Nutritional Status and Neurodevelopment in International Adoptees

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Abstract

Adequate intake and assimilation of all nutrients is important for brain health and function; however, several nutrients have more marked effects on brain development. Based on the timing, the extent of deprivation, and the pathophysiology of a given nutrient, specific hypothesis can be made regarding the effects of a given nutrient on specific neural systems. Internationally adopted children present a unique opportunity to study the effects of early nutrient deficiencies on neurodevelopment under relatively controlled conditions, given that the time of adoption into a stable environment clearly demarcates the end of a period of adversity. Although micronutrient deficiencies have an adverse impact on development in other populations, little is known about the nutritional status at arrival or the role of nutritional status in neurodevelopment in international adoptees, a population in which some neurobehavioral problems persist years after adoption. The goals of this set of studies were to investigate (1) the macro- and micronutritional status of internationally adopted children and (2) the association between nutritional status and neurodevelopment during the early adoption period. Studies one and two investigated iron status in children adopted from Eastern Europe. In study one, international adoptees had compromised iron status, with iron deficiency more prevalent in participants with *G. lamblia*, a parasite which may interfere with iron absorption. There was persistent iron deficiency at follow-up, likely due to the erythropoietic demands of catch-up growth. In study two, iron deficiency was associated with general cognitive and behavioral development during the early adoption period. Specifically, those with iron deficiency were more fearful at arrival and had problems
with activity and cooperation at the six-month follow-up. Cognitive performance was likely mediated by behaviors during testing. In study three, a comprehensive nutritional battery was completed for children adopted from Eastern Europe, Ethiopia, and China. 56% of the children had at least one micronutrient deficiency, with iron, zinc and vitamin D insufficiency/deficiency the most common deficiencies. Iron deficiency was associated with lower cognitive scores, slower speed of processing, as well as altered socioemotional behaviors and altered parent behaviors. Zinc deficiency was associated with lower quality exploratory behaviors and altered parent behaviors. These studies show that internationally adopted children are at risk for micronutrient deficiencies and that micronutrient status is associated with neurodevelopment during the early adoption period. Continued research will be important to understand the effects of these nutritional deficiencies on specific neurodevelopmental domains, to determine whether the developmental effects persist long-term, and to inform nutritional and neurodevelopmental principles that can be applied to develop interventions and services for children living in adverse and rehabilitating environments.
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Chapter I: The Role of Postnatal Nutrition in Developing Cognitive Systems:

**Direct and Indirect Effects**

Research in animal models and human populations show that certain nutrients are particularly important during the first years of life to support rapidly developing brain systems. There is convincing evidence that protein and energy malnutrition (PEM) during early development affects neurodevelopmental processes and impairs cognitive development (Pollitt, Gorman, Engle, Martorell, & Rivera, 1993; Pollitt, Gorman, Engle, Rivera, & Martorell, 1995). However, PEM is often accompanied by specific macronutrient deficiencies, and it is not known whether these developmental effects are due to PEM alone or to specific nutrient deficiencies which have also been linked to impaired cognitive development. It will be important to elucidate the roles of specific nutrients on neurodevelopmental processes and cognitive outcomes.

Specific nutrients are associated with neurodevelopmental functions based on the nutrients’ metabolic roles (Fuglestad, Georgieff, & Rao, 2008). Human studies and animal models support the hypothesis that nutritional deprivations during a period of rapid growth result in more profound structural, chemical, and physiological changes than if the same degree of deprivation is imposed during a more quiescent period. Furthermore, since the brain does not develop homogenously, brain regions that are growing particularly rapidly might be expected to be most affected. Therefore, specific hypotheses can be made regarding the neurodevelopmental effects of a given nutrient based on that nutrient’s metabolic role in regional brain development, the severity and
duration of the deficiency, and the timing of the deficiency (Fuglestad, Georgieff, & Rao, 2008). This chapter will discuss (1) the importance of measuring specific neurodevelopmental cognitive domains that are sensitive to specific nutrient deficiencies during the early postnatal years, (2) the roles of both direct and indirect effects of nutrient deficiencies on cognitive development, and (3) one population, internationally adopted children, who are at risk for both early nutritional deficiencies and developmental delays. While all nutrients are vital to support healthy development and brain function, several nutrients have more marked effects on brain development and will be highlighted in this chapter, specifically, LCPUFAs, iron, zinc, iodine, selenium, folate, vitamin B₁₂, and choline.

During the first three years of life, the brain is developing rapidly, undergoing dendritic arborization, synaptogenesis, myelogenesis, and apoptosis (Dobbing, 1990). This review will focus on nutrient deficiencies identified during the early postnatal years; however, deficiencies are likely not limited to this period as postnatal nutrient status is often associated with earlier (i.e., prenatal) nutrition. For instance, infants who have low iodine status due to living in an endemic region of iodine deficiency are likely exposed to prenatal iodine deficiency as well. Although iodine deficiency might be diagnosed postnatally, it is likely that neurodevelopmental processes that occur during gestation (e.g., neurogenesis) were affected during prenatal iodine deficiency. Moreover, fetal accumulation of numerous nutrients occurs during the third trimester, and infants born preterm may be at risk during the early postnatal period for nutrient deficiencies due to limited nutrient accrual prior to birth. For instance, infants are born with iron stores
proportional to their weight (Georgieff, 2006; Widdowson & Spray, 1951). Accordingly, low birth weight infants have reduced absolute iron stores at birth, placing them at a greater risk for postnatal iron deficiency compared to infants born normal weight.

Postnatal nutritional status is a marker for not only concurrent nutrient intake and metabolism, but also of nutritional history. Although this chapter will focus on postnatal nutrient deficiencies, it is difficult to isolate postnatal from prenatal nutritional status.

**Cognitive Domains that are Sensitive to Nutrient Deficiencies**

Cognitive domains that are sensitive to nutrient deficiencies include, but are not limited to, general cognitive development, hippocampal-based explicit memory, striatal-mediated implicit memory, and prefrontal-mediated working memory and inhibition. Nutrient deficiencies may also have indirect effects on these cognitive domains. For instance, specific nutrients are required for myelination, and deficiencies of these nutrients result in hypomyelination which may affect developing cognitive systems (Barnea-Goraly et al., 2005; McGraw, Liang, & Provenzale, 2002; Nagy, Westerberg, & Klingberg, 2004). A second potential indirect effect of nutrition on cognitive development is that exploration with the environment is necessary for typical neuro- and cognitive development (e.g., Bell & Fox, 1997; Black, Jones, Nelson, & Greenough, 1997). According to the functional isolation hypothesis, undernourished children explore and interact less with their physical and social environments, which may further delay cognitive development (Black et al., 2004; Lozoff et al., 2007; Lozoff et al., 1998).

**General Cognitive Development**

Many studies have used general cognitive development as outcome measures
[e.g., standardized assessments such as the Mental Development Index (MDI) of the Bayley Scales of Infant Development (BSID; Bayley, 1993)] to investigate the effects of nutrient deficiencies on development. More recent studies have begun, and it will be important to continue, to use specific cognitive outcomes based on the role of a given nutrient in neurodevelopment. Such lines of research will be important to continue to clarify the roles of specific nutrients on cognitive development. Furthermore, general cognitive measures may not be sensitive enough to identify the cognitive effects of mild to moderate nutrient deficiencies.

The associations of LCPUFAs, iron, zinc, and iodine and selenium with general cognitive development have been investigated

**LCPUFAs.** Poly-unsaturated fatty acids (PUFAs) are essential in all plasma membranes as phospholipids, and dietary intake of PUFAs alters the fatty acid composition of membrane phospholipids. Two long chain (LC)PUFAs, docosahexaenoic acid (DHA, 22:6n–3) and arachidonic acid (AA, 20:4n–6), are the major lipid components in membrane phospholipids, with high proportions of DHA in gray matter phospholipids (Innis, 2003). DHA and AA accumulate in the CNS during prenatal and early postnatal periods (Clandinin et al., 1980; Koletzko et al., 2001). LCPUFAs potentially affect neurodevelopment as differences in phospholipid composition alters membrane structure, which can affect the function of membrane-bound proteins (e.g., receptors, transport systems, ion channels) and the production and function of lipid-signaling molecules (e.g., eicosanoids). Furthermore, n-6 and n-3 fatty acids have differential effects on gene transcription (Innis, 2003). Animal studies show that dietary
intake of fatty acids alters brain lipid content, with DHA more affected by dietary intake than AA (Koletzko et al., 2001).

Results of early postnatal LCPUFA supplementation on general development are inconsistent in both preterm and term infants. One study in which preterm infants received either a formula with or without LCPUFAs for nine months reported higher scores on the MDI of the BSID at 18 months for boys in the supplemented group compared to the nonsupplemented group; however, no differences were found for girls (Fewtrell et al., 2004). In another supplementation trial, preterm infants received either a LCPUFA supplemented formula or a non-supplemented formula for three weeks during the stay on the neonatal unit, and no differences were found on the MDI at 18 months (Fewtrell et al., 2002). In another investigation, term infants were randomized to receive LCPUFA supplemented formula or non-supplemented formula for 12 months, and there were no differences in cognitive measures at 39 months (Auestad et al., 2003). However, term infants who were administered either DHA supplemented or DHA and AA supplemented formula for four weeks had higher scores on the MDI at age 18 months than the control group (non-supplemented formula), with a larger effect in those receiving the formula with both DHA and AA. Moreover, infant DHA concentrations at four months were correlated with MDI scores at 18 months (Birch, Garfield, Hoffman, Uauy, & Birch, 2000).

Although, the research on the effects of LCPUFAs on global development is mixed, investigations have begun to examine the effects of LCPUFAs on specific cognitive and neurodevelopmental domains. For instance, high concentrations of DHA
are found in the frontal cortex (Levant, Ozias, Jones, & Carlson, 2006), n-3 fatty acids affect dopamine metabolism (Innis, 2003; Levant, Radel, & Carlson, 2004), and LCPUFAs are present as phospholipids in myelin (Cockburn, 2003).

Iron. Iron-containing enzymes and proteins are involved in numerous neurodevelopmental processes (Beard & Connor, 2003; Rao, Tkac, Townsend, Gruetter, & Georgieff, 2003). Likewise, general development is altered by early iron deficiency. Iron deficiency most commonly occurs in infancy between 6 and 24 months due to low dietary intake or late introduction of iron-containing foods. Furthermore, infants born small for gestational age are at risk for iron deficiency during infancy due to reduced iron stores at birth (Georgieff, 2006; Widdowson & Spray, 1951). Accordingly, the association between iron deficiency during this period and general cognitive delays has been studied extensively. In a review of multiple well-controlled studies, infants with iron deficiency anemia score on average 6 to 15 points lower on developmental assessments compared to those without iron deficiency anemia (Lozoff et al., 2006). The role of iron deficiency on specific neurodevelopmental processes and cognitive outcomes is being investigated in animal models and human studies. Iron-containing enzymes are involved in energy metabolism (oxidative phosphorylation), synaptogenesis, dendritogenesis, myelination, and monoamine metabolism (Beard & Connor, 2003; Rao et al., 2003).

Zinc. Zinc is one of the most prevalent trace elements in the brain (Pfeiffer & Braverman, 1982). Zinc is present as a cofactor in proteins that interact with DNA and RNA (e.g., transcription factors, replication and transcription enzymes) and thus is
necessary for synthesis of nucleic acids and proteins. Zinc also affects insulin-like growth factor I (McNall, Etherton, & Fosmire, 1995). Accordingly, zinc deficiency has been shown to decrease brain growth, DNA, RNA, and protein concentrations in the rat pup (Sandstead, Frederickson, & Penland, 2000).

Results for the role of zinc in general cognitive performance are variable. Several well-controlled studies have not found an effect of zinc supplementation compared to placebo in infants who are at risk for zinc deficiency. For instance, in infants born small for gestational age in a low income region of India, no differences were found on the BSID between infants given a zinc supplement with a micronutrient mixture and infants given the micronutrient mixture without zinc from birth to nine months (Black et al., 2004). There was also no difference on the MDI of the BSID in term infants born small for gestational age from a low-income population in Brazil who were supplemented during the first year compared to those receiving placebo (Ashworth, Morris, Lira, & Grantham-McGregor, 1998). On the other hand, there is evidence that supplementation improves general cognitive development compared to placebo in infants at risk for zinc deficiency. Zinc supplementation during the first year in term infants from a low-income region of Chile did have a slight effect on the MDI of the BSID. In this study, there were no mean differences on the MDI between the supplemented group and the placebo group; however, compared to the placebo group, a smaller proportion of the supplemented group scored below the mean standardized score of 100 (Castillo-Duran et al., 2001).

It is likely that measures of general cognition are not sensitive enough to capture the effects of zinc on cognitive development (Sandstead et al., 2000), and it will be
paramount to investigate the effects of zinc deficiency on specific cognitive outcomes based on the known biochemical roles of zinc. Zinc is present in high concentrations in the cerebral cortex, hippocampus, and cerebellum (Frederickson & Danscher, 1990); therefore, developmental measures specific to these regions will be important in future research to clarify the effects of zinc deficiency on cognitive development. Zinc is also involved in neurotransmission as it is released into the synapse from zinc containing neurons and may modulate neuron excitability through effects on GABA and glutamate neurotransmission (Sandstead et al., 2000).

**Iodine and selenium.** Iodine is necessary for normal thyroid function, as it is a component of thyroid hormones. In general, thyroid hormones are involved in the regulation of cellular metabolism and in the regulation of hormones (e.g., growth hormone; Smith, Evans, Costall, & Smythe, 2002), and thus are important for cellular differentiation and growth. Selenium is required for the synthesis of the enzyme which activates thyroid hormone. Thus, selenium deficiency, like iodine deficiency, leads to hypothyroidism, and although the effects of selenium deficiency on development have yet to be researched in children, selenium deficiency may have similar effects to iodine deficiency on neuro- and cognitive development through depressed thyroid hormone. Iodine and selenium are both found in the soil, and food crops and pasture grasses grown in soils with low levels will have lower content of that mineral; thus, populations who depend on local food crops and pasture grasses grown in soils with low levels are at risk for deficiency.
The developmental effects of iodine deficiency are dependent on the timing and severity of the deficiency, with early prenatal development most vulnerable to iodine deficiency. Severe iodine deficiency during early pregnancy leads to cretinism, characterized by irreversible mental retardation, neurologic symptoms, and dwarfism (Cao et al., 1994; O'Donnell et al., 2002). While iodine deficiency that occurs later in development (i.e., third trimester, infancy, and childhood) or is mild or moderate does not cause cretinism, it is associated with reduced general cognitive performance. One study examined the timing of iodine deficiency in an endemic region of China by giving pregnant women and children iodine supplements during specific developmental periods. While the most positive developmental outcomes were associated with prenatal supplementation, those supplemented 3 to 12 months after birth had, on average, larger head circumferences and slightly higher scores on the BSID than the untreated group (Cao et al., 1994). There is also evidence that mild to moderate iodine deficiency negatively affects cognitive development. For instance, mild to moderate iodine deficiency in school-age children is associated with impaired cognitive development, with the degree of iodine deficiency associated with the severity of impairments (Azizi et al., 1993). Additionally, children from iodine deficient regions have cognitive impairments compared to children from similar regions that are iodine sufficient (Vermiglio et al., 1990).

While such studies support the role of iodine deficiency in cognitive functioning, it is unclear whether the cognitive impairments are caused by earlier iodine deficiency as the severity of iodine deficiency in childhood is likely correlated with the severity of
iodine deficiency earlier in development. Moreover, the association between cognitive impairments and iodine deficient regions may be due to confounding environmental factors. For instance, it is reasonable to expect that the parents’ iodine status will be correlated with their children’s iodine status from eating the same food. Parents who have more severe iodine deficiency may also have cognitive impairments and provide less cognitively-stimulating environments. Well-controlled supplement trials during postnatal periods are needed to clarify whether the postnatal cognitive impairments are due to irreversible effects of early iodine deficiency and/or environmental factors associated with the severity of iodine or whether iodine deficiency affects concurrent cognitive function. One well-controlled study found trends for better cognitive outcomes at age six in children whose mothers were supplemented early in pregnancy compared to children who were supplemented at two years of age (O'Donnell et al., 2002), supporting the critical importance of early iodine deficiency on later cognitive outcomes. However, another, double-blind randomized controlled study found improvements in several cognitive measures in children supplemented between the age of 10 and 12 (Zimmermann et al., 2006), highlighting the significance of iodine status and thyroid for normal brain function.

Although iodine is associated with general development, the research is limited on the role of (1) the effects of postnatal iodine deficiency independent of prenatal iodine status and independent of environmental correlates of iodine deficiency and (2) iodine deficiency in specific cognitive domains. Thyroid hormone acts at the level of transcription, and there is evidence that the effects of thyroid hormone on brain function
are due to thyroid hormone-mediated gene expression (Iniguez et al., 1996). Several
gene targets of thyroid hormone have been identified in the brain, and thus specific
hypotheses of the effects hypothyroidism from iodine or selenium deficiency may be
made based on the roles of these genes. Thyroid hormone has regional effects on the
cortex, hippocampus, striatum, and cerebellum (Iniguez et al., 1996; Smith et al., 2002)
and affects genes involved in myelination (Smith et al., 2002). Furthermore, examining
specific cognitive domains may be a more sensitive measure of the effects of more mild
iodine or selenium deficiency.

**Hippocampal-Dependent Explicit Memory**

The hippocampus develops rapidly during the late fetal and early postnatal period,
undergoing dendritogenesis and synaptogenesis, and reaching adult like volume during
the second half of the first year (reviewed in Nelson, 1995). However, development of
the dentate gyrus does not reach adult levels of synapses until the age of three or four
years (Eckenhoff & Rakic, 1991). Furthermore, while neurogenesis occurs primarily
during gestation, granule cell formation continues in the dentate gyrus postnatally
(Seress, Abraham, Tornoczky, & Kosztolanyi, 2001). Hippocampal circuitry is involved
in explicit memory formation as evidenced by lesion studies in animals and imaging
studies in humans (Nelson, 1995). Nutrients required for normal hippocampal function
include iron, zinc, iodine and selenium, folate and vitamin $\text{B}_12$, and choline.

**Iron.** Early iron deficiency is associated with altered hippocampal function and
poor performance on hippocampally-mediated memory tasks. In the rodent model, early
iron deficiency is associated with poor performance on hippocampal-mediated memory
tasks (e.g., spatial memory tasks; Felt & Lozoff, 1996). In an event related potential study, compared to iron sufficient infants, infants with iron deficiency anemia had delayed positive slow wave responses in a recognition memory paradigm during which they were presented with pictures of their mothers and picture of strangers, an electrophysiological response associated with hippocampal function (Burden et al., 2007). Although the effects of postnatal iron deficiency on behavioral measures of memory have not been investigated, prenatal iron deficiency (i.e., serum ferritin concentrations at birth) is associated with poorer performance on a delayed recall task (elicited imitation) at 12 months, consistent with altered hippocampal development and function (DeBoer, Wewerka, Bauer, Georgieff, & Nelson, 2005).

In animal models of perinatal deficiency, the hippocampus is particularly vulnerable to alterations in iron-dependent metabolic functions (deUngria et al., 2000), which may underlie the behavioral changes in memory. Gestational iron deficiency alters the neurochemical profile of the hippocampus indicating impaired myelination, decreased glutamatergic neurotransmission, and altered energy metabolism (Rao et al., 2003). Gestational iron deficiency is also associated with altered dendritic arborization in the hippocampus (Jorgenson, Wobken, & Georgieff, 2003) and altered long-term potentiation in area CA1 of the hippocampus (Jorgenson, Sun, O'Connor, & Georgieff, 2005). Accordingly, the hippocampus is particularly vulnerable to iron deficiency; however, many of these alterations in the hippocampus are associated with prenatal iron deficiency, and it will be interesting to see whether similar alterations occur with postnatal iron deficiency.
Zinc. Compared with other brain regions, zinc concentrations are particularly high in the hippocampus (Frederickson & Danscher, 1990). Consistent to its role in hippocampal function, zinc deficient rats perform more poorly in hippocampally-dependent spatial-working memory tasks compared to zinc sufficient rats (Frederickson, Frederickson, & Danscher, 1992). A potential mechanism of zinc in hippocampal-mediated memory is through its release into the synapse and its modulatory role on neurotransmission. Zinc is released into the synapse from zinc containing vesicles in the mossy fibers (which project from the dentate gyrus to area CA3) of the hippocampus (Howell, Welch, & Frederickson, 1984), a system involved in spatial orientation learning tasks (Ishihara, Mitsuno, Ishikawa, & Sasa, 1997). Studies that have inactivated mossy fibers by chelating zinc show impaired spatial learning in rats (Frederickson et al., 1992; Lassalle, Bataille, & Halley, 2000). However, knockout mice with zinc removed from the synaptic vesicles performed just as well on hippocampal-dependent tasks (Cole, Martyanova, & Palmiter, 2001). Although, not directly investigated, zinc deficiency likely affects hippocampal development and function in infants, and the role of zinc deficiency on hippocampal-dependent memory warrants further attention.

Iodine and selenium. Although the effects of either iodine or selenium deficiency on hippocampal development has not been directly investigated, hippocampal functioning and memory performance are dependent on thyroid hormone. In rodent models, adult rats supplemented with thyroid hormone performed better on spatial learning task compared to non-supplemented rats, and the effects seemed to be due to thyroid hormone alteration of acetylcholine neurotransmission in the hippocampus.
(Smith et al., 2002). Furthermore, in rodent adults, thyroid hormone is associated with altered dendritic spine density of pyramidal cells in CA1 region of the hippocampus (Gould, Allan, & McEwen, 1990). The specific effects of iodine or selenium deficiency on memory and hippocampal development have yet to be investigated.

**Folate and vitamin B<sub>12</sub>**. Folate and vitamin B<sub>12</sub> are both coenzymes in the methyl-transfer pathway. Folate and vitamin B<sub>12</sub> deficiencies are related to impaired memory in aging adults (Durga et al., 2007; Malouf & Grimley, 2008); however, the research is limited on the roles of folate and vitamin B<sub>12</sub> in hippocampally-dependent memory during development. In a rodent model, gestational folate deficiency was associated with impaired maze learning (Whitley, O’Dell, & Hogan, 1951). However in a recent longitudinal study, no differences were found between children born to mothers who had low gestational folate status during the last half of pregnancy on performance on numerous cognitive tests at age five years compared to infants born to mothers with normal folate status during pregnancy (Tamura et al., 2005).

A potential mechanism of altered methyl metabolism on impaired memory is elevated homocysteine levels. Gestational reduction of methyl sources (deficiency of vitamin B12, folate, and choline) increases levels of homocysteine in the hippocampus, and is associated with apoptosis and impaired maze learning (Blaise et al., 2007). High homocysteine levels are also associated with hippocampal atrophy in adults (Durga et al., 2007).

**Choline**. Like folate and vitamin B12, choline is involved in methyl metabolism; however, there is more evidence for the developmental effects of choline on
hippocampally-mediated memory. The research however has focused on choline deficiency or supplementation during late pregnancy. Prenatal choline supplementation results in better spatial memory performance in the adult rat compared to control rats (Meck & Williams, 1999). Because choline supplementation of adult animals does not seem to improve memory to the extent as supplementation during fetal development, it is likely that choline during early development alters the developmental trajectory of neural circuits that support memory, leading to long-term alterations in memory (Meck & Williams, 2003); however, it is unknown whether these effects are limited to the prenatal period or extend into the early postnatal years. Despite the mounting evidence from animal research for the role of choline in neurodevelopment and memory performance, questions still exist whether these findings translate to humans. However the evidence from animal models highly supports the hypothesis that dietary choline during pregnancy and perhaps the early postnatal period is necessary for normal cognitive development.

Several mechanisms for the effects of choline supplementation on memory development have been identified (Meck & Williams, 2003). Choline affects neuronal proliferation, neuronal and glial differentiation, and apoptosis in the hippocampus by altering gene expression (Zeisel & Niculescu, 2006). Yet another mechanism linking choline to memory is through hippocampal long-term potentiation (LTP); the stimulus threshold for LTP is inversely related to prenatal choline status (Pyapali, Turner, Williams, Meck, & Swartzwelder, 1998).

**Striatal-Mediated Implicit Memory**
During late gestation and early infancy, the striatum undergoes rapid development. The striatum is involved in implicit memory; specifically, memories that do not require conscious awareness or may not be stated explicitly (e.g., procedures; Nelson, 1995). Normal striatal function involves dopaminergic transmission, and striatal function may be disrupted by nutrients which alter dopaminergic metabolism. Although, the role of specific nutrients in striatal-mediated implicit memory is quite limited, there is evidence from iron deficiency studies in animal models that striatal development is altered. Furthermore, as the striatum develops rapidly during late gestation and the early postnatal period, this structure is vulnerable to deficiencies that occur during this time. The role of nutrients on striatal-mediated implicit memory deserves further attention.

**Iron.** In animal models of early iron deficiency, procedural behaviors which rely on striatal function are altered. Such changes in behaviors include altered forelimb placing and grooming sequences (Felt et al., 2006). These are accompanied by metabolomic changes (Ward et al., 2007) which imply significant myelination and/or fatty acid issues as being responsible. Others (Beard et al., 2006) have documented alterations in dopamine. The enzyme tyrosine hydroxylase, which is involved in dopamine synthesis, is iron-dependent, and animal studies which model ID in infancy show long-term dopamine alterations (Beard & Connor, 2003). Despite the evidence for altered implicit memory (i.e., procedural) performance in animal models of iron deficiency, such effects have yet to be studied in children.

**Prefrontal-Dependent Working Memory and Inhibitory Control**
Considerable development of the prefrontal cortex (PFC) occurs postnatally, undergoing synaptogenesis, dendritic and axonal growth, myelination, and synapse elimination (Huttenlocher & Dabholkar, 1997). Given the developmental changes that occur postnatally, this region and its connectivity to other structures, is vulnerable to early postnatal nutrient deficits that are critical for its normal development and function.

Development of the PFC is associated with acquisition of tasks of working memory and inhibitory control, such as the A not B task, which requires the ability to hold a representation in memory over time and to inhibit a motor response (Diamond, 1985). Evidence for the role of the PFC in such tasks is supported by both lesion studies in nonhuman primates (Diamond & Goldman-Rakic, 1989; Diamond, Zola-Morgan, & Squire, 1989) and in infant EEG studies. For instance, successful performance on the A not B task following a delay was associated frontal region EEG activity in infants ages seven to 12 months (Bell & Fox, 1992). Working memory tasks are also dependent on dopamine projections in the prefrontal cortex and striatum (Chudasama & Robbins, 2006; Diamond, 1996; Goldman-Rakic, Muly, & Williams, 2000). Development of working memory tasks, such as A not B, involves PFC development; however, performance may be associated with PFC development and/or the development of structures (e.g., striatum) and the projections between such structures and the PFC (Nelson, 1995). There is evidence for the role of LCPUFAs, iron, and zinc for PFC development and function.

**LCPUFAs.** N-3 fatty acids are involved in dopamine metabolism, and n-3 deficiency in animal models decreases dopamine concentrations in the frontal cortex (Innis, 2003). DHA concentrations in the frontal cortex are also particularly affected by
reduced DHA availability (B Levant et al., 2006). Although research is limited on the role of LCPUFAs in prefrontal-mediated tasks, term infants given LCPUFA supplemented formula for four months performed better on means-end problem solving tasks, which likely involve the frontal lobes, at 10 months of age than infants who received nonsupplemented formula (Willatts, Forsyth, DiModugno, Varma, & Colvin, 1998). These results may be due to other neurodevelopmental processes associated with LCPUFA status; future research is warranted to clarify the role of LCPUFAs in PFC development and function.

**Iron.** Consistent with the role of dopamine in PFC function, iron deficiency in infancy is associated with long-term effects on tasks associated with fronto-striatal circuitry. For instance, Chilean children, who were iron deficient with anemia during infancy had poorer performance on tasks requiring inhibition and planning at the ages of five and 10 years compared to children with no history of iron deficiency in infancy (Peirano, Algarin, Garrido, Nunez et al., 2004). Similar results were found in Costa Rican 19-year-olds who had a history of iron deficiency in infancy (Burden, Koss, & Lozoff, 2004). It will be important to investigate the effects of iron deficiency on PFC development and associated cognitive development.

**Zinc.** The cerebral cortex is particularly rich in zinc (Frederickson & Danscher, 1990). Although the effects of zinc deficiency on prefrontal-dependent tasks in infants and toddlers have not been studied, zinc deficiency is associated with such tasks later in development. For instance moderate zinc-deficient diets in prepubertal rhesus monkeys hamper working memory (Golub et al., 1994). Moreover, zinc deficiency in older,
school-aged children is associated with performance on prefrontal-dependent tasks. In supplementation studies of three separate populations, children ages 6-9 years who were supplemented with a micronutrient mixture with zinc performed better on a complex reasoning task compared to those who were supplemented with the micronutrient mixture without zinc (Penland, 2000). The biochemical mechanisms for the effects on PFC development and function are unknown, however because the PFC is particularly rich in zinc, and given the numerous roles of zinc in brain function and development, PFC function is vulnerable to zinc deficiency.

Cognitive Systems

Although, specific hypotheses can be made about the effect of nutrient deficiencies on specific structures (i.e., hippocampus, striatum, and PFC) and associated cognitive abilities (i.e., explicit memory, implicit memory, working memory), it is critical to note that these structures function within connected neural systems (Goldman-Rakic, 1987; Nelson, 1995). For instance, the PFC is part of a complex circuitry, and while the PFC is important for the normal development of working memory and inhibition, alterations in these behavioral tasks may be due to impaired functioning of the circuitry associated with the PFC. As an example, the alterations in PFC-mediated tasks in children who were iron deficient (Burden et al., 2004; Peirano, Algarin, Garrido, Nunez et al., 2004) may be related to altered dopamine metabolism in the striatum associated with disrupted circuitry with the PFC (Lozoff & Georgieff, 2006a). Although specific brain structures are identified with particular tasks, these structures are part of intricate neural systems.
Indirect Effects of Nutrition on Cognitive Domains

Myelination

Myelination is important for developing cognitive systems throughout childhood. Developing white matter improves signal transduction and may be associated with improved cognitive abilities. For instance, working memory performance is correlated with white matter maturation in the left frontal lobe whereas reading ability is correlated with white matter maturation in the temporal lobe (Nagy et al., 2004). Myelination begins prenatally and continues throughout childhood and adolescence. Diffusion tensor imaging (DTI) studies have shown that age-related changes in white matter (e.g., in PFC connections) throughout childhood and adolescence are associated with improved cognition (Barnea-Goraly et al., 2005; McGraw et al., 2002; Olesen, Nagy, Westerberg, & Klingberg, 2003). Additionally, in the hippocampus, an increase of proliferation of oligodendrocytes begins at around one year, likely associated with an increase in myelination (Seress et al., 2001). Therefore, cognitive domains, such as hippocampal-dependent explicit memory, striatal-mediated implicit memory, and prefrontal-mediated working memory may be affected by nutrient deficiencies through their roles in myelination. LCPUFAs, iron, iodine and selenium, vitamin B₁₂ and choline are implicated in myelination.

LCPUFAs. Dietary fatty acid composition can modify the composition of myelin (Cockburn, 2003); however, effects of LCPUFAs on myelination are mixed. Visual evoked potentials (VEP) have been used to assess LCPUFA-related differences in visual development; however, most have used VEP to measure visual acuity. Only a limited
number of investigations have reported VEP wave latencies, a measure hypothesized to reflect myelination of the visual pathway. One study found no differences in auditory (brainstem acoustic evoked potentials) or VEP wave latencies between preterm infants given PUFA supplemented formula (18 carbon) and infants given LCPUFA supplemented formula for 30 days (Bougle et al., 1999). Furthermore, preterm infants given an LCPUFA supplemented formula until six months adjusted age had no differences in global or visual myelination measured with MRI or in VEP latencies compared to preterm infants given non-supplemented formula (Van Wezel-Meijler et al., 2002). On the other hand, several studies have found electrophysiological differences associated with LCPUFAs in preterm infants. VEP wave latencies were shorter in preterm infants given LCPUFA supplemented formula compared to preterm infants given non-supplemented formula until three months adjusted age; however no differences were found in the auditory system (Faldella et al., 1996). In a study of prenatal supplementation with DHA, infants with higher DHA status at birth had shorter VEP latencies during the first four months of life (Malcolm, McCulloch, Montgomery, Shepherd, & Weaver, 2003). The association between LCPUFAs and VEP wave latencies is tenuous. Moreover, the evidence does not support that these limited positive findings in the visual system translate to myelination of other systems. It will be important to address the timing and duration of the LCPUFA supplementation on myelination in future research.

Iron. Iron deficiency in animal models results in loss of activity of enzymes involved in myelin lipid synthesis, accompanied by hypomyelination (Larkin & Rao,
Accordingly, there is electrophysiological evidence for iron-induced hypomyelination in children. Iron-deficient six-month-old infants have delayed latencies on auditory brainstem-evoked responses (Roncagliolo, Garrido, Walter, Peirano, & Lozoff, 1998), and children with a history of iron deficiency in infancy have delayed latencies in auditory brainstem responses and VEPs at ages three and four (Algarin, Peirano, Garrido, Pizarro, & Lozoff, 2003). Furthermore, striatal-dependent behavioral changes in the rodent model of early iron deficiency are associated with alterations in myelin-associated metabolites (Ward et al., 2007).

**Iodine and selenium.** Although myelination effects of iodine deficiency or selenium deficiency have not been investigated directly, hypothyroidism (which may be caused by iodine or selenium deficiency) is associated with abnormal myelination (Sethi & Kapil, 2004). Thyroid hormone is involved in regulating the transcription of proteins needed for myelination, including myelin basic protein and myelin associated glycoprotein (Farsetti, Desvergne, Hallenbeck, Robbins, & Nikodem, 1992; Rodriguez-Pena, 1999).

**Choline.** Choline, as phosphorylcholine, is found in sphingomyelin, a major lipid component of myelin (Colombo, Garcia-Rodenas, Guesry, & Rey, 2003). However, the effects of choline status on myelination have yet to be investigated.

**Vitamin B_{12}.** Developmental research on the role of folate and vitamin B12 on myelination is limited; however, both vitamin B_{12} and folate deficiencies in adults are associated with demyelination due to reduced methylation (Pandey, Kalita, & Misra, 2004; Surtees & Leonard, 1991). In a report of three cases of children with inborn errors
of either vitamin B₁₂ or folate metabolism, demyelination was associated with impaired methylation (Surtees & Leonard, 1991). Adults with vitamin B₁₂ deficiency have been shown to have delayed VEP latencies indicative of reduced myelination (Pandey et al., 2004).

**Functional Isolation Hypothesis: Motor Development, Activity, and Socioemotional and Exploratory Behaviors**

The association between undernutrition and cognitive development may be explained by the functional isolation hypothesis (e.g., Lozoff et al., 1998). According to this hypothesis, undernourished children have low levels of activity and reduced exploration with the environment which lead to, or exacerbate, hampered cognitive development. There is evidence for the role of motor skills in obtaining cognitive skills. For instance, infants who have more experience crawling perform better on A not B tasks compared to infants who have no or little experience crawling (Bell & Fox, 1997). Nutrient deficiencies that have marked effects on either motor development or activity and exploration include iron, zinc, and iodine and selenium. Deficiencies of these nutrients may have indirect effects on cognitive development.

The cerebellum and striatum are two structures involved in motor development and function and are vulnerable to several nutrient deficiencies. During late gestation and early infancy, both the striatum and cerebellum (Limperopoulos & du Plessis, 2006) undergo rapid development. However, motor development, the attainment of early motor milestones, and exploratory behaviors depend on more than neurodevelopment. Factors that are also altered by nutritional status, including energy, body proportionality and size,
neuromuscular strength, and practice, are important for motor development and exploration (Kariger et al., 2005). Despite the mechanisms, delayed motor development may impair cognitive development through decreased exploration and interaction with the physical and social environments and is important to consider when investigating the role of nutrient deficiencies on cognitive development.

**Iron.** Multiple well-controlled clinical studies in children ages 6-24 months with iron deficiency demonstrate significant decrements in motor achievement. Infants with iron deficiency anemia score on average 6 to 17 points lower on both gross and fine motor developmental assessments compared to infants without iron deficiency (Lozoff et al., 2006). Iron deficiency anemia is also associated with later attainment of walking in infants (Kariger et al., 2005). Furthermore, infants ages 5 to 19 months with iron deficiency, with or without anemia, were observed to spend less time in motor activity and locomotion at home, even after controlling for the attainment of walking (Olney et al., 2007).

In addition to iron’s effects on motor development, iron may contribute to decreased exploration with the environment through its role in monoamine metabolism. Monoamine metabolism involves iron dependent enzymes and animal studies which model iron deficiency in infancy show long-term monoamine alterations in the striatum (Beard & Connor, 2003). Decreased exploration as a result of early iron deficiency has been observed in rodents in a novel environment (Felt & Lozoff, 1996; Pinero, Jones, & Beard, 2001) and in one to two year old infants who were observed to be less playful, made fewer attempts on test items, were easily tired, and showed less pleasure (Lozoff et
al., 1998). Long-term attention problems have also been reported in adolescents who had ID anemia during infancy (Lozoff, Jimenez, Hagen, Mollen, & Wolf, 2000).

**Zinc.** Zinc is found in high concentrations in the cerebellum (Frederickson & Danscher, 1990), and zinc deficiency in rat pups affects cerebellum granule cells and purkinjie cells, reducing the number of cells and dendritic arborization (Dvergsten, Fosmire, Ollerich, & Sandstead, 1983, 1984; Dvergsten, Johnson, & Sandstead, 1984). Zinc is also found in the striatum (Vincent & Semba, 1989). Several animal models of zinc deficiency show reduced motor activity (Golub et al., 1994; Golub, Takeuchi, Keen, Hendrickx, & Gershwin, 1996). However, in a knockout study in mice which removed zinc from synaptic vesicles, no effects of were found on motor coordination (Cole, Martyanova, & Palmiter, 2001).

Zinc supplementation trials in children at risk for zinc deficiency have yielded variable results. During infancy, there is some implication that zinc supplementation improves motor development and promotes activity in the most severe cases of zinc deficiency (for review, see Black, 2003). Several randomized, double-blind, placebo-controlled studies have not found any effects of zinc on motor development. However, term infants born small for gestational age from a low-income population in Brazil who were supplemented with zinc during the first year had higher psychomotor developmental index (PDI) scores on the BSID compared to those receiving placebo (Ashworth, Morris, Lira, & Grantham-McGregor, 1998). Several other studies, which also did not find differences on motor development assessments, report qualitative differences in activity. For instance, supplementation for seven months in Guatemalan infants was associated
with differences in activity levels and patterns, despite no differences in the number of motor milestones (e.g., walking) attained between the zinc and control groups. Specifically, supplemented infants spent more time sitting up, less time lying down, and more time in play compared to the infants administered the placebo (Bentley et al., 1997). A similar study found children supplemented with zinc were more active and expended more energy (Sazawal et al., 1996).

Conversely, several randomized, double-blind, placebo-controlled studies have found effects of zinc on infant motor development in groups at risk for zinc deficiency. In a six month supplementation trial in very low birth weight infants, those receiving a zinc-copper supplementation had higher scores on the motor scale of the Griffiths Scales when followed every three months for one year (Friel et al., 1993). In another supplementation study, infants supplemented with iron and zinc (either with or without a micronutrient mix) for six months scored higher on the PDI of the BSID than infants given only iron, only zinc, or only the micronutrient mix (Black et al., 2004). There were no differences on the PDI of the BSID in a supplementation trial during the first year in term infants in Chile; however, a lower proportion in the supplemented group had motor dysfunction (below 11th percentile cutoff for BSID) than the placebo group, with gross & fine motor and control of movement affected (Castillo-Duran et al., 2001).

The mechanisms of zinc’s effect on motor development and activity are not currently known. While zinc is concentrated in the cerebellum and striatum, the mechanism of zinc’s effects on motor development and activity may be associated with non-neurodevelopmental factors; nonetheless reduced motor activity and exploration may
mediate the association between zinc deficiency and delayed cognitive development (Black et al., 2004).

**Iodine and selenium.** Thyroid hormone modulates gene expression in the cerebellum and in the striatum in perinatal rats (Iniguez et al, 1996). However, association of iodine and selenium deficiency with motor development is unclear. Mild to moderate iodine deficiency is associated with impairments in motor development (Aghini-Lombardi et al., 1995), and supplementation with iodine during childhood improves performance on fine motor tasks (Zimmermann et al., 2006). However, supplementation with iodine between 24 and 36 months did not improve fine motor skills at age six.

Although, the mediated effects of motor development and activity and exploration on cognitive development have not been studied directly, there is evidence for decreased activity and exploration in undernourished children, and future research in this area will be important.

**Conclusions: The Role of Postnatal Nutrition in Developing Cognitive Systems:**

**Direct and Indirect Effects**

Adequate intake and assimilation of all nutrients is important for brain health and function; however, several nutrients have more marked effects on brain development. Based on the timing, the extent of deprivation, and the pathophysiology of a given nutrient, specific hypothesis can be made regarding the effects of a given nutrient on specific cognitive systems. It will be important for future investigations to focus on precise cognitive outcomes as they may be more sensitive measures of the effects of
nutrient deficiencies. Furthermore, both direct and indirect effects of nutrition on developing cognitive systems need to be considered.

**Early Adverse Environment and Nutritional Risk in Internationally Adopted Children**

Children living in adverse environments are at an increased risk for nutrient deficiencies. However, it is difficult to isolate the role of *early* adversity on neurodevelopment in human populations, given that children raised in early adverse environments unfortunately continue to live in ongoing adversity that persistently threatens normal development. Internationally adopted children present a unique opportunity to study the effects of early nutritional deficiencies on neurodevelopment under relatively controlled conditions, given that the time of adoption into a stable environment clearly demarcates the end of a period of adversity.

Internationally adopted children often exhibit profound stunted growth and low weight for age at the time of adoption (Johnson et al., 1992; Miller & Hendrie, 2000). Although growth failure is well established in this population, little is known about the likely concurrent micronutrient deficiencies. Moreover, as a result of multiple risk factors experienced pre-adoption, many internationally adopted children arrive with neurodevelopmental delays (Johnson, 2000). Despite significant catch-up in general development, many of these children require special education services, suggesting that internationally adopted children continue to have deficits in more specific developmental domains (Gunnar, 2000; van Ijzendoorn & Juffer, 2005). There is marked heterogeneity in development in this population years after adoption. Most attempts to explain this
heterogeneity have focused on variations in duration (age at adoption) and the degree of adversity (Kreppner et al., 2007). However, recent longitudinal data have been unable to explain much of this variation. A potential mechanism to explain developmental outcomes in internationally adopted children which has not yet been investigated is micronutrient status.

Thus, the overall hypothesis of the following studies is that specific micronutrient deficiencies associated with early adversity will be present at arrival and will affect particular neurodevelopmental domains in internationally adopted children. The goals of this research are to assess (1) the nutritional status of internationally adopted children during the early adoption period and (2) the association between nutrient deficiencies and neurodevelopment during the early adoption period.

To meet these aims, three studies were performed. The first two studies, described in chapter II, examined iron status in children adopted from Eastern Europe. The first describes in detail the iron status at both arrival and at a six month follow-up in children under the age of 24 months at adoption. The second investigates the association between iron status and general cognitive and behavioral development in children adopted from Eastern Europe. The third study, described in chapter III, includes an assessment of both the macronutrient and micronutrient status at arrival of children adopted from three regions, Eastern Europe, Ethiopia, and China, and investigates the association between nutritional status and specific neurodevelopmental domains.

Although all the nutrients described above are important for neurodevelopment, these three studies focused on the overall macronutrient status and the micronutrients...
iron, zinc, folate, vitamin B₁₂, and iodine and selenium. Given the limited scope of this research, these studies do not include assessments of all of the aforementioned specific neurodevelopmental domains, but begin to investigate the role of nutrition in several focused domains and include both direct and indirect effects of nutrients on cognitive development. The developmental domains assessed included general cognitive and motor development as well as several specific neurodevelopmental domains, speed of processing and socioemotional and exploratory behaviors.
Chapter IIa: Iron Deficiency in International Adoptees from Eastern Europe

(Fuglestad et al., 2008)¹

In 2006, more than 19,000 children were adopted internationally into the U.S. (U.S. Department of State., 2008), with nearly 80% coming from countries which primarily offer institutional care for orphans. Growth failure is well-documented in international adoptees with approximately one month of linear growth lost for every three months in institutional care (Johnson et al., 1992). Following placement into more nurturing and nutritionally replete settings, the majority of stunted children experience a period of rapid physical catch-up growth (Johnson, 2000; Mason & Narad, 2005).

Despite the well-documented macronutrient abnormalities (i.e., stunted growth), information on micronutrient status of international adoptees is limited. Although it is one of the world’s most common nutrient deficiencies in children, iron deficiency (ID) has yet to be assessed in international adoptees following placement into their new adoptive homes. Previous studies in international adoptees from China and Guatemala indicated an anemia rate of approximately 30% at arrival in their adoptive country although the etiology of the anemia was undefined (Miller, Chan, Comfort, & Tirella, 2005; Miller & Hendrie, 2000). Furthermore, the rate of resolution of anemia after placement has not been investigated.

This study was conducted on a group of international adoptees from Eastern

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Europe to examine iron status at adoption and at follow-up six months post-adoption. At adoption, we expected a high rate of ID. Based on the documented macronutrient abnormalities of international adoptees it is likely that concurrent micronutrient deficiencies exist at adoption. At follow-up six months post-adoption, we expected iron status to improve due to placement into the nutrient-replete environments of their new homes. However, we also expected the high growth rates post-adoption, and the need for iron to support erythropoiesis during such high growth rates, to place additional stress on an already vulnerable iron system.

**Methods**

**Participants**

Participants were part of a larger longitudinal study on growth of children adopted into the U.S. from Eastern European institutionalization in orphanages or hospitals. This study was limited to participants entering at under 24 months of age. The study was approved by the Institutional Review Board of the University of Minnesota, and written informed consent was obtained from the adoptive parents. Participants who were between 6 and 24 months of age at adoption were recruited and sequentially enrolled through the International Adoption Clinic at the University of Minnesota between January 2005 and September 2006. Thirty two percent of the participants were low birth weight.

Exclusion criteria included major neurological problems, obvious congenital anomalies, non-iron-related hematologic disease, receipt of red blood cell supplementation or exchange transfusions, anemia not due to ID, and risk of fetal alcohol syndrome (FAS). Risk of FAS was based on assessment of facial features using FAS
Facial Photographic Analysis Software (FAS Diagnostic and Prevention Network, 2003) and routine clinical assessment at the International Adoption Clinic. None of the participants were identified as severe risk for FAS, and therefore none were excluded. The only participants excluded were three participants excluded for anemia not due to ID.

Of the 52 participants who met inclusion criteria, complete iron data sets at both time points were obtained for 37 participants. Five participants did not return for the follow-up assessment, and 10 participants had incomplete serum results or hemograms at either baseline or follow-up. Hemogram was missing for two participants due to unsatisfactory specimen collection or processing (clotting). The remaining eight did not have full iron batteries drawn. Although iron status was indeterminate for these 15 participants, there were no differences in demographics or anthropometrics between the 15 participants not included in analyses and the 37 participants whose data were analyzed.

**Study Design**

Participants were seen twice; the baseline assessment was done upon arrival into the U.S. and the follow-up assessment at six months post-adoption. Anthropometric and iron data were collected at both time points during the routine clinic visits for international adoptees at the International Adoption Clinic, University of Minnesota.

Iron status was measured using hemoglobin concentrations (Hgb), mean corpuscular volumes (MCV), percent transferrin saturation (%TS), and serum ferritin concentrations (SF). Mean values of each iron index were compared to the means for all children ages one and two years reported by the Third National Health and Nutrition
Examination Survey (NHANES III) (Hollowell et al., 2005). ID was defined as having two or more abnormal values for MCV, %TS, and/or SF. The cutoffs for the indices Hgb, %TS, and SF were based on NHANES III data (Looker, Dallman, Carroll, Gunter, & Johnson, 1997) and Healthy People 2010 (U.S. Department of Health and Human Services, 2000). Healthy people 2010 does not include cutoffs for MCV, and therefore, the cutoff for MCV was based on NHANES II data and Healthy People 2000 (Expert Scientific Working Group, 1985; National Center for Health Statistics, 2001). The cutoffs for the iron indices are as follows: Hgb < 11.0 g/dL, MCV < 74 fL, %TS < 10%, and SF < 10 µg/L. Treatment for frank iron deficiency anemia (IDA) was done at the discretion of the International Adoption Clinic, independent of the study results. The general practice of the International Adoption Clinic is to treat IDA, but not ID without anemia, with 2 to 3 mg/kg elemental iron for two months. Two children enrolled in the study were diagnosed with frank IDA and were treated with supplemental iron. The first had IDA at baseline and was treated with 1.4 mg/kg of iron daily using a multivitamin preparation with iron in addition to an iron supplemented formula, and her anemia resolved at the six month follow-up. The second was diagnosed at follow-up and was prescribed 2.2 mg/kg of iron daily as a ferrous sulfate solution but was not followed further in the International Adoption Clinic after treatment initiation.

Iron intake was assessed using parental report of a 3-day food diary for the participants at both assessments. Average iron intake was determined using the University of Minnesota’s Nutrition Coordinating Center’s Nutrition Data Systems for Research software (Nutrition Coordinating Center, 1998-1999) along with the Statistical
Analysis System (SAS Institute Inc, Cary, NC) for the computation of final averages. The resulting averages for iron intake were compared to Recommended Dietary Allowances (RDAs) (Food and Nutrition Board, 2001) and U.S. population intake norms (Alaimo et al., 1994).

Anthropometry was performed by the staff at the International Adoption Clinic at baseline and follow-up and included length (taken in triplicate and averaged), weight (in single), and occipitofrontal circumference (in single). Z scores were calculated based on CDC 2000 norms using Epi Info 3.3 (CDC, 2004). Catch-up growth was defined as a change in length $z$ score from baseline to follow-up of greater than +0.5.

Intestinal parasitic infections, including *Giardia lamblia* (*G. lamblia*), were screened as part of the routine medical screening at the International Adoption Clinic. Stool for ova & parasites or assays for *G. lamblia* antigen was used to detect infection. The only positive finding was for *G. lamblia*. *G. lamblia* was assessed only at baseline and was known for 32 of the 37 participants. Thirty-one percent ($n = 10$) were positive for *G. lamblia* at baseline. Children positive for *G. lamblia* were treated with Nitazoxanide (Alinia) suspension 100mg twice daily for three days and were rechecked two to four weeks later to be sure the infection had cleared.

**Biochemical Assays**

The battery of iron status indices comprised SF, %TS, hematocrit, Hgb, MCV, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and red cell distribution width (RDW). SF, %TS, MCV, and Hgb were used to evaluate iron status. Whole blood was used by the Fairview Hematology Laboratory of the University of
Minnesota Children’s Hospital to determine Hgb and MCV with a Beckman Coulter® LH system (Beckman Coulter, Fullerton, CA). The serum was centrifuged and assayed for SF and %TS by the Fairview Protein Laboratory of the University of Minnesota Children’s Hospital. SF was determined by chemiluminescent immunoassay, and %TS was measured by nephelometry. Hgb, MCV, SF, and %TS were done in single using random assay instruments. Assays were done using standard CLIA certified techniques, all of which have interassay variabilities <5%.

As a part of an acute phase response, the elevation of ferritin concentration has been known to cause underestimation of the prevalence of IDA (Wieringa, Dijkhuizen, West, Northrop-Clewes, & Muhilal, 2002). Thus, C-reactive Protein (CRP), an inflammatory marker, was assessed at both baseline and follow up to determine whether the SF concentrations were reliable reflections of iron stores. All CRP levels were <2.0mg/dL at baseline ($M = 0.18, SD = 0.35$) and at follow-up ($M = 0.25, SD = 0.19$).

The participants were screened for elevated lead levels at baseline. All lead concentrations were < 10μg/dL ($M = 1.65, SD = 0.99$), and lead concentrations were not correlated with the iron indices.

**Data Analyses**

Comparisons of parametric variables were assessed by $t$-tests, while non-parametric variables were assessed by $\chi^2$ analyses. Multiple regression was used to assess the relationship between growth and iron status. Statistical significance was set at an alpha of 0.05.

**Results**
Demographics (Table 1)

Baseline assessment was completed within 30 days of arrival into the U.S. \((M = 17, SD = 6)\) and follow-up assessments were completed six months post-arrival \((M = 6.14, SD = 0.41)\). Age at baseline was correlated with duration of institutionalization, \(r = 0.67, p < 0.001 \) \((n = 35; \) pre-adoption histories were not known for two participants\), indicating that the majority of time prior to adoption was spent in institutional care.

Iron Deficiency at Baseline and Follow-up (Table 2)

SF at baseline was included in analyses for 36 of the 37 participants; one result that fell more than seven standard deviations above the mean was excluded. Baseline \%TS, MCV, and Hgb for all 37 participants were included in analyses.

At baseline and follow-up, mean MCV and \%TS were lower than the mean values reported by NHANES III. At follow-up only, mean SF was lower than the NHANES III mean, while mean Hgb was higher than the NHANES III mean (Table 2). The rate of ID was 25% at baseline and 16% at follow-up (Table 2). ID did not change significantly between baseline and follow-up, \(t(36) = 0.90, p = 0.37 \).

To potentially explain the high rate of ID at baseline and the lack of significant improvement in iron status during the first six months post-adoption, daily iron intake, post-adoption catch-up growth, and the presence of parasitic disease (specifically \(G. lamblia\)) were considered as etiologies.

Daily Iron Intake

Daily iron intake was available for all participants at baseline and for 33 participants at follow-up; the missing data were due to incomplete food diaries. Mean
daily iron intake at both baseline and follow-up were more than the RDA and more than
the U.S. average daily intake (Table 1). Mean differences in daily iron intake did not
differ between those who were iron deficient \( (n = 9) \) compared to those who were iron
sufficient \( (n = 27) \) at baseline \( (M = 15.7 \text{ mg/day}, SD = 7.9 \) and \( M = 13.8 \text{ mg/day}, SD =
6.2 \) respectively, \( t(34) = -0.76, ns \) \) nor did mean daily iron intake differ between those
who were iron deficient \( (n = 5) \) and those who were iron sufficient \( (n = 28) \) at follow-up
\( (M = 14.2 \text{ mg/day}, SD = 5.5 \) and \( M = 12.3 \text{ mg/day}, SD = 7.6 \) respectively, \( t(31) = -0.54,
ns \)). Iron intake at baseline was positively correlated with change in Hgb, \( r = 0.47, p < 0.01 \),
and change in MCV, \( r = 0.50, p < 0.01 \), and was negatively correlated with change
in RDW, \( r = -0.38, p < 0.05 \), over the six months.

**Anthropometrics/ Post-adoption Catch-up Growth**

Ninety-five percent \( (n = 35) \) demonstrated positive change in length \( z \) score
between baseline and follow-up (Table 1). Mean growth rate (change in length \( z 
\) score/months between assessments) was 0.12 \( z \) score \( (SD = 0.08) \), with 65\% \( (n = 24) \)
demonstrating catch-up growth (>0.5 change in \( z \) score) in length from baseline to
follow-up. To test whether iron stores at baseline predicted the amount of post-adoption
growth, the correlation between SF at baseline with growth rate was analyzed, and no
correlation was found \( (r = -0.14, ns) \). To test the specific effect of rapid growth rate on
iron stores indexed by SF, multiple regression analyses were used, and only those who
had positive change in length \( z \) score were included \( (n = 35) \). Growth rate was negatively
associated with change in SF between baseline and follow-up, \( \beta = -0.34, t(32) = -2.05, p 
< 0.05 \) (Figure 1). This association was effectively unchanged when controlling for daily
iron intake at baseline, \( \beta = -0.32, t(31) = -1.80, p = 0.08 \), and iron intake did not predict changes in SF, \( \beta = 0.09, \text{ns} \).

**Infectious Disease**

Of the 32 participants for whom *G. lamblia* was known, 31% \((n=10)\) were positive. Those positive for *G. lamblia* had more compromised iron status than those negative for *G. lamblia* (Table 3).

**Discussion**

This study found compromised iron status in international adoptees at adoption, and over the ensuing six months international adoptees failed to show significant improvement in their iron status in spite of adoption into presumably nutritionally adequate environments and rapid correction of growth deficits. This lack of recovery in iron status was exacerbated by rapid post-adoption growth rates and *G. lamblia* infection at baseline.

The higher rate of ID at the time of adoption may be attributed to several factors. The first is inadequate endowment of iron stores at birth. Infants are born with iron stores proportional to their birth weight. Premature and intrauterine growth retarded infants are born with lower absolute iron stores than term infants, placing them at higher risk for earlier postnatal ID (Georgieff, Mills, Gordon, & Wobken, 1995; Widdowson & Spray, 1951). Thirty-two percent of the children in this study were low birth weight for either reason and would have been at risk for low fetal iron endowment (Georgieff, 2006). Second, dietary intake of iron prior to adoption would influence iron status at adoption.
Unfortunately, little reliable information was available on the prenatal, birth, and institutional dietary histories of this sample and therefore was not addressed in this study.

Iron status is not only a function of iron intake but also of iron absorption and distribution. For example, the anemias of chronic inflammation and chronic disease occur in individuals with chronic inflammatory diseases or immune responses. These anemias are characterized by hepcidin–mediated reduced intestinal iron absorption resulting in less available iron for erythropoiesis (Horl, 2007). International adoptees experience a wide range of social and health adversities prior to adoption which may affect iron metabolism, similar to that in anemias of chronic inflammation and chronic disease. In our cohort, iron status at adoption was partly determined by parasitic infection as those infected with the parasite *G. lamblia* had a more compromised iron status than those not infected. *G. lamblia* likely causes micronutrient deficiencies by decreasing absorption (Luján et al., 1995; Troeger et al., 2007). Although the *G. lamblia* infection cleared prior to the follow-up assessment, those infected at baseline continued to have compromised iron status at follow-up likely due to the compromised iron status at baseline paired with the increased iron requirements of post-adoption catch-up growth. Thus, continued surveillance of iron status post-adoption is necessary regardless of gastrointestinal infestation status.

During the first six months-post adoption, this sample experienced the typical catch-up growth in both length and weight that has been previously documented (Johnson, 2000; Mason & Narad, 2005). In accordance with this improvement in macronutrient status, we anticipated iron status to also improve. Instead, despite
placement into post-adoption environments where the RDA for iron was met, compromised iron status persisted six months post-adoption. The lack of improvement may be explained by the degree of ID at adoption, iron demand and utilization, and ongoing *G. lamblia* infection.

Iron stores as indexed by SF were on average below normal at follow-up, and despite mean daily iron intakes above the RDA at both one month- and six months-post-adoption, iron intake was not associated with improvements in iron stores (SF). However, iron intake was related to slight improvements in functional indices (Hgb, MCV, RDW), indicating that dietary iron was likely being used for erythropoiesis. The dietary intake was not sufficient to also replete iron stores (SF) during post-adoption catch-up growth.

Consistent with other studies that have shown rapid growth to increase iron demand (Georgieff, Wewerka, Nelson, & deRegnier, 2002) this study found that the high rates of post-adoption catch-up growth reduced iron stores as indexed by the SF concentration; the greater the catch-up growth during the first six months post-adoption, the more SF decreased from baseline to follow-up. This cohort maintained compromised iron status without anemia as evidenced by low iron stores and saturations in order to maintain erythropoiesis during the rapid blood volume expansion accompanying high rates of catch-up growth. An alternative explanation for this negative association between change in SF and catch-up growth is that excessive iron intake, and thus the presence of greater iron stores, inhibits growth in children who are already iron sufficient (Iannotti, Tielsch, Black, & Black, 2006; Idjradinat, Watkins, & Pollitt, 1994; Majumdar, Paul, Talib, & Ranga, 2003). However, SF at baseline in this cohort was relatively low (<50th
percentile) suggesting that children were not iron overloaded at arrival. Furthermore, SF at study entry was not related to the amount of subsequent post-adoption catch-up growth indicating that iron status did not suppress growth in this sample.

As a micronutrient critical for both erythropoiesis and brain development, iron is vital particularly in the first few years of life when rapid growth and development are occurring. During periods of rapid catch-up growth following growth restriction, iron is preferentially shunted away from storage (e.g. liver) and non-storage (e.g. heart and brain) tissues as it is put towards the increased requirements for erythropoiesis that accompanies the growth spurt (Alaimo et al., 1994). ID, not only IDA but also deficiency of lesser severity, may have long-term effects on brain development and subsequent cognitive and behavioral development (Lozoff et al., 2006; Lozoff et al., 2000). Given the effects of ID on brain development, the compromised iron status we found in Eastern European adoptees, and the developmental delays reported in international adoptees (Beckett et al., 2006; Gunnar, 2000), screening for ID without anemia and IDA is warranted in international adoptees who are at risk for compromised iron endowment followed by a period of rapid catch-up growth. Furthermore, international adoptees should be screened for iron status not only at the time of adoption but subsequently after catch-up growth has been observed. A reassessment of dietary iron requirements during the period of rapid growth in this population may be indicated since it appears that the standard RDA and the average intake are not adequate to improve iron status in the immediate period following adoption. A trial of medicinal iron in children who have ID without anemia will be important to address whether the compromised iron
status in international adoptees is due to the limited amount of dietary iron available to them or to their inability to absorb it adequately. In addition, it is important to screen for and treat *G. lamblia* in international adoptees. Infants who are positive for *G. lamblia* at the time of adoption need to have their iron status closely monitored and even though the treatment of *G lamblia* may appear successful, affected children still need their iron status monitored post-adoption.
Table 1
Summary Demographics, Daily Iron Intake, and Anthropometrics

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Range</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time in Institution (months)</td>
<td>9 – 24</td>
<td>15 ± 3</td>
</tr>
<tr>
<td>Age at Baseline (months)</td>
<td>8.94 - 22.63</td>
<td>15.70 ± 3.45</td>
</tr>
<tr>
<td>Age at Follow-up (months)</td>
<td>14.91 - 29.20</td>
<td>21.84 ± 3.57</td>
</tr>
<tr>
<td>Time in US before baseline (days)</td>
<td>7 - 30</td>
<td>17 ± 6</td>
</tr>
<tr>
<td>Time between baseline &amp; follow-up (months)</td>
<td>5.09 - 6.97</td>
<td>6.14 ± 0.41</td>
</tr>
<tr>
<td>Percent Low Birth Weight (&lt;2500 grams)</td>
<td>32%</td>
<td></td>
</tr>
<tr>
<td>Sex (n)</td>
<td>Female: 22, Male: 15</td>
<td></td>
</tr>
<tr>
<td>Country of origin (n)</td>
<td>Russia: 32, Ukraine: 3, Kazakhstan: 2</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Daily Iron Intake (mg/day)</th>
<th>Baseline (n=37)</th>
<th>Follow-up (n=33)</th>
<th>RDA</th>
<th>US average daily intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (months)</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-12</td>
<td>19.55 ± 8.05; n=5</td>
<td>12.58 ± 7.29</td>
<td>11</td>
<td>15.5</td>
</tr>
<tr>
<td>12+</td>
<td>13.54 ± 6.01; n=32</td>
<td>9.53</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Percent below RDA</td>
<td>11% (n=4)</td>
<td>21% (n=7)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Anthropometrics (z scores)</th>
<th>Baseline</th>
<th>Follow-up</th>
<th>Rate of change between baseline and follow-up (change in z score/months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length</td>
<td>-1.24 ± 0.89</td>
<td>-0.49 ± 0.87</td>
<td>0.12 ± 0.08</td>
</tr>
<tr>
<td>Weight</td>
<td>-1.73 ± 1.00</td>
<td>-0.53 ± 1.07</td>
<td>0.19 ± 0.14</td>
</tr>
<tr>
<td>Weight for Length</td>
<td>-0.63 ± 1.06</td>
<td>-0.02 ± 1.18</td>
<td>0.11 ± 0.19</td>
</tr>
<tr>
<td>OFC</td>
<td>-0.67 ± 1.03</td>
<td>0.11 ± 1.20</td>
<td>0.13 ± 0.11</td>
</tr>
</tbody>
</table>

Note: RDA is based on Food and Nutrition Board (2001) and US average daily intake is based on Alaimo et al., (1994).
Table 2
Iron Indices at Baseline and Follow-up

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Follow-up</th>
<th>NHANES III&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hgb (g/dL)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD (range)</td>
<td>12.3 ± 0.9 (10.4–14.5)</td>
<td>12.5 ± 0.9 (10.8–14.9)&lt;sup&gt;**&lt;/sup&gt;</td>
<td>12.0 ± 0.8 (10.5 –13.6)</td>
</tr>
<tr>
<td>% (n) &lt; 11.0 g/dL</td>
<td>8% (&lt;i&gt;n&lt;/i&gt;=3)</td>
<td>3% (&lt;i&gt;n&lt;/i&gt;=1)</td>
<td></td>
</tr>
<tr>
<td><strong>MCV (fl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD (range)</td>
<td>76 ± 5 (64–84)&lt;sup&gt;***&lt;/sup&gt;</td>
<td>78 ± 4 (69–87)&lt;sup&gt;**&lt;/sup&gt;</td>
<td>80 ± 5 (69–87)</td>
</tr>
<tr>
<td>% (n) &lt; 74 fl</td>
<td>30% (&lt;i&gt;n&lt;/i&gt;=11)</td>
<td>14% (&lt;i&gt;n&lt;/i&gt;=5)</td>
<td></td>
</tr>
<tr>
<td><strong>%TS (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD (range)</td>
<td>13 ± 7 (1–28)&lt;sup&gt;***&lt;/sup&gt;</td>
<td>13 ± 7 (3–33)&lt;sup&gt;***&lt;/sup&gt;</td>
<td>18 ± 10 (4–39)</td>
</tr>
<tr>
<td>% (n) &lt; 10%</td>
<td>35% (&lt;i&gt;n&lt;/i&gt;=13)</td>
<td>35% (&lt;i&gt;n&lt;/i&gt;=13)</td>
<td></td>
</tr>
<tr>
<td><strong>SF&lt;sup&gt;b&lt;/sup&gt; (µg/L)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD (range)</td>
<td>23 ± 15 (8–86)</td>
<td>20 ± 12 (5–65)&lt;sup&gt;**&lt;/sup&gt;</td>
<td>26 ± 23 (3–73)</td>
</tr>
<tr>
<td>% (n) &lt; 10 µg/L</td>
<td>3% (&lt;i&gt;n&lt;/i&gt;=1)</td>
<td>16% (&lt;i&gt;n&lt;/i&gt;=6)</td>
<td></td>
</tr>
</tbody>
</table>

Iron Deficiency: 2 or more abnormal indicators (MCV, %TS, SF)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24% (&lt;i&gt;n&lt;/i&gt;=9)</td>
<td>16% (&lt;i&gt;n&lt;/i&gt;=6)</td>
</tr>
</tbody>
</table>

Note.  
<sup>a</sup> Range for NHANES III is 2.5<sup>th</sup> percentile to 97.5<sup>th</sup> percentile;  
<sup>b</sup> <i>n</i>=36 at baseline for SF  
Using one sample <i>t</i>-test to compare the means for the iron indices at baseline and follow-up to the means reported in NHANES III, differences are indicated as follows: * <i>p</i> < 0.05, **<i>p</i> < 0.01, ***<i>p</i> < 0.001.
Table 3
Iron Indices for Those With and Without G. lamblia

<table>
<thead>
<tr>
<th>Mean ± SD</th>
<th>Baseline</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive for G. lamblia (n=10)</td>
<td>Negative for G. lamblia (n=22)</td>
</tr>
<tr>
<td>Hgb (g/dL)</td>
<td>12.2 ± 0.9</td>
<td>12.3 ± 1.0</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>76 ± 4</td>
<td>77 ± 5</td>
</tr>
<tr>
<td>TS (%)</td>
<td>9 ± 3*</td>
<td>15 ± 8*</td>
</tr>
<tr>
<td>SF (μg/L)^a</td>
<td>26 ± 24</td>
<td>24 ± 10</td>
</tr>
</tbody>
</table>

Iron Deficiency ^b 30% (n=3) 14% (n=3) 40% (n=4)* 9% (n=2)*

Note. Using independent samples t-test to compare the means for the iron indices between those positive and those negative for G. lamblia, differences are indicated as follows: * p < 0.05. ^a n=36 at baseline for SF; ^b 2 (+) abnormal indicators.
Figure 1. Correlation between growth rate and change in SF between baseline and follow-up in participants with positive change in $z$ scores between baseline and follow-up ($r^2 = 0.12$).
Chapter IIb: The Role of Iron Deficiency in General and Behavioral Development in Children Adopted from Eastern Europe

At the time of adoption, many internationally adopted children arrive with delays in general neurobehavioral development (Johnson, 2000). Although they demonstrate considerable resiliency and exhibit improvements in general development (Rutter & the English and Romanian Adoptees Study Team, 1998), they nevertheless have marked heterogeneity in outcome with specific long-term neurobehavioral and cognitive problems (Kreppner et al., 2007). Problems that seem to be particularly sensitive to the experience of early institutional care include multiple mental health problems, emotional issues, and learning problems, including poor school performance (Beckett et al., 2007; Colvert, et al., 2008; Kreppner et al., 2007). However, the most consistent behavioral finding in this population is inattention, overactivity, and executive function impairments (Colvert, et al., 2008; Stevens et al., 2008).

Multiple risk factors prior to adoption, such as poor health care, prenatal alcohol exposure, social deprivation, lack of cognitive stimulation, and malnutrition may interrupt normal development and contribute to the heterogeneity of outcomes. Protein-energy malnutrition is well-documented in internationally adopted children and is evidenced by growth failure (i.e., stunted linear growth and low weight for age) at the time of arrival into the U.S. (Johnson, 2000). However, the degree of macronutrient malnutrition, as indexed by weight for age, is a poor predictor of developmental outcomes in this group (Sonuga-Barke et al., 2008).
Populations with protein-energy malnutrition usually have concurrent micronutrient deficiencies, defined as imbalances of particular vitamins, elements, or minerals. Micronutrient malnutrition may also occur independently of macronutrient malnutrition, thus affecting development in children who are of normal weight. Compared to macronutrient status, much less is known about micronutrient malnutrition at the time of adoption. There is however evidence that iron deficiency (ID) is common during the early post-adoption period (Fuglestad, et al., 2008).

ID has an adverse impact on neurobehavioral development in other populations (for review, see Lozoff & Georgieff, 2006). ID is associated with behavioral and cognitive deficits that are similar to those reported in internationally adopted children. These include emotional problems, such as increased wariness and fearfulness and decreased positive affect (Lozoff et al., 2008; Lozoff et al., 1998), poor school performance, executive function impairments, and increased attention problems years after iron repletion (Lozoff et al., 2000; Peirano, Algarin, Garrido, & Nunez, 2004).

The goal of this study was to investigate whether ID has an effect on cognitive and behavioral development in internationally adopted children during the early post-adoption period and to assess the relationship between behavioral abnormalities and cognitive performance. It has been suggested that impaired behavioral regulation in children with ID negatively affects their cognitive performance (Lozoff et al., 1998). Some studies find that short-term iron therapy in infants with ID improves their cognitive performance (Oski & Honig, 1978; Walter, Kovalskys, & Stekel, 1983), as well as their cooperation and attention (Walter et al., 1983) and alertness and responsiveness (Oski &
Honig, 1978) during testing, but others do not. In internationally adopted children, there is emerging evidence that their poorer school performance is in part mediated by behavioral problems, specifically inattention and overactivity (Beckett et al., 2007; van Ijzendoorn, Juffer, & Poelhuis, 2005).

**Methods**

**Participants**

Participants were part of a larger longitudinal study on neuroendocrine functions of children adopted into the U.S. from Eastern European institutionalization in orphanages or hospitals (Miller et al., 2009). The study was approved by the Institutional Review Board of the University of Minnesota, and written informed consent was obtained from the parents. Participants who were adopted from Eastern Europe were recruited and enrolled through the Adoption Medicine Program and Clinic (formerly the International Adoption Clinic) at the University of Minnesota between January, 2005, and March, 2007.

The inclusion criteria were having iron data at both the baseline and follow-up assessments at the Adoption Medicine Program and Clinic. Exclusion criteria were major neurological problems and major health problems. Three were excluded due to a high risk for fetal alcohol syndrome (FAS). Risk of FAS was based on assessment of facial features by using FAS Facial Photographic Analysis Software (FAS Diagnostic and Prevention Network, 2003) and routine clinical assessment at the Adoption Medicine Program and Clinic. Participants included in analyses (N=57) were between the ages of 8.9–45.6 months at the initial assessment. The mean age was 19.4 (SD=8.7), and the
majority were under 24 months of age, consistent with previous ID research. The iron status of 37 was previously reported (Fuglestad, et al., 2008).

**Study Design**

Participants were seen twice. The baseline assessment was within one month of arrival into the U.S., and the follow-up assessment was six months later. At both time points, nutritional data were collected during the routine medical evaluations at the Adoption Medicine Program and Clinic and the Mullen Scales of Early Learning (Mullen, 1995) was administered during a research session on the same day. Parents completed the Toddler Behavior Assessment Questionnaire-R (Goldsmith, 1996) at home and returned the questionnaire at both time points.

**Measures**

**Nutritional assessments.** Anthropometry was used to assess macronutrient status and included height (recumbent length under 24 months of age), weight, and occipitofrontal circumference (OFC). Z scores, including height-for-age z score (HAZ), weight-for-age z score (WAZ), weight-for-height z score (WHZ), and OFC-for-age z score (OFCZ) were calculated using CDC 2000 norms with Epi info 3.3 (CDC, 2004).

ID was defined as two or more abnormal indices: transferrin saturation (TS) < 12%, serum ferritin (SF) < 12 µg/L, mean corpuscular volume (MCV) < 74 fL, and hemoglobin (Hgb) < 11.0g/dL. ID with anemia was defined as ID with Hgb < 11.0g/dL. Threshold values to define ID were based on NHANES II (Expert Scientific Working Group, 1985), NHANES III (Looker, Dallman, Carroll, Gunter, & Johnson, 1997), and Healthy People 2000 (National Center for Health Statistics, 2001) and 2010 (U.S.)
Department of Health and Human Services, 2000). The thresholds are consistent with the definition of ID used in recent studies examining ID and child development (e.g., Lozoff et al., 2008). Treatment for frank iron deficiency anemia (IDA) was done at the discretion of the Adoption Medicine Program and Clinic, independent of the study results. The general practice of the Adoption Medicine Program and Clinic is to treat IDA, but not ID without anemia, with 2 to 3 mg/kg elemental iron for two months.

**Mullen Scales of Early Learning.** Four subscales (fine motor, visual reception, expressive language, and receptive language) of the Mullen Scales of Early Learning were administered to assess general development. At the baseline assessment, the language scales were not administered as these scales are specific to the English language, and participants had only been in the U.S. and exposed to English for a short time. The individual subscales use a t score distribution with a mean of 50 (SD=10). At the follow-up assessment, the four subscales were used to calculate the Mullen Early Learning Composite Score, which is on a scale with a mean of 100 (SD=15). Scores below 85 (-1 SD) are considered below average.

**Examiner Behavior Rating.** For a subsample at the follow-up assessment (n=47), the examiner rated the child’s behavior immediately following administration of the Mullen Scales of Early Learning to document behaviors during testing. The Mullen Scales of Early Learning does not include a behavior rating scale. Thus, a behavior scale was created using items from the Behavior Rating Scale of the Bayley Scales of Infant Development II (Bayley, 1993) as well as several additional items (Table 4). The
behaviors were rated on a scale of zero to five, with higher scores indicating more optimal behaviors.

**Toddler Behavior Assessment Questionnaire-R (TBAQ-R).** Parents completed the TBAQ-R, a 110 item questionnaire, from which ten temperament scores are calculated. Scores range from one to seven, with higher scores indicating a greater frequency of the given behavior. The TBAQ-R is designed for children between the ages of 16 and 36 months. To justify using this questionnaire for a broader age range, reliability of each of the scales was calculated using Crohnbach’s alpha, and values ranged from 0.68 to 0.83 for each scale.

**Data Reduction**

Prior to comparing the behavioral variables between those with ID and those with iron sufficiency, the behavioral variables from the TBAQ-R and the examiner behavior ratings were combined respectively to create behavioral composites. Groupings were made to address the behaviors that have been reported in previous research and to address the potential neurobiology of ID. Three behavior domains from the examiner ratings were determined a priori and were created to assess specific behavior domains that have been found in previous research to be associated with iron status, specifically, activity and cooperation, fearfulness, and positive affect. Four behavior domains from the TBAQ-R were also determined a priori, and included activity and impulsivity, attention, fearfulness, and positive affect. The behavior domains from the TBAQ-R differed from the examiner-rating in that attention and activity were separated into two domains. The behavior composites were calculated using the average of the behavioral variables which
fit theoretically with each behavior domain. Cronbach’s alpha was used to assess the reliability of each scale and are presented in Table 4. TBAQ-R scores and examiner ratings were combined separately (Table 4).

**Missing Behavioral Data**

Not all participants had complete behavioral data. Missing data on the TBAQ-R were due to parents not completing and returning the questionnaire (baseline: \( n = 14 \); follow-up: \( n = 2 \)). Missing data on the Mullen Scales of Early Learning were due to the child not being able to finish the test (follow-up: \( n = 1 \)). There were no differences in age at adoption or nutritional assessments (anthropometry or iron data) between those with missing data and those with complete data. \( Ns \) are reported for all analyses.

**Statistical analysis**

\( T \) tests and analysis of variance (ANOVA) were used for continuous variables. McNemars test of related proportions was used to compare proportions of ID between the initial and the six month assessment. Multiple regression analyses were used to examine a meditational model between iron status, behavior, and general development. Binary logistic regression was used to predict binary outcomes. All results with an alpha level of 0.10 are reported.

Age at adoption, baseline HAZ, and baseline OFCZ were included as covariates in analyses when examining behavioral outcomes based on iron status. Given that most of the children (more than 85% of the sample) were placed in institutional care at birth, age at adoption was used as a marker of the length of deprivation. The degree of growth failure at adoption has been used as a marker of the severity of deprivation experienced
prior to adoption (Kertes, Gunnar, Madsen, & Long, 2008). Thus, HAZ at baseline was included in analyses. Finally, due to the small mean OFCZ at baseline (i.e., below zero z score), the presence of microcephaly (<-2 z scores) in some participants, and the potential effects of microcephaly on cognitive and behavioral development (Dolk, 1991; Stoler-Poria, Lev, Schweiger, Lerman-Sagie, & Malinger, 2010), baseline OFCZ was included in all analyses.

Results

Sample Characteristics and Nutritional Assessments

Sample characteristics and results of the nutritional assessments are presented in Table 5. There were significant improvements in HAZ, \( t(56) = -10.42, p<0.001 \), WAZ, \( t(56) = -6.82, p<0.001 \), WHZ, \( t(56) = -2.43, p<0.05 \), and OFCZ, \( t(56) = -6.73, p<0.001 \), between baseline and follow-up. At the baseline assessment, 26% (n=15) were iron deficient, the majority without anemia. There were no differences between those with ID and those with iron sufficiency at the initial assessment in HAZ, WAZ, WHZ, or OFCZ. Those with ID at arrival were younger at adoption than those with iron sufficiency, \( t(55) = 2.84, p<0.01 \).

Unlike the improvements in macronutrient status, no improvement in iron status occurred from baseline to follow-up, \( \chi^2(1) = 1.23, ns \). Of those who had ID at follow-up, six had ID at baseline and four developed ID from baseline to follow-up. Only WHZ was different at the follow-up assessment between those who were iron deficient and those who were iron sufficient at follow-up, \( t(55) = -2.07, p<0.05 \) (Table 6). Similar to our previous finding in a subsample who were under 24 months of age at adoption
(Fuglestad, et al., 2008), iron status was associated with post-adoption catch-up growth. Increased weight gain over the six months was negatively correlated with changes in iron stores (SF), \( r = -0.34, p<0.05 \), and those with ID at follow-up demonstrated more catch-up growth in WAZ, \( t(55) = -3.50, p=0.001 \), WHZ, \( t(55) = -3.80, p<0.001 \), and OFCZ, \( t(56) = -2.50, p<0.05 \), from baseline to follow-up (Table 6).

**Behavioral Assessments**

Results for the behavioral assessments and baseline iron status are presented in Table 7. For the Mullen Scales of Early Learning, ID at baseline was not associated with any of the subscales or the Early Learning Composite at either the baseline or the follow-up assessment. There was a trend for those with ID at baseline to be rated by the examiner as more fearful during testing at follow-up, \( F(1,42) = 2.90, p=0.096 \). There was also a trend for participants with ID at baseline to be rated by their parents on the TBAQ-R as more fearful at baseline than those with iron sufficiency, \( F(1,38) = 3.58, p=0.066 \).

Results for the behavioral assessments and follow-up iron status are presented in Table 8. Participants with ID at follow-up had lower mean scores on the Mullen Early Learning Composite, \( F(1,51) = 7.09, p=0.010 \). Logistic regression was used to examine the clinical significance (i.e., scoring in the ‘below average’ range) of this difference on the Mullen Early Learning Composite. Iron status at follow-up, age at adoption, baseline HAZ, and baseline OFCZ were used as predictors and whether or not the participants scored below average on the Mullen Early Learning Composite at follow-up as the dependent variable. This model was statistically significant when compared to the model
with intercept only, $\chi^2(4)=20.71$, $p<0.001$. ID at follow-up was associated with a greater likelihood to score below average on the Mullen Early Learning Composite, odds ratio (OR) =16.06, 95% confidence interval (CI) = 2.51 – 102.67, $p < 0.01$. Eighty percent ($n=8$) of those with ID scored below average compared to 32% ($n=15$) of those who were iron sufficient. Older age at adoption, OR=1.1, 95% CI=1.02 – 1.19, $p < 0.05$, and smaller OFCZ, OR=0.42, 95% CI=0.18 – 0.97, $p < 0.05$, were also associated with a greater likelihood to score below average,

For the examiner behavior rating, those with ID at follow-up scored lower on activity and cooperation (high scores indicate more optimal functioning), $F(1,42) = 9.74$, $p<0.010$. Specifically, those with ID displayed more activity and impulsivity with less cooperation and attention during testing than those with iron sufficiency. Those with ID also exhibited less positive affect during testing, $F(1,42) = 4.02$, $p=0.05$. On the TBAQ-R at follow-up, those with ID at follow-up were rated higher by parents on the activity and impulsivity behavior composite, indicating more activity with less inhibitory control in those with ID, $F(1,50) = 4.18$, $p<0.05$. There was also a trend for those with ID to be rated more fearful by their parents on the TBAQ-R than those with iron sufficiency, $F(1,50) = 3.37$, $p=0.072$.

Given that iron status at follow-up was associated with both the Mullen Early Learning Composite and behaviors during testing, the association between iron status, Mullen scores, and behaviors was examined more closely using multiple regression analysis. We hypothesized that the association between iron status at follow-up and the Mullen Early Learning Composite would be mediated by examiner-rated behaviors
during testing. Examiner-rated activity and cooperation was the only behavior composite correlated with the Mullen Early Learning Composite, $r = 0.48$, $p=0.001$, and there is evidence that such behaviors mediate cognitive performance in both infants with ID (Oski & Honig, 1978; Walter et al., 1983) and in international adoptees (Beckett et al., 2007; van Ijzendoorn et al., 2005). Thus, the activity and cooperation behavior composite was tested as a mediator.

To test this association, three regression equations were computed consistent with the procedures described by Baron and Kenny (1986) to test a mediation effect. In model one, iron status was used to predict the Mullen Early Learning Composite. In model two, iron status was used to predict the activity and cooperation behavior composite during testing. Finally, in model three, both iron status and the examiner-rated activity and cooperation behavior composite were used to predict the Mullen Early Learning Composite. Age at adoption, baseline HAZ, and baseline OFCZ were included as predictors in all three models.

The regression equation for model one, was significant, $F(4,42) = 4.31$, $p<0.01$, with ID, $\beta = -0.29$, $p < 0.05$ and older age at adoption, $\beta = -0.32$, $p < 0.05$, independently predicting lower scores on the Mullen Early Learning Composite. There was also a trend for smaller OFCZ to predict lower Mullen scores, $\beta = 0.30$, $p= 0.069$. In model two, the regression equation was significant, $F(4,42) = 3.17$, $p<0.05$, with ID predicting lower scores on the activity and cooperation behavior composite, $\beta = -0.42$, $p < 0.01$. In model three, when iron status and the activity and cooperation behavior composite were entered as independent predictors of the Mullen Early Learning Composite, the regression
equation was significant, \( F(5,41) = 6.89, p<0.001 \). In this model, iron status was no longer significant, and lower scores on the activity and cooperation behavior composite predicted lower scores on the Mullen Early Learning Composite, \( \beta = 0.46, p = 0.001 \). Older age at adoption, \( \beta = -0.28, p < 0.05 \), and smaller OFCZ, \( \beta = 0.29, p < 0.05 \) also predicted lower scores on the Mullen Early Learning Composite in this final model. These results are consistent with a mediational model (Figure 2).

**Discussion**

Internationally adopted children remain at risk for ID at six months post-adoption despite the improvements in their nutritional and social environments. We have demonstrated that ID in this population is related to their rapid rate of growth post-institutionalization (Fuglestad, et al., 2008). In this study, we found that this persistent ID is associated with behavioral alterations and with general developmental delays. The effect size on the standardized test in our study is similar to that which has been reported in infants with a history of ID with anemia. Infants with a history of ID with anemia score on average between 6 and 15 points lower than their iron sufficient peers (Lozoff et al., 2006), and in our sample, those with ID at the six months follow-up scored 12 points lower than those with iron sufficiency. In addition, we demonstrated that the general delays seem to be mediated through the behavioral effects of ID.

The association between iron status and developmental outcomes was greater at the six month follow-up compared to the baseline assessment within one month post-adoption. At baseline, there was a trend for the iron deficient children to be rated by their parents as more fearful. There was also a trend for those with ID at baseline to be rated
by the examiner as more fearful at follow-up. There was no association between iron status and general development on the Mullen Scales of Early Learning. In contrast, iron status at the six month follow-up was associated with behavioral alterations rated by both parents and the examiner. The iron deficient children were rated as more active, displaying less inhibitory control, by their parents at follow-up. There was a trend to be rated as more fearful by their parents. They were also less cooperative and more active and displayed less positive affect during standardized testing. As a group they performed poorly on the Mullen Early Learning Scales, with 80% scoring below average and in the range for clinical concern.

The contrast between the baseline and follow-up findings was striking. The paucity of differences between the iron deficient and iron sufficient children at arrival may be attributable to both groups having multiple risk factors active so soon after adoption (e.g., macronutrient abnormalities, lack of cognitive and social stimulation during institutionalization) affecting development that may have masked the iron effect. At the six month follow-up, these other risk factor may have lessened, while the rate of ID did not, thus allowing iron status at follow-up to differentiate between the groups. Also likely, the additional six months of ID increased the duration of the deficiency sufficiently to lead to more prominent developmental differences; however, not all of those with ID at follow-up were deficient at baseline.

Some of the behaviors of the iron deficient adoptees are consistent with those observed in other populations with ID. The perturbed affective behaviors such as increased wariness and fearfulness and decreased positive affect are consistent with
iron’s role in regulating monoamine metabolism (e.g., dopamine). Likewise, the
behaviors we observed during testing, such as being less attentive and cooperative, are
consistent with some previous descriptions of iron deficient children (Lozoff et al.,
1998a; Oski & Honig, 1978; Walter et al., 1983). However, these behaviors during
testing do not replicate in many available studies.

An interesting finding of the current study is that the iron deficient children in this
study had increased activity particularly at the follow-up visit. This contrasts with other
studies that have described the behavioral profile of ID during infancy and toddlerhood as
a hesitant child who in addition to being wary, has decreased activity and fewer social
interactions (Lozoff et al., 1998). At five years of age following iron repletion, such
children continue to have decreased physical activity with less positive affect and poorer
social interactions (Corapci, Radan, & Lozoff, 2006). It is not until adolescence that
more externalizing and attention problems emerge in those who have a history of ID
during infancy (Corapci, Calatroni, Kaciroti, Jimenez, & Lozoff, 2009; B. Lozoff et al.,
2000). There are several potential explanations for this discrepancy. The duration and
severity of the deficiency may have differential effects on neurodevelopment and
subsequent behaviors. The behavior profile described in the literature is derived
primarily from infants who have experienced chronic and severe ID with anemia. Most
of the children in our sample did not have severe ID, as few had anemia. Their ID may
not have been as chronic since several who were not ID at one month developed ID at six
months post-adoption. Animal models show an overcompensation of the dopamine
system with moderate cases of ID (Beard et al., 2006), whereas in severe ID, compensation may be inadequate, thus causing differential behavioral effects.

It is also possible that ID may affect behaviors at one age, which then alter the child’s developmental trajectory. Based both on the functional isolation hypothesis and the integrated biological/environmental model, Lozoff and colleagues (Corapci et al., 2009) have suggested that wary behaviors early in life may lead to externalizing behaviors later. According to the functional isolation hypothesis, children with ID have behavioral and affective alterations which cause them to seek less stimulation from their physical and social environments, and such behaviors elicit fewer developmentally-facilitating interactions from their caregivers. This may lead to fewer learning experiences during which the child interacts with his/her environment, which in turn may result in increased emotionality in response to novel situations (Barnes, 1976). The wary and hesitant child with decreased activity may also require less caregiver guidance necessary for developing self-regulation skills, thus leading to an increase in externalizing behaviors over time (Corapci et al., 2009).

Consistent with these models, the severe social deprivation during institutionalization in addition to ID may potentiate the development of difficulties with self-regulation in internationally adopted children. We speculate that this isolation may well have begun in the institutions prior to adoption resulting in a more wary and hesitant phenotype at the time of arrival. The transition to a less structured, more stimulating, and perhaps overwhelming environment, combined with ongoing ID-induced perturbations of the monoamine neurotransmitter systems may have lead to an earlier expression of the
hyperactive phenotype, particularly for those who may have sought less physical and social stimulation both during and soon after institutionalization. Moreover, the behavioral pattern we found in which baseline ID was associated with increased fearful behaviors whereas ID six months later was associated with decreased attention and cooperation is consistent with past research on internationally adopted children. They exhibit passive behaviors immediately following adoption followed by later problems with inattention and overactivity (Kreppner et al., 2007; Zeanah et al., 2009).

A major finding of our study was that poor performance on standardized testing in those with ID was mediated by behavioral problems during testing. There are several interpretations of this association between behavior and cognitive performance. First, given the altered behaviors during testing, standardized testing scores may not be an accurate indication of cognitive competence in these populations. Van Ijzendoorn et al. (2006) suggest that there may be a disconnect between cognitive competence and performance in international adoptees as adoptees with normal IQs have problems with school performance. Another study found poor school performance in internationally adopted children to be mediated in part by behavioral problems, specifically inattention and overactivity (Beckett et al., 2007). Likewise, cognitive deficits in children with ID may be due to specific cognitive disruptions (e.g., altered recognition memory due to hippocampal effects; Georgieff, 2008), as well as behavioral alterations (e.g., wariness, Lozoff et al., 1998a), findings consistent with the numerous roles of iron in the central nervous system (Lozoff & Georgieff, 2006b). Iron deficient children have been observed to be more wary and hesitant during testing on the Bayley Scales of Infant Development,
paying less attention to the tester and making fewer attempts on test items (Lozoff et al., 1998). Similarly, an accurate score of cognitive ability on standardized tests in iron deficient children may have been difficult to obtain due to poor attention and cooperation (Walter et al., 1983) or decreased alertness and responsiveness (Oski & Honig, 1978) during testing. Another possibility is that problem behaviors in general may lead to fewer and/or poorer quality learning experiences in the children’s natural environments. If children are more wary to explore their environments or have attention problems in their environments, learning experiences may be disrupted (Lozoff et al., 2008). Future studies are needed to understand the association between behavior and cognition in both internationally adopted children and in children with ID.

This study was not designed to address the differences between ID with and without anemia. However, an important finding was that the developmental effects were seen in a group of children with ID, even though there was very little anemia in this sample. During ID early in life, iron is prioritized to red blood cells for hemoglobin synthesis over other tissues (Georgieff, 2008). If brain ID occurs prior to frank anemia, developmental effects may also appear without anemia. Although the developmental effects of ID with anemia are well-documented, there is less research on the effects of less severe non-anemic ID. However, recent animal models and studies in human populations are beginning to provide evidence for neurobehavioral effects of ID without anemia (Carlson et al., 2009; Siddappa et al., 2004).

This study has several limitations. The sample included only adoptees coming from Eastern Europe. It will be important to continue to investigate ID in internationally
adopted children from other regions. Since this was a correlational study, caution must be used assigning a causal association between ID and the neurobehavioral effects. For instance, ID may be an indicator of the severity of deprivation prior to adoption, and the developmental deficits and behavioral differences are due to such factors rather than iron status.

It will be important in future research to address whether these effects are due to concurrent ID or whether ID during the early adoption period leads to persistent developmental problems even if iron status improves. Understanding the role of early ID within the larger scale of neurobehavioral abnormalities in internationally adopted children is of particular interest for the development of effective early intervention programs. Unlike many risk factors (i.e., fetal alcohol exposure) experienced by internationally adopted children, nutrition is amendable through intervention during the early adoption period. Furthermore, the role of ID in transitioning to a new less structured environment following institutionalization may have important implications for parents and clinicians.
Table 4

**Behavior Composites**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Examiner-Rated Behavior Composites</th>
<th>TBAQ-R Behavior Composites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity &amp; Cooperation</td>
<td>Fearfulness</td>
<td>Positive Affect</td>
</tr>
<tr>
<td>($\alpha = 0.91$)</td>
<td>($\alpha = 0.76$)</td>
<td>($\alpha = 0.59$)</td>
</tr>
<tr>
<td>Activity &amp; Impulsivity</td>
<td>Attention</td>
<td>Object fear</td>
</tr>
<tr>
<td>($\alpha = 0.61; 0.83$)</td>
<td>($\alpha = 0.77; 0.80$)</td>
<td>Pleasure</td>
</tr>
<tr>
<td>Attention to tasks</td>
<td>Fear of novelty (objects)</td>
<td></td>
</tr>
<tr>
<td>Persistence in attempting to complete tasks</td>
<td>Hypersensitivity to test materials and stimuli</td>
<td></td>
</tr>
<tr>
<td>Cooperation</td>
<td>Social fearfulness</td>
<td></td>
</tr>
<tr>
<td>Hyperactivity</td>
<td>Enthusiasm toward tasks</td>
<td></td>
</tr>
<tr>
<td>Inhibitory control/Impulsivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative affect</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note.** Behavior composites were calculated as the average of the individual behavior variables. The first alpha level for each TBAQ-R behavior composite is for the baseline assessment and the second is for the follow-up assessment.
Table 5
Sample Characteristics, Anthropometrics, and Iron Status

<table>
<thead>
<tr>
<th>Sample Characteristics</th>
<th>Range</th>
<th>M (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at adoption (months)</td>
<td>8.9 – 45.6</td>
<td>19.4 (8.7)</td>
</tr>
<tr>
<td>Time in institution (months)</td>
<td>7.3 – 45.5</td>
<td>16.2 (6.8)</td>
</tr>
<tr>
<td>Time in US before assessment (days)</td>
<td>6 – 37</td>
<td>17 (6)</td>
</tr>
<tr>
<td>Sex ((n))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female: 32; Male: 25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Country of origin ((n))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Russia: 49; Kazakhstan: 5; Ukraine: 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macronutrient Status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAZ</td>
<td>-1.14 (1.06)</td>
<td>-0.46 (0.95)***</td>
</tr>
<tr>
<td>WAZ</td>
<td>-1.55 (1.40)</td>
<td>-0.62 (1.13)***</td>
</tr>
<tr>
<td>WHZ</td>
<td>-0.65 (1.41)</td>
<td>-0.22 (1.16)*</td>
</tr>
<tr>
<td>OFCZ</td>
<td>-0.67 (1.08)</td>
<td>-0.02 (1.22)***</td>
</tr>
<tr>
<td>Iron Deficiency</td>
<td>26%</td>
<td>18%</td>
</tr>
<tr>
<td>(n=15;)</td>
<td>(n=10)</td>
<td></td>
</tr>
<tr>
<td>((n=11) without anemia)</td>
<td>((n=8) without anemia)</td>
<td></td>
</tr>
</tbody>
</table>

*Note.* Paired samples \(t\) tests were used for comparisons between baseline and follow-up.
Table 6
Sample Characteristics as a Function of Iron Status

<table>
<thead>
<tr>
<th></th>
<th>Iron status at baseline</th>
<th></th>
<th>Iron status at follow-up</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IS</td>
<td>ID</td>
<td>IS</td>
<td>ID</td>
</tr>
<tr>
<td></td>
<td>M (SD)</td>
<td>M (SD)</td>
<td>M (SD)</td>
<td>M (SD)</td>
</tr>
<tr>
<td>Age at adoption (months)</td>
<td>20.7 (9.6)</td>
<td>15.8 (3.5)**</td>
<td>20.0 (9.4)</td>
<td>17.0 (3.8)</td>
</tr>
<tr>
<td>Sex (n)</td>
<td>23 female</td>
<td>9 female</td>
<td>28 female</td>
<td>4 female</td>
</tr>
<tr>
<td></td>
<td>19 male</td>
<td>6 male</td>
<td>19 male</td>
<td>6 male</td>
</tr>
<tr>
<td>HAZ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>-1.19 (1.09)</td>
<td>-0.98 (0.98)</td>
<td>-1.15 (1.09)</td>
<td>-1.08 (0.95)</td>
</tr>
<tr>
<td>Follow-up</td>
<td>-0.56 (0.92)</td>
<td>-0.20 (1.03)</td>
<td>-0.47 (0.99)</td>
<td>-0.43 (0.80)</td>
</tr>
<tr>
<td>Change from Baseline to Follow-up</td>
<td>0.64 (0.50)</td>
<td>0.79 (0.45)</td>
<td>0.68 (0.49)</td>
<td>0.66 (0.53)</td>
</tr>
<tr>
<td>WAZ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>-1.49 (1.41)</td>
<td>-1.73 (1.40)</td>
<td>-1.45 (1.39)</td>
<td>-2.01 (1.39)</td>
</tr>
<tr>
<td>Follow-up</td>
<td>-0.65 (1.12)</td>
<td>-0.54 (1.17)</td>
<td>-0.72 (1.12)</td>
<td>-0.13 (1.07)</td>
</tr>
<tr>
<td>Change from Baseline to Follow-up</td>
<td>0.84 (1.01)</td>
<td>1.19 (1.08)</td>
<td>0.73 (0.93)</td>
<td>1.87 (1.00)****</td>
</tr>
<tr>
<td>WHZ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>-0.54 (1.45)</td>
<td>-0.95 (1.30)</td>
<td>-0.51 (1.20)</td>
<td>-1.29 (2.13)</td>
</tr>
<tr>
<td>Follow-up</td>
<td>-0.21 (1.17)</td>
<td>-0.23 (1.19)</td>
<td>-0.36 (1.13)</td>
<td>0.46 (1.13)*</td>
</tr>
<tr>
<td>Change from Baseline to Follow-up</td>
<td>0.33 (1.31)</td>
<td>0.72 (1.43)</td>
<td>0.15 (1.11)</td>
<td>1.74 (1.59)****</td>
</tr>
<tr>
<td>OFCZ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>-0.70 (1.15)</td>
<td>-0.57 (0.90)</td>
<td>-0.68 (1.15)</td>
<td>-0.60 (0.75)</td>
</tr>
<tr>
<td>Follow-up</td>
<td>-0.12 (1.29)</td>
<td>0.22 (0.96)</td>
<td>-0.14 (1.23)</td>
<td>0.54 (1.03)</td>
</tr>
<tr>
<td>Change from Baseline to Follow-up</td>
<td>0.59 (0.72)</td>
<td>0.79 (0.74)</td>
<td>0.54 (0.70)</td>
<td>1.14 (0.63)*</td>
</tr>
</tbody>
</table>

Note. Paired samples \( t \) tests were used for comparisons between baseline and follow-up. Independent samples \( t \) tests for comparisons between IS and ID. \( *p<0.05, **p<0.01, ***p<0.001 \)
Table 7

*General Development and Behavior as a Function of Baseline Iron Status*

<table>
<thead>
<tr>
<th>Iron Status at Baseline</th>
<th>Significant Covariates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IS</td>
</tr>
<tr>
<td></td>
<td>$M \pm SE (n)$</td>
</tr>
<tr>
<td><strong>Mullen Scales of Early Learning</strong></td>
<td></td>
</tr>
<tr>
<td>Fine Motor</td>
<td></td>
</tr>
</tbody>
</table>
| Baseline               | 41 ± 1, (42)           | 38 ± 2, (15)           | Age (-)**
| Follow-up              | 46 ± 2, (41)           | 46 ± 3, (15)           | Age (-)**
| Visual Reception       |                        |                        |
| Baseline               | 40 ± 2, (42)           | 34 ± 3, (15)           | Age (-)**
| Follow-up              | 45 ± 2, (41)           | 42 ± 3, (15)           | Age (-*)
| Early Learning Composite |                        |                        |
| Follow-up              | 89 ± 2, (41)           | 87 ± 3, (15)           | Age (-*)
| **Examiner Behavior Rating (Follow-up)** |                        |
| Activity & Cooperation | 3.7 ± 0.2 (35)         | 3.3 ± 0.3 (12)         |                        |
| Fearfulness            | 4.6 ± 0.1 (35)         | 4.3 ± 0.2 (12)†        | OFCZ (+)†               |
| Positive Affect        | 2.3 ± 0.1 (35)         | 2.2 ± 0.2 (12)         |                        |
| **TBAQ-R**             |                        |                        |
| Activity & Impulsivity |                        |                        |
| Baseline               | 4.03 ± 0.15 (31)       | 4.24 ± 0.24 (12)       | OFCZ (-)†               |
| Follow-up              | 3.91 ± 0.14 (41)       | 4.12 ± 0.24 (14)       |                        |
| Attention              |                        |                        |
| Baseline               | 2.97 ± 0.16 (31)       | 2.72 ± 0.26 (12)       |                        |
| Follow-up              | 3.48 ± 0.13 (41)       | 3.40 ± 0.22 (14)       | Age (+)*                |
| Fearfulness            |                        |                        |
| Baseline               | 3.04 ± 0.15 (31)       | 3.60 ± 0.25 (12)†      | Age (+)*                |
| Follow-up              | 2.96 ± 0.12 (41)       | 2.84 ± 0.20 (14)       | Age (-*)                |
| Positive Affect        |                        |                        |
| Baseline               | 4.48 ± 0.15 (31)       | 4.18 ± 0.25 (12)       |                        |
| Follow-up              | 4.95 ± 0.10 (41)       | 5.04 ± 0.17 (14)       |                        |

Note. Results of ANOVAs comparing general development and behavior composites based on iron status. All means are adjusted for the covariates HAZ, OFCZ, and age at baseline. Higher scores on the TBAQ indicate a greater frequency of the given behavior. Higher Scores on the examiner’s behavior-rating indicate more optimal functioning. Direction of the association between the dependent variables and the covariates are noted in parentheses, (+) or (-). †$p<0.10$; *$p<0.05$; **$p<0.01$; ***$p<0.001$. 

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Table 8
General Development and Behavior as a Function of Follow-up Iron Status

<table>
<thead>
<tr>
<th>Mullen Scales of Early Learning (Follow-up)</th>
<th>Iron Status at Follow-up</th>
<th>Significant Covariates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early Learning Composite</td>
<td>IS: 91 ± 2, (46)</td>
<td>ID: 79 ± 4, (10)**</td>
</tr>
</tbody>
</table>

| Examiner Behavior Rating (Follow-up)      | IS: 3.8 ± 0.2 (38)       | ID: 2.7 ± 0.3 (9)**     |            |
| Activity & Cooperation                    |                          |                         |            |
| Fearfulness                               | 4.5 ± 0.1 (38)           | 4.4 ± 0.2 (9)           |            |
| Positive Affect                           | 2.3 ± 0.1 (38)           | 2.0 ± 0.2 (9)*          |            |

| TBAQ-R (Follow-up)                        | IS: 3.86 ± 0.12 (46)    | ID: 4.49 ± 0.28 (9)*   |            |
| Activity & Impulsivity                    |                          |                         |            |
| Attention                                 | 3.45 ± 0.12 (46)         | 3.51 ± 0.27 (9)         | Age (+)*   |
| Fearfulness                               | 2.86 ± 0.11 (46)         | 3.33 ± 0.24 (9)†        | Age (-)†   |
| Positive Affect                           | 4.94 ± 0.09 (46)         | 5.13 ± 0.21 (9)         |            |

Note. Results of ANOVAs comparing general development and behavior composites based on iron status. All means are adjusted for the covariates HAZ, OFCZ, and age at baseline. Higher scores on the TBAQ indicate a greater frequency of the given behavior. Higher Scores on the examiner’s behavior-rating indicate more optimal functioning. Direction of the association between the dependent variables and the covariates are noted in parentheses, (+) or (-). †p<0.10; *p<0.05; **p<0.01; ***p<0.001.
Figure 2. The association between iron status at follow-up and the Mullen Early Learning Composite at follow-up is mediated through activity and cooperation behaviors during testing. All coefficients are standardized. Model one: iron status predicts Mullen Early Learning Composite. Model two: iron status predicts activity and cooperation during testing. Model three: the association between iron status and the Mullen Early Learning Composite is mediated by activity cooperation during testing. *p<0.05; **p<0.01; ***p<0.001.
Chapter III: Nutritional Status and Neurodevelopment in Internationally Adopted Children at the Time of Arrival: Preliminary Data

In the previous studies, we reported that iron deficiency (ID) is fairly common in children adopted from Eastern Europe, with approximately one quarter iron deficient at the time of arrival into the United States (Fuglestad et al., 2008), and we found that ID, primarily without anemia, is associated with both behavioral alterations and hampered general cognitive development in children adopted from Eastern Europe. Little is known about the iron status of children adopted from other regions. Previous studies have reported that approximately 30% of children adopted from China and Guatemala are anemic at the time of arrival (Miller et al., 2005; Miller & Hendrie, 2000), with fewer adoptees from Ethiopia anemic at arrival (Miller, Tseng, Tirella, Chan, & Feig, 2008). However, the etiology of the anemia was not specified as ID in these studies. Furthermore, there is a lack of data on the incidence of pre-anemic ID. Additionally, given the prevalence of ID and anemia in international adoptees, it is likely that they are at risk for other micronutrient deficiencies. Of particular concern are those nutrients that are important for growth and neurodevelopment.

Risk for Micronutrient Deficiencies

Macronutrient abnormalities are well-documented in international adoptees at the time of arrival, and similar patterns of growth failure are seen in children across different countries of origin (Johnson, 2000; Miller & Hendrie, 2000). At arrival, all internationally adopted children are likely to be at risk for micronutrient deficiencies that occur commonly in children and/or frequently coexist with macronutrient abnormalities.
[i.e., protein energy malnutrition (PEM)]. ID is common worldwide (30% of the developing world’s population and 9% of U.S. toddlers are iron-deficient) and most frequently occurs during infancy, between 6 and 24 months of age (Joint FAO/WHO Expert Consultation on Human Vitamin and Mineral Requirements, 2004). The availability of zinc, which is essential for growth, and therefore especially critical for infants and children, tracks with protein intake (Caulfield & Black, 2004). Folate deficiency is common in developing countries, and vitamin B$_{12}$ deficiency is common during pregnancy and infancy (Joint FAO/WHO Expert Consultation on Human Vitamin and Mineral Requirements, 2004).

Internationally adopted children may also be at risk for nutrient deficiencies based on the pre-adoption environment specific to the region from which they are adopted. Internationally adopted children from regions that consume less meat and more grains can be expected to have higher rates of iron, zinc, and vitamin B$_{12}$ deficiency (Joint FAO/WHO Expert Consultation on Human Vitamin and Mineral Requirements, 2004). Iodine and selenium deficiency are expected to be greater in children adopted from regions with low soil content of these minerals (e.g., China; Cao et al., 1994). Children adopted from Ethiopia may be at an increased risk for vitamin A deficiency, which is common in regions of Africa (Joint FAO/WHO Expert Consultation on Human Vitamin and Mineral Requirements, 2004). International adoptees may also be at risk for vitamin D deficiency from limited sun exposure due to institutionalization (e.g., China, Russia) and due to living in northern climates (e.g., Russia).
Thus, the first goal of this study was to assess the nutritional status, of both macronutrients and micronutrients, in internationally adopted children at the time of arrival into the U.S., specifically in children adopted from Eastern Europe, Ethiopia, and China. In 2009, these three regions were the most common regions of international adoption into the U.S., with children adopted from these regions making up just over half of all international adoptions into the U.S. (U.S. Department of State, 2010). By including children adopted from three regions, the goal of the study was to focus on patterns of nutrient abnormalities common to the pre-adoption early adverse experiences. Specifically, we hypothesized that international adoptees, irrespective of geographic origin (i.e., Eastern Europe, Ethiopia, or China) would demonstrate a common pattern of growth failure (i.e., low weight and length z-scores) at arrival. We also expected similarities in micronutrient deficiencies across regions; however, regional differences may occur based on the aforementioned regional environmental risks.

**Association between Nutritional Status and Neurodevelopment**

At the time of adoption, many international adoptees arrive with delays in general development (Johnson, 2000). They demonstrate considerable resiliency, exhibiting improvements in general development years after adoption (Beckett et al., 2006; O'Connor et al., 2000; Rutter & the English and Romanian Adoptees Study Team, 1998). Despite this tremendous resiliency, some neurodevelopmental problems continue long-term, with several domains at particular risk for problems in this population.

International adoptees experience many pre-adoption risk factors that may contribute to these persistent problems, and little research has focused on the role of
nutrition. Furthermore, in the previous study, we found that ID was associated with more fearful behaviors at arrival and with developmental delays and increased problems with activity and cooperation during standardized testing six months later. Thus, the second aim of the study was to assess the association between nutrient deficiencies and several neurodevelopmental domains that are sensitive to nutrient deficiencies during the first few years of life. Specifically, we expected general development to be compromised in international adoptees at the time of arrival. Moreover, we expected the status of specific nutrients to map onto the neurodevelopmental profile of internationally adopted children. Specific hypotheses can be made given each nutrient’s metabolic roles in neurodevelopment.

**General cognitive and motor development.** Many international adoptees experience catch-up in general cognitive development (van Ijzendoorn & Juffer, 2006; van Ijzendoorn et al., 2005); however, some children continue to have lower IQs years after adoption (Beckett et al., 2007), as well as specific cognitive deficits, such as learning problems, poor school performance (Beckett et al., 2007; van Ijzendoorn et al., 2005) and executive function impairments (Colvert et al., 2008). General cognitive development is particularly vulnerable to PEM that occurs under the age of three due to the rapid development during this time (for review, see Fuglestad et al., 2008). PEM is often accompanied by micronutrient deficiencies, and therefore, it is not known whether these developmental effects are due to PEM alone or to micronutrient deficiencies. Iron, zinc, iodine and selenium have also been linked to general cognitive development.
Years after adoption, some children also continue to show difficulty in cerebellum and striatum dependent motor skills, such as balance and coordination (Tober & Pollak, 2005) and have smaller cerebellar volumes (Bauer, Hanson, Pierson, Davidson, & Pollak, 2009). Iron, zinc, iodine, and vitamin B_{12} (Allen, 2006) are important for motor development in infants and toddlers. Multiple well-controlled clinical studies in children ages 6-24 months with ID demonstrate significant decrements in motor achievement (Lozoff, 1990; Lozoff et al., 2006; Walter et al., 1983). Zinc is found in high concentrations in the cerebellum (Frederickson & Danscher, 1990), and during infancy, zinc supplementation improves motor development and promotes activity in the most severe cases of zinc deficiency (Black, 2003). Finally, children living in areas of endemic iodine deficiency demonstrate alterations in psychomotor development (Azizi et al., 1993).

**Speed of processing.** Although not directly observed in international adoptees, speed of processing, which may be altered by myelination, may be affected by nutritional status. Such alterations may affect cognitive functioning and lead to some delays reported in international adoptees. Macronutrients and iron are required for myelination. PEM alters the fatty acid profile and may result in hypomyelination (Faldella et al., 1996). ID in animal models results in loss of activity of enzymes involved in myelin lipid synthesis, accompanied by hypomyelination (Larkin & Rao, 1990). Accordingly, there is electrophysiological evidence for iron-induced hypomyelination in children, as ID is associated with delayed latencies on auditory brainstem-evoked responses (Roncagliolo et al., 1998) and visual evoked potentials (Algarin et al., 2003).
Socioemotional and exploratory behaviors. Internationally adopted children exhibit socioemotional and behavioral alterations. Children who have lived in particularly deprived environments have been observed to be more passive and explore less (Bowlby, 1951). Similar behaviors have been observed in children living in orphanages abroad (Johnson, 2000). Some studies, but not all, have reported internalizing behaviors, such as increased anxiety, fearfulness, and unhappiness, in adoptees years after adoption (Colvert, Rutter, Beckett et al., 2008). However, one of the most consistent findings in internationally adoptees is problems with inattention and overactivity (Stevens et al., 2008).

Early ID is associated with socioemotional alterations. Iron-dependent enzymes (tyrosine hydroxylase and tryptophan hydroxylase) are required in monoamine metabolism and animal studies which model ID in infancy show long-term monoamine alterations (Beard & Connor, 2003; Lozoff et al., 2006), which may be associated with altered behaviors. Preschoolers with ID anemia have been observed to display less positive affect and less social referencing to their mothers (Lozoff et al., 2007).

Both ID and zinc deficiency have been linked to inhibited exploratory behaviors. Decreased exploration as a result of early ID has been observed in rodents (Felt & Lozoff, 1996; Pinero, Jones, & Beard, 2001) and in toddlers (Lozoff et al., 1998b). Zinc supplementation in deficient children increases activity and energy expenditure, (Sazawal et al., 1996), and increases the time spent in play (Bentley et al., 1997), behaviors that may lead to greater environmental exploration. Such reduced activity and inhibited
exploration in iron or zinc deficient infants and toddlers may exacerbate hampered cognitive abilities.

Methods

Participants

**International adoptees.** Participants who met inclusion criteria were recruited and enrolled through the Adoption Medicine Program and Clinic at the University of Minnesota between May 2008 and March 2010. Inclusion criteria were being between 9 and 18 months at the time of arrival into the U.S. and being adopted from Eastern Europe, Ethiopia, or China. Forty-one internationally adopted participants were enrolled in the study and were between 8.71 and 18.92 months of age at the clinic assessment.

The medical records from the Adoption Medicine Program and Clinic of participants were screened for exclusion criteria. Exclusion criteria for internationally adopted children were congenital abnormalities, major neurological problems, high risk for fetal alcohol syndrome (FAS), and serious medical conditions (e.g., heart failure, kidney disease, cystic fibrosis). No participants were excluded due to the exclusion criteria.

**Non-adopted controls.** A control group of children raised by their biological parents matched for age and sex to the 36 international adoptees who completed the neurodevelopmental assessment were recruited from the low-risk Infant Participant Pool (IPP) at the Institute of Child Development, University of Minnesota, for developmental assessments only. The IPP is a database of children born in the Twin Cities metro area. Parents of all children born in the Twin Cities hospitals at the time of birth are given the
option to include their child in the IPP database to potentially be contacted to participate in research at the University of Minnesota. One control participant was matched to each internationally adopted participant for sex and scheduled to be within two weeks of age to the internationally adopted participant for the neurodevelopmental assessment. Thirty-three non-adopted control participants were enrolled in the study and were between 9.56 and 19.45 months of age at the neurodevelopmental assessment.

Exclusion criteria for the non-adopted controls included pre-term birth, low birth weight (<2500g), a history of anemia (hemoglobin < 11.0), weight for height below two z scores at the developmental assessment, and any major health issues that would affect growth or development. Medical records were available and screened for anemia in 16 non-adopted controls (medical records have not yet been collected in the ongoing study for the remainder of the controls). Five non-adopted controls were excluded due to anemia (n=4) or due to low WHZ (n=1). Thus, 28 non-adopted controls were included in analyses.

Study Design

Internationally adopted participants had both nutritional and neurodevelopmental assessments done within approximately one month of arrival into the U.S. The nutritional assessment was done during the routine medical evaluations at the Adoption Medicine Program Clinic between 5 and 40 days post-arrival (M=21 days, SD=9). The developmental assessment was done at the Center of Neurobehavioral Development (CNBD), University of Minnesota, within two weeks of the medical visit (M=11 days, SD=7).
Control participants were enrolled to assess typical development in the neurodevelopmental domains. There was a single research visit for control participants during which the developmental assessment and a brief nutritional assessment (anthropometry only) were completed.

The study was approved by the Institutional Review Board of the University of Minnesota, and written informed consent was obtained from the parents.

**Nutritional Measures**

**Anthropometry.** Anthropometry was performed to assess macronutrient status and was completed for both internationally adopted participants and non-adopted control participants. Anthropometry for international adoptees was completed as part of the routine medical visit at the Adoption Medicine Program and Clinic. Anthropometry was completed for the control group to screen for exclusion criteria and was done during the developmental assessment by an examiner who was trained by the staff at the Adoption Medicine Program and Clinic. Anthropometry included recumbent length, weight, and occipitofrontal circumference (OFC). Z scores, including height-for-age z score (HAZ), weight-for-age z score (WAZ), weight-for-height z score (WHZ), and OFC-for-age z score (OFCZ) were calculated using CDC 2000 norms with Epi info 3.3 (Centers for Disease Control and Prevention, Atlanta, GA).

**Biochemical analyses.** Additional blood was drawn on international adoptees during their medical visit for biochemical analyses of nutrients. Nutrient levels for all analyses were analyzed at Fairview Laboratory of the University of Minnesota Medical Center using standard CLIA certified techniques, all of which have interassay
variabilities <5%. Specific nutrient assessments and normal values are described in Table 9.

All abnormal laboratory values were communicated to the health care providers at the Adoption Medicine Program and Clinic. The general practice of the Adoption Medicine Program and Clinic is to treat ID with anemia with 3 to 6 mg/kg elemental iron per day for six to eight weeks, and to treat ID without anemia with 3 to 6 mg/kg elemental iron per day for four weeks, followed by iron rich foods and a multivitamin with iron. Zinc deficiency was treated with a multivitamin with zinc. Those with elevated thyroid stimulating hormone (TSH) were treated with iodized salt. Vitamin D deficiency was treated with 2,000 IU vitamin D for eight weeks, along with 30 to 75 mg/kg of elemental calcium per day tapered over four weeks to the RDA. Vitamin D insufficiency was treated with 1,000 IU vitamin D per day for eight weeks along with 500mg elemental calcium per day for those over 12 months.

Information regarding health status that may be associated with the nutritional indices was obtained from the participants’ medical records at the Adoption Medicine Program and Clinic. These included measures of inflammation, serum lead, intestinal parasites, and chronic infections. C-reactive protein (CRP) was used as an inflammatory marker. Iron, zinc, and retinol binding protein (RBP) have been shown to be affected by the acute phase response. The elevation of ferritin concentration as part of an acute phase response has been known to cause underestimation of the prevalence of ID (Wieringa et al., 2002). Thus, CRP was assessed to determine whether the serum ferritin concentrations were reliable reflections of iron stores. During the acute phase response,
serum zinc decreases (Keen, Taubeneck, Daston, Rogers, & Gershwin, 1993). RBP concentrations also fall during the acute phase response (Fuhrman, Charney, & Mueller, 2004). Seven percent (2 of 27) had elevated CRP levels (>8mg/L). Serum lead levels are inversely related to both zinc and iron status (Bressler, Olivi, Cheong, Kim, & Bannona, 2004; Bressler et al., 2007; Cerklewski & Forbes, 1976). Three percent (1 of 36) had elevated lead levels (>10µg/dL) at arrival. Intestinal parasites may interfere with both macro- and micronutrient status (Casapia, Joseph, Núñez, Rahme, & Gyorkos, 2006; Luján et al., 1995; Troeger et al., 2007). Stool of for ova and parasites or assays for *Giardia lamblia* antigen were used to detect infection of intestinal parasites including *G lamblia*. Seventeen percent (6 of 35) had intestinal parasites. Five were positive for *G lamblia* and one for *Blastocystis hominis*. The medical records were also screened for the presence of infections including hepatitis A, hepatitis B, hepatitis C, HIV, and tuberculosis (TB). The presence of chronic infections may exacerbate zinc deficiency (Keen et al., 1993). Seventeen percent (6 of 36) had hepatitis A. There were no infections of hepatitis B (of 38), and 2% (1 of 41) had hepatitis C. There were no cases of HIV (0 of 41), and 25% (6 of 24) were positive for latent TB. Overall, 17% (7 of 41) of the sample had one or more of the above infections. Health indices of inflammation, serum lead, intestinal parasites, and chronic infections were examined in relation to nutritional status.

**Neurodevelopmental Measures.**

**Bayley Scales of Infant Development III (BSID III).** The BSID III (Bayley, 2006) was completed to assess both general cognitive and motor development. Specifically, the
cognitive and motor subscales were administered, and composite scores for each subscale were used in analyses. The motor subscale assesses both gross and fine motor skills. The composite scores are on a scale with a mean of 100 and standard deviation of 15. Scores below -1 SD (85) are considered below average.

**Visual Evoked Potentials (VEPs).** VEP was used to assess speed of processing. Myelination of the visual system can be assessed by measuring the latency of the event-related potential (ERP) component associated with the visual system (P100) following the presentation of visual stimuli (Algarin et al., 2003; Odom et al., 2004). As myelination increases conductance rates, shorter latencies are indicative of myelination. Children were seated on the parent’s lap in front of a computer monitor and presented reversed checks, during which VEPs were recorded. Visual stimuli were presented for 100 ms for 100 trials of 500 ms each. VEP recordings used Ag AgCl electrodes, fastened to the scalp with adhesive foam padding, Grass electrode paste, and headbands. Data were recorded from the scalp site Oz according to the International 10/20 system. A ground electrode was placed over the forehead. All electrodes were referenced to Cz. Electro-ocular activity (EOG) was recorded from a transverse position above and below the eye to allow for detection and deletion of blink artifacts. Electrode impedances were kept below 10 kΩ.

A Grass Model 12A5 amplifier was used to record and filter both EEG and EOG signals. EEG amplifier gain was set to 20,000 and EOG was set to 5000, and digitized using a 12-bit A/D converter. Lower and upper cut off frequencies were 0.01 to 30 Hz respectively, with a 60 Hz notch filter. All channels were sampled at 200 Hz beginning
100 msec before stimulus onset and continuing to the end of the record epoch.

Processing and analysis of the EEG signal was carried out using the ERPW analysis software package (New Boundary Technologies, Minneapolis, MN).

Electrophysiological data were excluded if the EEG signal exceeded +/-250 mV in any 100 ms window. EOG artifacts were corrected (Gratton, Coles, & Donchin, 1983).

Individual averages were constructed using 100 ms prior to stimulus onset for baseline correction. A minimum of 20 trials was required for inclusion in the final sample.

**Socioemotional and Exploratory Child Behaviors and Parent Behaviors.**

Behaviors were videotaped and coded for a 15 minute play session. The play session included five minutes of child-only free play, five minutes during which portions (i.e., fear, joy/pleasure, interest, and activity episodes) of the Laboratory Temperament Assessment Battery (Lab-TAB; Goldsmith & Rothbart, 1999) were administered, and five minutes of parent-child social play. During the free play, the child was in a room with one parent, and the parent was instructed to allow the child to play on his/her own. Specifically, the parent was instructed to not to initiate any communication or play, but was permitted to respond to his/her child. During the Lab-TAB, novel toys were presented to the child, and the examiner interacted with the child. During the social play, the parent was instructed to play with his/her child as he/she does at home. During the free play and social play, a consistent set of toys was available. An examiner with a video camera was in the corner of the room throughout the 15 minute play session.

Trained personnel coded specific behaviors of the child and of the parent after watching videos of the 15 minute play session. Socioemotional behaviors of the child
included fear, both of new people and new situations and objects, positive affect, negative affect, social communication (i.e., initiating and responding to), and regulatory behaviors, including both cooperation and hyperactivity/impulsivity. Exploratory behaviors of the child included both quality and quantity of exploration. The two parent behaviors coded were sensitivity versus intrusiveness and limit setting. Behaviors were coded on a scale of zero to five with higher scores indicating greater evidence of the given behavior. Behavior coding was based on descriptions from the Lab-TAB, the Behavior Rating Scale of the BSID II (Bayley, 1993) and unpublished data (Gunnar). The parent behavior limit setting was not applicable for 65% ($n=11$ of 17), indicating that these children did not display any behavior that required parents to set limits. Thus, this behavior was analyzed as whether or not limit setting was needed during the session. Inter-rater percent agreement was calculated for 20% of videos coded and was 77%.

**Missing Data**

Of the 41 internationally adopted participants, not all had complete nutritional data. Four did not have the full nutritional battery completed due to limited funding at the beginning of the study. From May 2008 until December, 2008, nutritional assessments for children adopted from China were funded by a separate grant which covered fewer nutritional analyses. Nutritional data were also missing due to a limited volume of blood drawn. To keep the risk of the study minimal for the young children, blood samples were collected for nutritional analyses only if the participants were already having blood drawn as part of the routine medical evaluation at the Adoption Medicine Program and Clinic. Several participants ($n=4$) did not have any blood drawn during
their Adoption Medicine Program and Clinic visit due to having the routine laboratory assessments recently completed at another clinic (e.g., the child’s general pediatrician’s clinic). Of these four participants, complete information for one participant was available for ID anemia from the other clinic. Second, only a limited blood sample was collected for nutritional assessments, and thus the full nutritional battery was incomplete for seven participants. Four participants had incomplete nutritional data due to unsatisfactory specimen collection or processing; one due to clotting and three due to collection in the incorrect tube, increasing risk of contamination for zinc analyses. Children with and without missing nutritional data did not differ significantly \((p<0.05)\) in region adopted from or age at adoption. The only difference in anthropometry was those with missing nutritional data had lower mean HAZ \((M=-1.60, SD=1.20)\) compared to those with complete data \((M=-0.75, SD=1.09)\), \(t(39)=2.37, p<0.05\).

Eighty-eight percent \((n=36)\) of the 41 internationally adopted participants returned for the neurodevelopmental assessment. Three families chose not to return for the developmental assessments due to the nutritional labs not being complete at the clinic visit, one family did not return due to time constraints, and one family did not give a reason. One child returned, but was unable to complete any of the developmental assessments. There were no significant differences \((p<0.05)\) in region of origin, age at adoption, or any nutritional indices between those who returned for the developmental assessment and those who did not.

Of those included in the developmental analyses, socioeconomic information was available for 89\% \((n=31)\) of the international adoptees and 79\% \((n=22)\) of the non-
adopted controls. Socioeconomic data was missing due to parents not completing and returning the demographic questionnaire \((n=5)\) and data not yet collected in the ongoing study \((n=6)\). There were no significant differences \((p<0.05)\) in region of origin, age, or nutritional markers for those who returned the demographic information and those who did not.

Of those who completed the neurobehavioral developmental assessment, VEP data were available for 29 international adoptees and 18 non-adopted controls. Missing data were due to having data on fewer than 20 trials, the child being unable to complete the VEP testing, or technical problems (international adoptees: \(n=7\); non-adopted controls: \(n=10\)). There were no differences \((p<0.05)\) in group (international adoptees or non-adopted controls), region of origin, age, or nutritional indices between those who had complete VEP data and those who did not. Socioemotional and exploratory behaviors were coded for a subsample of internationally adopted children, \((n=17)\), four from Eastern Europe, nine from Ethiopia, and four from China. Behavior for the remaining participants has not yet been coded in the ongoing study.

**Statistical Analyses**

\(T\) tests and analysis of variance (ANOVA) were used for comparisons of continuous variables. Fisher LSD post-hoc analyses were completed for ANOVAs used to compare means between three or more groups. Non-parametric variables were assessed using \(\chi^2\) analyses. All results with an alpha level of 0.10 are reported.

**Results**

**Sample Characteristics**
**International adoptees.** Descriptive data for the international adoptees sample are shown in Table 10. Participants were between 8.71 months and 18.92 months at their initial clinical visit. There was a trend for those from Eastern Europe to be older than those adopted from Ethiopia, $F(2,38) = 3.04, p=0.06$. Time spent in institutional care also differed across region, $F(2,38) = 7.36, p<0.01$. Post hoc analyses revealed that those from Eastern Europe spent more time in institutional care than children adopted both Ethiopia, $p<0.001$, and China, $p=0.05$. There was a trend for those adopted from China to have spent more time in institutional care than those from Ethiopia, $p=0.074$. Results were similar when comparing the percent of pre-adoption time spent in institutional care, $F(2,38) = 3.44, p<0.05$. Those from Eastern Europe spent a greater percentage of their pre-adoption time in institutional care than those adopted from Ethiopia, $p<0.05$, and there was a trend for those from Eastern Europe to spend a greater percentage of time in institutional care than those from China, $p=0.096$. However, there was no difference between those adopted from Ethiopia and those adopted from China. Ratio of males to females differed across region of origin, $\chi^2(2) = 7.09, p<0.05$. Follow-up chi-square analyses revealed that Eastern Europe and China differed, with China having more females to males than Eastern Europe, $\chi^2(1) = 7.08, p<0.01$.

**Non-adopted controls.** Descriptive data for the non-adopted controls compared to the international adoptees is presented in Table 11. There were no differences between age at developmental assessment or male to female ratio between the non-adopted controls and the international adoptees. The international adoptees had lower HAZ, $t(61)=4.01, p<0.001$, WAZ, $t(61)=3.89, p<0.001$, and OFCZ, $t(61)=2.52, p<0.05$,
compared to the non-adopted controls. There were no differences in parental race, education level, or marital status between the international adoptees and the non-adopted controls. However, parental income was higher for the adopted group compared to the control group, with a greater percentage of the international adopted group earning over $100,000 annually, $\chi^2(1)=7.14, p<0.01.$

**Macronutrient Assessments**

Summary data for all the nutritional measures are presented in Table 12.

**Anthropometry.** Growth failure was evidenced by mean HAZ and WAZ below zero. There were no differences in HAZ, WAZ, OFCZ across regions; however, there was a trend for WHZ to differ across regions, $F(2,38)=2.50, p=0.096.$ Post-hoc analyses revealed that there were trends for those from China to have lower WHZ than those from Eastern Europe, $p=0.055,$ and those from Ethiopia, $p=0.067.$ Those with $z$ scores below -2 were considered in the below normal range. There were no differences in the rates of those below -2 $z$ scores for any of the anthropometry indices across regions.

**Serum protein.** There were no abnormal albumin levels. Thirty-three percent had low serum RBP concentrations. The incidence of low serum RBP did not differ across region of origin. Low RBP concentrations could be due to vitamin A deficiency, protein malnutrition, or the acute phase response. RBP is a carrier protein for retinol, and RBP was correlated with retinol, $r = 0.64, p<0.001;$ however low RBP was not likely due to vitamin A deficiency as there were no low plasma retinols in this cohort. RBP was positively correlated with WHZ, $r=0.39, p<0.05,$ suggesting that RBP may be associated
with protein malnutrition. RBP concentrations also decline during the acute phase response. However, RBP was not associated with CRP.

**Micronutrient Assessments**

Overall, 19% were iron deficient. Out of the seven children with ID, two (5%) had anemia. The rate of ID differed across regions, $\chi^2(2) = 12.65, p<0.01$. The rate of ID was higher in those from Eastern Europe than those adopted from Ethiopia, $\chi^2(1) = 9.00, p<0.01$, and from China, $\chi^2(1) = 7.29, p<0.01$. Of the entire sample, 37% were zinc deficient. The rate of zinc deficiency did not differ across region of origin. Iron and zinc deficiency were examined in association to neurodevelopment.

Twenty-three percent of the entire sample had low vitamin D. Eight percent ($n=2$) had frank deficiency and 15% ($n=4$) had an insufficient level. The rate of vitamin D deficiency/insufficiency differed across regions, $\chi^2(2) = 8.40, p<0.05$. Those from Eastern Europe had a higher rate than children adopted from Ethiopia, $\chi^2(1) = 6.79, p<0.01$, and China $\chi^2(1) = 4.38, p<0.05$.

There were no cases of vitamin A deficiency as indexed by serum retinol. There was one case of low folic acid (4%) and no cases of low vitamin B12. Overall, 9% had elevated TSH, which may be caused by iodine or selenium deficiency. Of the three participants with elevated TSH, two had free thyroxine (T4) levels within normal limits and one participant had a borderline free T4 level. The rate of elevated TSH did not differ across region of origin. Given the low incidence of these deficiencies, vitamin D, vitamin A, folic acid, vitamin B12, and TSH were not examined in association with neurodevelopment.
The association between micronutrient status and sample characteristics, nutritional status, and health indices. Analyses between specific micronutrient deficiencies (i.e., iron, zinc, and vitamin D) and sample characteristics, nutritional status, and health status were completed to identify risk factors for a given deficiency (Table 13). Specifically, independent t tests and chi-square analyses were used to compare age at adoption, time in institution, sex, nutritional status, serum lead levels, inflammation (elevated CRP), intestinal parasites, and chronic infection between those with a given deficiency and those with sufficient levels.

Those with ID spent a greater percent of pre-adoption time in institutional care, \( t(35) = -4.22, p < 0.001 \). However, it is unknown whether ID was associated with institutional care or region of origin, as children adopted from Eastern Europe had both a higher incidence of ID and spent more time in institutional care compared to those adopted from the other two regions. There were no differences found in these measures for zinc deficiency. Those with vitamin D insufficiency/deficiency were older at arrival compared to those with sufficient vitamin D, \( t(24) = -2.03, p = 0.05 \). Children from Eastern Europe had a higher rate for vitamin D insufficiency/deficiency, and they were also older than those adopted from the other two regions. Thus, it is not clear whether the risk for vitamin D insufficiency/deficiency was due to being older at the time of adoption or being adopted from Russia.

Finally, having one micronutrient deficiency did not seem to increase the likelihood of having other deficiencies. Of the 27 micronutrient deficiencies reported, 20 (74%) were in children with only one micronutrient deficiency. Two participants had
two micronutrient deficiencies; one child had both ID and vitamin D insufficiency, and another child had vitamin D insufficiency and folic acid deficiency. One participant had three deficiencies (ID, vitamin D insufficiency, and elevated TSH).

Neurodevelopmental Assessment

BSID III. Age at arrival, time spent in institutional care, and anthropometry were not correlated with either the BSID III cognitive composite score or the motor composite score. There were also no differences in the cognitive or motor composite scores between regions (Figure 3). Thus, all internationally adopted participants were analyzed together when examining performance on the BSID III. The international adoptees had lower BSID III scores, scoring approximately 1 SD (15 points) lower than the non-adopted controls on the cognitive composite scores (international adoptees: $M=89$, $SD=12$; controls: $M=104$, $SD=13$), $t(61)=4.70$, $p<0.001$, and motor composite scores (international adoptees: $M=83$, $SD=12$; controls: $M=97$, $SD=10$), $t(61)=5.00$, $p<0.001$.

Macronutrients. ANOVA was used to identify mean differences on the BSID III between non-adopted controls, international adoptees in the normal range, and international adoptees with low macronutrient status for each of the macronutrient indices (Figure 4). The analysis was not completed for WHZ as only one child had low WHZ. The ANOVA was significant for HAZ for both cognitive, $F(2,60)=12.12$, $p<0.001$, and motor scores, $F(2,60)=13.75$, $p<0.001$. The non-adopted controls differed from all the international adoptees, despite HAZ status, for both cognitive, $p<0.001$, and motor composite scores, $p<0.001$. The ANOVA for WAZ was also significant for both cognitive, $F(2,60)=10.91$, $p<0.001$, and motor scores, $F(2,60)=13.20$, $p<0.001$.  

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Specifically, the non-adopted controls differed from all the international adoptees, despite WAZ status, for both cognitive, normal WHZ: $p<0.001$; low WHZ, $p=0.01$, and motor scores, $p<0.001$. The ANOVA results for OFCZ was significant for cognitive, $F(2,60)=11.44$, $p<0.001$, and motor scores, $F(2,60)=13.94$, $p<0.001$. Again, the only mean difference was between the non-adopted controls and all international adoptees, despite OFCZ status, for both cognitive, normal OFCZ: $p<0.001$; low OFCZ: $p<0.01$, and motor scores, $p\leq0.001$. Overall, there were no mean differences on the BSID III cognitive composite score or the motor composite score between international adoptees in the normal range and those below the normal range for each of the markers of macronutrient status.

**Micronutrient.** The association between BSID III scores and the micronutrients iron and zinc were examined independently (Figure 4). The ANOVA for iron status was significant for cognitive, $F(2,56)=13.22$, $p<0.001$, and motor scores, $F(2,60)=11.88$, $p<0.001$. There were several mean differences in cognitive scores. First, non-adopted controls had higher scores than all international adoptees, $p<0.001$. Second, international adoptees with ID had lower scores than those with iron sufficiency on the cognitive composite, $p<0.05$. Because there was a higher rate of ID in those adopted from Eastern Europe, a two-way ANOVA was conducted which included only the internationally adopted participants and used both iron status and whether or not participants were adopted from Eastern Europe as independent variables. There was a trend toward significance for iron status, $F(1,27)=3.64$, $p=0.067$; however, neither the main effect for Eastern Europe or the interaction was significant. Thus, it is likely that the difference on
cognitive scores was due to iron status rather than region of origin. For the motor composite, the non-adopted controls had higher scores than the international adoptees despite iron status, iron sufficient: \( p<0.001 \); iron deficient: \( p=0.001 \). There was no mean difference in motor scores between adoptees with ID and those with iron sufficiency.

The ANOVA for zinc status was significant for cognitive, \( F(2,50)=8.53, p=0.001 \), and motor scores, \( F(2,50)=10.64, p<0.001 \). Non-adopted controls had higher scores than all international adoptees, despite zinc status, for both cognitive, zinc sufficiency: \( p<0.001 \); zinc deficiency: \( p<0.05 \), and motor scores, zinc sufficiency: \( p<0.001 \); zinc deficiency: \( p<0.05 \). There were no mean differences on the cognitive or motor composites between those with zinc deficiency and those with zinc sufficiency.

**VEP**. Oz P100 latency was not correlated with age, time in institution, or anthropometry. There were no differences in mean latencies across regions (Figure 3). Thus, all internationally adopted participants were analyzed together when examining VEPs. International adoptees who scored below average on the cognitive subtest of the BSID III (\(<85\) ) had longer Oz P100 latencies (\( M=142.50 \) ms, \( SD=24.44 \) ) compared to those who had cognitive scores in the normal range (\( M=123.00 \) ms, \( SD=17.05 \) ), \( t(24)=-2.23, p<0.05 \).

**Macronutrients**. There were no differences in the mean P100 latency for Oz between the international adopted participants and the non-adopted controls. There were also no mean differences on the Oz P100 latency between those in the normal range and those below the normal range on each of the of macronutrient status categories (Figure 5).
**Micronutrients.** Iron status was the only micronutrient examined in association to Oz P100 latencies because it was the only one expected to affect speed of processing. The ANOVA comparing mean latencies between non-adopted controls, iron sufficient adoptees, and iron deficient adoptees was not significant. Given the preliminary nature of the data analyses and the limited statistical power, an independent samples $t$ test between the iron sufficient adoptees and the iron deficient adoptees was done, and there was a trend for those with ID to have slower latencies, $t(22)=-1.85, p=0.08$ (Figure 5).

**Socioemotional and exploratory child behaviors and parent behaviors.** Given that there was a wide range of ages and age was correlated with several child behaviors, age at behavioral assessment was included as a covariate in all the behavioral analyses. The only trend for a difference across regions, while controlling for age, was for social communication, $F(3,13)=3.41, p=0.064$. Children adopted from Eastern Europe had lower social communication scores than those adopted from Ethiopia, $p<0.05$, and China, $p<0.05$ (Figure 3). Thus, all internationally adopted participants were analyzed together when examining behaviors, and region of origin was considered for the social communication behaviors.

**Macronutrients.** Although macronutrient status was not expected to be associated with behaviors, differences in behavior based on macronutrient status were screened to ensure there was no association between macronutrients and child or parent behaviors. There were no mean differences in child or parent behaviors between those in the normal range and those below the normal range on each of the of macronutrient indices, while controlling for age at behavioral assessment.
**Micronutrients.** The association between behaviors and the micronutrients iron and zinc were examined independently, and the results for the child behaviors are presented in Figure 6. Iron status was associated with several child behaviors. Those with ID were more fearful of new situations and objects, $F(2,14)=4.75, p<0.05$, and those with ID displayed less social communication, $F(2,14)=5.19, p<0.05$. Because participants adopted from Eastern Europe displayed less social communication, a two-way ANOVA was conducted using both iron status and whether or not participants were adopted from Eastern Europe as independent variables. Neither iron status nor region of origin was significant. Thus, given this small sample it is not known whether the difference in social communication is due to being adopted from Eastern Europe or due to iron status. Iron status was also associated with the parent behavior limit setting. Those with ID were less likely to require limit setting, $\chi^2(1)=3.86, p<0.05$. Fifty percent of adoptees with iron sufficiency required limit setting compared to 0% of those with ID.

There were differences in both child and parent behaviors based on zinc status. Those with zinc deficiency had poorer quality exploration, $F(2,10)=5.45, p<0.05$. Parents of children with zinc deficiency displayed less sensitivity with greater intrusiveness ($M=2.89, SD=0.18$) compared to those with zinc sufficiency ($M=4.13, SD=0.33$), $F(2,10)=10.66, p<0.01$.

**Discussion**

**Nutritional Status**

**Macronutrients.** Similar to previous studies in international adoptees (e.g., Johnson, 2000; Miller & Hendrie, 2000), we found macronutrient abnormalities as
evidenced by growth failure. Children adopted from Eastern Europe, Ethiopia, and China were on average approximately one standard deviation below normal for length for age and weight for age, and this did not differ across region. Mean weight for height was near average; however, there was a trend for children from China to have lower weight for height than the other two regions. OFC growth failure occurred to a lesser degree than length and weight for age, which was likely due to selections for children with larger head circumferences during the pre-adoption screening. These anthropometric indices were similar to our previous studies in children adopted from Eastern Europe, where height and weight for age were more affected than weight for height and OFC (Fuglestad et al., 2008). However, contrary to our findings, a recent study did not find growth failure in children adopted from Ethiopia (Miller et al., 2008). This discrepancy may be due to the fact that the precise birth dates of many children coming from Ethiopia are uncertain and ages which are estimated based on growth and development (Miller et al., 2008) may cause an underestimation of growth failure.

Further evidence for macronutrient abnormalities was that one-third had low concentrations of the serum protein RBP. However, the etiology of the low RBP was unclear. Low RBP may be due to both vitamin A deficiency and protein malnutrition, (Baeten et al., 2004; Fuhrman et al., 2004). RBP values were correlated with both weight for height and serum retinol, indicating that RBP values were likely affected by both macronutrient and vitamin A status. RBP is also a negative acute phase reactant, and concentrations decrease during inflammatory states. A recent review suggests that low concentrations of hepatic proteins, including RBP, may be an indicator of the seriousness
of inflammation and disease (Fuhrman et al., 2004). We did not find any association between RBP and other indicators of inflammation (i.e., elevated CRP) or disease (i.e., presence of infections).

**Micronutrients.** Micronutrient deficiencies were common, with 56% of the international adoptees having at least one micronutrient deficiency. Zinc and iron were the most prevalent deficiencies. Vitamin D insufficiency/deficiency was also common. There were only a few cases other micronutrient deficiencies. There was little comorbidity among the micronutrient deficiencies; only three children had more than one deficiency. Although all adoptees, despite region, were at risk for micronutrient deficiencies, there were regional differences in the status of particular micronutrients.

Similar to our previous studies in children adopted from Eastern Europe (Fuglestad et al., 2008), ID, primarily without anemia, was common. Nineteen percent of children adopted from Eastern Europe, Ethiopia, and China were iron deficient. However, there was a regional difference with children adopted from Eastern Europe having a much higher rate of ID than children adopted from the other two regions. Approximately two-thirds of the children adopted from Eastern Europe had ID compared to less than 10% of children adopted from China or Ethiopia. This rate of ID in children from Eastern Europe was much higher than the rate we reported in our previous studies (i.e., approximately one quarter). This could be due to the age difference in the samples. We previously found that children from Eastern Europe who had ID were younger than those who were iron sufficient (chapter IIb). The sample of adoptees in the current study
was younger at adoption (9 to 18 months) than the previous samples (9 to 24 months and 9 to 46 months).

Contrary to our expectations, ID rates were not similar across regions, and the reason for this difference was not clear. Iron status in infants and toddlers is a function of both iron stores at birth and postnatal dietary intake. Infants born with low iron stores at birth (e.g., premature and intrauterine growth restricted infants) are at an increased risk for postnatal ID (Georgieff et al., 1995; Widdowson & Spray, 1951). Pre-adoption information regarding birth weight, gestation length, and postnatal dietary intake prior to adoption was not available for participants in this study, but may have varied across regions.

In addition to iron stores and postnatal dietary intake, iron status is a function of iron absorption and utilization. Several indices of iron absorption and utilization were measured at the time of adoption. Although, there was no significant difference in the rate of parasitic infections, which may interfere with intestinal iron absorption (Luján et al., 1995; Troeger et al., 2007), 33% of those with ID were infected with an intestinal parasite compared to 15% of those with good iron status. As we continue to enroll participants in this study, it will be interesting to see if our previous finding in which the presence of intestinal parasites increased the risk for ID is replicated. The only difference between those with ID and those with iron sufficiency was that participants with ID had spent more time in institutionalization. We hypothesized in the previous study that ID in this population may in part be associated with the pre-adoption adversity, which may create a hepcidin-mediated deficiency similar to the anemias of chronic inflammation and
chronic disease (Fuglestad et al., 2008). Consistent with this hypothesis, longer duration of institutionalization increased the risk for ID. However, because children adopted from Eastern Europe had spent more time in institutionalization than the other two regions, it is difficult to distinguish whether higher rates of ID were due to institutionalization or to being adopted from Eastern Europe.

Zinc deficiency was more prevalent than ID; 37% of the participants were zinc deficient at the time of arrival. There were no significant differences in the rate of zinc deficiency across regions even though the rate in children adopted from China was over twice that than in those adopted from Eastern Europe. Fifty percent of children adopted from China were zinc deficient.

The risk for zinc deficiency was not evident from our data. Studies in other populations show that risk for zinc deficiency is highest in those who have inadequate zinc intake due primarily to low consumption of animal foods (the best source of zinc) and/or consume diets with low bioavailability due to high intakes of foods which interfere with zinc absorption (such as phytates or fiber; Joint FAO/WHO Expert Consultation on Human Vitamin and Mineral Requirements, 2004). However, information on pre-adoption dietary intake was not available in this study, and therefore not assessed. Secondary zinc deficiency may occur despite adequate zinc intake. During the acute phase response, zinc is sequestered to the liver, decreasing its availability for other tissues (Keen et al., 1993). Zinc deficiency may also occur due to increased losses during diarrhea (Joint FAO/WHO Expert Consultation on Human Vitamin and Mineral Requirements, 2004). The risk for zinc deficiency increases during periods of growth.
since zinc is critical for growth. However, macronutrient status, measures of the acute phase response and inflammation (i.e., CRP), and presence of intestinal parasites or chronic infections at arrival were not associated with zinc deficiency.

Vitamin D insufficiency/deficiency was common. Twenty-three percent had either insufficient or deficient levels (eight percent had frank vitamin D deficiency). Adoptees from Russia had the highest rate, with two-thirds having insufficient vitamin D. Rates in Ethiopia and China were both under 10%. These rates of vitamin D insufficiency/deficiency, other than in children adopted from Russia, were lower than expected given the estimated rates of 12% with vitamin D deficiency and 40% with insufficiency in healthy toddlers growing up in the northern U.S. (Gordon et al., 2008).

Other than iron, zinc, and vitamin D, there were few other micronutrient deficiencies. There were only a few cases of elevated TSH. Although iodine and selenium were not assessed directly, this was likely due to low iodine. There was one case of low folic acid and no vitamin B₁₂ deficiencies. Contrary to our expectations, there was no vitamin A deficiency identified by low serum retinol. However, low RBP, another indicator of vitamin A deficiency, was observed.

**Association between Nutritional Status and Neurodevelopment**

Given that over half of international adoptees had a micronutrient deficiency, it is important to understand the role of nutrient status in neurodevelopment, especially since the majority of the deficiencies were iron and zinc, both nutrients that are important for neurodevelopment. Similar to previous studies in international adoptees (Sonuga-Barke et al., 2008), macronutrient abnormalities were not associated with neurodevelopment.
However, micronutrient abnormalities were associated with several neurodevelopmental domains.

**General cognitive and motor development.** The international adoptees, despite region of origin, were delayed in general cognitive and motor development compared to non-adopted controls who were growing up in similar home environments to the post-adoption environments of the adopted group. On average, the adopted children scored approximately one standard deviation below the non-adopted group on both the cognitive and motor subtests of the BSID III. There were no differences in cognitive or motor scores across regions for the adopted group.

Iron status, but not zinc status, was associated with cognitive scores. International adoptees with ID scored 10 points lower than the adoptees with iron sufficiency. This difference seemed to be due to iron status rather than whether or not the children were adopted from Eastern Europe. Although, the etiology of the ID in this population is not known, there is emerging evidence that cognitive effects are greater when the deficiency is experience prenatally rather than postnatally (Golub, 2010). Thus, it is probable that many of those with ID at arrival have experienced perinatal ID, during which low iron stores at birth would increase the risk for ID at the time of adoption. Unlike studies in other populations with ID (Lozoff et al., 2006), we did not find any differences in motor scores between those with ID and those with iron sufficiency. It is possible that other risk factors such as lack of pre-adoption opportunity for motor practice or decreased muscle tone may mask any effects of ID on motor development.
Although we expected zinc deficiency to be associated with general cognitive and motor development, there were no differences in BSID III scores. However, the evidence for the role of zinc deficiency on general development has been mixed, with some studies reporting mean differences on standardized measures of development (Castillo-Duran et al., 2001), while others do not (Ashworth et al., 1998). It is likely that the general measures of development are not very sensitive to the specific neurodevelopmental effects of zinc deficiency.

**Electrophysiological speed of processing.** There was no difference in speed of processing (i.e., VEP latencies) between the international adoptees and the non-adopted controls. Nor were there differences among the international adoptees across regions of origin. Macronutrient abnormalities were also not associated with VEP latencies even though altered lipid profile may affect myelination (Faldella et al., 1996). It may be that a more precise measure of lipids (e.g., specific fatty acids), rather than a general macronutrient measure, such as height and weight, will be more predictive of latencies.

Similar to studies of ID with anemia, ID in the international adoptees was associated with longer latencies, perhaps due to disruptions in myelination. This finding is unique in that it is the first study to show delayed speed of processing in associated with ID, primarily without anemia. However, the sample size was small, and this finding needs to be replicated with more participants. Measuring VEP is important in that it yields human evidence consistent with the hypomyelination observed in animal models of ID (Larkin & Rao, 1990) and it helps in understanding the neurodevelopmental processes interrupted by ID. However, VEP is a measure of the speed of processing only
in the visual system. It is reasonable to speculate that if myelination in the visual system is disrupted by ID, other systems undergoing myelination at the same time (e.g., cognitive systems) may also be affected. For instance, those with BSID III cognitive scores in the below average range had slower VEP latencies. Future studies that investigate the neurodevelopmental processes underlying the general cognitive delays associated with ID are needed.

**Socioemotional and exploratory behaviors.** Preliminary data for child socioemotional and exploratory behaviors and parent behaviors during a play session was available for a subsample of the international adoptees. Those with ID were more fearful to new situations and objects. Increased fearfulness in international adoptees is consistent with the parent-reported behaviors in the previous study of Eastern European adoptees who were described as more fearful within one month of arrival. During the free play, those with ID also displayed less social communication, both initiating and responding to, than their iron sufficient peers. These socioemotional behaviors are similar to those that have been described in other populations who have experienced early ID (Lozoff et al., 2007).

We did not find problems with cooperation in adoptees with ID. However, in our previous study, activity and cooperation problems were not observed until six months post-adoption. We had hypothesized that problems with activity and cooperation may have been due to a transactional effect during which a wary child elicits less parental interaction and requires less parental help with behavior regulation, leading to increased externalizing behaviors later on. Consistent with this hypothesis, those with ID required
less limit setting by their parents during the play session than those with good iron status. Follow-up studies will be necessary to determine whether such child and parent behaviors do indeed lead to attention and activity problems, behaviors that are well-documented in international adoptees.

International adoptees with zinc deficiency displayed different exploratory behaviors from those with zinc sufficiency. Specifically, those with zinc deficiency had poorer quality exploration (i.e., less manipulation and active exploration of toys) during the free play session. Although quality of exploration has not been directly assessed in children with zinc deficiency, previous studies do show that infants and toddlers with zinc deficiency spend less time in active and energetic behaviors (Sazawal et al., 1996). The role these behaviors play in cognitive and social development in children with zinc deficiency is not clear. However, according to the functional isolation hypothesis, undernourished children with low levels of activity, reduced exploration, and decreased social interaction may lead to, or worsen, hampered cognitive development (Lozoff et al., 1998b). Future studies are needed to clarify the association between exploratory behavior and cognitive development in zinc deficient children.

Parents of adoptees with zinc deficiency displayed less sensitivity and more intrusiveness during the play session. Specifically, parents of those with zinc deficiency were less child-centered in their behaviors and less successful in reading their child’s cues and responding effectively. Rather, their behaviors were more adult-centered, being more directive and controlling. Although the explanation for this difference in parent behavior is uncertain, a symptom of zinc deficiency is lethargy (Prasad, 1985), and one
hypothesis it that more directive, adult-centered parenting is required with a child who is more lethargic. Although, lethargy specifically was not measured in this study, the exploratory behaviors of those with zinc deficiency were less active. A child who expends less energy and is less active may require more parent-centered behaviors rather than child-centered.

Although the data on socioemotional and exploratory behaviors is preliminary and was completed for only a subsample, continued research on the role of both iron and zinc deficiency in child behaviors, parent behaviors, and parent-child interaction will be important in understanding the role of nutritional status on cognitive, behavioral, and social development.

There are several limitations to this study. First, a small number of children have been enrolled in this study. We are continuing to enroll participants and collect data and will have more power to distinguish developmental differences due to nutritional status as well as to increase the strength of our current findings. Second, the associations between nutritional status and neurodevelopment are correlational, and it is difficult to assign a causal relationship between a specific deficiency and the neurodevelopmental outcomes.

Furthermore, this study only included one time point. Follow-up studies are needed to track the resolution of the nutritional deficiencies and to determine the appropriate nutritional intervention for each nutrient. For instance, in our previous study (Fuglestad et al., 2008), post-adoption catch-up growth increased the risk for ID. Zinc, which is also necessary for growth, will need to be followed during the early adoption period. Follow-up studies are also needed to determine whether or not the developmental
correlates of nutritional deficiencies persist beyond the resolution of the deficiencies. Moreover, it will be important to understand how nutritional deficiencies affect the adoptees’ interactions with their physical and social environments, which may alter long-term cognitive, social, and behavioral outcomes. Another area of future research will be to investigate specific cognitive domains, such as hippocampal-dependent explicit memory, prefrontal-dependent working memory and inhibitory control, and striatal-mediated implicit memory, which may also be affected by iron or zinc deficiency.

This research has important clinical implications for both international adoptees as well as other populations who may experience nutritional deficiencies. We found that ID, the majority without anemia, was associated with adverse effects on general cognitive development, slower speed of processing (perhaps due to disruptions in myelination), and alterations in both child and parent behaviors. ID without anemia is rarely screened in infants and toddlers, and this study along with others are beginning to show that pre-anemic ID may have similar effects on neurodevelopment as ID with anemia. In addition, this is the first study to investigate zinc status in internationally adopted children, and we found high rates of zinc deficiency as well as an association with altered child and parent behaviors. Screening for and treating both nonanemic ID and zinc deficiency may be an effective intervention to best support neurodevelopment during the early post-adoption period in international adoptees.
Table 9
*Nutritional Assessments and Norms*

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Measure</th>
<th>Normal Value</th>
<th>Assay Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>Anthropometry</td>
<td>≥2 z-scores (CDC)</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Serum Albumin</td>
<td>7-12m: 3.2-5.7 g/dL</td>
<td>Colorimetric reflectance spectrophotometry (bromocresol green)/Ortho 5.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13-24 m: 1.9-5.0 g/dL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Retinol Binding Protein (RBP)</td>
<td>3-6 mg/dL</td>
<td>Nephelometry</td>
</tr>
<tr>
<td>Iron*</td>
<td>Hemoglobin (Hgb)</td>
<td>&gt;11 mg/dL</td>
<td>Spectrophotometric/Coulter</td>
</tr>
<tr>
<td></td>
<td>MCV</td>
<td>&gt;74 fl</td>
<td>Electrical impedance; Spectrophotometric; Laser optical scatter/Coulter</td>
</tr>
<tr>
<td></td>
<td>Serum Ferritin (SF)</td>
<td>&gt;12 µg/L</td>
<td>Chemiluminescent immunoassay; Immunometric assay</td>
</tr>
<tr>
<td></td>
<td>Transferrin Saturation (TS)</td>
<td>&gt;12%</td>
<td>Rate nephelometry</td>
</tr>
<tr>
<td>Zinc</td>
<td>Serum Zinc</td>
<td>60 -120 µg/dL</td>
<td>Atomic absorption spectrophotometry</td>
</tr>
<tr>
<td>Vitamin D**</td>
<td>Plasma 25-OH</td>
<td>&gt;30 ng/mL</td>
<td>Radioimmunoassay</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>Plasma Retinol</td>
<td>13-50 µg/dL</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>Folate</td>
<td>Serum Folate</td>
<td>&gt;5.4 ng/mL</td>
<td>Chemiluminescent immunoassay</td>
</tr>
<tr>
<td></td>
<td>Red Blood Cell Folate</td>
<td>280-791 ng/mL</td>
<td>Chemiluminescent immunoassay</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>Serum Vitamin B12</td>
<td>200-900 pg/mL</td>
<td>Chemiluminescent immunoassay</td>
</tr>
<tr>
<td>Iodine &amp; Selenium</td>
<td>Thyroid Stimulating Hormone (TSH)</td>
<td>0.4-5.0 mU/L</td>
<td>Chemiluminescent immunoassay</td>
</tr>
<tr>
<td></td>
<td>Free T4 (Thyroxine)</td>
<td>&lt;12 m: 0.80-1.90 ng/dL</td>
<td>Chemiluminescent immunoassay; competitive immunoassay</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;12 m: 0.7-1.85 ng/dL</td>
<td></td>
</tr>
</tbody>
</table>

*Note.* * Iron deficiency was defined as having two abnormal values. Iron deficiency with anemia was defined as having iron deficiency as well as low Hgb. Threshold values to define ID were based on NHANES II (Expert Scientific Working Group, 1985), NHANES III (Looker et al., 1997a), and Healthy People 2000 (National Center for Health Statistics, 2001) and 2010 (U.S. Department of Health and Human Services, 2000).

**Vitamin D deficiency was defined as <20 ng/mL, and vitamin D insufficiency was defined as >20 ng/mL and <30 ng/mL (Holick, 2007).**
Table 10
Sample Characteristics for International Adoptees

<table>
<thead>
<tr>
<th></th>
<th>Eastern Europe $n=10$</th>
<th>Ethiopia $n=18$</th>
<th>China $n=13$</th>
<th>Total Sample $n=41$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>M (SD)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (months)</td>
<td>14.20 (3.18)</td>
<td>11.66 (2.36)</td>
<td>12.94 (2.60)</td>
<td>12.68 (2.78)</td>
</tr>
<tr>
<td>Time in Institution (months)</td>
<td>12.10 (3.89)</td>
<td>6.03 (2.00)</td>
<td>8.73 (5.87)</td>
<td>8.37 (4.63)</td>
</tr>
<tr>
<td>Preadoption Time in Institution</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (% female)</td>
<td>30 (3)</td>
<td>61 (11)</td>
<td>85 (11)</td>
<td>61 (25)</td>
</tr>
</tbody>
</table>

Note. Groups having the same subscript are not significantly different ($p<0.10$).
Table 11
Sample Characteristics of Non-Adopted Controls Compared to International Adoptees

<table>
<thead>
<tr>
<th></th>
<th>International Adoptees</th>
<th>Non-adopted Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( n=35 )</td>
<td>( n=28 )</td>
</tr>
<tr>
<td><strong>M (SD)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at Developmental Assessment</td>
<td>12.74 (2.56)</td>
<td>12.94 (2.68)</td>
</tr>
<tr>
<td>Time in Institution (months)</td>
<td>7.91 (4.55)</td>
<td>---</td>
</tr>
<tr>
<td>HAZ</td>
<td>-1.09 (1.23)</td>
<td>0.00 (0.85)***</td>
</tr>
<tr>
<td>WAZ</td>
<td>-1.17 (1.12)</td>
<td>-0.12 (0.99)***</td>
</tr>
<tr>
<td>WHZ</td>
<td>-0.03 (1.03)</td>
<td>0.34 (0.97)</td>
</tr>
<tr>
<td>OFCZ</td>
<td>-0.12 (1.23)</td>
<td>0.56 (0.81)*</td>
</tr>
<tr>
<td></td>
<td>% (n)</td>
<td>% (n)</td>
</tr>
<tr>
<td>Sex (% Female)</td>
<td>63 (22 of 35)</td>
<td>57 (16 of 28)</td>
</tr>
<tr>
<td>Socioeconomic Status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parent 1 Race (% Caucasian)</td>
<td>97 (30 of 31)</td>
<td>96 (21 of 22)</td>
</tr>
<tr>
<td>Parent 2 Race (% Caucasian)</td>
<td>100 (28 of 28)</td>
<td>96 (21 of 22)</td>
</tr>
<tr>
<td>Marital Status (% Married)</td>
<td>90 (28 of 31)</td>
<td>96 (21 of 22)</td>
</tr>
<tr>
<td>Parent 1 Education Level (% Bachelors Degree)</td>
<td>94 (29 of 31)</td>
<td>82 (18 of 22)</td>
</tr>
<tr>
<td>Parent 2 Education Level (% Bachelors Degree)</td>
<td>79 (22 of 28)</td>
<td>78 (17 of 22)</td>
</tr>
<tr>
<td>Annual Household Income (% over $100,000)</td>
<td>65 (20 of 31)</td>
<td>27 (6 of 22)**</td>
</tr>
</tbody>
</table>

*Note. Data is for all those who completed the neurobehavioral developmental assessment.
†\( p<0.10 \). * \( p<0.05 \). ** \( p<0.01 \). *** \( p<0.001 \).
Table 12
Macro- and Micronutritional Status of International Adoptees

<table>
<thead>
<tr>
<th></th>
<th>Eastern Europe</th>
<th>Ethiopia</th>
<th>China</th>
<th>Total Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=10</td>
<td>n=18</td>
<td>n=13</td>
<td>n=41</td>
</tr>
<tr>
<td><strong>Macronutrient Status</strong> M (SD)</td>
<td><strong>M (SD)</strong></td>
<td><strong>M (SD)</strong></td>
<td><strong>M (SD)</strong></td>
<td><strong>M (SD)</strong></td>
</tr>
<tr>
<td>HAZ</td>
<td>-1.01 (1.38)</td>
<td>-1.37 (1.21)</td>
<td>-0.77 (1.06)</td>
<td>-1.12 (1.20)</td>
</tr>
<tr>
<td>WAZ</td>
<td>-0.95 (1.13)</td>
<td>-1.27 (1.16)</td>
<td>-1.37 (0.84)</td>
<td>-1.23 (1.05)</td>
</tr>
<tr>
<td>WHZ</td>
<td>0.25 (1.14)</td>
<td>0.10 (0.92)</td>
<td>-0.58 (0.98) b</td>
<td>-0.08 (1.03)</td>
</tr>
<tr>
<td>OFCZ</td>
<td>-0.46 (1.26)</td>
<td>0.23 (1.19)</td>
<td>-0.57 (1.08)</td>
<td>-0.19 (1.20)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>% Below Normal (n)</th>
<th>% Below Normal (n)</th>
<th>% Below Normal (n)</th>
<th>% Below Normal (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAZ</td>
<td>30 (3)</td>
<td>33 (6)</td>
<td>23 (3)</td>
<td>29 (12)</td>
</tr>
<tr>
<td>WAZ</td>
<td>20 (2)</td>
<td>22 (4)</td>
<td>15 (2)</td>
<td>20 (8)</td>
</tr>
<tr>
<td>WHZ</td>
<td>0 (0)</td>
<td>6 (1)</td>
<td>0 (0)</td>
<td>2 (1)</td>
</tr>
<tr>
<td>OFCZ</td>
<td>10 (1)</td>
<td>6 (1)</td>
<td>8 (1)</td>
<td>7 (3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Serum Proteins</strong></th>
<th>% Below Normal (n)</th>
<th>% Below Normal (n)</th>
<th>% Below Normal (n)</th>
<th>% Below Normal (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>0 (0 of 6)</td>
<td>0 (0 of 12)</td>
<td>0 (0 of 12)</td>
<td>0 (0 of 30)</td>
</tr>
<tr>
<td>RBP</td>
<td>33 (2 of 6)</td>
<td>39 (5 of 13)</td>
<td>25 (2 of 8)</td>
<td>33 (9 of 27)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Micronutrient Status</strong></th>
<th>% Deficient (n)</th>
<th>% Deficient (n)</th>
<th>% Deficient (n)</th>
<th>% Deficient (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron</td>
<td>63 (5 of 8) a</td>
<td>6 (1 of 16) b</td>
<td>8 (1 of 13) b</td>
<td>19 (7 of 37)</td>
</tr>
<tr>
<td>Zinc</td>
<td>20 (1 of 5)</td>
<td>31 (4 of 13)</td>
<td>50 (5 of 10)</td>
<td>37 (10 of 28)</td>
</tr>
<tr>
<td>Vitamin D1</td>
<td>67 (4 of 6) a</td>
<td>8 (1 of 12) b</td>
<td>13 (1 of 8) b</td>
<td>23 (6 of 26)</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>0 (0 of 0)</td>
<td>0 (0 of 12)</td>
<td>0 (0 of 8)</td>
<td>0 (0 of 26)</td>
</tr>
<tr>
<td>Folic Acid</td>
<td>20 (1 of 5)</td>
<td>0 (0 of 11)</td>
<td>0 (0 of 11)</td>
<td>4 (1 of 27)</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>0 (0 of 0)</td>
<td>0 (0 of 13)</td>
<td>0 (0 of 12)</td>
<td>0 (0 of 31)</td>
</tr>
<tr>
<td>TSH (elevated) (Iodine &amp; Selenium)</td>
<td>14 (1 of 7)</td>
<td>0 (0 of 16)</td>
<td>20 (2 of 10)</td>
<td>9 (3 of 33)</td>
</tr>
</tbody>
</table>

*Note.* Groups having the same subscript are not significantly different (p<0.10). 1Includes both vitamin D deficiency and insufficiency.
Table 13
Sample Characteristics, Nutritional Status, and Health Indices as Possible Risk Factors for Micronutrient Deficiencies

<table>
<thead>
<tr>
<th></th>
<th>Sufficient</th>
<th>Deficient</th>
<th>Sufficient</th>
<th>Deficient</th>
<th>Sufficient</th>
<th>Insufficient/Deficient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$M$ (SD)</td>
<td>$M$ (SD)</td>
<td>$M$ (SD)</td>
<td>$M$ (SD)</td>
<td>$M$ (SD)</td>
<td>$M$ (SD)</td>
</tr>
<tr>
<td>Age at Arrival (months)</td>
<td>12.48 (2.85)</td>
<td>14.35 (2.78)</td>
<td>13.02 (2.93)</td>
<td>12.15 (1.8)</td>
<td>11.50 (2.63)</td>
<td>14.33 (4.13)*</td>
</tr>
<tr>
<td>Preadoption Time in</td>
<td>64.97 (32.15)</td>
<td>91.16 (5.28)**</td>
<td>63.68 (33.55)</td>
<td>74.47 (29.70)</td>
<td>58.87 (33.32)</td>
<td>76.83 (29.60)</td>
</tr>
<tr>
<td>Institutional Care (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAZ</td>
<td>-0.89 (1.10)</td>
<td>-1.17 (0.84)</td>
<td>-0.86 (1.06)</td>
<td>-0.75 (1.26)</td>
<td>-0.81 (1.16)</td>
<td>-0.79 (0.86)</td>
</tr>
<tr>
<td>WAZ</td>
<td>-1.10 (1.02)</td>
<td>-1.45 (0.74)</td>
<td>-1.02 (0.78)</td>
<td>-1.24 (1.50)</td>
<td>-1.03 (1.07)</td>
<td>-0.92 (0.86)</td>
</tr>
<tr>
<td>WHZ</td>
<td>-0.17 (1.08)</td>
<td>-0.29 (0.62)</td>
<td>-0.08 (1.07)</td>
<td>-0.49 (1.19)</td>
<td>-0.17 (0.88)</td>
<td>-0.01 (1.23)</td>
</tr>
<tr>
<td>OFCZ</td>
<td>-0.05 (1.21)</td>
<td>-0.36 (1.28)</td>
<td>-0.01 (1.28)</td>
<td>-0.16 (1.47)</td>
<td>-0.03 (1.50)</td>
<td>0.28 (0.55)</td>
</tr>
<tr>
<td>Lead (µg/dL)</td>
<td>3.29 (6.69)</td>
<td>1.83 (1.17)</td>
<td>2.00 (1.10)</td>
<td>2.10 (1.10)</td>
<td>2.00 (1.05)</td>
<td>8.80 (15.82)**</td>
</tr>
<tr>
<td>Sex (% Female)</td>
<td>67 (20 of 30)</td>
<td>43 (3 of 7)</td>
<td>50 (9 of 18)</td>
<td>80 (2 of 10)</td>
<td>65 (13 of 20)</td>
<td>50 (3 of 6)</td>
</tr>
<tr>
<td>Elevated CRP (&gt;8mg/L )</td>
<td>5 (1 of 22)</td>
<td>20 (1 of 5)</td>
<td>7 (1 of 15)</td>
<td>0 (0 of 6)</td>
<td>6 (1 of 16)</td>
<td>0 (0 of 5)</td>
</tr>
<tr>
<td>Intestinal Parasite</td>
<td>15 (4 of 27)</td>
<td>33 (2 of 6)</td>
<td>12 (2 of 17)</td>
<td>13 (1 of 8)</td>
<td>11 (2 of 18)</td>
<td>33 (2 of 6)</td>
</tr>
<tr>
<td>Presence of Infection</td>
<td>20 (6 of 30)</td>
<td>0 (0 of 7)</td>
<td>11 (2 of 18)</td>
<td>20 (2 of 10)</td>
<td>13 (3 of 20)</td>
<td>0 (0 of 6)</td>
</tr>
</tbody>
</table>

Note. Comparisons were done between the sufficient and deficient groups for each micronutrient. †$p<0.10$. *$p<0.05$. **$p<0.01$. ***$p<0.001$. 
Figure 3. Neurodevelopmental outcomes as a function of region of origin. †p<0.10. * p<0.05. ** p<0.01. *** p<0.001.
Figure 4. Bayley Scales of Infant Development (BSID) III as a function of macro- and micronutrient status. IA: international adoptees. †p<0.10. * p<0.05. ** p<0.01. *** p<0.001.
Figure 5. VEPs as a function of macro- and micronutrient status. IA: international adoptees. †p<0.10. * p<0.05. ** p<0.01. *** p<0.001.
Figure 6. Child behaviors as a function of micronutrient status. Means are adjusted for age at assessment. Higher scores indicate greater evidence of the given behavior. IA: international adoptees. †p<0.10. * p<0.05. ** p<0.01. *** p<0.001.
Chapter IV. General Summary, Clinical Implications, and Future Directions

In the three studies, we found high rates of both iron and zinc deficiency, as well as some vitamin D insufficiency/deficiency. There was a risk for micronutrient deficiencies in all internationally adopted children adopted from Eastern Europe, Ethiopia, and China, with 56% having at least one micronutrient deficiency. However, children adopted from Eastern Europe had higher rates of both iron deficiency and vitamin D insufficiency/deficiency than children coming from the other two regions. We also found that micronutrient status during the early adoption period is associated with cognitive development, speed of processing, and both child behaviors and parent behaviors. These research findings are based on a limited sample of international adoptees. However, continued research in this area will have important clinical implications and will yield greater understanding on the role of nutrition in neurodevelopment.

The findings in these three studies, as well as continued research in this area, have several important clinical implications. Approximately 250,000 children under the age of three years in the United States are reported to be maltreated (Child Welfare Information Gateway, 2006). Growth failure and long-term learning problems are a hallmark of such children living in early adverse environments (e.g., King & Taitz, 1985; Olivan, 2003) and are similar to those seen in international adoptees. Continued research may inform nutritional and neurodevelopmental principles which can be applied to developing interventions and services for children living in adverse environments, including children adopted internationally into the U.S. Assessment of nutritional status is not part of the regular clinical evaluation of maltreated children under the age of three. Ultimately, this
research will be useful in developing clinical screening for maltreated populations to
determine optimal nutritional interventions to support growth and neurodevelopmental
recovery. Unlike many risk factors (i.e., fetal alcohol exposure) experienced by
international adoptees and other maltreated populations that are not amendable, nutrition
is amendable through intervention.

A second clinical implication is that this research shows that ID, primarily
without anemia, has similar associations with neurodevelopment as ID with anemia.
During ID, iron is prioritized to red blood cells for hemoglobin synthesis over other
tissues, and brain ID occurs prior to anemia (Georgieff, 2008). Thus, anemia, which is
routinely assessed in infants and toddlers, may not be an accurate indication of brain ID.

Future research will be important to understand metabolism in populations
experiencing early adversity. Both macronutrient delivery (dietary intake) and nutrient
assimilation and distribution are required to support growth. A potential mechanism for
growth failure due to early adversity is altered nutrient assimilation due to alterations in
the hypothalamic-pituitary-adrenocortical axis (HPA) which may down-regulate growth
hormones. Early adversity may also affect micronutrient metabolism. For example,
individuals with chronic inflammatory diseases or immune responses commonly develop
the anemias of chronic inflammation and chronic disease. Internationally adopted
children experience a wide range of social and health adversities prior to adoption which
may affect micronutrient metabolism, similar to that in the anemias of chronic
inflammation and chronic disease (e.g., Coe, Lubach, & Shirtcliff, 2007). Given the
evidence from animal models and a growing body of evidence from studies in children
which show that that early adverse experiences are associated with disturbance in HPA
functioning (Bruce, Fisher, Pears, & Levine, 2009; E.R. De Kloet, Rosenfeld, Van Eekelen, Sutanto, & Levine, 1988; De Kloet, Vreugdenhil, Oitzl, & Joels, 1998; Meaney & Szyf, 2005), one possibility to be explored is that altered metabolism due to HPA-driven down-regulation of growth hormones increases the risk for nutrient deficiencies. Future studies are needed to investigate the association between the HPA axis and nutritional status, as well as the role of both HPA and nutritional status in neurodevelopment.

Another area of future research will be to investigate the interactions between cognitive systems, interactions between cognitive and social and behavioral domains (all of which may be affected by nutritional status), as well as the indirect effects of nutrition on cognitive development. It is important to note that neural systems may compete with, cooperate with, and/or compensate for activity of other neural systems (Kim & Baxter, 2001; White & McDonald, 2002). Thus, while specific hypotheses can be made on the neurodevelopmental effects of a nutrient deficiency based on that nutrient’s role(s) in a particular neural system, neural systems do not function in isolation. Furthermore, in the three studies, we have shown that iron and zinc deficiency are associated with both child and parent behaviors, and in Eastern European adoptees, cognitive performance seemed to be mediated by behaviors during testing. Not only will continued research in the interactions of neural systems and in the interactions between domains improve our understanding of the role of specific micronutrients on development, but will also inform interventions. For instance, many of the developmental effects of iron deficiency persist after iron is repleted (Lozoff & Georgieff, 2006). Research is needed to investigate whether the cognitive and behavioral effects of iron and zinc deficiency we observed are
reversed with nutritional treatment post-adoption, and if not, whether behavioral interventions are effective in ameliorating some of the effects of the deficiency.
References


