23 Cognitive and Behavioral Consequences of Iron Deficiency

Laura E. Murray-Kolb and Amrita Arcot

Keywords

Iron deficiency · Cognition · Behavior · Affect Neurophysiology · Women · Children Neurobiology

Introduction

Iron deficiency is the most prevalent single nutrient deficiency worldwide primarily affecting infants, children, and women of reproductive age [1]. Although individuals of all ages and classes are susceptible to iron deficiency, those who are poor and less educated are most vulnerable. Iron deficiency has been identified as a biological risk factor for the failure of >250 million children in reaching their full developmental potential, resulting in reductions in economic productivity [2]. The association between iron status and brain functioning has been examined through investigations of central nervous system biochemical changes using animal studies and through the assessment of cognitive functioning and behavior in humans. There is clear evidence from animal studies that iron is critical for myelination, neuronal morphology, neuronal metabolic activity, and the synthesis of monoamines [3]. In humans, the evidence points to cognitive, motor, behavioral, and affective alterations in iron-deficient individuals [4]. In most age groups, iron repletion appears to ameliorate these alterations. The exception is infancy where the negative consequences of iron deficiency seem to persist despite iron repletion, perhaps indicating critical periods of development during which a deficiency in iron leads to irreversible effects.

A heterogeneous distribution of iron exists in the human brain with differential patterns in children versus adults [5]. The accumulation of iron in different brain regions is a function of the stage of brain development [6]. The highest concentrations of brain iron are found in the substantia nigra, deep cerebellar nuclei, the red nucleus, the nucleus accumbens, and portions of the hippocampus [7, 8]. Dopaminergic, serotonergic, and noradrenergic systems have been identified as sensitive to brain iron status [9].

This chapter summarizes cognitive and behavioral alterations resulting from iron deficiency and iron deficiency anemia in children and women of reproductive age. Multiple reviews have been published on these topics [10, 11], so here we focus on reviewing randomized controlled studies published in the past decade,

The original version of this book was revised. An erratum to this book can be found at https://doi.org/10.1007/978-3-031-14521-6_28

L. E. Murray-Kolb (✉)
Department of Nutrition Science, Purdue University, West Lafayette, IN, USA
e-mail: lmurrayk@purdue.edu

A. Arcot
Department of Nutritional Sciences, The Pennsylvania State University, University Park, PA, USA
e-mail: avr5374@psu.edu

C. D. Karakochuk et al. (eds.), *Nutritional Anemia*, Nutrition and Health,
https://doi.org/10.1007/978-3-031-14521-6_23

examining both the short- and long-term cognitive and behavioral consequences of iron deficiency. Studies where the control group contained approximately the same levels of iron as the treatment group (for instance, control group given a multiple micronutrient (MMN) treatment or comparing oral iron to intravenous iron) were excluded from evaluation.

Effects of Iron Deficiency on Cognition and Behavior in Children

Most studies examining the association between iron status and cognitive/behavioral outcomes have been conducted in infants and young children. Over 40 years of accumulated evidence from infant studies reveals that iron deficiency anemia is associated with impaired performance on developmental tests as well as behavioral differences such that iron-deficient anemic infants are more wary, hesitant, and clingy when compared to their iron-sufficient counterparts [12]. During the preschool years, iron deficiency anemia has been associated with impairments in learning and language acquisition; motor development; and in school-aged children, iron deficiency anemia has been shown to be related to impaired academic performance (especially on verbal and math tests) and memory [13]. Many of the recent studies on the association between iron status and cognition/behavior have focused on the long-term consequences of early-life iron treatment. The studies that we include here were conducted in apparently healthy children; we have divided the studies by primary outcome of interest (cognitive or behavioral outcomes).

Effects of Iron Treatment on Child Cognitive Outcomes

Ten recent publications were identified in which children or adolescents received iron treatment or a true control to assess cognitive outcomes [14–23]. Sample sizes across all ten studies ranged from 140 to 1933 with supplementation periods ranging from 4.5 to 8.5 months. As far as vehicle of supplementation, four provided iron supplements (drops or tablets) [14, 16, 19, 21], three provided iron via infant formula [15, 22, 23], and three provided iron through either fortification [17, 18] or biofortification [20] of food. Five of the studies assessed outcomes immediately following the end of the treatment period [14, 17, 18, 20, 21] and the other five assessed outcomes several years later [15, 16, 19, 22, 23]. As far as data analysis, eight used the original randomized groups [14–20, 23] while two ran the analyses with children stratified by iron status in infancy [22] or by iron status in the fetal-neonatal and infancy periods [21]. All studies used multiple biomarkers specific to iron to determine iron status although the 3.5-year follow-up study conducted in Sweden [16] did not measure iron status. Since iron status at follow-up may have affected the interpretation of the findings, contextualization of those results are more difficult. Of note, the study in China included measures of serum ferritin concentrations but did not collect a measure of inflammation and, therefore, ferritin levels were not adjusted [21].

Table 23.1 summarizes the main outcome variables and findings from these studies. Full scale intelligence quotient (IQ), memory, and motor function were the main outcome domains assessed using both manual and computerized tasks. Of the eight studies which used the original randomized groups, three reported no significant differences between the intervention and the control group at endline or follow-up [16, 17, 19]. Two of these studies provided supplementation (iron drops) to infants in Sweden [16, 19] while the other used fortified biscuits, provided to Moroccan school-aged children [17]. All three of these studies used measures of cognition that require highly trained administrators and that are subjective in nature. In addition, these measures were developed and standardized in a Western context. As such, using Western-based standardized scores may not have been appropriate for the study conducted in Morocco. Three other studies which ran analyses using the original randomization groups reported improvements in cognition favorable to the iron treatment group, and there was indication that those with a lower iron status

Table 23.1 Studies examining the effects of iron treatment on cognitive domains in children and adolescents (2010–2020)

Location	*n*	Age at enrollment into original study	Intervention duration (months)	Age at follow-up (years)	Control group	Intervention group(s)	Outcome domain	Analyses groups	Main findings
South Africa (2012) [14]	321[a]	6–11 years	8.5	Same range as enrollment (immediately after 8.5-month intervention)	Placebo	Iron (50 mg iron sulfate) plus DHA/EPA (420/80 mg) or Iron (50 mg iron sulfate) plus placebo or Placebo plus a mixture of DHA/EPA (420/80 mg)	Memory Visuospatial cognition	As per original randomization	• Iron supplemented groups had higher memory scores compared to placebo group; subanalyses reveal this is likely driven by the children who were anemic at baseline • DHA/EPA supplemented group had lower memory scores compared to placebo group • A significant difference in baseline scores were seen between children who were iron-deficient and iron-deficient anemic with the anemic children having lower scores
Chile (2012) [15]	573	6 months	6	10	Formula with 2.3 mg/L iron	Formula with 12.7 mg/L iron	Intelligence quotient (IQ) Spatial memory Achievement (math) Visual perception and motor coordination	As per original randomization	• Spatial memory and visual motor integration were significantly higher in the low-iron formula group than the high-iron formula group • When accounting for hemoglobin level at enrollment, higher scores were seen with high-iron formula consumption in children with the lowest hemoglobin levels but lower scores for children with the highest hemoglobin levels

(continued)

Table 23.1 (continued)

Location	*n*	Age at enrollment into original study	Intervention duration (months)	Age at follow-up (years)	Control group	Intervention group(s)	Outcome domain	Analyses groups	Main findings
Sweden (2013) [16]	285[a] (birth weight between 2000 and 2500 g)	1.5 months	4.5 (until child was 6 months of age)	3.5	0 mg/kg/d iron drops	1 mg/kg/d iron drops or 2 mg/kg/d iron drops	Intelligence quotient (IQ)	As per original randomization	• No significant differences in IQ between control and intervention groups nor between the two intervention groups
Morocco (2016) [17]	457[a]	Third-sixth graders (~7 years on average)	7 (2 or 3 biscuits per day for 6 days/week)	Same range as enrollment (immediately after 7-month intervention)	Placebo biscuits	Biscuits containing the following: ~8 mg Fe as ferrous sulfate or ~8 mg Fe as sodium iron EDTA that contained ~41 mg EDTA or ~41 mg sodium EDTA	Memory Manual activity Visuospatial cognition	As per original randomization	• No significant improvement was found on cognitive outcomes, with iron or EDTA
Cambodia (2018) [18]	1933	6–16 years	6 (during school days, 6 days/week excluding national holiday)	Same range as enrollment (immediately after 6-month intervention)	Local conventional rice	UltraRice original (10.7 mg Fe/100 g) or UltraRice new (7.6 mg Fe/100 g) or NutriRice (7.5 mg Fe/100 g)	Fluid and crystalized intelligence	As per original randomization	• The UltraRice original group improved in fluid intelligence to a greater degree than the placebo group • No differences found between the UltraRice new and placebo groups nor between the NutriRice and placebo groups

Sweden (2018) [19]	285[a] (birth weight between 2000 and 2500 g)	1.5 months	4.5 (until child was 6 months of age)	7	0 mg/kg/d iron drops	1 mg/kg/d iron drops or 2 mg/kg/d iron drops	Intelligence quotient (IQ)	As per original randomization	• No significant differences in IQ between control and intervention groups nor between the two intervention groups
India (2018) [20]	140[a]	12–16 years	6	Same range as enrollment (immediately after 6-month intervention)	Conventional pearl millet (21 ppm Fe from baseline-4 mo: 52 ppm Fe from 4–6 mo)	Iron biofortified pearl millet (86 ppm Fe)	Attention Memory	As per original randomization	• Biofortified group improved in attention and memory to a greater degree than the control group
China (2018) [21]	1482	1.5 months	7.5	9 months	Placebo (in utero, mom received 0.40 mg folate/d or 300 mg Fe and 0.40 mg folate/d)	Iron (~1 mg/kg of elemental iron/d) (in utero, mom received 0.40 mg folate/d or 300 mg Fe and 0.40 mg folate/d)	Motor	• Iron-deficient in both fetal-neonatal and infancy periods • Fetal-neonatal iron deficiency only • Infancy iron deficiency only • No iron deficiency	• Fetal-neonatal and/or infancy iron deficiency groups had lower motor scores than children without iron deficiency
Chile (2019) [22]	875[b]	6 months	6 (until child was 12 months of age)	5.5 and 10	Formula with no added iron	Formula with 2.3 mg/L iron or Formula with 12.7 mg/L iron	Language	• Iron sufficient in infancy • Iron deficient in infancy • Iron deficient anemic in infancy[b]	• More severe iron deficiency in infancy related to lower language abilities at 5 and 10 years of age (resulting from higher child dull affect/social reticence and parental unresponsiveness at 5 years)

(continued)

Table 23.1 (continued)

Location	*n*	Age at enrollment into original study	Intervention duration (months)	Age at follow-up (years)	Control group	Intervention group(s)	Outcome domain	Analyses groups	Main findings
Chile (2019) [23]	405[b]	6 months	6 (until child was 12 months of age)	16	Formula with 2.3 mg/L iron	Formula with 12.7 mg/L iron	Cognitive ability Visual-motor Memory Achievement (math, vocabulary, and comprehension)	As per original randomization	• Lower memory and achievement (math and comprehension) scores for those randomized to the higher iron formula vs. the lower iron formula • For visual-motor outcome, group who consumed higher iron formula had better scores if hemoglobin was low at 6 months and lower scores if hemoglobin was high at 6 months, compared to group who consumed lower iron formula

[a]Children who were severely anemic at enrollment were excluded from the study
[b]Children identified as anemic during infancy were treated with iron

at baseline experienced the greatest gains in terms of cognitive scores [14, 18, 20]. It may be noteworthy that supplementation occurred during the school years in these studies and not during the infancy period, when supplementation was not related to improvements in cognition [16, 19]. The study conducted in India [20] used computerized tests which are less subjective and allow for a finer assessment of cognition (for instance, measuring time in milliseconds). Nevertheless, two studies [14, 18] used more subjective measures, similar to those used in the Sweden and Morocco studies described above and yet, reported significant differences after supplementation. Again, timing of supplementation may be at play, but it may also be important to consider the way in which the cognitive test scores were calculated. The study in Cambodia used raw scores for the three administered tests (Raven's Colored Progressive Matrices and the Block Design and Picture Completion subtests from the Wechsler Intelligence Scale for Children III), citing that no standardized scores appropriate to that setting were available [18]. The study in South Africa used Western standardized scores for some of the outcomes (subscales of the Kaufman Assessment Battery for Children) and raw scores for others (where no standardized scores exist; Hopkins Verbal Learning Test) [14]. Interestingly, the differences between groups were only seen for tests where the authors used raw scores. Whether or not a lack of association when using Western-based standardized scores is an indication that applying these scores in these contexts is inappropriate is a question that remains to be answered. One final possibility should be mentioned here. The iron treatments used in the studies conducted in Cambodia [18] and India [20] provided higher levels of other micronutrients (such as vitamin B12 or zinc) compared to the placebo groups. The possibility that these nutrients positively influenced cognition cannot be ruled out. Of the remaining studies that conducted analyses using the original randomization groups, both reported worse cognitive outcomes for children who had been treated with higher iron formula vs. those treated with a low iron formula during infancy [15, 23]. These follow-up studies assessed children at 10 and 16 years of age and used cognitive assessments that require a highly trained administrator. Interestingly, when accounting for hemoglobin levels at enrollment, both studies reported higher scores (for spatial memory assessed with the Kaufman Assessment Battery for Children at 10 years of age and for visual motor integration assessed with the Beery-Buktenica Developmental Test of Visual-Motor Integration at both 10 and 16 years of age) after iron treatment for those children whose hemoglobin was low at baseline (6 months of age) but lower scores after iron treatment for those children whose hemoglobin was high at baseline.

The two studies that ran analyses based on iron status in infancy both reported lower scores (motor or language) in children who were iron deficient during the fetal/neonatal and/or infancy periods [21, 22]. One of these studies assessed outcomes immediately at the end of the treatment (9 months of age) [21] while the other assessed outcomes several years after the treatment ended (at 5.5 and 10 years of age) [22].

Overall, results from recent studies, which assessed the association between iron and cognition in children and adolescents, are mixed. In general, the studies point to an association between poor iron status and lower cognitive scores. However, the benefits of treating with iron on cognitive outcomes are not clearly established. Studies that provided iron treatment during infancy seem to indicate no benefit or even worse outcomes with higher doses of iron. Alternatively, studies that provided the iron treatment during the school-age years appear to show a benefit of the supplementation. Given the findings that early life iron deficiency may have irreversible effects, it appears that preventing iron deficiency in infancy should be a top priority. While a limited number of observational studies that assess the association between iron status in children and adolescents and neurophysiology exist, no such randomized controlled trials were found in our assessment of articles published in the past decade. Additional studies are needed to better understand the role of timing, duration, and severity of iron deficiency on cognitive outcomes and neurophysiology as well as the effect of timing, duration, dose, and vehicle of supplementation on these outcomes in children. Type of testing conducted and manner in which the tests are scored may also affect the findings.

Long-Term Effects of Iron Treatment in Early Life on Child Behavioral and Affective Outcomes

Over the past decade, seven publications were identified in which children had received iron treatment or a control during infancy and were then followed up at later ages during childhood to assess behavioral and affective (outward expression of an individual's internal emotions) outcomes [16, 19, 24–28]. The seven included studies represent follow-up from three original studies [29–31] with follow-up sample sizes ranging from 161 to 1116 and supplementation periods of approximately 6 months for six of the studies [16, 18, 25–28] and 12–20 months for one [24]. As far as the vehicle for supplementation, Chun-Ming et al. provided a sachet of multiple micronutrients to be added to complementary foods (comparison group received a sachet of rice flour and vegetable oil), Berglund et al. provided iron drops (1 or 2 mg/kg body weight/day with comparison group receiving 0 mg/kg body weight/day), and Lozoff et al. provided a high or low-iron formula (12.7 mg/L and 2.3 mg/L, respectively; comparison group received a formula with no added iron). Chun-Ming and colleagues only measured hemoglobin and, as such, there is no indication of whether or not the anemia measured in their study was due to iron deficiency. At baseline [29], no child was excluded due to their hemoglobin concentration while at follow-up [24], the authors excluded individuals who were anemic. The studies conducted by Berglund et al. and Lozoff et al. used multiple biomarkers which are specific for iron status and, as such, were able to classify the children as iron-sufficient, iron-deficient, and iron-deficient anemic. Of importance, the 3.5-year follow-up [16] conducted by Berglund et al. did not assess iron status at follow-up; as such, the interpretation of the findings is less clear. The study conducted by Berglund et al. excluded anemic children at baseline [30] and the study conducted by Lozoff et al. [31] was a prevention trial, randomizing children who were iron sufficient or iron deficient but not anemic but supplementing all children who were anemic at baseline. Age of children at follow up ranged from 3.5 to 17 years. Of the seven follow-up studies assessing behavior, four analyzed the data by using the original randomized groups [16, 19, 25, 27]. The others ran the analyses with the children stratified by iron status in infancy (iron-sufficient, iron-deficient, or iron-deficient anemic [26, 28] or by whether or not the deficiency was corrected by the original treatment [24]) to assess the impact of early-life iron status on later behavioral outcomes.

Table 23.2 summarizes the main outcome variables and findings from these follow-up studies. Affect, behavior, and social difficulties were the three main outcome domains assessed through researcher observation, parental report, and/or child self-report, depending on the study. The studies that utilized the original randomization groups in their analyses reported a higher prevalence of behavioral problems (overall behavior as assessed with the Child Behavior Checklist (CBCL), externalizing problems (CBCL), and conduct disorder (CBCL)) in the control group (no additional iron provided), vs. the groups who received iron when behavior was assessed through parental report [16, 19, 27], and more positive affect in children who received iron vs. those who did not when assessed via observer rating [25]. These studies assessed the children years after the original supplementation (at 3.5, 7, 10, and 14 years of age). However, when behavior was assessed through child self-report (at ~14 years of age), higher scores on the ADHD symptoms subscale were found in the groups who received iron vs. the group who did not [27]. As only one study used child self-report to assess behavior, this finding will need to be replicated in future studies before it can be properly interpreted. All of the remaining analyses assessed the association of early-life iron status to later behavioral/affective outcomes (regardless of randomization group although they controlled for group) and reported that iron deficiency ane-

Table 23.2 Long-term follow-up of studies examining the effect of iron treatment in infancy on child and adolescent behavior and affect (2010–2020)

Location	*n*	Age at enrollment into original study (months)	Intervention duration (months)	Age at follow-up (years)	Control group	Intervention group(s)	Outcome domain	Analyses groups	Main findings
China (2011) [24]	161	4–12	12–20 months (until child was 24 months of age)	4	Sachet of rice flour + vegetable oil (matched total energy of treatment sachet)	Sachet of 6 mg iron, 4.1 mg zinc, 385 mg calcium, 0.2 mg vit B_2, 7 ug vit D, 3.8 g protein)	Affect and behavior	• Chronic IDA[a] (anemic in infancy and not corrected with treatment) • Corrected IDA[a] (anemia in infancy that was corrected by 24 months) • Non-IDA in infancy and at 24 months[a]	• Chronic IDA children showed less positive affect, less frustration tolerance, more passive behavior and more physical self-soothing compared to non-IDA in infancy children • The behavior and affect of children in the corrected IDA group did not differ from those in the non-IDA in infancy group
Sweden (2013) [16]	285[b] (birth weight between 2000 and 2500 g)	1.5	4.5 (until child was 6 months of age)	3.5	0 mg/kg/d iron drops	1 mg/kg/d iron drops or 2 mg/kg/d iron drops	Behavior	As per original randomization	• Higher behavioral problem scores in control group vs. all other groups
Chile (2014) [25]	1032	6	6 (until child was 12 months of age	10	Formula with no added iron	Formula with 2.3 mg/L iron or Formula with 12.7 mg/L iron	Affect and behavior	As per original randomization comparing any (high + low iron formula) vs. no iron formula	• Iron supplemented children were more cooperative, confident, persistent, coordinated, direct, and worked harder after praise compared to non-supplemented children • In a positive affect task, iron supplemented children spent more time laughing/smiling with their mothers compared to non-supplemented children • In a social stress task, the iron supplemented children smile/laughed more and required less prompting compared to non-supplemented children

(continued)

Table 23.2 (continued)

Location	*n*	Age at enrollment into original study (months)	Intervention duration (months)	Age at follow-up (years)	Control group	Intervention group(s)	Outcome domain	Analyses groups	Main findings
Chile (2017) [26]	873	6	6 (until child was 12 months of age)	5.5, 10, ~14	Formula with no added iron	Formula with 2.3 mg/L iron or Formula with 12.7 mg/L iron	Social difficulties and behavior	• Iron sufficient in infancy • Iron deficient in infancy • Iron deficient anemic in infancy[c]	• Iron-deficient anemic in infancy related to child dull affect and maternal unresponsiveness at 5.5 years which, in turn, related to higher child peer rejection at 10 years and subsequent problem behaviors and deviant friends in adolescence (~14 years)
Sweden (2018) [19]	285[b] (birth weight between 2000 and 2500 g)	1.5	4.5 (until child was 6 months of age)	7	0 mg/kg/d iron drops	1 mg/kg/d iron drops or 2 mg/kg/d iron drops	Behavior	As per original randomization but combining the two iron supplemented groups	• Lower externalizing problems in iron supplemented groups vs. control group
Chile (2018) [27]	1116	6	6 (until child was 12 months of age)	11–17 (mean: ~ 14 years)	Formula with no added iron	Formula with 2.3 mg/L iron or Formula with 12.7 mg/L iron	Social difficulties and behavior	As per original randomization comparing any (high + low iron formula) vs. no iron formula and comparing high vs. low iron formula groups AND by iron status in infancy: • Iron sufficient in infancy • Iron deficient in infancy • Iron deficient anemic in infancy[c]	• Lower conduct problems in iron formula vs. no iron formula groups • Higher ADHD symptoms in iron formula vs. no iron formula groups • No differences when comparing high vs. low iron formula groups • Both iron-deficient and iron-deficient anemic infants in infancy had higher adolescent behavior problems compared to iron sufficient in infancy

Chile (2018) [28]	1116	6	6 (until child was 12 months of age)	11–17 (mean: ~14 years)	Formula with no added iron	Formula with 2.3 mg/L iron or Formula with 12.7 mg/L iron	Behavior	• Iron sufficient in infancy • Iron deficient in infancy • Iron-deficient anemic in infancy[c]	• Iron-deficient anemic in infancy related to excessive alcohol use and risky sexual behavior in adolescence (resulting from poor emotional regulation at 10 years) • Iron-deficient anemic in infancy related to higher risk taking in adolescence (resulting from attention control deficits at 10 years)

[a]Although the authors classify the children as chronic IDA (iron-deficient anemic), corrected IDA, or non-IDA, hemoglobin was the only biomarker assessed. Anemic children at follow-up (4 years of age) were excluded
[b]Children who were anemic at enrollment were excluded from the study
[c]Children identified as anemic during infancy were treated with iron

mia in infancy was related to worse affect (less positive affect, more dull affect) and worse behavior (higher externalizing problems, excessive alcohol use, risky sexual behavior) even after controlling for possible confounders (such as SES, maternal education, maternal depressive symptoms, child sex, family stressors, the home environment) when compared to children who were iron sufficient in infancy [24, 26, 28]. One study compared children whose anemia was corrected in infancy to those who were never anemic in infancy and found no differences in behavior or affect at the 4-year follow-up [24]. Another study found that children who were iron deficient (but not anemic) in infancy had higher adolescent behavior problems compared to children who were iron sufficient in infancy [28].

Together, these studies indicate negative consequences of iron deficiency and iron deficiency anemia in infancy on behavior and affect, years later. The differences reported among adolescents who were formerly iron deficient are especially troubling, given the serious potential consequences of the behaviors (excessive alcohol consumption and risky sexual behaviors). The finding that behavioral differences might persist in children who were iron deficient, but not anemic, in infancy is cause for concern as the prevalence of iron deficiency without anemia is high and iron deficiency, in the absence of anemia, typically goes undetected. Whether or not iron supplementation in early life can reverse these negative consequences is still in question since few randomized controlled trials exist and most of the long-term studies have assessed the outcomes based on early-life status as opposed to randomization group.

Effects of Iron Deficiency on Cognition and Behavior in Women of Reproductive Age

While decades of research indicate an association between iron status and cognitive/behavioral outcomes in infants and young children, few studies have been conducted in adults. This was due to the belief that the brain was resistant to changes in iron once the blood–brain barrier reached maturity [32]. However, animal studies revealed that the uptake of iron into the brain is dependent on iron status, as there is an increased rate with low iron status and a decreased rate with high iron status [33]. Furthermore, the uptake process is not reflective of overall blood–brain barrier permeability [34, 35]. Since this knowledge emerged, there has been an increased interest in understanding the association between iron status and cognition/behavior in adults, particularly in women of reproductive age, given their susceptibility to iron deficiency. Studies conducted prior to the past 10 years included both observational and intervention designs and most were conducted in developed countries. The observational studies suggested a relation between iron status and cognition such that higher iron levels are associated with better cognitive functioning, specifically, spatial ability, attention, memory, and executive functioning. These studies also report a relation between iron status and affect such that higher iron levels are associated with fewer depressive symptoms and a higher quality of life [4]. All of the intervention studies assessing cognition as the outcome, reported an improvement in cognitive functioning with iron supplementation. Likewise, in intervention studies assessing affect as the outcome, improvements were reported following iron supplementation with the greatest improvements found among women who had poor baseline iron status. However, few studies were randomized controlled trials and few studies specifically examined the effect of iron deficiency, in the absence of anemia, on cognition, behavior, or affect. Here, we focus on recent randomized controlled studies that examined the association between iron supplementation and cognition/behavior in women of reproductive age.

Effects of Iron Treatment on Cognitive and Neurophysiological Outcomes in Women of Reproductive Age

Four manuscripts, representing three randomized controlled trials, of the effects of iron treatment on cognition in adult women of reproductive age have been published over the past decade [36–39]. Three recruited university-attending women as the participants [36, 38, 39] and one recruited women who worked on a tea plantation [37]. The vehicle for supplementation was beef in one study (comparison group received non-beef foods), provided for 16 weeks [36], double-fortified salt (comparison group received single-fortified salt), provided for 10 months [37], and biofortified beans (comparison group received conventional beans), provided for 18 weeks [38, 39]. All studies included multiple iron status biomarkers which were assessed at baseline and endline and the study in Rwanda was restricted to women with a ferritin ≤20 ng/mL at baseline. The studies analyzed the data using an intent to treat approach and also included secondary data analysis approaches.

Table 23.3 summarizes the main outcome variables and findings from these randomized controlled trials. The main outcome domains were memory and attention and one study also used electroencephalogram (EEG) measurements to assess electrophysiology [39]. The study conducted in the United States did not find any difference in cognitive outcomes between the groups at endline [36]. Both groups improved their iron status over time but changes in cognitive outcomes did not differ by group. On the other hand, when classifying the women as those who had a positive change in ferritin vs. those who did not, the authors report greater improvements in all three cognitive domains tested (memory, attention, spatial planning) in the "ferritin responders" vs. the "ferritin non-responders." In contrast, the studies conducted in India and Rwanda found significant differences between groups at endline on the cognitive domains tested (memory, attention, perception) with women in the treatment arms having greater improvements [37, 38]. Although the exact tests given differed in these studies, all studies utilized computerized cognitive tests. The study in Rwanda was limited to women who were iron deficient at baseline, the study in India included only the subgroup of women who had the lowest ferritin values from the larger parent trial, and the study conducted in the United States did not limit enrollment based on ferritin concentrations. Indeed, at baseline, the mean ferritin concentrations of those in the US study were nearly four times higher than the mean ferritin concentrations of those in the Rwanda study and almost 40% higher than those in the India study. It is possible that women with a lower iron status at baseline experience a greater benefit of increased iron status. The Rwanda study also included measures of electrophysiology and found greater improvements in EEG amplitude and spectral power in the group who consumed the biofortified beans vs. those who consumed the conventional beans [39]. An important contribution of this study is the finding that changes in brain activity (EEG) mediate the relation between changes in iron biomarkers and changes in cognition (memory and attention). Studies which use electrophysiological measurements and relate the findings to changes in cognition are especially helpful in terms of providing a link between the mechanistic studies conducted with animal models and the behavioral and affective outcomes that are typically measured in human studies.

Recent observational studies (not reviewed in-depth here) are supportive of an association between iron status and cognitive functioning in women of reproductive age, indicating that better executive functioning and attention scores are found in women who are iron sufficient vs. those who are deficient [40–42]. Two recent observational studies have also examined the association between iron status and neurobiology. One revealed differences in left EEG alpha activity in prefrontal regions between iron-deficient (non-anemic) and iron-sufficient women of reproductive age [43]. The other study found differences in brain connectivity (using functional magnetic resonance imaging) between women who had been iron-deficient anemic in infancy vs. controls (iron sufficient or mild iron deficiency in infancy) [44]. Specifically, formerly iron deficient anemic subjects had decreased connectivity from the posterior Default Mode Network (DMN) to the left posterior cingulate cortex (PCC) and increased connectivity from the anterior DMN to the right PCC. They also exhibited differences in the left medial frontal gyrus.

Table 23.3 Randomized controlled trials assessing the effect of iron treatment on cognition and neurophysiology in women of reproductive age (2010–2020)

Location	*n*	Participant age (years)	Intervention duration	Control group	Intervention group(s)	Outcome domain	Analyses groups	Main findings
USA (2014) [36]	43	18–30	16 weeks	Non-beef foods containing levels of calories and protein similar to the beef lunches	Beef lunches (3 oz., 3 times per week; approximately 2.4 mg iron/3 oz. of beef)	Memory, attention, and spatial planning	As per original randomization and comparing ferritin responders vs. non-responders	• No differences on any cognitive outcomes between original randomization groups • Ferritin responders had greater improvements in working memory, attention, and spatial planning compared to ferritin non-responders
India (2017) [37]	126	18–55	10 months	Iodized salt (47 mg potassium iodate/kg)	Double fortified salt (47 mg potassium iodate/kg and 3.3 mg microencapsulated ferrous fumarate/g)	Memory, attention, and perception	As per original randomization and assessing association between change in iron biomarker and change in cognitive performance	• Greater improvements on memory, attention, and perception tasks for the double fortified salt vs. the iodized only salt group • Increases in ferritin were significantly related to better performance on all three domains tested
Rwanda (2017) [38]	150[a]	18–27	18 weeks	Conventional beans (50.1 ppm iron)	Biofortified beans (86.1 ppm iron)	Memory and attention	As per original randomization and assessing association between change in iron biomarker and change in cognitive performance	• Greater improvements on attention and memory tasks for the biofortified group vs. the conventional group • Increases in ferritin were significantly related to better performance on the attention and memory tasks
Rwanda (2019) [39]	55[a]	18–27	18 weeks	Conventional beans (50.1 ppm iron)	Biofortified beans (86.1 ppm iron)	EEG	As per original randomization	• Greater improvements in measures of EEG amplitude and spectral power for the biofortified group vs. the conventional group

[a]Only included women with a ferritin ≤20 ng/mL. The larger, parent trial included 195 women

Although these observational studies are supportive of a relation between iron and cognition in women of reproductive age, the number of recent randomized controlled trials assessing the effects of iron treatment on cognition in this population is extremely limited. While the studies provide evidence that iron deficiency is related to cognitive alterations and changes in electrophysiology, more work is needed to fully understand these associations in this age group. Optimal cognitive functioning is necessary for performing day-to-day duties. Alterations in maternal cognitive functioning may have significant implications for maternal–child interactions with subsequent negative effects on child development, as women are often the primary caregiver for children. It is therefore crucial that these types of studies continue to be conducted in women of reproductive age.

Effects of Iron Treatment on Behavioral and Affective Outcomes in Women of Reproductive Age

Three recent randomized controlled trials were identified in which women of reproductive age received iron treatment or a control and behavioral/affective variables were assessed as the outcomes of interest [45–47]. Two of the studies were conducted in developed countries [45, 46] and the other was conducted in Iran [47], a semi-developed country. Sample sizes ranged from 70–198 with intervention periods ranging from 2 weeks (intravenous iron) to 12 weeks. As for the vehicle for supplementation, the study conducted in Switzerland utilized intravenous iron (comparison group received intravenous placebo) while the other two provided oral iron supplements as tablets (with comparison groups receiving oral placebo tablets). As far as iron status assessment and inclusion criteria, all of the studies included multiple iron status biomarkers which were assessed both at baseline and endline and all of the studies excluded women who were anemic at baseline. Additionally, the studies conducted in Switzerland and France included only women whose baseline ferritin levels were ≤ 50 ng/mL and the study conducted in Iran included only women with postpartum depression. All of the studies analyzed the data using an intent to treat approach and it is important to note that the study conducted in France was observer blinded while the other studies were double blinded.

Table 23.4 summarizes the main outcome variables and findings from the randomized controlled trials conducted over the past decade. The main outcome domains were affect (specifically, anxiety, depression, and quality of life) and fatigue. In both studies where fatigue was assessed, the authors found significantly larger decreases in fatigue in the women who received iron vs. those who received a placebo [45, 46]. For one of these studies, this association was only true for women whose ferritin concentrations were ≤ 15 ng/mL at baseline [45]. For the studies that assessed affect, one reported no significant differences between groups on measures of anxiety, depression, or quality of life [46]. The other study reported significant improvements in depression scores in women who were treated with iron compared to those treated with placebo (improvement rate of 42.8% vs. 20.0% for iron vs. placebo treated, respectively; $p = 0.03$) [47]. Several differences exist between these studies which may contribute to the discrepant finding: (1) different instruments were used to assessed depression, (2) participants in the Iran study were all 1-week postpartum while the participants in France were not in the postpartum period, and (3) the study conducted in Iran only enrolled women who had been diagnosed with postpartum depression.

Although the number of randomized controlled trials assessing iron status and behavior in women of reproductive age is limited, the studies provide evidence that iron status is related to behavior and that iron repletion may ameliorate the negative findings. Of importance, all of these studies excluded anemic women and, therefore, the findings indicate alterations in behavior in those who are iron deficient but not anemic. In other words, mild iron deficiency has behavioral and affective consequences in women of repro-

Table 23.4 Randomized controlled trials assessing the effect of iron treatment on behavior and affect in women of reproductive age (2010–2020)

Location	*n*	Participant age (years)	Intervention duration	Control group	Intervention group(s)	Outcome domain	Analyses groups	Main findings
Switzerland (2011) [45]	90[a]	≥18	2 weeks (outcomes assessed at 6 weeks)	Intravenous placebo for 2 weeks (4 infusions with 200 mL of 0.9% saline, period of 10 min each)	800 mg of iron (cumulative dose) as iron (III)-hydroxide sucrose for 2 weeks (4 infusions containing 200 mg of solution) in 200 mL of 0.9% saline, period of 10 min each)	Fatigue	As per original randomization	• Fatigue decreased significantly more in IV iron vs. placebo group; this was true only for those whose baseline ferritin levels were ≤ 15 ng/mL
France (2012) [46]	198[a]	18–53	12 weeks	Placebo	80 mg iron/d (prolonged release ferrous sulfate)	Affect and fatigue	As per original randomization	• Fatigue decreased significantly more in iron vs. placebo group • No significant difference between groups on anxiety, depression, or quality of life scores
Iran (2017) [47]	70[b]	20–40	6 weeks	Placebo	50 mg elemental iron/d (ferrous sulfate)	Affect	As per original randomization	• Significant improvement in depression scores in iron treated vs. placebo group

[a]Only included women with a ferritin ≤50 ng/mL and hemoglobin ≥120 g/L
[b]Only included mothers with postpartum depression and who were not anemic

ductive age. As mentioned above in our review of the findings in children, this is especially concerning, given the lack of identification of iron deficiency in the absence of anemia, in most settings.

Conclusion

The brief review provided in this chapter reveals an association between iron deficiency (with and without anemia) and alterations in cognition and behavior for both children and women of reproductive age. Findings of this association in the absence of anemia are particularly troubling, given the magnitude of iron deficiency without anemia and the fact that it goes largely undetected. The findings of long-term cognitive and behavioral consequences of iron deficiency in infancy despite iron repletion are also of particular concern. Finally, the fact that higher iron doses used for repletion may be related to worse outcomes when supplementation occurs in infancy needs to be further investigated. These findings indicate that preventing iron deficiency in infancy should be a top priority. The magnitude of cognitive and affective changes reported with iron deficiency vary by study but, in general, indicate levels that are likely to impact daily activities. Although studies of the functional consequences of iron deficiency continue, there is a clear need for well-designed randomized controlled studies in order to better understand the effects of timing, duration, and severity of the deficiency as well as the optimal treatment timing, duration, and dose. Studies that link changes in neurophysiology to changes in these cognitive and behavioral outcomes are especially needed.

References

1. Kassebaum NJ, Jasrasaria R, Naghavi M, Wulf SK, Johns N, Lozano R, Regan M, Weatherall D, Chou DP, Eisele TP, Flaxman SR, Pullan RL, Brooker SJ, Murray CJL. A systematic analysis of global anemia burden from 1990 to 2010. Blood. 2014;123:615–24.
2. Lu C, Black MM, Richter LM. Risk of poor development in young children in low-income and middle-income countries: an estimation and analysis at the global, regional, and country level. Lancet Glob Health. 2016;4:e916–22.
3. Murray-Kolb LE. Iron and brain functions. Curr Opin Clin Nutr Metab Care. 2013;16:703–7.
4. McClung JP, Murray-Kolb LE. Iron nutrition and premenopausal women: effects of poor iron status on neuropsychological and physical performance. Annu Rev Nutr. 2013;33:271–88.
5. Beard JL, Connor JR, Jones BC. Iron in the brain. Nutr Rev. 1993;51:157.
6. Pinero DJ, Li NQ, Connor JR, Beard JL. Alterations in brain iron metabolism in response to dietary iron changes. J Nutr. 2000;130:254–63.
7. Aoki S, Okada Y, Nishimura K, Barkovich AJ, Kjos BO, et al. Normal deposition of brain iron in childhood and adolescence: MR imaging at 1.5 T. Radiology. 1989;172:381–5.
8. Hill JM. The distribution of iron in the brain. In: Youdim MBH, editor. Brain iron: neurochemistry and behavioural aspects. London: Taylor & Francis; 1988. p. 1–24.
9. Beard JL, Connor JR. Iron status and neural functioning. Annu Rev Nutr. 2003;23:41–58.
10. Pivina L, Semenova Y, Doşa MD, Dauletyarova M, Bjørklund G. Iron deficiency, cognitive functions, and neurobehavioral disorders in children. J Mol Neurosci. 2019;68:1–10.
11. Ferreira A, Neves P, Gozzelino R. Multilevel impacts of iron in the brain: the cross talk between neurophysiological mechanisms, cognition, and social behavior. Pharmaceuticals (Basel). 2019;12:126.
12. Lozoff B, Klein NK, Nelson EC, McClish DK, Manuel M, Chacon ME. Behavior of infants with iron-deficiency anemia. Child Dev. 1998;69:24–36.
13. Grantham-McGregor S, Ani C. A review of studies on the effect of iron deficiency on cognitive development in children. J Nutr. 2001;131(2S-2):649S–66S; discussion 666S–668S
14. Baumgartner J, Smuts CM, Malan L, Kvalsvig J, van Stuijvenberg ME, Hurrell RF, Zimmermann MB. Effects of iron and n-3 fatty acid supplementation, alone and in combination, on cognition in school children: a randomized, double-blind, placebo-controlled intervention in South Africa. Am J Clin Nutr. 2012;96:1327–38.
15. Lozoff B, Castillo M, Clark MK, Smith BJ. Iron-fortified vs low-iron infant formula: developmental outcome at 10 years. Arch Pediatr Adolesc Med. 2012;166:208.
16. Berglund SK, Westrup B, Hägglöf B, Hernell O, Domellöf M. Effects of iron supplementation of LBW infants on cognition and behavior at 3 years. Pediatrics. 2013;131:47–55.
17. Bouhouch RR, El-Fadeli S, Andersson M, Aboussad A, Chabaa L, Zeder C, Kippler M, Baumgartner J, Sedki A, Zimmermann MB. Effects of wheat-flour biscuits fortified with iron and EDTA, alone and in combination, on blood lead concentration, iron status,

and cognition in children: a double-blind randomized controlled trial. Am J Clin Nutr. 2016;104:1318–26.
18. Fiorentino M, Perignon M, Kuong K, de Groot R, Parker M, Burja K, Dijkhuizen MA, Sokjom S, Chamnan C, Berger J, Wieringa FT. Effect of multi-micronutrient-fortified rice on cognitive performance depends on premix composition and cognitive function tested: results of an effectiveness study in Cambodian schoolchildren. Public Health Nutr. 2018;21:816–27.
19. Berglund SK, Chmielewska A, Starnberg J, Westrup B, Hägglöf B, Norman M, Domellöf M. Effects of iron supplementation of low-birth-weight infants on cognition and behavior at 7 years: a randomized controlled trial. Pediatr Res. 2018;83:111–8.
20. Scott SP, Murray-Kolb LE, Wenger MJ, Udipi SA, Ghugre PS, Boy E, Haas JD. Cognitive performance in Indian school-going adolescents is positively affected by consumption of iron-biofortified pearl millet: a 6-month randomized controlled efficacy trial. J Nutr. 2018;148:1462–71.
21. Santos DCC, Angulo-Barroso RM, Li M, Bian Y, Sturza J, Richards B, Lozoff B. Timing, duration, and severity of iron deficiency in early development and motor outcomes at 9 months. Eur J Clin Nutr. 2018;72:332–41.
22. East P, Delker E, Blanco E, Encina P, Lozoff B, Gahagan S. Effect of infant iron deficiency on children's verbal abilities: the roles of child affect and parent unresponsiveness. Matern Child Health J. 2019;23:1240–50.
23. Gahagan S, Delker E, Blanco E, Burrows R, Lozoff B. Randomized controlled trial of iron-fortified versus low-iron infant formula: developmental outcomes at 16 years. J Pediatr. 2019;212:124–130.e1.
24. Chang S, Wang L, Wang Y, Brouwer ID, Kok FJ, Lozoff B, Chen C. Iron-deficiency anemia in infancy and social emotional development in preschool-aged Chinese children. Pediatrics. 2011;127:e927–33.
25. Lozoff B, Castillo M, Clark KM, Smith JB, Sturza J. Iron supplementation in infancy contributes to more adaptive behavior at 10 years of age. J Nutr. 2014;144:838–45.
26. East P, Lozoff B, Blanco E, Delker E, Delva J, Encina P, Gahagan S. Infant iron deficiency, child affect, and maternal unresponsiveness: testing the long-term effects of functional isolation. Dev Psychol. 2017;53:2233–44.
27. Doom JR, Richards B, Caballero G, Delva J, Gahagan S, Lozoff B. Infant iron deficiency and iron supplementation predict adolescent internalizing, externalizing, and social problems. J Pediatr. 2018;195:199–205.
28. East P, Delker E, Lozoff B, Delva J, Castillo M, Gahagan S. Association among infant iron deficiency, childhood emotion and attention regulation, and adolescent problem behaviors. Child Dev. 2018;89:593–608.
29. Chun-Ming C, Wang Y, Chang S. Effect of in-home fortification of complementary feeding on intellectual development of Chinese children. Biomed Environ Sci. 2010;23:83–91.
30. Berglund S, Westrup B, Domellöf M. Iron supplements reduce the risk of iron deficiency anemia in marginally low birth weight infants. Pediatrics. 2010;126:e874–83.
31. Lozoff B, DeAndraca I, Castillo M, Smith JB, Walter T, Pino P. Behavioral and development effects of preventing iron-deficiency anemia in health full-term infants. Pediatrics. 2003;112:846–54.
32. Murray-Kolb LE. Iron status and neuropsychological consequences in women of reproductive age: what do we know and where are we headed? J Nutr. 2011;141:747S–55S.
33. Taylor EM, Crowe A, Morgan EH. Transferrin and iron uptake by the brain: effects of altered iron status. J Neurochem. 1991;57:1584–92.
34. Malecki EA, Cook B, Devenyl AG, Beard JL, Connor JR. Transferrin is required for normal distributions of ^{59}Fe and ^{54}Mn in mouse brains. J Neurol Sci. 2000;170:112–8.
35. Pinero DJ, Jones B, Beard JL. Alterations in brain iron metabolism in response to dietary iron changes. J Nutr. 2000;130:254–63.
36. Blanton C. Improvements in iron status and cognitive function in young women consuming beef or non-beef lunches. Nutrients. 2014;9:90–110.
37. Wenger MJ, Murray-Kolb LE, Nevins JEH, Venkatramanan S, Reinhart GA, Wesley A, Haas JD. Consumption of a double-fortified salt affects perceptual, attentional, and mnemonic functioning in women in a randomized controlled trial in India. J Nutr. 2017;147:2297–308.
38. Murray-Kolb LE, Wenger MJ, Scott SP, Rhoten SE, Lung'aho MG, Haas JD. Consumption of iron-biofortified beans positively affects cognitive performance in 18 to 27-year-old Rwandan female college students in an 18-week randomized controlled efficacy trial. J Nutr. 2017;147:109–17.
39. Wenger MJ, Rhoten SE, Murray-Kolb LE, Scott SP, Boy E, Gahutu JB, Haas JD. Changes in iron status are related to changes in brain activity and behavior in Rwandan female university students: results from a randomized controlled efficacy trial involving iron-biofortified beans. J Nutr. 2019;149:687–97.
40. Blanton CA, Green MW, Kretsch MJ. Body iron is associated with cognitive executive planning function in college women. Br J Nutr. 2013;109:906–13.
41. Scott SP, Murray-Kolb LE. Iron status is associated with performance on executive functioning tasks in nonanemic young women. J Nutr. 2016;146:30–7.
42. Cook RL, O'Dwyer NJ, Parker HM, Donges CE, Cheng HL, Steinbeck KS, Cox EP, Franklin JL, Garg ML, Rooney KB, O'Connor HT. Iron deficiency anemia, not iron deficiency, is associated with reduced attention in healthy young women. Nutrients. 2017;9:1216.
43. Dziembowska I, Kwapisz J, IZdebski P, Zekanowska E. Mild iron deficiency may affect female endurance and behavior. Physiol Behav. 2019;205:44–50.

44. Algarin C, Karunakaran KD, Reyes S, Morales C, Lozoff B, Peirano P, Biswal B. Differences on brain connectivity in adulthood are present in subjects with iron deficiency anemia in infancy. Front Aging Neurosci. 2017;9:54.
45. Krayenbuehl PA, Battegay E, Breymann C, Furrer J, Schulthess G. Intravenous iron for the treatment of fatigue in non-anemic, premenopausal women with low serum ferritin concentrations. Blood. 2011;118:3222–7.
46. Vaucher P, Druais PL, Waldvogel S, Favrat B. Effect of iron supplementation on fatigue in nonanemic menstruating women with low ferritin: a randomized controlled trial. CMAJ. 2012;184:1247–54.
47. Sheikh M, Hantoushzadeh S, Shariat M, Farahani Z, Ebrahiminasab O. The efficacy of early iron supplementation on postpartum depression, a randomized double-blind placebo-controlled trial. Eur J Nutr. 2017;56:901–8.