study was that there was a high follow up rate (95%) from the original preschool trial, decreasing the likelihood of bias. A second strength was that the sample size was large which allowed our statistical analyses to have sufficient power to detect subtle differences between groups and, yet again, decreased the likelihood of bias. Thirdly, the study sample was part of a randomized, double-blind, controlled trial, increasing the reliability of the results and decreasing the likelihood of bias. Fourthly, conducting follow up assessments and observations at seven to nine years allowed for greater ease of testing and time for behavior to more fully develop. Finally, this is the only study we are aware of that has observed the effects of

early life micronutrient supplementation on child behavior at seven to nine years, making this a unique look into the long term effects of supplementation.

A limitation of this study is that there has been limited use of the PCIRS in scientific literature. While the scale has in depth descriptions of each behavioral dimension and how they should be scored, there are no established normal values and no studies in the context of nutrition to compare results. Another limitation of this study is that the children were not followed continuously between the preschool supplementation trial and the follow-up study. This makes it possible that, between the two time points, there were variables that could have affected outcomes but could not be accounted for. However, because of the use of randomization the probability of one supplementation group being affected by unknown variables and the others not, is very low. Additionally, while the large sample size can be interpreted as a strength, it can also be interpreted as a limitation since the large power to detect subtle differences can lead to finding statistically significant differences that are not clinically significant. Finally, although supplementation began early in the children's lives, no iron or zinc supplementation occurred during infancy or in utero, a period of especially rapid brain development (24). It is possible that supplementation during infancy would have shown a greater effect on childhood behavior.

VI. Conclusion

In conclusion, this follow up on preschool micronutrient supplementation found that iron plus folic acid supplementation was associated with higher scores in demandingness and zinc supplementation was associated with higher scores in sociability in children 7 to 9 years of age in this rural Nepali population. These findings add to the growing literature of micronutrient supplementation on behavior and suggest that more research is warranted to elucidate the

specific effects and behavioral domains associated with iron and zinc supplementation. Research which explores the effects of iron and zinc supplementation on child behavior, as rated by the PCIRS, would be of particular interest and a natural next step. Additionally, studies which utilize other or multiple measures of behavior would be especially useful in the determination of effects of iron and zinc supplementation in preschool children. Finally, further studies such as longitudinal research assessing school achievement, school completion and professional success would be required to determine the functional consequences of such behavioral differences during adolescence and adulthood.

Appendix

Behavioral Rating Scale: Child Ratings

Positive Mood

This scale assesses the extent to which the child is satisfied, content, and pleased with the situation overall. Ratings should attempt to balance both the intensity of the child's positive affect, and the relative amount of time positive behavior is displayed. Measures of child positive affect include smiles, laughs, physical affection, (e.g. hugs, snuggling with parent, kisses), and positive comments and tone of voice. A lack of positive affect does not mean a negative mood.

Positivity Ratings

1 = Unhappy or no affect – no signs of positive mood. (Remember: lack of positive affect does not mean a negative mood)

2 = Minimally positive – fleeting smiles or positive vocalizations; lukewarm, explicit positive affect shown only occasionally and with very low levels of intensity; positive affect more absent than present.

3 = Somewhat positive – generally content, explicit positive affect is more frequent and intense than a rating of 2, but certainly not as much as would warrant a rating of 4. This is the "average happy child."

4 = Moderately happy, laughing, smiling, talking, and having a good time for most of the observation, not simply content

5 = Gleeful, joyful, having a great time during virtually all of the observation and for the remainder of the time, obviously content and/or happy.

Negative Mood

This scale assesses the extent to which the child cries, fusses, tenses body while crying, throws "temper tantrums," and otherwise expresses his/her discontent. Bear in mind the frequency and intensity of negatively affective behavior when making this rating.

Negativity Ratings

1 = Not at all discontented – no signs of negative affect.

2 = Occasional mild distress – minimal level of fussiness, easily soothed, may be upset for brief periods of time. Mild negative affect.

3 = Moderately distressed – upset and negatively affective for under half of the epoch; increased levels of intensity during episodes of negative affect; child is soothable.

4 = Noticeably distressed – displays negative affect for half or more than half of the epoch; periods of negative affect are characterized by increased intensity and frequency from a rating of 3; child needs persistent parental soothing to calm, and thus can show some periods of calmness.

5 = Constantly negative – crying or angry most of the observation; much stronger and more explicit expressions of anger or distress, which could include, but is not limited to, more screaming, hostile verbalizations, or intense body language; resistant to parental attempts to soothe; rarely to never content or positive.

Lively/Active

The extent to which the child is motorically active during the observation. This includes: the speed of motor activity (moving fast, whether walking, crawling, squirming, or running), the frequency of motor activity (spending a lot of time in high-energy activities), the amplitude or

intensity of motor activity (jumping high, bouncing vigorously), the duration of motor activity (persisting in energetic activity longer than other children), the preference for motor activity (choosing high-energy games, activities), and a negative reaction to enforced non-activity (reacting with restlessness). Be aware that these ratings are context-sensitive within each episode, different activities pull for a different level of motor activity (e.g., dinner table vs. playing outside). Structured activities (e.g. board game) may look different.

Activity Ratings

1 = Not at all active/lively – child typically stays in one place not moving arms, legs, hands or feet; sits quietly.

2 = Minimally active/lively – child exhibits some active movements but periods of non-movement exceed those of movement.

3 = Average – about average in activity, sometimes active, sometimes inactive; difficult to characterize.

4 = Moderately active – child is predominantly active but has a few periods of inactivity. Periods of movement exceed those of non-movement.

5 = Highly active – child is constantly moving some body part, something is moving at all times; Child prefers active games and activities to non-active ones.

Sociability

The degree to which the child initiates social interactions with the parent or any other person and responds to their social initiations. Frequency, intensity, and variety of initiations and responses are considered. Behaviors must be construed to have social interactive intent. A child

who spends most of the time demanding to be held, although this is technically initiating, will not receive a high sociability score. The following behaviors are considered initiating behaviors: looking at parent (when preceded by a parent behavior eliciting looking), looking and/or commenting, looking at parent and pointing, offering parent an object (spontaneously, without prompting), approaching parent, and inviting parent to participate in play or activity.

Sociability Ratings

1 = Not sociable – no social initiations by child and no responsiveness to other's overtures. Oblivious to social environment; engaged in own activity, including non-activity, for most of observation.

2 = Minimally sociable – social initiations by child are rare or ambiguous. Child largely uninterested in other people. Child is usually engaged in own activity. May be brief, low intensity responses (e.g., a look) occasionally, but these usually occur in response to persistence on part of the parent.

3 = Moderately sociable – child may initiate (e.g., looks, comments), but less frequently than a 4 or 5; child does typically respond to other people; initiations lack intensity and responses to others are lacking in spontaneity.

4 = Frequently sociable – child initiates frequently, with more persistence or intensity, and/or demonstrates more spontaneity than a rating of 3, responses to others' bids are also often timely, appropriate, and contingent.

5 = Highly sociable – child initiates frequently, using a variety of signals. Multiple instances of child directing social situations, positive signals to other people, and appropriately responding to

their initiations. Child clearly initiates and responds to developmentally appropriate social bids, such as smiling, waving, commenting, looking, pointing, and approaching.

Sustained Attention

This scale assesses the child's sustained involvement with the physical world and objects. The involved child initiates contact with objects and sustains it. If objects are within reach, the child seeks the toys out, looks at them, touches them, explores them; and may comment on them. He/she seems interested in the objects and what can be done with them. Sustained attention or involvement can also include attention to the parent. Enjoyment and interest are separate, but related constructs to higher levels of sustained attention. Therefore, enjoyment/interest and sustained attention do not need to co-occur, but quite often will and this can be used for discriminating judgments. The uninvolved child may appear apathetic, bored, distracted, or distressed. Be aware that these ratings are both context-sensitive and age-dependent.

Attention Ratings

1 = Not characteristic – child does not display sustained attention. Instead, she/he moves from object to object in a non-systematic manner, without seeming to focus on what the objects have to offer.

2 = Minimally characteristic – child is minimally involved with objects and sustains attention for only brief periods of time, or displays only one incident of any marked attention.

3 = Somewhat characteristic – child maintains involvement for relatively longer periods of time than a rating of 2, but does experience some periods of distraction.

4 = Moderately characteristic – child maintains more time involved in interactions with things and seems to enjoy them. Child is more involved than not.

5 = Highly characteristic – the child is clearly involved, interested, and/or focused for most of the time. Child is interested, and/or focused for most of the time. When child is playing with objects, he/she is interested in playing with objects; when eating, he/she is interested in eating.

Demandingness

This scale measures the extent to which the child makes, or appears to make, excessive persistent and/or negative bids for attention, as characterized by demanding attention above and beyond when basic needs have already been met or initial appropriate requests have already been addressed by the parent. This code is different from negative mood, as the child's actions are attention seeking rather than an expression of needs. Routine requests and bids that are neither particularly frequent, intense, or aversive are only considered in this rating when the child also engages in intense, aversive demandingness. Child is not simply discontent. The child's actions may be perceived as negatively demanding or intrusive by the observer or the parent, but not by the child. A very demanding child allows very little behavior by the parent(s) to take place that does not involve him/her in some way.

Behaviors seen as especially demanding are those interrupting ongoing activities of parents (e.g., spousal interactions, reading, cooking, talking on the phone) and directing parent's behavior (i.e., telling parent what to do). Demanding behaviors are generally socially undesirable and inappropriate. They may be characterized as rude or inconsiderate. A child will receive a higher rating of demandingness for the quality (more inappropriate bids would "bump" a score up); persistence on the part of the child, continuing with actions even after parent has tried to

appropriately redirect the child, or after child has been given the feedback that they need to stop; also look at the intensity and frequency. This code is context-dependent – have the child's basic needs already been met?

Demandingness Ratings

1 = Not at all demanding – no evidence of demandingness; for the most part the child displays only appropriate bids for attention. Communicates needs in a prosocial manner.

2 = Minimally demanding – an occasional whine or cry, but will stop after parent's redirection or prompting. Intensity and frequency remain low.

3 = Moderately demanding – intensity and frequency are increased. Child uses verbal commands or directives with another or cries/whines more persistently, however may comply after more intense reprimands by parent.

4 = Very demanding – child is very persistent and intense. Child continues to make demands even after redirecting or reprimands by the parent(s), or being ignored by the parent(s). child is more often demanding than not, but frequency and intensity are lower than a 5.

5 = Predominately demanding – continuous crying or tantruming for the sole purpose of getting what he/she wants, or getting parent's attention. The child spends most of the 10-minute period crying or demanding. The child persists and/or the behavior is highly intensive and aversive.

References

1. Gropper SS, Smith JL, Groff JL. Advanced Nutrition and Human Metabolism. 5th ed. Canada: Wadsworth, Cengage Learning; 2009.

2. Tandara L, Salamunic I. Iron metabolism: current facts and future directions. Biochem Med. 2012;22(3):311–28.

3. Beard JL, Dawson H, Pinero DJ. Iron metabolism: A comprehensive review. Nutr Rev. 1996;54(10):295–317.

4. Zimmermann MB, Hurrell RF. Nutritional iron deficiency. Lancet. 2007;370(9586):511–20.

5. Grantham-McGregor S, Cheung YB, Cueto S, Glewwe P, Richter L, Strupp B. Developmental potential in the first 5 years for children in developing countries. Lancet. 2007;369:60–70.

6. Ramakrishnan U. Prevalence of micronutrient malnutrition worldwide. Nutr Rev. 2002;60(5):S46–52.

7. Beard JL, Dawson HD. Iron. In: O'Dell B, Sunde RA, editors. Handb Nutr Essent Miner Elem. New: Marcel Dekker, Inc; 1997.

8. Bjorn-Rasmussen E, Hallberg L, Isaksson B, Arvidsson B. Food iron absorption in man. Application of the two-pool extrinsic tag method to measure heme and non-heme iron absorption. J Clin Invest. 1974;53:247–56.

9. Teucher B, Olivares M, Cori H. Enhancers of iron absorption: Ascorbic acid and other organic acids. Int J Vitam Nutr Res. 2004;74(6):403–19.

10. Monsen E. Iron nutrition and absorption: Dietary factors which impact iron bioavailability. J Am Diet Assoc. 1988;88:786–90.

11. Kumar V, Sinha AK, Makkar HPS, Becker K. Dietary roles of phytate and phytase in human nutrition: A review. Food Chem. 2010;120:945–59.

12. McLean E, Cogswell M, Egli I, Wojdyla D, de Benoist B. Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System, 1993-2005. Public Heal Nutr. 2009;12:444–54.

13. Stoltzfus RJ. Iron deficiency: global prevalence and consequences. Food Nutr Bull. 2003 Dec;24(4 Suppl):S99–103.

14. Lukowski AF, Koss M, Burden MJ, Jonides J, Nelson CA, Kaciroti N, et al. Iron deficiency in infancy and neurocognitive functioning at 19 years: evidence of long-term deficits in executive function and recognition memory. Nutr Neurosci. 2010;13(2):54–70.

15. Felt BT, Peirano P, Algarin C, Chamorro R, Sir T, Kaciroti N, et al. Long-term neuroendocrine effects of iron-deficiency anemia in infancy. Pediatr Res. 2012;71(6):707–12.

16. Lozoff B, Beard JL, Connor JR, Felt BT. Long-lasting neural and behavioral effects of iron deficiency in infancy. Nutr Rev. 2006;64:S34–43.

17. Scrimshaw NS. Iron deficiency. Sci Am. 1991;265:46–52.

18. Benton D. Micronutrient status, cognition and behavioral problems in childhood. Eur J Nutr. 2008;47(Suppl 3):38–50.

19. Bruner AB, Joffe A, Duggan AK, Casella JF, Brandt J. Randomised study of cognitive effects of iron supplementation in non-anaemic iron-deficient adolescent girls. Lancet. 1996;348:992–6.

20. Murray-Kolb LE, Beard JL. Iron treatment normalizes cognitive functioning in young women. Am J Clin Nutr. 2007;85:778–87.

21. McClung JP, Murray-Kolb LE. Iron Nutrition and Premenopausal Women: Effects of Poor Iron Status on Physical and Neuropsychological Performance. Annu Rev Nutr. 2013 Apr 29;

22. Fuqua BK, Vulpe CD, Anderson GJ. Intestinal iron absorption. J Trace Elem Med Bio. 2012;26:115–9.

23. Pantopoulos K. Iron metabolism and the IRE/IRP regulatory system: An update. Ann NY Acad Sci. 2004;1012:1–13.

24. Gerlach M, Ben-Shachar D, Riederer P, Youdim MBH. Altered brain metabolism of iron as a cause of neurodegenerative diseases? J Neurochem. 1994;63(3):793–807.

25. Rouault TA, Cooperman S. Brain iron metabolism. Semin Pediatr Neurol. 2006;13:142–8.

26. Burdo JR, Connor JR. Brain iron uptake and homeostatic mechanisms: An overview. BioMetals. 2003;16:63–75.

27. Crichton RR, Dexter DT, Ward RJ. Brain iron metabolism and its perturbation in neurological diseases. J Neural Transm. 2011;118:301–14.

28. Ke Y, Qian ZM. Brain iron metabolism: Neurobiology and neurochemistry. Prog Neurobiol. 2007;83:149–73.

29. Beard JL, Connor J, Jones BC. Brain iron: Location and function. Prog Food Nutr Sci. 1993;17:183–221.

30. Beard JL. Iron deficiency alters brain development and functioning. J Nutr. 2003;133(5):1468S–72S.

31. Youdim MBH. Brain iron deficiency and excess; Cognitive impairment and neurodegenration with involvement of striatum and hippocampus. Neurotox Res. 2008;14(1):45–56.

32. Beard JL, Connor JR. Iron status and neural functioning. Annu Rev Nutr. 2003;23:41–58.

33. Connor JR, Menzies SL. Relationship of iron to oligodendrocytes and myelination. Glia. 1996;17:83–93.

34. Lozoff B. Iron deficiency and child development. Food Nutr Bull. 2007;28(Suppl 4):S560–71.

35. Felt BT, Beard JL, Schallert T, Shao J, Aldridge JW, Connor JR, et al. Persistent neurochemical and behavioral abnormalities in adulthood despite early iron supplementation for perinatal iron deficiency anemia in rats. Behav Brain Res. 2006;171(2):261–70.

36. Hambridge M. Biomarkers of trace mineral intake and status. J Nutr. 2003;133:948S–55S.

37. Wish JB. Assessing iron status: Beyond serum ferritin and transferrin saturation. Clin J Am Soc Nephrol. 2006;1:S4–8.

38. WHO. Serum ferritin concentrations for the assessment of iron status and iron deficiency in populations. Geneva: World Health Organization; 2011.

39. Thurnham DI, McCabe LD, Haldar S, Wieringa FT, Northrop-Clewes CA, McCabe GP. Adjusting plasma ferritin concentrations to remove the effects of subclinical inflammation in the assessment of iron deficiency: a meta-analysis. Am J Clin Nutr. 2010;92:546–55.

40. Engle-Stone R, Nankap M, Ndjebayi AO, Erhardt JG, Brown KH. Plasma ferritin, soluble transferrin receptor, and body iron stores identify similar risk factors for iron deficiency but result in different estimates of the national prevalence of iron deficiency and iron-deficiency anemia among women and children in Cameroon. J Nutr. 2013;143(3):369–77.

41. WHO. Assessing the iron status of populations: including literature reviews: report of a joint World Health Organization?Centers for Disease Control and Prevention technical consultation on the assessment of iron status at the population level. 2nd ed. Geneva: World Health Organization; 2007.

42. Beerenhout C, Bekers O, Kooman JP, van der Sande FM, Leunissen KM. A comparison between the soluble transferrin receptor, transferrin saturation and serum ferritin as markers of iron state in hemodialysis patients. Nephron. 2002;92:32–5.

43. Verhoef H, West C, Ndeto P, Burema J, Beguin Y, Kok FJ. Serum transferrin receptor concentration indicates increased erythropoiesis in Kenyan children with asymptomatic malaria. Am J Clin Nutr. 2001;74:767–75.

44. WHO. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Geneva: World Health Organization; 2011.

45. Koury MJ, Ponka P. New insights into erythropoiesis: the roles of folate, vitamin B12, and iron. Annu Rev Nutr. 2004;24:105–31.

46. Shah A. Causes of anemia. Indian J Med Sci. 2004;58(1):24–5.

47. Chesters J. Zinc. In: O'Dell BL, Sunde RA, editors. Handb Nutr Essent Miner Elem. New York: Marcel Dekker, Inc; 1997.

48. Wessells KR, Brown KH. Estimating the global prevalence of zinc deficiency: Results based on zinc availability in national food supplies and the prevalence of stunting. PloS One. 2012;7:e50568.

49. Hotz C, Brown KH. Assessment of the risk of zinc deficiency in populations and options for its control. Food Nutr Bull. 25:94–204.

50. Ahmed T, Hossain M, Sanin KI. Global burden of maternal and child undernutrition and micronutrient deficiencies. Ann Nutr Metab. 2012;61(Suppl 1):8–17.

51. Lönnerdal B. Dietary factors influencing zinc absorption. J Nutr. 2000;130:1378S–83S.

52. Whittaker P. Iron and zinc interactions in humans. Am J Clin Nutr. 1998;68:442S–46S.

53. Hambidge M. Human zinc deficiency. J Nutr. 2000;130:1344S–9S.

54. Hambridge KM, Walravens PA. Disorders of mineral metabolism. J Clin Gastroenterol. 1982;11:87–118.

55. Ford D. Intestinal and placental zinc transport pathways. Proc Nutr Soc. 2004;63:21–9.

56. Hempe JM, Cousins RJ. Cysteine-rich intestinal protein and intestinal metallothionein: an inverse relationship as a conceptual model for zinc absorption in rats. J Nutr. 1992;122(1):89–95.

57. Sekler I, Sensi SL, Hershfinkel M, Silverman WF. Mechanism and regulation of cellular zinc transport. Mol Med. 2007;13:337–43.

58. Andrews GK. Cellular zinc sensors: MTF-1 regulation of gene expression. BioMetals. 2001;14:223–37.

59. Gower-Winter SD, Levenson CW. Zinc in the central nervous system: From molecules to behavior. BioFactors. 2012;38(3):186–93.

60. Frederickson CJ, Koh J-Y, Bush AI. The neurobiology of zinc in health and disease. Nat Rev Neurosci. 2005;6(6):449–62.

61. Takeda A. Zinc homeostasis and functions of zinc in the brain. BioMetals. 2001;14:343–51.

62. Takeda A. Movement of zinc and its functional significance in the brain. Brain Res Rev. 2000;34:137–48.

63. Rahman MT. Dietary zinc and the brain. In: Preedy VR, Watson RR, Martin CR, editors. Handb Behav Food Nutr. New York: Springer Science+Business Media, LLC; 2011. p. 2357–73.

64. Cuajungco MP, Lees GJ. Zinc metabolism in the brain: Relevance to human neurodegenerative disorders. Neurobiol Dis. 1997;4:137–69.

65. Sensi SL, Paoletti P, Bush AI, Sekler I. Zinc in the physiology and pathology of the CNS. Nat Rev Neurosci. 2009;10:780–91.

66. Hess SY, Lönnerdal B, Hotz C, Rivera JA, Brown KH. Recent advances in knowledge of zinc nutrition and human health. Food Nutr Bull. 2009;30(Suppl 1):S5–11.

67. Wensink J, Lenglet WJM, Vis RD, Van den Hamer CJA. The effect of dietary zinc deficiency on the mossy fiber zinc content of the rat hippocampus. Histochemistry. 1987;87:65–9.

68. Palmiter RD. Constitutive expression of metallothionein-III (MT-III), but not MT-I, inhibits growth when cells become zinc deficient. Toxicol Appl Pharmacol. 1995;135:139–46.

69. McCall KA, Huang C, Fierke CA. Function and mechanism of zinc metalloenzymes. J Nutr. 2000;130:1437S–46S.

70. Shankar AH, Prasad AS. Zinc and immune function: the biological basis of altered resistance to infection. Am J Clin Nutr. 1998;68:447S–63S.

71. King JC, Shames DM, Lowe NM, Woodhouse LR, Sutherland B, Abrams SA, et al. Effect of acute zinc depletion on zinc homeostasis and plasma zinc kinetics in men. Am J Clin Nutr. 2001;74(1):116–24.

72. Lowe NM, Fekete K, Desci T. Methods of assessment of zinc status in humans: a systematic review. Am J Clin Nutr. 2009;89:2040S–51S.

73. Wood RJ. Assessment of marginal zinc status in humans. J Nutr. 2000;130:1350S–4S.

74. Gibson RS, Hess SY, Hotz C, Brown KH. Indicators of zinc status at the population level: a review of the evidence. Brit J Nutr. 2008;99(Suppl 3):S14–S23.

75. Hotz C. Dietary indicators for assessing the adequacy of population zinc intakes. Food Nutr Bull. 2007;28(Suppl 3):S430–53.

76. De Benoist B, Darnton-Hill I, Davidsson L, Fontaine O, Hotz C. Conclusions of the joint WHO/UNICEF/IAEA/IZiNCG interagency meeting on zinc status indicators. Food Nutr Bull. 2007;28(Suppl 3):S480–4.

77. Eliot L. What's going on in there? New York: Bantam Books; 1999.

78. De Haan M, Johnson MH, editors. The cognitive neuroscience of development. New York: Psychology Press; 2003.

79. McCann JC, Ames BN. An overview of evidence for a causal relation between iron deficiency during development and deficits in cognitive or behavioral function. Am J Clin Nutr. 2007;85:931–45.

80. Knudsen EI. Sensitive periods in the development of the brain and behavior. J Cogn Neurosci. 2004;16(8):1412–25.

81. Webb SJ, Monk CS, Nelson CA. Mechanisms of postnatal neurobiological development: Implications for human development. Dev Neuropsychol. 2001;19(2):147–71.

82. Thomas DG, Grant SL, Aubuchon-Endsley NL. The role of iron in neurocognitive development. Dev Neuropsychol. 2009;34(2):196–222.

83. Walker SP, Wachs TD, Meeks Gardner J, Lozoff B, Wasserman GA, Pollitt E, et al. Child development: risk factors for adverse outcomes in developing countries. Lancet. 2007;369:145–57.

84. Black MM. Micronutrient deficiencies and cognitive functioning. J Nutr. 2003;133:3927S–31S.

85. Salgueiro MJ, Zubillaga MB, Lysionek AE, Caro RA, Weill R, Boccio JR. The role of zinc in the growth and development of children. Nutrition. 2002;18:510–9.

86. Shrestha NR. Nepal and Bangladesh: A global studies handbook. Santa Barbara, CA: ABC-CLIO, Inc; 2002.

87. Nepal. World Factb 2013-2014. Washington, DC: Central Intelligence Agency; 2013.

88. Lawoti M. Informal institutions and exclusion in democratic Nepal. Himalaya. 2010;28:17–32.

89. Aasland A, Haug M. Perceptions of social change in Nepal: Are caste, ethnicity, and region of relevance? J Asian Afr Stud. 2011;46(2):184–201.

90. Malik K. Human Development Report 2013: The rise of the south: Human progress in a diverse world. New York: United Nations Development Programme;

91. Whelpton J. A history of Nepal. New York: Cambridge University Press; 2005.

92. Hausmann R, Tyson LD, Zahidi S. The global gender gap report 2012. World Economic Forum; 2012.

93. Berglund SK, Westrup B, Hägglöf B, Hernell O, Domellöf M. Effects of iron supplementation of LBW infants on cognition and behavior at 3 years. Pediatrics. 2013 Jan;131(1):47–55.

94. Lozoff B, De Andraca I, Castillo M, Smith J, Walter T, Pino P. Behavioral and developmental effects of preventing iron-deficiency anemia in healthy full-term infants. Pediatrics. 2003;112:846–54.

95. Chang S, Wang L, Wang Y, Brouwer ID, Kok FJ, Lozoff B, et al. Iron-deficiency anemia in infancy and social emotional development in preschool-aged Chinese children. Pediatrics. 2011 Apr;127(4):e927–33.

96. Konofal E, Lecendreux M, Deron J, Marchand M, Cortese S, Zaim M, et al. Effects of iron supplementation on attention deficit hyperactivity disorder in children. Pediatr Neurol. 2008;38(1):20–6.

97. Ashworth A, Morris SS, Lira PIC, Grantham-McGregor SM. Zinc supplementation, mental development and behaviour in low birth weight term infants in northeast Brazil. Eur J Clin Nutr. 1998 Mar;52(3):223–7.

98. Sazawal S, Bentley M, Black R, Dhingra P, George S, Bhan M. Effect of zinc supplementation on observed activity in low socioeconomic Indian preschool children. Pediatrics. 1996;98:1132–7.

99. Bilici M, Yildirim F, Kandil S, Bekaroglu M, Yildirimis S, Deger O, et al. Double-blind, placebo-controlled study of zinc sulfate in the treatment of attention deficit hyperactivity disorder. Progr Neuro-Psychopharmacol Biol Pschiatry. 2004;28:181–90.

100. Lozoff B, Jimenez E, Hagen J, Mollen E, Wolf A. Poorer behavioral and developmental outcome more than 10 years after treatment for iron deficiency in infancy. Pediatrics. 2000;105:E51.

101. Hamadani JD, Fuchs GJ, Osendarp SJ, Khatun F, Huda SN, Grantham-McGregor SM. Randomized controlled trial of the effect of zinc supplementation on the mental development of Bangladeshi infants. Am J Clin Nutr. 2001 Sep;74(3):381–6.

102. Pongcharoen T, DiGirolamo A, Ramakrishnan U, Winichagoon P, Flores R, Martorell R. Long-term effects of iron and zinc supplementation during infancy on cognitive function at 9 y of age in northeast Tahi children: a follow-up study. Am J Clin Nutr. 2011;93:636–43.

103. Siegel E, Kordas K, Stoltzfus R, Katz J, Khatry S, LeClerq S, et al. Inconsistent effects of iron-folic acid and/or zinc supplementation on the cognitive development of infants. J Heal Popul Nutr. 2011;29:593–604.

104. Rico J, Kordas K, Lopez P, Rosado J, Vargas G, Ronquillo D, et al. Efficacy of iron and/or zinc supplementation on cognitive performance of lead-exposed Mexican schoolchildren: a randomized, placebo-controlled trial. Pediatrics. 2006;117:e518–27.

105. Olney D, Pollitt E, Kariger P, Khalfan S, Ali N, Tielsch J, et al. Combined iron and folic acid supplementation with or without zinc reduces time to walking unassisted among Zanzibari infants 5- to 11-mo old. J Nutr. 2006;136:2427–34.

106. Black MM, Baqui AH, Zaman K, Ake Persson L, El Arifeen S, Le K, et al. Iron and zinc supplementation promote motor development and exploratory behavior among Bangladeshi infants. Am J Clin Nutr. 2004 Oct;80(4):903–10.

107. Kordas K, Stoltzfus R, López P, Rico J, Rosado J. Iron and zinc supplementation does not improve parent or teacher ratings of behavior in first grade Mexican children exposed to lead. Pediatrics. 2005;147:632–9.

108. Tielsch JM, Khatry SK, Stoltzfus RJ, Katz J, Leclerq SC, Adhikari R, et al. Effect of routine prophylactic supplementation with iron and folic acid on preschool child mortality in southern Nepal: community-based, cluster-randomised, placebo-controlled trial. Lancet. 2006;367(9505):144–52.

109. Tielsch J, Khatry S, Stoltzfus R, Katz J, LeClerq S, Adhikar R, et al. Effect of daily zinc supplementation on child mortality in southern Nepal: a community-based, cluster randomised, placebo-controlled trial. Lancet. 2007;370(9594):1230–9.

110. De Onis M, Onyango A, Borghi E, Siyam A, Nishida C, Siekmann J. Development of a WHO growth reference for school-aged children and adolescents. Bull World Heal Organ. 7AD;85(9):660–7.

111. Caldwell B, Bradley R. Home Observation for the Measurement of the Environment. Little Rock: University of Arkansas at Little Rock; 1984.

112. Raven J, Court J. Manual for Raven's progressive matrices and vocabulary scales. Oxford: Oxford Psychologists Press, LTD; 1992.

113. Bracken BA, McCallum RS. Universal Nonverbal Intelligence Test. Itasca, IL: Riverside; 1998.

114. Henderson SE, Sugden DA. Movement Assessment Battery for Children. London: Psychological Corp; 1992.

115. Murray-Kolb LE, Khatry SK, Katz J, Schaefer BA, Cole PM, LeClerq SC, et al. Preschool micronutrient supplementation effects on intellectual and motor function in school-aged Nepalese children. Arch Pediar Adolesc Med. 2012;166(5).

116. Belsky J, Crnic K, Gable S. The determinants of coparenting in families with toddler boys: Spousal differences and daily hassles. Child Dev. 1995;66:629–42.

117. Churchich JE, Scholz G, Kwok F. Activation of pyridoxal kinase by metallothionein. Biochim Biophys Acta. 1989;996:181–6.

118. Carter CS, Grippo AJ, Pournajafi-Nazarloo H, Ruscio MG, Porges SW. Oxytocin, vasopressin and sociality. Prog Brain Res. 170:331–6.

119. Caldwell HK. Neurobiology of Sociability. Adv Exp Med Biol. 2012;739:187–205.

120. Liu D, Seuthe AB, Ehrler OT, Zhang X, Wyttenbach T, Hsu JF, et al. Oxytocin-receptor binding: Why divalent metals are essential. J Am Chem Soc. 2005;23(7):2024–5.

121. Nuttall JR, Oteiza PI. Zinc and the ERK kinases in the developing brain. Neurotox Res. 2012;21:128–41.

122. Satoh Y, Endo S, Nakata T, Kobayashi Y, Ikeda T, Takeuchi A, et al. ERK2 contributes to the control of social behaviors in mice. J Neurosci. 2011;31(33):11953–67.

123. Golub MS, Keen CL, Gershwin ME, Hendrickx AG. Developmental zinc deficiency and behavior. J Nutr. 1995 Aug;125(8):2263S.

124. Chen X, Rubin KH, Li D. Relation between academic achievement and social adjustment: evidence from Chinese children. Dev Psychol. 1997 May;33(3):518–25.

125. Malecki S, Elliot S. Children's social behaviors as predictors of academic achievement: A longitudinal analysis. Sch Psychol Q. 2002;17:1–23.

126. Green K, Forehand R, Beck S, Vosk B. An assessment of the relationship among measures of children's social competence and children's academic achievement. Child Dev. 1980;51:1149–56.

127. Chen X, Li D, Li Z, Li B, Liu M. Sociable and prosocial dimensions of social competence in Chinese children: Common and unique contributions to social, academic, and psychological adjustment. Dev Psychol. 2000;36(3):302–14.

128. Chen X, Liu M, Rubin KH, Cen G, Gao X, Li D. Sociability and prosocial orientation as predictors of youth adjustment: A seven-year longitudinal study in a Chinese sample. Int J Behav Dev. 2002;26(2):128–36.

129. Rubin KH, Coplan RJ. Paying attention to and not neglecting social withdrawal and social isolation. Merrill Palmer Quart. 2004;50(4):506–34.

130. Katz SJ, Conway CC, Hammen CL, Brennan PA, Najman JM. Childhood social withdrawal, interpersonal impairment, and young adult depression: A mediational model. J Abnorm Child Psychol. 2011;39(8):1227–38.

131. Prior M, Smart D, Sanson A, Oberklaid F. Does shy-inhibited temperament in childhood lead to anxiety problems in adolescence? J Am Acad Child Psy. 2000;39(4):461–8.

132. Rubin KH, Chen X, McDougall P, Bowker A, McKinnon J. The Waterloo Longitudinal Project: Predicting internalizing and externalizing problems in adolescence. Dev Psychopathol. 1995;7(4):751–64.

133. Benton D. Micronutrient status, cognition and behavioral problems in childhood. Eur J Nutr. 2008 Aug;47 Suppl 3:38–50.

134. Lozoff B, Klein NK, Nelson EC, McClish DK, Manuel M, Chacon ME. Behavior of infants with iron-deficiency anemia. Child Dev. 1998;69(1):24–36.

135. Cole PM, Tamang BL, Shrestha S. Cultural variations in the socialization of young children's anger and shame. Child Dev. 2006;77(5):1237–51.

136. Cole PM, Bruschi CJ, Tamang BL. Cultural differences in children's emotional reactions to difficult situations. Child Dev. 2002;73(3):983–96.

137. Cole PM, Tamang BL. Nepali children's ideas about emotional displays in hypothetical challenges. Dev Psychol. 1998;34(4):640–6.

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Education

August 2009 – December 2013 (expected), Schreyer Honors College, The Pennsylvania State University, University Park, PA

- Nutritional Sciences B.S., Basic Option, The College of Health and Human Development
- Psychology B.S., Neuroscience Option, The College of the Liberal Arts
- Honors in Nutritional Sciences

Recognitions

Fall 2009 – Spring 2013, Academic Excellence Award, Schreyer Honors College

- Four year merit scholarship awarded to freshman admits to the Schreyer Honors College

April 2013, The Pennsylvania State University Undergraduate Research Exhibition

- Honorable Mention, Health and Life Sciences
- Honorable Mention, University Libraries Awards for Information Literacy
- Digital copy of poster found at: *http://publications.libraries.psu.edu/scholarship/bahnfleth*

Fall 2009 – Present, Dean's List, The Pennsylvania State University

Spring 2010, The President's Freshman Award, The Pennsylvania State University

Experience

August 2011 – Present, Laboratory Assistant, Murray-Kolb Laboratory, Department of Nutritional Sciences, The Pennsylvania State University

- Adviser: Dr. Laura Murray-Kolb, Assistant Professor of Nutritional Sciences
- Undergraduate Honors Thesis: Micronutrient supplementation in preschool and its effect on behavior in Nepali children at 7 to 9 years
- Experience in data analysis, scientific writing, basic statistics, SAS
- Currently helping to restart pilot study exploring systemic iron status, brain iron status and cognitive function in women of reproductive age

Fall 2013, Undergraduate Teaching Assistant, NUTR 251: Introductory Principles of Nutrition, The Pennsylvania State University

- Instructor: Dr. Jill Patterson, Assistant Professor of Nutritional Sciences
- Responsibilities include grading student assignments, holding review sessions and assisting students during weekly classes

April 2013, Experimental Biology 2013 Conference, Boston, MA

- Presented poster of original research
- "Long-term effects of micronutrient supplementation on school age child behavior"

Spring 2012, The University of Melbourne, Parkville, VIC, AUS

- Study abroad
- Recipient of Schreyer Ambassador Travel Grant
- Courses in Australian history, non-conscious psychology, modern food concerns and modern famine
- Accepted as a residential student at Trinity College

Summer 2011, International Program in Nutrition, Rome, IT

- Study abroad
- Recipient of Schreyer Ambassador Travel Grant
- Courses in Italian architecture, international nutrition and the Mediterranean diet

Association Membership

Spring 2013 – Present, ASN, American Society for Nutrition

Community Service

Fall 2009 – Present, The Student United Way, The Pennsylvania State University,

- Treasurer, Fall 2012 – Spring 2013
- Plan and run the annual winter Trash to Treasure Sale
- Participate in service projects associated with the Centre County United Way and The Pennsylvania State University

Fall 2010 – Fall 2011, Summer 2013 – Present, International Conversation Partner, Global Connections, The Pennsylvania State University

- Help international undergraduate and graduate students practice conversational English
- Participate in and facilitate cultural exchange

June 2010 – November 2011, Mount Nittany Medical Center, State College, PA

- Provided assistance to employees and visitors on patient floors

Fall 2009 – Spring 2011, Oriana Singers THON, The Pennsylvania State University

- THON Weekend Chair, Fall 2009 – Spring 2010
    - Coordinated activities related to THON weekend including group attendance, floor pass use and communication with the organization's THON family
- THON Co-chairwoman, Fall 2010 – Spring 2011
    - Led weekly planning meetings, coordinated fundraising trips and events, organized group's donations
- Official fundraising group of the Oriana Singers benefiting the Four Diamonds Fund through the Penn State Dance Marathon

Activities

Spring 2009 – Present, Long Distance Running

- Philadelphia Rock-n-Roll Half Marathon, September 2011
- Tussey Mountainback 50 Mile Relay, October 2011
- Great Ocean Road Half Marathon, May 2012
- Rochester Half Marathon, September 2013

Fall 2012 - Present, Essence II, Ltd., University Park, PA

- Community choir
- Music from the African and African American tradition

Fall 2013 – Present, Penn State Vegetarian Club

Fall 2009 – Fall 2011, Oriana Singers, The Pennsylvania State University

- Advanced women's choir
- Alto I Section Leader, Fall 2011