Psychological Medicine

cambridge.org/psm

Iron deficiency and internalizing symptom severity in unmedicated adolescents: a pilot study

Malak Abbas[1], Kellen Gandy[2], Ramiro Salas[3], Sridevi Devaraj[4] and Chadi A. Calarge[5]

[1]The Rockefeller University, New York, NY 10065, USA; [2]St. Jude Children's Research Hospital, Houston, Texas 77027, USA; [3]Baylor College of Medicine – Center for Translational Research on Inflammatory Diseases, Michael E DeBakey VA Medical Center, Houston, Texas 77030, USA; [4]Baylor College of Medicine, Houston, Texas 77030, USA and [5]Baylor College of Medicine – The Menninger Department of Psychiatry and Behavioral Sciences, 1102 Bates Ave, Suite 790, Houston, Texas 77030, USA

Original Article

Cite this article: Abbas M, Gandy K, Salas R, Devaraj S, Calarge CA (2023). Iron deficiency and internalizing symptom severity in unmedicated adolescents: a pilot study. *Psychological Medicine* **53**, 2274–2284. https://doi.org/10.1017/S0033291721004098

Received: 3 May 2021
Revised: 12 September 2021
Accepted: 17 September 2021
First published online: 16 December 2021

Key words:
Adolescents; anxiety disorders; basal ganglia; ferritin; iron deficiency; major depressive disorder

Author for correspondence:
Chadi A. Calarge,
E-mail: chadi.calarge@bcm.edu

Abstract

Background. Iron plays a key role in a broad set of metabolic processes. Iron deficiency is the most common nutritional deficiency in the world, but its neuropsychiatric implications in adolescents have not been examined.

Methods. Twelve- to 17-year-old unmedicated females with major depressive or anxiety disorders or with no psychopathology underwent a comprehensive psychiatric assessment for this pilot study. A T1-weighted magnetic resonance imaging scan was obtained, segmented using Freesurfer. Serum ferritin concentration (sF) was measured. Correlational analyses examined the association between body iron stores, psychiatric symptom severity, and basal ganglia volumes, accounting for confounding variables.

Results. Forty females were enrolled, 73% having a major depressive and/or anxiety disorder, 35% with sF < 15 ng/mL, and 50% with sF < 20 ng/mL. Serum ferritin was inversely correlated with both anxiety and depressive symptom severity ($r = -0.34$, $p < 0.04$ and $r = -0.30$, $p < 0.06$, respectively). Participants with sF < 15 ng/mL exhibited more severe depressive and anxiety symptoms as did those with sF < 20 ng/mL. Moreover, after adjusting for age and total intracranial volume, sF was inversely associated with left caudate (Spearman's $r = -0.46$, $p < 0.04$), left putamen ($r = -0.58$, $p < 0.005$), and right putamen ($r = -0.53$, $p < 0.01$) volume.

Conclusions. Brain iron may become depleted at a sF concentration higher than the established threshold to diagnose iron deficiency (i.e. 15 ng/mL), potentially disrupting brain maturation and contributing to the emergence of internalizing disorders in adolescents.

Background

Iron deficiency is the most common nutritional deficiency in the world (CDC, 2002; Looker, Cogswell, & Gunter, 2002). After initially decreasing with the introduction of food enrichment in the United States, the prevalence of iron deficiency has resurged, particularly among certain age, sex, and racial/ethnic groups (Gupta, Hamner, Suchdev, Flores-Ayala, & Mei, 2017; Looker et al., 2002; Sun & Weaver, 2021). For instance, between the 1988–1994 and the 1999–2000 NHANES survey, the prevalence of iron deficiency increased from about 1% to 5% in 12- to 15-year-old males. Moreover, while the prevalence of iron deficiency ranges between 9% and 16% in 12- to 19-year-old females, it is nearly twice as prevalent in non-Hispanic Black and Mexican American females compared to their non-Hispanic White counterparts (Gupta et al., 2017; Looker et al., 2002; WHO, 2001).

Iron deficiency may have significant implications for mental health. Iron is an essential micronutrient, involved in oxygen transport, cellular respiration, and DNA synthesis (Beard & Connor, 2003; Youdim, 2008). The main mechanism for the brain to uptake iron primarily involves endocytosis of transferrin bound to its receptor (TfR1), with a significant contribution by the divalent metal transporter 1 (DMT1) (Rouault & Cooperman, 2006; Wade, Chiou, & Connor, 2019). Ferritin can also be directly transported across the blood-brain barrier (BBB) (Wade et al., 2019). Oligodendrocytes have both a high content and utilization rate of iron (Moller et al., 2019). In contrast, while neurons have a high iron requirement, they have little capacity to store it (Connor & Menzies, 1996), making them particularly vulnerable to iron deficiency. Additionally, brain iron content differs by anatomical region and age, with the basal ganglia and red nucleus containing the most iron, while the cortical gray and white matter have low iron content (Haacke et al., 2005; Hallgren & Sourander, 1958). This distribution, already apparent in childhood and adolescence (Peterson et al., 2019), accentuates with age (Sedlacik et al., 2014).

© The Author(s), 2021. Published by Cambridge University Press

CAMBRIDGE UNIVERSITY PRESS

Mechanistic studies have implicated iron deficiency in monoaminergic signaling impairment, partially mediated by the fact that iron is a cofactor for tyrosine hydroxylase (Anderson et al., 2009; Baumgartner et al., 2012a, 2012b; Baumgartner, Smuts, & Zimmermann, 2014; Beard, Erikson, & Jones, 2002; Burhans et al., 2005; Coe, Lubach, Bianco, & Beard, 2009; Jellen et al., 2013). Iron deficiency is associated with alterations in the expression of dopamine-related genes and decreased density of dopamine transporters and dopamine D_1 and D_2 receptors in the basal ganglia (Beard, Chen, Connor, & Jones, 1994; Burhans et al., 2005; Erikson, Jones, & Beard, 2000; Erikson, Jones, Hess, Zhang, & Beard, 2001; Jellen et al., 2013; Nelson, Erikson, Pinero, & Beard, 1997; Pino et al., 2017). Iron deficiency is also associated with disrupted serotoninergic and noradrenergic function as well as with impaired total mitochondrial oxidative capacity at the beginning of peak dendritic growth (Bastian, von Hohenberg, Georgieff, & Lanier, 2019; Baumgartner et al., 2012a, 2012b, 2014; Mohamed, Unger, Kambhampati, & Jones, 2011). These abnormalities result in cognitive and behavioral deficits, including inattention and anxiety-like behaviors (Beard et al., 1994, 2002; Carlson, Stead, Neal, Petryk, & Georgieff, 2007; Fretham et al., 2012; Golub, Hogrefe, & Germann, 2007; Kennedy et al., 2014; Mohamed et al., 2011; Schmidt, Waldow, Grove, Salinas, & Georgieff, 2007; Tran et al., 2016).

Consistent with these preclinical findings, low body iron has been associated with attention-deficit hyperactivity disorder (ADHD) and several observational and intervention studies in women of reproductive age have implicated iron deficiency in depressive symptoms (Beard et al., 2005; Corwin, Murray-Kolb, & Beard, 2003; Fordy & Benton, 1994; Karl et al., 2010; Low, Speedy, Styles, De-Regil, & Pasricha, 2016; Rangan, Blight, & Binns, 1998; Vahdat Shariatpanaahi, Vahdat Shariatpanaahi, Moshtaaghi, Shahbaazi, & Abadi, 2007). Surprisingly, however, little research has examined the association of iron deficiency with internalizing (i.e. depressive and anxiety) symptoms in school-aged children and adolescents (Matsuo et al., 2008; Vulser et al., 2015). One retrospective Japanese study in 6- to 15-year-old children with serum ferritin concentration (sF) <50 ng/mL, referred for a child and adolescent psychiatric evaluation, found iron supplementation effective at increasing sF and reducing psychiatric symptoms (Mikami et al., 2019). Two randomized double-blind placebo-controlled iron supplementation studies in adolescent females examining cognitive outcomes reached divergent conclusions, with only one reporting an improvement in 'mood, lassitude, and concentration' following replenishment of iron stores (Ballin et al., 1992; Bruner, Joffe, Duggan, Casella, & Brandt, 1996). Finally, one study utilized the Taiwanese national health insurance database, finding that iron deficiency anemia was associated with more than twofold increased risk for depressive or anxiety disorders, compared to those without anemia (Chen et al., 2013).

Importantly, anemia (regardless of its etiology) is known to be associated with irritability, apathy, fatigue, low mood, and concentration difficulties (Murray-Kolb, 2011), complaints that overlap with internalizing symptoms, making it necessary to characterize the psychiatric effects of iron deficiency in the absence of anemia.

In this pilot study, we examined the prevalence and clinical correlates of iron deficiency in adolescent girls with and without anxiety or depressive disorders, who were otherwise healthy. We hypothesized that iron deficiency would be associated with more severe internalizing symptoms. Given that altered basal ganglia morphometry, metabolism, and perfusion have been implicated in internalizing disorders (Bastian et al., 2019; Beard et al., 1994; Bourre et al., 1984; Cammer, 1984a; Connor & Menzies, 1996; Matsuo et al., 2008; Tansey & Cammer, 1988; Vulser et al., 2015) and that these structures are key nodes in the fronto-subcortical neural circuits, underlying various processes relevant to mood regulation (Williams, 2016) and because iron deficiency disrupts neurotransmitter signaling in the basal ganglia (Baumgartner et al., 2012a, 2012b, 2014), we further sought to examine the association between iron deficiency and basal ganglia volumes.

Methods

Participants

This analysis used data collected in the context of two studies, with the first participant enrolled on 02/12/2016. Both enrolled participants with the same demographic and clinical characteristics, with one focused on examining gut permeability in major depressive disorder (MDD) (Calarge, Devaraj, & Shulman, 2019), while the other focused on brain imaging (Calarge et al., 2017). In both studies, 12- to 17-year-old unmedicated females with MDD, anxiety disorders (i.e. separation anxiety disorder, generalized anxiety disorder, or social phobia), or with no psychiatric disorders (i.e. healthy controls) were enrolled from general pediatrics clinics if they had a normal body mass index (BMI, i.e. between the 5th and 85th percentile for age and sex). The presence of bipolar disorder, autistic disorder, schizophrenia, obsessive-compulsive disorder, ADHD, and/or eating disorder led to exclusion. Additional exclusionary criteria included the presence of intellectual disability or language barrier due to inability to complete study procedures, treatment with psychotropics within 6 months before study entry, and presence of a serious general medical condition (e.g. involving a vital organ) or pregnancy. Individuals exposed to major traumatic events (e.g. death of loved ones, natural disaster, etc.) in the prior 6 months were also excluded (Calarge et al., 2019). Furthermore, the use of non-steroidal anti-inflammatory drugs or medications for seasonal allergies or asthma in the prior week, of pre/probiotics in the prior 6 weeks, or of antibiotics in the prior 6 months; or a major change in diet (e.g. switching to vegetarian or excluding a food group, like eggs or dairy products) in the prior 6 weeks or the presence of functional gastrointestinal disorders all led to exclusion from the first study, as these factors may alter gut permeability. Participants wearing braces or having any contraindication for undergoing magnetic resonance imaging (MRI) were excluded from the brain imaging study.

The study was approved by the Baylor College of Medicine Institutional Review Board. After the study details were reviewed, written consent was obtained from parents or legal guardians and verbal assent from the participants.

Procedures

A board-certified child and adolescent psychiatrist conducted an unstructured interview with the adolescent and parent. The MINI International Psychiatric Interview V6.0, a structured clinical interview based on the fifth edition of the Diagnostic and Statistical Manual for Mental Disorders (DSM-5) (American Psychiatric Association, 2013), was administered to the parent by trained research staff. Also, the participants completed the Center for Epidemiological Studies Depression Scale for Children (CESD-C) and the Screen for Child Anxiety-Related Disorders (SCARED), both well-validated and widely used

https://doi.org/10.1017/S0033291721004098 Published online by Cambridge University Press

measures of depressive and anxiety symptoms, respectively (Birmaher et al., 1999, 1997; Faulstich, Carey, Ruggiero, Enyart, & Gresham, 1986; Weissman, Orvaschel, & Padian, 1980). Best-estimate DSM-5-based diagnoses were generated, using all available clinical information.

Race and ethnicity were self-reported. The parents also reported their household income and educational level. Participants rated their sexual maturity using a validated form (Calarge, Acion, Kuperman, Tansey, & Schlechte, 2009; Calarge, Mills, Ziegler, & Schlechte, 2018). When applicable, they also noted the first day of their last menstrual period, the typical duration of their cycle, and the average number of sanitary pads used per day, during their menses. Participants 13 years of age and older were also queried about the use of hormonal contraception. The parents completed a questionnaire about birth history, including prenatal care, *in-utero* prescribed or illicit drug exposure, and pre/perinatal complications. The parents were also asked to rate their confidence level in the information recalled. Additionally, medical records were reviewed, since birth when available, to extract information related to body iron status (i.e. hemoglobin and history of anemia or transfusion).

Height was measured to the nearest 0.1 cm with a wall-mounted stadiometer (Ayrton Model S100, Hamburg, Germany) and weight was recorded to the nearest 0.1 kg (Seca 220 digital scale, Hamburg, Germany) with participants in indoor clothes without shoes. These measurements were obtained in duplicate, and the average was used.

The 2004 Block Food Frequency Questionnaire (FFQ) for Ages 8–17 was completed by the participants, with parents assisting as needed (D'Occhio, Fordyce, Whyte, Aspden, & Trigg, 2000). The FFQ includes 77 food items, developed based on the NHANES 1999–2002 dietary recall data. The nutrient database was developed from the USDA Nutrient Database for Dietary Studies, version 1.0. Individual portion size is asked, and pictures are provided to enhance the accuracy of quantification. When available, the FFQ allowed estimating daily iron intake. Poor iron intake was defined as an estimated daily intake <8 mg/day for 12- and 13-year-olds and <15 mg/day for older female participants (Trumbo, Schlicker, Yates, & Poos, 2002).

Participants underwent a venous blood draw, in the morning, after at least a 9 h fast. Serum was used to measure sF (Immunoassay on Vitros 5600 Chemistry System, Ortho Clinical Diagnostics, Raritan, NJ, USA).

Brain imaging acquisition and segmentation

MRI of the brain was obtained using a 3 T Siemens PRISMA scanner equipped with a 64-channel head-neck coil. Anatomical imaging included a 3D MPRAGE T1-weighted scan sequence (TR/TI/TE = 2400/1000/2.24 ms, 0.8 mm isotropic resolution). The raw MRI data were inspected by a trained operator for scanner-related artifacts (including head motion) immediately following scan acquisition. T1-weighted MRI scans were preprocessed and analyzed using Freesurfer version 6.0 (http://surfer.nmr.mgh.harvard.edu), a brain imaging software designed to characterize the morphometric properties of the brain (Fischl et al., 2002, 2004). Subcortical brain volumes were segmented based on the standardized Aseg atlas. For this study, we focused on basal ganglia volume including the putamen, globus pallidus, and the caudate (online Supplementary Fig.), given these structures' higher iron content and to minimize the risk for type 1 error (Haacke et al., 2005; Hallgren & Sourander, 1958). Total intracranial volume was adjusted for in the analysis of these basal ganglia subregions.

Statistical analysis

BMI was computed as weight/height^2 (kg/m^2) and age-sex-specific BMI *Z*-scores were generated based on the 2000 Centers for Disease Control and Prevention normative data (Ogden et al., 2002). Following published guidelines, iron deficiency was defined as sF < 15 ng/mL (WHO, 2011). However, in light of evidence suggesting that such cutoff may be too conservative (Garcia-Casal et al., 2018; Mast, Blinder, Gronowski, Chumley, & Scott, 1998; North, Dallalio, Donath, Melink, & Means, 1997), we also examined the association of iron deficiency with outcomes of interest using the more liberal cutoff of 20 ng/mL.

Given the comorbidity between depressive and anxiety disorders, we computed an internalizing symptoms composite *z*-score capturing the symptoms severity on both the SCARED and CESD-C (Song, Lin, Ward, & Fine, 2013). Group differences between participants with and those without internalizing disorders were compared using the Wilcoxon rank-sum test for continuous variables and χ^2 or Fisher's exact test for categorical variables. Multivariable regression analyses examined the associations between iron deficiency status and outcomes of interest (e.g. depression or anxiety symptom severity or basal ganglia volume), accounting for relevant confounders. Cohen's *d* effect size was computed (Cohen, 1988). Analyses used procedures from SAS version 9.4 for Windows (SAS Institute Inc, Cary, NC, USA).

Results

Participants

Table 1 summarizes the demographic and clinical characteristics of the 40 participants contributing data to this analysis. Although no significant differences in demographic characteristics between participants were found, participants with internalizing disorders tended to be older, and more likely to be Hispanic and post-menarchal. Although participants with internalizing disorders had lower sF and a numerically higher prevalence of iron deficiency (whether defined as sF < 15 or 20 ng/mL), these differences did not reach statistical significance (Table 1).

Association between iron markers and internalizing symptoms

Serum ferritin concentration was inversely correlated with internalizing symptom severity as captured by the composite *z*-score ($r = -0.36$, $p < 0.03$), the SCARED ($r = -0.34$, $p < 0.04$), and the CESD-C ($r = -0.30$, $p < 0.06$). Notably, compared to those without iron deficiency (defined as sF < 20 ng/mL), participants with iron deficiency had significantly higher internalizing symptom composite *z*-score (*z*-score = 0.73 *v.* −0.73, respectively, Cohen's $d = 0.82$, $p < 0.02$) as well as higher scores on the SCARED ($p < 0.002$) and the CESD-C ($p < 0.03$) (Fig. 1*A*). Moreover, the magnitude of this association was even greater in participants with sF < 15 ng/mL compared to those with sF ⩾ 15 ng/mL (Cohen's $d = 1.01$, $p < 0.005$ for the composite *z*-score; $d = 1.08$ for the SCARED, $p < 0.003$; and $d = 0.83$ for the CESD-C, $p < 0.02$; Fig. 1*B*).

Iron markers and basal ganglia morphology

Of the 24 participants who underwent brain imaging, 16 (67%) had an internalizing disorder and nine (38%) had sF < 15 ng/ml. Differences in demographic variables or in iron deficiency prevalence between participants with *v.* those without internalizing disorders were not statistically significant (all *p* values >0.05).

https://doi.org/10.1017/S0033291721004098 Published online by Cambridge University Press

Table 1. Demographic and clinical characteristics of female adolescents ($n = 40$) with internalizing disorders *v.* healthy controls

	Healthy controls $N = 11$	Internalizing $N = 29$	p value
Age, yrs	14.5 ± 1.6	15.2 ± 1.6	>0.10
White race, *n* (%)	7 (64%)	20 (69%)	>0.50
Hispanic, *n* (%)	2 (18%)	14 (48%)	>0.10
Median household income, $\$ \times 10^3$/yr	70 (39–140)	80 (25–140)	>0.40
BMI *Z*-score[a]	0.64 ± 0.43	0.33 ± 0.67	>0.10
Sexual maturity rating, % in stages I, II, III, IV, V	9/9/27/18/36	0/7/17/45/31	>0.20
Had menarche, *n* (%)	9 (82%)	28 (97%)	>0.10
Time since menarche, yrs	3.3 ± 2.5	3.5 ± 1.8	>0.80
Poor iron intake,[b] *n* (%)	4 (67%)	11 (73%)	>0.90
Duration of last menses, days	5.6 ± 1.6	5.7 ± 3.4	>0.90
Number of daily sanitary pads	3.6 ± 0.7	3.6 ± 1.1	>0.80
Luteal phase[c], *n* (%)	5 (56%)	18 (64%)	>0.70
Menorrhagia present[d], *n* (%)	1 (13)	2 (7)	>0.50
Hormonal contraception[e], *n* (%)	1 (11)	3 (11)	>0.90
Serum ferritin, ng/mL	29.5 ± 20.2	22.1 ± 14.4	>0.10
Serum ferritin <15 ng/mL, *n* (%)	2 (18%)	12 (41%)	>0.10
Serum ferritin <20 ng/mL, *n* (%)	4 (36%)	16 (55%)	>0.20
SCARED	12.9 ± 9.1	37.9 ± 15.2	<0.0001
CESD-C	5.3 ± 3.8	30.0 ± 12.4	<0.0001
Internalizing symptoms composite *Z*-score	−2.18 ± 0.67	0.83 ± 1.55	<0.0001

Mean ± s.d., unless otherwise specified.
SCARED, Screen for Child Anxiety Related Disorders; CESD-C, Center for Epidemiological Studies Depression Scale for Children.
[a]BMI *Z*-score: age-sex-specific body mass index.
[b]Dietary data were available for only 15 participants with internalizing disorders and 6 healthy controls.
[c]Luteal phase was defined as within 14 days of the end of each participant's 'average' menstrual cycle length.
[d]Menorrhagia was defined as duration of menses of >7 days or use of >12 sanitary pads per day.
[e]Only participants ⩾13 years old ($n = 35$) were queried about hormonal contraceptives use.

After adjusting for age and total intracranial volume, sF was inversely associated with the volume of the left caudate (Spearman's $r = -0.46$, $p < 0.04$, Table 2), left putamen ($r = -0.58$, $p < 0.005$), and the right putamen ($r = -0.53$, $p < 0.01$). Similarly, there was a statistical trend for the left putamen and caudate volumes to be larger in participants with iron deficiency, as defined by sF < 15 ng/mL (Fig. 2*A*). The effect sizes were somewhat smaller for the right basal ganglia structures, compared to the left (Fig. 2*B*).

Discussion

To our knowledge, this pilot study is the first to examine the association between body iron stores, internalizing symptoms severity, and brain structure in unmedicated adolescent females, who have undergone a thorough psychiatric assessment. Body iron stores were inversely associated with more severe anxiety and depressive symptoms and positively associated with basal ganglia morphometry.

Iron deficiency and internalizing disorders in youth

Internalizing disorders in adolescents are common and impairing, with recent data suggesting increasing incidence (Twenge, Cooper, Joiner, Duffy, & Binau, 2019). Many factors are likely implicated, given the heterogeneous nature of depressive and anxiety disorders. That iron deficiency would contribute to the recent change in the prevalence of internalizing disorders in adolescents is plausible for several reasons: (1) iron plays a critical role in the brain, potentially impacting the structure and function of mood-relevant areas, (2) iron deficiency is common in adolescence (CDC, 2002, 2014; Gupta et al., 2017; Looker et al., 2002), and (3) available evidence suggests that replenishing iron stores may improve internalizing symptoms (Mikami et al., 2019).

To our knowledge, however, the prevalence of iron deficiency in adolescents with internalizing disorders has not been examined. We found that 32% of our participants had sF < 15 ng/mL. This high prevalence of iron deficiency, which may be partially accounted for by the over-representation of Hispanic females in our study (Gupta et al., 2017; Looker et al., 2002; WHO, 2001), is particularly troubling given that our participants had undergone a thorough screening to rule out many general medical conditions that could cause iron deficiency. Moreover, the pre/perinatal history and the medical record review exclude the possibility that our findings represent chronic sequelae of early-life or concurrent anemia (online Supplementary Data). As such, to the best of our knowledge, our participants were healthy, had received good prenatal care, had not had perinatal anemia, but did have low iron intake despite coming from households with a median income nearly double the national average (Table 1).

https://doi.org/10.1017/S0033291721004098 Published online by Cambridge University Press

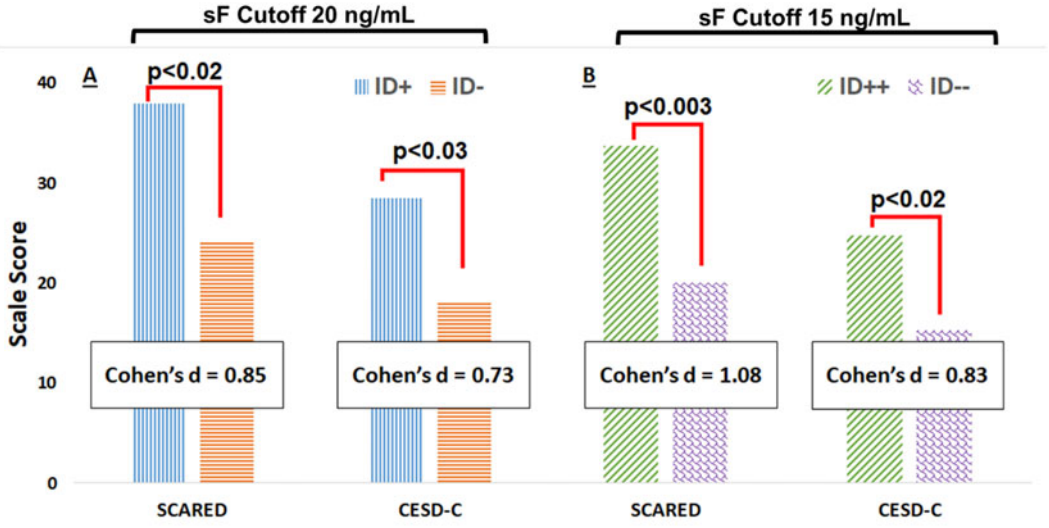


Fig. 1. (*A*) Least squares means for internalizing symptom severity in unmedicated adolescent females with sF < (ID+, blue) *v*. ⩾20 ng/mL (ID−, orange), adjusted for age. (*B*) Same comparison but between those with sF < (ID++, green) *v*. ⩾15 ng/mL (ID−−, purple).

Table 2. Least squares means for basal ganglia structures volumes (mL) in unmedicated female adolescents ($n = 24$) with iron deficiency *v*. those without, adjusted for age and intracranial volume

	Serum ferritin (sF) cutoff of 15 ng/mL				Serum ferritin (sF) cutoff of 20 ng/mL			
	sF ⩾ 15 $N = 15$	sF < 15 $N = 9$	Cohen's *d*	*p* value	sF ⩾ 20 $N = 11$	sF < 20 $N = 13$	Cohen's *d*	*p* value
Left basal ganglia	10.48	11.08	0.80	<0.08	10.48	10.90	0.53	>0.20
Left putamen	4.93	5.23	0.74	<0.10	4.88	5.17	0.71	<0.10
Left caudate	3.59	3.84	0.74	<0.10	3.61	3.74	0.352	>0.40
Left pallidum	1.96	2.01	0.24	>0.50	1.98	1.98	0.0055	>0.90
Right basal ganglia	10.54	10.96	0.73	>0.10	10.52	10.85	0.54	>0.20
Right putamen	5.011	5.200	0.70	>0.10	4.49	5.16	0.62	>0.10
Right caudate	3.71	3.88	0.55	>0.20	3.71	3.82	0.32	>0.40
Right pallidum	1.81	1.87	0.34	>0.40	1.81	1.86	0.26	>0.50

This low iron intake is consistent with recent data showing a trend for reduction in iron intake in the US population, with an associated increase in the prevalence of iron deficiency anemia. The large effect sizes we found for the association between iron deficiency and internalizing symptoms highlight the potential for replenishing iron stores to reverse the recently documented increase in the prevalence of depressive and anxiety disorders in adolescents (Mojtabai & Olfson, 2020; Mojtabai, Olfson, & Han, 2016)

Brain iron homeostasis during iron deficiency

Understanding the interplay between iron deficiency and brain structure and function across the lifespan requires one to consider three inter-related factors: (1) iron transport into the brain, (2) hierarchy of iron distribution to bodily systems, and (3) how the presence of iron deficiency has been defined. Once the BBB matures during infancy, iron transport into the brain becomes tightly regulated, protecting it from daily fluctuations in systemic iron levels (Wade et al., 2019). Preclinical and clinical studies have established that iron is prioritized for erythropoiesis, for evident survival advantage. As the body is faced with insufficient iron intake to meet its needs, iron reserves are tapped in a relatively hierarchical order (e.g. liver, skeletal muscle, heart, etc.) and, with increasing iron deficiency severity, iron is eventually diverted even from the brain to the bone marrow (Ennis, Dahl, Rao, & Georgieff, 2018; Guiang, Georgieff, Lambert, Schmidt, & Widness, 1997; Zamora, Guiang, Widness, & Georgieff, 2016). Finally, historically, iron deficiency has been the focus of public health interventions due to its association with anemia. Current guidelines to diagnose ID have been based on anemia-relevant markers, such as bone marrow iron content and sF below

https://doi.org/10.1017/S0033291721004098 Published online by Cambridge University Press

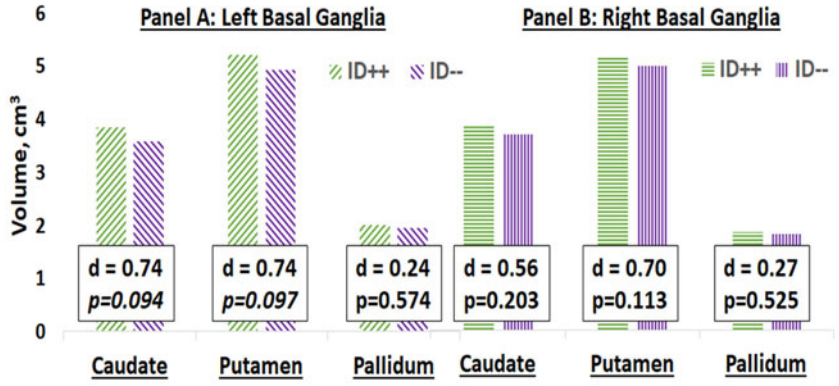


Fig. 2. Cohen's *d* for the differences in least squares means for basal ganglia structures volumes in females with sF < (ID++, green) *v.* ⩾15 ng/mL (ID−−, purple), accounting for age and total intracranial volume.

which anemia develops (WHO, 2011). They are not based on the assessments of brain iron content or the emergence of neuropsychiatric manifestations. Considering these three factors together, it thus follows that, compared to infants who may not benefit from the protective effect of the BBB yet, brain iron content in adolescents will only be impacted once a threshold (in terms of severity of body iron stores depletion) has been crossed. However, what severity of iron deficiency is needed to overcome the homeostatic mechanisms of the BBB, resulting in brain iron depletion, is unknown. What appears certain is that brain iron is drawn upon before anemia develops. This is supported by studies in adolescents and young adults with iron deficiency but without anemia finding improvement in neurocognitive and emotional functioning following iron supplementation (Ballin et al., 1992; Beard et al., 2005; Corwin et al., 2003; Fordy & Benton, 1994; Karl et al., 2010; Low et al., 2016; Rangan et al., 1998; Vahdat Shariatpanaahi et al., 2007; Verdon et al., 2003). This appears consistent with our findings both concerning internalizing symptom severity and subcortical structures volumes.

Notably, we found a larger association between internalizing symptom severity and iron deficiency (a categorical variable) compared to sF (a continuous variable). This is consistent with the BBB's role in protecting the brain from fluctuations in body iron until the homeostatic mechanisms are overwhelmed (Wade et al., 2019). When iron deficiency is defined more liberally, as sF < 20 ng/mL, the association with symptom severity remains significant, albeit of a smaller magnitude, suggesting that the cut-off for sF of <15 ng/mL to define iron deficiency may be overly conservative, having been established based on hematological outcomes without regard to the function of other organs, like the brain. In fact, for those participants with a hemoglobin level obtained within one year before study entry, anemia was quite uncommon with only one person affected (online Supplementary Data). In patients with restless leg syndrome, iron supplementation aims to raise sF > 50 ng/mL. However, the association between peripheral and brain iron content has not been well examined in general and not at all in adolescents. Identifying the threshold below which iron deficiency starts drawing on brain iron is an area that requires urgent attention, with potentially widespread clinical implications.

Iron deficiency and brain structure and function

Brain iron is also critical for myelin formation (Bastian et al., 2019; Beard & Connor, 2003; Lange & Que, 1998). Oligodendrocytes are enriched with iron-requiring enzymes involved in lipid metabolism, needed for initial myelin deposition as well as for maintaining its integrity (Bourre et al., 1984; Cammer, 1984a, 1984b; Connor & Menzies, 1996; Tansey & Cammer, 1988). Two brain imaging studies have explored the association between brain iron content and brain function in children. Peterson et al. used data collected in healthy 12- to 21-year-olds, enrolled in the National Consortium on Alcohol and Neurodevelopment in Adolescence study (Peterson et al., 2019). They repurposed scans obtained for functional MRI and diffusion tensor imaging (DTI) to track brain iron distribution, replicating the fact that several subcortical nuclei are iron-rich, and that iron accumulates with increasing age but plateaus by early adulthood. Additionally, working memory speed was inversely associated with iron signal in the left dentate nucleus and substantia nigra (Peterson et al., 2019). Similarly, Carpenter et al. used brain imaging to estimate brain iron content in 39 healthy children (56% female, mean age 9.5 ± 1.3 years), finding again a positive association between age and iron content in the

https://doi.org/10.1017/S0033291721004098 Published online by Cambridge University Press

basal ganglia (Carpenter et al., 2016). Moreover, the right caudate iron content was positively associated with spatial IQ (Carpenter et al., 2016).

Using structural brain imaging, we found an inverse association between body iron status and the volumes of the putamen and the left caudate. The effect sizes were smaller for the right hemisphere structures, perhaps reflecting the lateralization in brain iron content observed in some studies (Langkammer et al., 2012; Xu, Wang, & Zhang, 2008). This inverse association may reflect the fact that brain iron moderates the decrease in subcortical structures volume observed during late childhood and adolescence (Raznahan et al., 2014; Wierenga et al., 2014). It may also reflect iron deficiency-induced impairment in dopaminergic signaling (Beard et al., 1994; Burhans et al., 2005; Erikson et al., 2000, 2001; Jellen et al., 2013; Nelson et al., 1997; Pino et al., 2017). Preclinical studies have linked stimulant-induced reduction in dopamine D_2 receptor density in the ventral striatum with an increase in putamen volume (Chang et al., 2005; Churchwell, Carey, Ferrett, Stein, & Yurgelun-Todd, 2012; Groman, Morales, Lee, London, & Jentsch, 2013; Jan, Lin, Miles, Kydd, & Russell, 2012; Jernigan et al., 2005). Similarly, clinical studies have found enlarged putamen in patients with stimulant use disorders (Ersche et al., 2017, 2011, 2012; Jacobsen, Giedd, Gottschalk, Kosten, & Krystal, 2001). Finally, our finding is also consistent with increased basal ganglia volumes following extended treatment with 'typical' antipsychotics, characterized by potent dopamine D_2 antagonist activity (Corson, Nopoulos, Miller, Arndt, & Andreasen, 1999; Navari & Dazzan, 2009). The basal ganglia are a key node in the cortico-striato-thalamo-cortical loops, subserving a multitude of neuropsychological processes implicated in psychopathologies, such as inhibitory control and reward processing (Drysdale et al., 2017; Janiri et al., 2019; Pizzagalli et al., 2009; Wei & Wang, 2016; Williams, 2016). Whether iron supplementation would reverse these structural changes requires future studies.

Timing of iron deficiency and persistence of neuropsychiatric sequelae

Given the key role iron plays in a broad set of metabolic processes, it is not surprising that iron deficiency would be associated with cognitive and neuropsychiatric deficits (Vulser et al., 2016). However, the nature, severity, and chronicity of these effects are closely tied to the time during development when iron deficiency ensues (Barks, Hall, Tran, & Georgieff, 2019; Georgieff, 2017). The earlier the exposure, the broader the impact on neurocognitive functioning given the brain's substantial iron-dependent metabolic needs early in life, when brain structures underlying basic neurocognitive processes are rapidly developing (Barks et al., 2019; Georgieff, 2017; Georgieff, Brunette, & Tran, 2015; Lozoff & Georgieff, 2006). This is thought to involve irreversible impairment in gene expression, affecting neuronal growth and plasticity (Georgieff et al., 2015). These 'sensitive periods' have been shown both in animal and clinical studies (Barks et al., 2019; Georgieff, 2017; Mudd et al., 2018). For instance, children exposed to iron deficiency *in-utero* or in infancy, show motor, cognitive, emotional, and social deficits long after iron stores had been replenished (Barks et al., 2019; Doom et al., 2018; Lozoff, Jimenez, Hagen, Mollen, & Wolf, 2000; Lozoff et al., 2013). Additionally, in children with ADHD, Turner et al. found that a small mean corpuscular volume (a marker of iron deficiency) in the toddler years predicted poor response to psychostimulant treatment in elementary school (Turner, Xie, Zimmerman, & Calarge, 2010). However, in contrast to what appears as persistent sequelae when iron deficiency occurs early in life, cognitive deficits in children and adults with iron deficiency, with or without anemia, can improve with iron repletion (Chmielewska et al., 2019; Grantham-McGregor & Ani, 2001; Low et al., 2016; McCann & Ames, 2007). For example, iron supplementation in 5- to 8-year-old children with ADHD and women with postpartum depression reduces symptom severity, particularly in those with iron deficiency (Konofal et al., 2008; Sever, Ashkenazi, Tyano, & Weizman, 1997; Wassef, Nguyen, & St-Andre, 2019). In other words, which sequelae arise closely depend on which brain areas are most metabolically actively and/or rapidly developing at the time of iron deficiency, making them particularly vulnerable to its impact (Lozoff & Georgieff, 2006; Vulser et al., 2016).

Some limitations of our pilot study must be acknowledged. First, our findings are based on a relatively small sample size, requiring replication in a larger study. Second, given that internalizing disorders disproportionately affect adolescent females, we did not recruit males. As such, whether our findings extend to males remains to be seen. Third, because this was a cross-sectional evaluation, the direction of the causal association between iron deficiency and our outcomes of interest cannot be established. While low sF is the most specific non-invasive marker of iron deficiency, with excellent reproducibility (Belza, Ersboll, Henriksen, Thilsted, & Tetens, 2005), neither the duration of iron deficiency was available, nor the presence of anemia ruled out as we did not measure hemoglobin. Because some symptoms associated with anemia may overlap with internalizing symptoms (Murray-Kolb, 2011), future studies should exclude the confounding effect of anemia to best examine the independent effect of iron deficiency on the brain. Given the exploratory nature of this study, no correction for multiple comparisons was made. As shown in Table 1, 62% of the participants were estimated to be in the luteal phase of their menstrual cycle, a time when ferritin tends to be higher (Kim, Yetley, & Calvo, 1993). As such, the rate of iron deficiency may have been even higher had we enrolled the participants upon the onset of their menstrual phase. Finally, measuring C-reactive protein would have ruled out cases of inflammation, where ferritin would have been elevated. However, the stringent inclusion/exclusion criteria and the high prevalence of iron deficiency suggest that our participants did not have acute inflammation.

In summary, given iron's role in multiple metabolic processes affecting brain structure and function, iron deficiency can have a wide-ranging impact on brain development. In youth, this may compound their proclivity to develop internalizing disorders and compromise treatment response. Future studies, ideally longitudinal, should examine how changes in iron status during pubertal maturation may moderate brain development and the emergence of psychopathology. This risk may impact males and females differently, given that iron deficiency disproportionately affects menstruating females, particularly of minority background. Moreover, future interventions should seek to examine the clinical benefits of replenishing iron stores.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0033291721004098.

Acknowledgements. The authors thank the families and the research team members.

Financial support. This research received no specific grant from any funding agency, commercial or not-for-profit sectors.

Conflict of interest. None.

References

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.). Arlington, VA: American Psychiatric Publishing.

Anderson, J. G., Fordahl, S. C., Cooney, P. T., Weaver, T. L., Colyer, C. L., & Erikson, K. M. (2009). Extracellular norepinephrine, norepinephrine receptor and transporter protein and mRNA levels are differentially altered in the developing rat brain due to dietary iron deficiency and manganese exposure. *Brain Research*, *1281*, 1–14. doi: 10.1016/j.brainres.2009.05.050

Ballin, A., Berar, M., Rubinstein, U., Kleter, Y., Hershkovitz, A., & Meytes, D. (1992). Iron state in female adolescents. *American Journal of Diseases of Children*, *146*(7), 803–805. doi: 10.1001/archpedi.1992.02160190035015

Barks, A., Hall, A. M., Tran, P. V., & Georgieff, M. K. (2019). Iron as a model nutrient for understanding the nutritional origins of neuropsychiatric disease. *Pediatric Research*, *85*(2), 176–182. doi: 10.1038/s41390-018-0204-8

Bastian, T. W., von Hohenberg, W. C., Georgieff, M. K., & Lanier, L. M. (2019). Chronic energy depletion due to iron deficiency impairs dendritic mitochondrial motility during hippocampal neuron development. *Journal of Neuroscience*, *39*(5), 802–813. doi: 10.1523/JNEUROSCI.1504-18.2018

Baumgartner, J., Smuts, C. M., Malan, L., Arnold, M., Yee, B. K., Bianco, L. E., … Zimmermann, M. B. (2012a). Combined deficiency of iron and (n-3) fatty acids in male rats disrupts brain monoamine metabolism and produces greater memory deficits than iron deficiency or (n-3) fatty acid deficiency alone. *Journal of Nutrition*, *142*(8), 1463–1471. doi: 10.3945/jn.111.156281

Baumgartner, J., Smuts, C. M., Malan, L., Arnold, M., Yee, B. K., Bianco, L. E., … Zimmermann, M. B. (2012b). In male rats with concurrent iron and (n-3) fatty acid deficiency, provision of either iron or (n-3) fatty acids alone alters monoamine metabolism and exacerbates the cognitive deficits associated with combined deficiency. *Journal of Nutrition*, *142*(8), 1472–1478. doi: 10.3945/jn.111.156299

Baumgartner, J., Smuts, C. M., & Zimmermann, M. B. (2014). Providing male rats deficient in iron and n-3 fatty acids with iron and alpha-linolenic acid alone affects brain serotonin and cognition differently from combined provision. *Lipids in Health and Disease*, *13*, 97. doi: 10.1186/1476-511X-13-97

Beard, J. L., Chen, Q., Connor, J., & Jones, B. C. (1994). Altered monamine metabolism in caudate-putamen of iron-deficient rats. *Pharmacology, Biochemistry and Behavior*, *48*(3), 621–624.

Beard, J. L., & Connor, J. R. (2003). Iron status and neural functioning. *Annual Review Nutrition*, *23*, 41–58. doi: 10.1146/annurev.nutr.23.020102.075739020102.075739

Beard, J. L., Erikson, K. M., & Jones, B. C. (2002). Neurobehavioral analysis of developmental iron deficiency in rats. *Behavioural Brain Research*, *134*(1-2), 517–524.

Beard, J. L., Hendricks, M. K., Perez, E. M., Murray-Kolb, L. E., Berg, A., Vernon-Feagans, L., … Tomlinson, M. (2005). Maternal iron deficiency anemia affects postpartum emotions and cognition. *Journal of Nutrition*, *135*(2), 267–272. doi: 10.1093/jn/135.2.267

Belza, A., Ersboll, A. K., Henriksen, M., Thilsted, S. H., & Tetens, I. (2005). Day-to-day variation in iron-status measures in young iron-deplete women. *British Journal of Nutrition*, *94*(4), 551–556. doi: 10.1079/bjn20051461

Birmaher, B., Brent, D. A., Chiappetta, L., Bridge, J., Monga, S., & Baugher, M. (1999). Psychometric properties of the screen for child anxiety related emotional disorders (SCARED): A replication study. *Journal of the American Academy of Child and Adolescent Psychiatry*, *38*(10), 1230–1236. doi: 10.1097/00004583-199910000-00011

Birmaher, B., Khetarpal, S., Brent, D., Cully, M., Balach, L., Kaufman, J., & Neer, S. M. (1997). The screen for child anxiety related emotional disorders (SCARED): Scale construction and psychometric characteristics. *Journal of the American Academy of Child and Adolescent Psychiatry*, *36*(4), 545–553. doi: 10.1097/00004583-199704000-00018

Bourre, J. M., Pascal, G., Durand, G., Masson, M., Dumont, O., & Piciotti, M. (1984). Alterations in the fatty acid composition of rat brain cells (neurons, astrocytes, and oligodendrocytes) and of subcellular fractions (myelin and synaptosomes) induced by a diet devoid of n-3 fatty acids. *Journal of Neurochemistry*, *43*(2), 342–348. doi: 10.1111/j.1471-4159.1984.tb00906.x

Bruner, A. B., Joffe, A., Duggan, A. K., Casella, J. F., & Brandt, J. (1996). Randomised study of cognitive effects of iron supplementation in non-anaemic iron-deficient adolescent girls. *Lancet (London, England)*, *348*(9033), 992–996. doi: 10.1016/S0140-6736(96)02341-0

Burhans, M. S., Dailey, C., Beard, Z., Wiesinger, J., Murray-Kolb, L., Jones, B. C., & Beard, J. L. (2005). Iron deficiency: Differential effects on monoamine transporters. *Nutritional Neuroscience*, *8*(1), 31–38.

Calarge, C. A., Acion, L., Kuperman, S., Tansey, M., & Schlechte, J. A. (2009). Weight gain and metabolic abnormalities during extended risperidone treatment in children and adolescents. *Journal of Child and Adolescent Psychopharmacology*, *19*(2), 101–109. doi: 10.1089/cap.2008.007

Calarge, C. A., Devaraj, S., & Shulman, R. J. (2019). Gut permeability and depressive symptom severity in unmedicated adolescents. *Journal of Affective Disorders*, *246*, 586–594. doi: 10.1016/j.jad.2018.12.077

Calarge, C. A., Gandy, K., Barba Villalobos, G., Nguyen, J., Kim, S. Y., & Maletic-Savatic, M. (2017). *In-vivo measurement of a neurogenic signal and pattern separation in adolescent depression*. Paper presented at the American College of Neuropsychopharmacology Annual Meeting, Palm Springs, CA.

Calarge, C. A., Mills, J. A., Ziegler, E. E., & Schlechte, J. A. (2018). Calcium and vitamin D supplementation in boys with risperidone-induced hyperprolactinemia: A randomized, placebo-controlled pilot study. *Journal of Child and Adolescent Psychopharmacology*, *28*(2), 145–150. doi: 10.1089/cap.2017.0104

Cammer, W. (1984a). Carbonic anhydrase in oligodendrocytes and myelin in the central nervous system. *Annals of the New York Academy of Sciences 429*, 494–497. https://doi.org/10.1111/j.1749-6632.1984.tb12376.x.

Cammer, W. (1984b). Oligodendrocyte associated enzymes. In Norton, W. T. (Ed.), *Oligodendroglia* (pp. 199–232). New York: Plenum Press.

Carlson, E. S., Stead, J. D., Neal, C. R., Petryk, A., & Georgieff, M. K. (2007). Perinatal iron deficiency results in altered developmental expression of genes mediating energy metabolism and neuronal morphogenesis in hippocampus. *Hippocampus*, *17*(8), 679–691. doi: 10.1002/hipo.20307

Carpenter, K. L. H., Li, W., Wei, H., Wu, B., Xiao, X., Liu, C., … Egger, H. L. (2016). Magnetic susceptibility of brain iron is associated with childhood spatial IQ. *Neuroimage*, *132*, 167–174. doi: 10.1016/j.neuroimage.2016.02.028

CDC, Centers for Disease Control and Prevention. (2002). Iron deficiency — United States, 1999–2000. *Morbidity and Mortality Weekly Report*, *51*, 897–899. http://www.cdc.gov/MMWR/PDF/wk/mm5140.pdf.

CDC, Centers for Disease Control and Prevention. (2014). Trace elements. *Second National Report on Biochemical Indicators of Diet and Nutrition in the U.S. Population* 2012. Retrieved from https://www.cdc.gov/nutritionreport/summary_2012.html.

Chang, L., Cloak, C., Patterson, K., Grob, C., Miller, E. N., & Ernst, T. (2005). Enlarged striatum in abstinent methamphetamine abusers: A possible compensatory response. *Biological Psychiatry*, *57*(9), 967–974. doi: 10.1016/j.biopsych.2005.01.039

Chen, M. H., Su, T. P., Chen, Y. S., Hsu, J. W., Huang, K. L., Chang, W. H., … Bai, Y. M. (2013). Association between psychiatric disorders and iron deficiency anemia among children and adolescents: A nationwide population-based study. *BMC Psychiatry*, *13*, 161. doi: 10.1186/1471-244X-13-161

Chmielewska, A., Dziechciarz, P., Gieruszczak-Bialek, D., Horvath, A., Piescik-Lech, M., Ruszczynski, M., … Szajewska, H. (2019). Effects of prenatal and/or postnatal supplementation with iron, PUFA or folic acid on neurodevelopment: Update. *British Journal of Nutrition*, *122*(Suppl. 1), S10–S15. doi: 10.1017/S0007114514004243

Churchwell, J. C., Carey, P. D., Ferrett, H. L., Stein, D. J., & Yurgelun-Todd, D. A. (2012). Abnormal striatal circuitry and intensified novelty seeking among adolescents who abuse methamphetamine and cannabis. *Developmental Neuroscience*, *34*(4), 310–317. doi: 10.1159/000337724

Coe, C. L., Lubach, G. R., Bianco, L., & Beard, J. L. (2009). A history of iron deficiency anemia during infancy alters brain monoamine activity later in juvenile monkeys. *Developmental Psychobiology*, *51*(3), 301–309. doi: 10.1002/dev.20365

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences* (2nd ed.). New York: Routledge.

Connor, J. R., & Menzies, S. L. (1996). Relationship of iron to oligodendrocytes and myelination. *Glia*, *17*(2), 83–93. doi: 10.1002/(SICI)1098-1136(199606)17:2<83::AID-GLIA1>3.0.CO;2-7

Corson, P. W., Nopoulos, P., Miller, D. D., Arndt, S., & Andreasen, N. C. (1999). Change in basal ganglia volume over 2 years in patients with schizophrenia: Typical versus atypical neuroleptics. *American Journal of Psychiatry*, *156*(8), 1200–1204. doi: 10.1176/ajp.156.8.1200

Corwin, E. J., Murray-Kolb, L. E., & Beard, J. L. (2003). Low hemoglobin level is a risk factor for postpartum depression. *Journal of Nutrition*, *133*(12), 4139–4142. doi: 10.1093/jn/133.12.4139

D'Occhio, M. J., Fordyce, G., Whyte, T. R., Aspden, W. J., & Trigg, T. E. (2000). Reproductive responses of cattle to GnRH agonists. *Animal Reproduction Science*, *60–61*, 433–442. doi: 10.1016/s0378-4320(00)00078-6

Doom, J. R., Richards, B., Caballero, G., Delva, J., Gahagan, S., & Lozoff, B. (2018). Infant iron deficiency and iron supplementation predict adolescent internalizing, externalizing, and social problems. *Journal of Pediatrics*, *195*, 199–205.e192. doi: 10.1016/j.jpeds.2017.12.008

Drysdale, A. T., Grosenick, L., Downar, J., Dunlop, K., Mansouri, F., Meng, Y., … Liston, C. (2017). Resting-state connectivity biomarkers define neurophysiological subtypes of depression. *Nature Medicine*, *23*(1), 28–38. doi: 10.1038/nm.4246

Ennis, K. M., Dahl, L. V., Rao, R. B., & Georgieff, M. K. (2018). Reticulocyte hemoglobin content as an early predictive biomarker of brain iron deficiency. *Pediatric Research*, *84*(5), 765–769. doi: 10.1038/s41390-018-0178-6

Erikson, K. M., Jones, B. C., & Beard, J. L. (2000). Iron deficiency alters dopamine transporter functioning in rat striatum. *Journal of Nutrition*, *130*(11), 2831–2837.

Erikson, K. M., Jones, B. C., Hess, E. J., Zhang, Q., & Beard, J. L. (2001). Iron deficiency decreases dopamine D1 and D2 receptors in rat brain. *Pharmacology, Biochemistry and Behavior*, *69*(3–4), 409–418. doi: S0091-3057(01)00563-9

Ersche, K. D., Acosta-Cabronero, J., Jones, P. S., Ziauddeen, H., van Swelm, R. P., Laarakkers, C. M., … Williams, G. B. (2017). Disrupted iron regulation in the brain and periphery in cocaine addiction. *Translational Psychiatry*, *7*(2), e1040. doi: 10.1038/tp.2016.271

Ersche, K. D., Barnes, A., Jones, P. S., Morein-Zamir, S., Robbins, T. W., & Bullmore, E. T. (2011). Abnormal structure of frontostriatal brain systems is associated with aspects of impulsivity and compulsivity in cocaine dependence. *Brain*, *134*(Pt 7), 2013–2024. doi: 10.1093/brain/awr138

Ersche, K. D., Jones, P. S., Williams, G. B., Turton, A. J., Robbins, T. W., & Bullmore, E. T. (2012). Abnormal brain structure implicated in stimulant drug addiction. *Science (New York, N.Y.)*, *335*(6068), 601–604. doi: 10.1126/science.1214463

Faulstich, M. E., Carey, M. P., Ruggiero, L., Enyart, P., & Gresham, F. (1986). Assessment of depression in childhood and adolescence: An evaluation of the center for epidemiological studies depression scale for children (CES-DC). *American Journal of Psychiatry*, *143*(8), 1024–1027. doi: 10.1176/ajp.143.8.1024

Fischl, B., Salat, D. H., Busa, E., Albert, M., Dieterich, M., Haselgrove, C., … Dale, A. M. (2002). Whole brain segmentation: Automated labeling of neuroanatomical structures in the human brain. *Neuron*, *33*(3), 341–355. doi: 10.1016/s0896-6273(02)00569-x

Fischl, B., Salat, D. H., van der Kouwe, A. J., Makris, N., Segonne, F., Quinn, B. T., & Dale, A. M. (2004). Sequence-independent segmentation of magnetic resonance images. *Neuroimage*, *23*(Suppl. 1), S69–S84. doi: 10.1016/j.neuroimage.2004.07.016

Fordy, J., & Benton, D. (1994). Does low iron status influence psychological functioning? *Journal of Human Nutrition and Dietetics*, *7*, 127–133.

Fretham, S. J., Carlson, E. S., Wobken, J., Tran, P. V., Petryk, A., & Georgieff, M. K. (2012). Temporal manipulation of transferrin-receptor-1-dependent iron uptake identifies a sensitive period in mouse hippocampal neurodevelopment. *Hippocampus*, *22*(8), 1691–1702. doi: 10.1002/hipo.22004

Garcia-Casal, M. N., Pena-Rosas, J. P., Urrechaga, E., Escanero, J. F., Huo, J., Martinez, R. X., & Lopez-Perez, L. (2018). Performance and comparability of laboratory methods for measuring ferritin concentrations in human serum or plasma: A systematic review and meta-analysis. *PLoS ONE*, *13*(5), e0196576. doi: 10.1371/journal.pone.0196576

Georgieff, M. K. (2017). Iron assessment to protect the developing brain. *American Journal of Clinical Nutrition*, *106*(Suppl. 6), 1588S–1593S. doi: 10.3945/ajcn.117.155846

Georgieff, M. K., Brunette, K. E., & Tran, P. V. (2015). Early life nutrition and neural plasticity. *Development and Psychopathology*, *27*(2), 411–423. doi: 10.1017/S0954579415000061

Golub, M. S., Hogrefe, C. E., & Germann, S. L. (2007). Iron deprivation during fetal development changes the behavior of juvenile rhesus monkeys. *Journal of Nutrition*, *137*(4), 979–984. doi: 10.1093/jn/137.4.979

Grantham-McGregor, S., & Ani, C. (2001). A review of studies on the effect of iron deficiency on cognitive development in children. *The Journal of Nutrition*, *131*(2S-2), 649S–666S; discussion 666S-668S.

Groman, S. M., Morales, A. M., Lee, B., London, E. D., & Jentsch, J. D. (2013). Methamphetamine-induced increases in putamen gray matter associate with inhibitory control. *Psychopharmacology*, *229*(3), 527–538. doi: 10.1007/s00213-013-3159-9

Guiang, S. F., 3rd, Georgieff, M. K., Lambert, D. J., Schmidt, R. L., & Widness, J. A. (1997). Intravenous iron supplementation effect on tissue iron and hemoproteins in chronically phlebotomized lambs. *The American Journal of Physiology*, *273*(6 Pt 2), R2124–R2131.

Gupta, P. M., Hamner, H. C., Suchdev, P. S., Flores-Ayala, R., & Mei, Z. (2017). Iron status of toddlers, nonpregnant females, and pregnant females in the United States. *American Journal of Clinical Nutrition*, *106*(Suppl. 6), 1640S–1646S. doi: 10.3945/ajcn.117.155978

Haacke, E. M., Cheng, N. Y., House, M. J., Liu, Q., Neelavalli, J., Ogg, R. J., … Obenaus, A. (2005). Imaging iron stores in the brain using magnetic resonance imaging. *Magnetic Resonance Imaging*, *23*(1), 1–25. doi: 10.1016/j.mri.2004.10.001

Hallgren, B., & Sourander, P. (1958). The effect of age on the non-haemin iron in the human brain. *Journal of Neurochemistry*, *3*(1), 41–51. doi: 10.1111/j.1471-4159.1958.tb12607.x

Jacobsen, L. K., Giedd, J. N., Gottschalk, C., Kosten, T. R., & Krystal, J. H. (2001). Quantitative morphology of the caudate and putamen in patients with cocaine dependence. *American Journal of Psychiatry*, *158*(3), 486–489. doi: 10.1176/appi.ajp.158.3.486

Jan, R. K., Lin, J. C., Miles, S. W., Kydd, R. R., & Russell, B. R. (2012). Striatal volume increases in active methamphetamine-dependent individuals and correlation with cognitive performance. *Brain Sciences*, *2*(4), 553–572. doi: 10.3390/brainsci2040553

Janiri, D., Moser, D. A., Doucet, G. E., Luber, M. J., Rasgon, A., Lee, W. H., … Frangou, S. (2019). Shared neural phenotypes for mood and anxiety disorders: A meta-analysis of 226 task-related functional imaging studies. *JAMA Psychiatry*, *77*(2), 172–179. 10.1001/jamapsychiatry.2019.3351.

Jellen, L. C., Lu, L., Wang, X., Unger, E. L., Earley, C. J., Allen, R. P., … Jones, B. C. (2013). Iron deficiency alters expression of dopamine-related genes in the ventral midbrain in mice. *Neuroscience*, *252*, 13–23. doi: 10.1016/j.neuroscience.2013.07.058

Jernigan, T. L., Gamst, A. C., Archibald, S. L., Fennema-Notestine, C., Mindt, M. R., Marcotte, T. D., … Grant, I. (2005). Effects of methamphetamine dependence and HIV infection on cerebral morphology. *American Journal of Psychiatry*, *162*(8), 1461–1472. doi: 10.1176/appi.ajp.162.8.1461

Karl, J. P., Lieberman, H. R., Cable, S. J., Williams, K. W., Young, A. J., & McClung, J. P. (2010). Randomized, double-blind, placebo-controlled trial of an iron-fortified food product in female soldiers during military training: Relations between iron status, serum hepcidin, and inflammation. *American Journal of Clinical Nutrition*, *92*(1), 93–100. doi: 10.3945/ajcn.2010.29185

Kennedy, B. C., Dimova, J. G., Siddappa, A. J., Tran, P. V., Gewirtz, J. C., & Georgieff, M. K. (2014). Prenatal choline supplementation ameliorates the long-term neurobehavioral effects of fetal-neonatal iron deficiency in rats. *Journal of Nutrition*, *144*(11), 1858–1865. doi: 10.3945/jn.114.198739

Kim, I., Yetley, E. A., & Calvo, M. S. (1993). Variations in iron-status measures during the menstrual cycle. *American Journal of Clinical Nutrition*, *58*(5), 705–709. doi: 10.1093/ajcn/58.5.705

Konofal, E., Lecendreux, M., Deron, J., Marchand, M., Cortese, S., Zaim, M., … Arnulf, I. (2008). Effects of iron supplementation on attention deficit hyperactivity disorder in children. *Pediatric Neurology*, *38*(1), 20–26. doi: S0887-8994(07)00417-1

Lange, S. J., & Que, L., Jr. (1998). Oxygen activating nonheme iron enzymes. *Current Opinion in Chemical Biology*, *2*(2), 159–172. doi: 10.1016/s1367-5931(98)80057-4

Langkammer, C., Schweser, F., Krebs, N., Deistung, A., Goessler, W., Scheurer, E., … Reichenbach, J. R. (2012). Quantitative susceptibility mapping (QSM) as a means to measure brain iron? A post mortem validation study. *Neuroimage*, *62*(3), 1593–1599. doi: 10.1016/j.neuroimage.2012.05.049

https://doi.org/10.1017/S0033291721004098 Published online by Cambridge University Press

Looker, A. C., Cogswell, M. E., & Gunter, E. W. (2002). *Iron deficiency – United States*, 1999–2000. Retrieved from http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5140a1.htm#tab1.

Low, M. S., Speedy, J., Styles, C. E., De-Regil, L. M., & Pasricha, S. R. (2016). Daily iron supplementation for improving anaemia, iron status and health in menstruating women. *Cochrane Database of Systematic Review*, *4*, CD009747. doi: 10.1002/14651858.CD009747.pub2

Lozoff, B., & Georgieff, M. K. (2006). Iron deficiency and brain development. *Seminars in Pediatric Neurology*, *13*(3), 158–165. doi: 10.1016/j.spen.2006.08.004

Lozoff, B., Jimenez, E., Hagen, J., Mollen, E., & Wolf, A. W. (2000). Poorer behavioral and developmental outcome more than 10 years after treatment for iron deficiency in infancy. *Pediatrics*, *105*(4), E51.

Lozoff, B., Smith, J. B., Kaciroti, N., Clark, K. M., Guevara, S., & Jimenez, E. (2013). Functional significance of early-life iron deficiency: Outcomes at 25 years. *Journal of Pediatrics*, *163*(5), 1260–1266. doi: 10.1016/j.jpeds.2013.05.015

Mast, A. E., Blinder, M. A., Gronowski, A. M., Chumley, C., & Scott, M. G. (1998). Clinical utility of the soluble transferrin receptor and comparison with serum ferritin in several populations. *Clinical Chemistry*, *44*(1), 45–51.

Matsuo, K., Rosenberg, D. R., Easter, P. C., MacMaster, F. P., Chen, H. H., Nicoletti, M., … Soares, J. C. (2008). Striatal volume abnormalities in treatment-naïve patients diagnosed with pediatric major depressive disorder. *Journal of Child and Adolescent Psychopharmacology*, *18*(2), 121–131. doi: 10.1089/cap.2007.0026

McCann, J. C., & Ames, B. N. (2007). An overview of evidence for a causal relation between iron deficiency during development and deficits in cognitive or behavioral function. *American Journal of Clinical Nutrition*, *85*(4), 931–945.

Mikami, K., Okazawa, H., Kimoto, K., Akama, F., Onishi, Y., Takahashi, Y., … Matsumoto, H. (2019). Effect of oral iron administration on mental state in children with low serum ferritin concentration. *Global Pediatric Health*, *6*, 2333794X19884816. doi: 10.1177/2333794X19884816

Mohamed, W. M., Unger, E. L., Kambhampati, S. K., & Jones, B. C. (2011). Methylphenidate improves cognitive deficits produced by infantile iron deficiency in rats. *Behavioural Brain Research*, *216*(1), 146–152. doi: 10.1016/j.bbr.2010.07.025

Mojtabai, R., & Olfson, M. (2020). National trends in mental health care for US adolescents. *JAMA Psychiatry*, *77*(7), 703–714. doi: 10.1001/jamapsychiatry.2020.0279

Mojtabai, R., Olfson, M., & Han, B. (2016). National trends in the prevalence and treatment of depression in adolescents and young adults. *Pediatrics*, *138*(6), e20161878. doi: 10.1542/peds.2016-1878.

Moller, H. E., Bossoni, L., Connor, J. R., Crichton, R. R., Does, M. D., Ward, R. J., … Ronen, I. (2019). Iron, myelin, and the brain: Neuroimaging meets neurobiology. *Trends in Neurosciences*, *42*(6), 384–401. doi: 10.1016/j.tins.2019.03.009

Mudd, A. T., Fil, J. E., Knight, L. C., Lam, F., Liang, Z. P., & Dilger, R. N. (2018). Early-life iron deficiency reduces brain iron content and alters brain tissue composition despite iron repletion: A neuroimaging assessment. *Nutrients*, *10*(2), 135. doi: 10.3390/nu10020135.

Murray-Kolb, L. E. (2011). Iron status and neuropsychological consequences in women of reproductive age: What do we know and where are we headed? *Journal of Nutrition*, *141*(4), 747S–755S. doi: 10.3945/jn.110.130658

Navari, S., & Dazzan, P. (2009). Do antipsychotic drugs affect brain structure? A systematic and critical review of MRI findings. *Psychological Medicine*, *39*(11), 1763–1777. doi: 10.1017/S0033291709005315

Nelson, C., Erikson, K., Pinero, D. J., & Beard, J. L. (1997). In vivo dopamine metabolism is altered in iron-deficient anemic rats. *Journal of Nutrition*, *127*(12), 2282–2288.

North, M., Dallalio, G., Donath, A. S., Melink, R., & Means, R. T., Jr. (1997). Serum transferrin receptor levels in patients undergoing evaluation of iron stores: Correlation with other parameters and observed versus predicted results. *Clinical and Laboratory Haematology*, *19*(2), 93–97. doi: 10.1046/j.1365-2257.1997.00041.x

Ogden, C. L., Kuczmarski, R. J., Flegal, K. M., Mei, Z., Guo, S., Wei, R., … Johnson, C. L. (2002). Centers for disease control and prevention 2000 growth charts for the United States: Improvements to the 1977 National Center for Health Statistics version. *Pediatrics*, *109*(1), 45–60.

Peterson, E. T., Kwon, D., Luna, B., Larsen, B., Prouty, D., De Bellis, M. D., … Pfefferbaum, A. (2019). Distribution of brain iron accrual in adolescence: Evidence from cross-sectional and longitudinal analysis. *Human Brain Mapping*, *40*(5), 1480–1495. doi: 10.1002/hbm.24461

Pino, J. M. V., da Luz, M. H. M., Antunes, H. K. M., Giampa, S. Q. C., Martins, V. R., & Lee, K. S. (2017). Iron-restricted diet affects brain ferritin levels, dopamine metabolism and cellular prion protein in a region-specific manner. *Frontiers in Molecular Neuroscience*, *10*, 145. doi: 10.3389/fnmol.2017.00145

Pizzagalli, D. A., Holmes, A. J., Dillon, D. G., Goetz, E. L., Birk, J. L., Bogdan, R., … Fava, M. (2009). Reduced caudate and nucleus accumbens response to rewards in unmedicated individuals with major depressive disorder. *American Journal of Psychiatry*, *166*(6), 702–710. doi: 10.1176/appi.ajp.2008.08081201

Rangan, A. M., Blight, G. D., & Binns, C. W. (1998). Iron status and non-specific symptoms of female students. *Journal of the American College of Nutrition*, *17*(4), 351–355. doi: 10.1080/07315724.1998.10718774

Raznahan, A., Shaw, P. W., Lerch, J. P., Clasen, L. S., Greenstein, D., Berman, R., … Giedd, J. N. (2014). Longitudinal four-dimensional mapping of subcortical anatomy in human development. *Proceedings of the National Academy of Sciences of the USA*, *111*(4), 1592–1597. doi: 10.1073/pnas.1316911111

Rouault, T. A., & Cooperman, S. (2006). Brain iron metabolism. *Seminars in Pediatric Neurology*, *13*(3), 142–148. doi: 10.1016/j.spen.2006.08.002

Schmidt, A. T., Waldow, K. J., Grove, W. M., Salinas, J. A., & Georgieff, M. K. (2007). Dissociating the long-term effects of fetal/neonatal iron deficiency on three types of learning in the rat. *Behavioral Neuroscience*, *121*(3), 475–482. doi: 10.1037/0735-7044.121.3.475

Sedlacik, J., Boelmans, K., Lobel, U., Holst, B., Siemonsen, S., & Fiehler, J. (2014). Reversible, irreversible and effective transverse relaxation rates in normal aging brain at 3T. *Neuroimage*, *84*, 1032–1041. doi: 10.1016/j.neuroimage.2013.08.051

Sever, Y., Ashkenazi, A., Tyano, S., & Weizman, A. (1997). Iron treatment in children with attention deficit hyperactivity disorder. A preliminary report. *Neuropsychobiology*, *35*(4), 178–180.

Song, M. K., Lin, F. C., Ward, S. E., & Fine, J. P. (2013). Composite variables: When and how. *Nursing Research*, *62*, 45–49. https://doi.org/10.1097/NNR.0b013e3182741948.

Sun, H., & Weaver, C. M. (2021). Decreased iron intake parallels rising iron deficiency anemia and related mortality rates in the US population. *Journal of Nutrition*, *151*(7), 1947–1955. https://doi.org/10.1093/jn/nxab064.

Tansey, F. A., & Cammer, W. (1988). Acetyl-CoA carboxylase in rat brain. I. Activities in homogenates and isolated fractions. *Brain Research*, *471*(1), 123–130. doi: 10.1016/0165-3806(88)90157-5

Tran, P. V., Kennedy, B. C., Pisansky, M. T., Won, K. J., Gewirtz, J. C., Simmons, R. A., & Georgieff, M. K. (2016). Prenatal choline supplementation diminishes early-life iron deficiency-induced reprogramming of molecular networks associated with behavioral abnormalities in the adult rat hippocampus. *Journal of Nutrition*, *146*(3), 484–493. doi: 10.3945/jn.115.227561

Trumbo, P., Schlicker, S., Yates, A. A., & Poos, M. (2002). Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. *Journal of the American Dietetic Association*, *102*(11), 1621–1630.

Turner, C. A., Xie, D., Zimmerman, B. M., & Calarge, C. A. (2010). Iron status in toddlerhood predicts sensitivity to psychostimulants in children. *Journal of Attention Disorders*, *16*(4), 295–303. 1087054710385067.

Twenge, J. M., Cooper, A. B., Joiner, T. E., Duffy, M. E., & Binau, S. G. (2019). Age, period, and cohort trends in mood disorder indicators and suicide-related outcomes in a nationally representative dataset, 2005–2017. *Journal of Abnormal Psychology*, *128*(3), 185–199. doi: 10.1037/abn0000410

Vahdat Shariatpanaahi, M., Vahdat Shariatpanaahi, Z., Moshtaaghi, M., Shahbaazi, S. H., & Abadi, A. (2007). The relationship between depression and serum ferritin level. *European Journal of Clinical Nutrition*, *61*(4), 532–535. doi: 10.1038/sj.ejcn.1602542

Verdon, F., Burnand, B., Stubi, C. L., Bonard, C., Graff, M., Michaud, A., … Favrat, B. (2003). Iron supplementation for unexplained fatigue in non-anaemic women: Double blind randomised placebo controlled trial. *BMJ*, *326*(7399), 1124. doi: 10.1136/bmj.326.7399.1124

https://doi.org/10.1017/S0033291721004098 Published online by Cambridge University Press

Vulser, H., Lemaitre, H., Artiges, E., Miranda, R., Penttilä, J., Struve, M., … Paillère-Martinot, M. L. (2015). Subthreshold depression and regional brain volumes in young community adolescents. *Journal of the American Academy of Child and Adolescent Psychiatry*, *54*(10), 832–840. doi: 10.1016/j.jaac.2015.07.006

Vulser, H., Wiernik, E., Hoertel, N., Thomas, F., Pannier, B., Czernichow, S., … Lemogne, C. (2016). Association between depression and anemia in otherwise healthy adults. *Acta Psychiatrica Scandinavica*, *134*(2), 150–160. doi: 10.1111/acps.12595

Wade, Q. W., Chiou, B., & Connor, J. R. (2019). Iron uptake at the blood-brain barrier is influenced by sex and genotype. *Advances in Pharmacology*, *84*, 123–145. doi: 10.1016/bs.apha.2019.02.005

Wassef, A., Nguyen, Q. D., & St-Andre, M. (2019). Anaemia and depletion of iron stores as risk factors for postpartum depression: A literature review. *Journal of Psychosomatic Obstetrics and Gynaecology*, *40*(1), 19–28. doi: 10.1080/0167482X.2018.1427725

Wei, W., & Wang, X. J. (2016). Inhibitory control in the cortico-basal ganglia-thalamocortical loop: Complex regulation and interplay with memory and decision processes. *Neuron*, *92*(5), 1093–1105. doi: 10.1016/j.neuron.2016.10.031

Weissman, M. M., Orvaschel, H., & Padian, N. (1980). Children's symptom and social functioning self-report scales. Comparison of mothers' and children's reports. *Journal of Nervous and Mental Disease*, *168*(12), 736–740.

WHO, World Health Organization. (2001). Iron deficiency anaemia – Assessment, prevention, and control. A guide for programme managers. https://www.who.int/nutrition/publications/en/ida_assessment_prevention_control.pdf.

WHO, World Health Organization. (2011). Serum ferritin concentrations for the assessment of iron status and iron deficiency in populations. Retrieved from http://www.who.int/vmnis/indicators/serum_ferritin.pdf.

Wierenga, L., Langen, M., Ambrosino, S., van Dijk, S., Oranje, B., & Durston, S. (2014). Typical development of basal ganglia, hippocampus, amygdala and cerebellum from age 7 to 24. *Neuroimage*, *96*, 67–72. doi: 10.1016/j.neuroimage.2014.03.072

Williams, L. M. (2016). Precision psychiatry: A neural circuit taxonomy for depression and anxiety. *The Lancet. Psychiatry*, *3*(5), 472–480. doi: 10.1016/S2215-0366(15)00579-9

Xu, X., Wang, Q., & Zhang, M. (2008). Age, gender, and hemispheric differences in iron deposition in the human brain: An in vivo MRI study. *Neuroimage*, *40*(1), 35–42. doi: 10.1016/j.neuroimage.2007.11.017

Youdim, M. B. (2008). Brain iron deficiency and excess; cognitive impairment and neurodegeneration with involvement of striatum and hippocampus. *Neurotoxicity Research*, *14*(1), 45–56.

Zamora, T. G., Guiang, S. F., 3rd, Widness, J. A., & Georgieff, M. K. (2016). Iron is prioritized to red blood cells over the brain in phlebotomized anemic newborn lambs. *Pediatric Research*, *79*(6), 922–928. doi: 10.1038/pr.2016.20

https://doi.org/10.1017/S0033291721004098 Published online by Cambridge University Press