JOURNAL OF CHILD AND ADOLESCENT PSYCHOPHARMACOLOGY
Volume 20, Number 6, 2010

Pp. 495–502
DOI: 10.1089/cap.2010.0053

Serum Ferritin and Amphetamine Response in Youth with Attention-Deficit/Hyperactivity Disorder

Chadi Calarge, M.D.,[1] Cristan Farmer, M.A.,[2] Robert DiSilvestro, Ph.D.,[3] and L. Eugene Arnold, M.D., Ph.D.[4]

Abstract

Introduction: Iron deficiency (ID) has been associated with attention and behavioral problems, in general, and with attention-deficit/hyperactivity disorder (ADHD), in particular. The study aim was to explore whether iron stores, as reflected by serum ferritin concentration, predicted response to psychostimulants.

Methods: Six- to 14-year-old children with ADHD enrolled in a multiphase, double-blind, randomized, placebo-controlled trial investigating zinc supplementation in treating ADHD and optimizing response to psychostimulants. The Swanson, Nolan, and Pelham (SNAP) ADHD rating scale was the primary clinical instrument. Serum ferritin concentration was obtained at baseline and 8 weeks later. Partial correlations, adjusting for age and sex, were computed.

Results: Fifty-two participants (83% males) had a mean age of 10 years. Their ADHD symptoms were moderately severe at baseline (SNAP item mean = 2.1). Their mean ferritin concentration was 18.4 ng/mL, with 23% of the participants having a level below 7, the assay-defined threshold for ID. Serum ferritin was inversely correlated with baseline inattention, hyperactivity/impulsivity, and total ADHD symptom scores (Partial Spearman's $r = -0.31$, $p = 0.04$; $r = -0.42$, $p < 0.006$; and $r = -0.43$, $p < 0.004$, respectively) and with the weight-adjusted dose of amphetamine used to optimize clinical response (Partial Spearman's $r = -0.45$, $p < 0.007$). Psychotropic-treatment history moderated some, but not all, of these associations, with previously medicated children showing a stronger association between ferritin concentration and ADHD symptom severity.

Conclusion: These findings add to the growing literature implicating ID in ADHD. The prediction of amphetamine optimal dose by ferritin concentration suggests that iron supplementation should be investigated as a potential intervention to optimize response to psychostimulants at a lower dose in individuals with low iron stores and ADHD.

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is one of the most common psychiatric disorders in children (American Psychiatric Association 2000). It is characterized by age-inappropriate levels of inattention, hyperactivity, and impulsivity that impair one's functioning (American Psychiatric Association 2000). With the heritability of ADHD estimated at around 0.8 (Faraone 2000), there has been great interest in identifying a genetic etiology for this disorder, leading to a relative neglect of the environmental contribution to its emergence. Some of the genes involved undoubtedly predispose to environmental vulnerability, so that the environmental contribution may be more than the 20% implied by the heritability figure. Consequently, with the prevalence of ADHD ranging between 5% and 10% in school-aged children (Pliszka 2007), the variance in ADHD involving environmental factors, either alone or in consort with genetic determinants, translates into a substantial number of patients. Thus, identifying environmental risk factors associated with ADHD remains in the public health interest. Arguably, these factors are more amenable to preventive interventions, compared to the genetic predisposition.

Over the last two decades, iron deficiency (ID) has emerged as a potential risk factor for inattention and externalizing disorders (Lozoff et al. 2000; Oner et al. 2008). ID is the most common single-nutrient condition in the world (Beard and Connor 2003). It disproportionately affects pregnant women and children from infancy to adolescence (Marks et al. 1998). In the United States, ID remains a public health concern with up to 7% of young children

Aspects of this work were submitted for presentation at the 57th Annual Meeting of the American Academy of Child and Adolescent Psychiatry, October 2010, New York, New York.
[1]Department of Psychiatry, The University of Iowa Carver College of Medicine, Iowa City, Iowa.
[2]The Nisonger Center, Ohio State University, Columbus, Ohio.
Departments of [3]Human Nutrition and [4]Psychiatry, Ohio State University, Columbus, Ohio.
This study was funded by the National Institute of Mental Health (5R34MH071683 and K23MH085005). And by Award Number UL1RR025755 from the National Center for Research Resources. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center for Research Resources or the National Institutes of Health.

and 16% of adolescent women afflicted (Centers for Disease Control and Prevention 2002). This is of particular importance since this developmental period is characterized by substantial brain growth and maturation.

Converging evidence from *in vitro*, animal, and clinical studies supports a potential role for ID, with or without anemia, in the emergence of ADHD. First, iron is a cofactor for many enzymes and is incorporated in various structural and transport proteins in the brain (Beard and Connor 2003). More specifically, it is a cofactor for tyrosine hydroxylase, the rate-limiting enzyme for catecholamine synthesis (Sachdev 1993). In addition, the basal ganglia, a group of brain nuclei often implicated in ADHD (Dickstein et al. 2006), are particularly rich in iron (Beard and Connor 2003) and when rats experience ID, they exhibit a reduction in the density of the dopamine transporter and the D1 and D2 dopamine receptors in the basal ganglia (Beard et al. 1994; Nelson et al. 1997; Erikson et al. 2000, 2001; Burhans et al. 2005). ID rats also exhibit a reversal in the circadian rhythms of dopamine-mediated locomotor activity and thermoregulation and an altered pattern of response to d-amphetamine (Youdim et al. 1981). Further, the response to cocaine, a potent inhibitor of the dopamine transporter, is attenuated in ID rats (Nelson et al. 1997; Erikson et al. 2000). Additional support comes from studies in children with ID showing reduced prolactin response to a clonidine challenge, again reflecting dysfunctional dopaminergic signaling (Felt et al. 2006a).

With iron playing a key role in neurotransmission, dendritogenesis, synaptogenesis, and myelination (Connor and Menzies 1996; Beard and Connor 2003), concerns have been raised about the impact ID might have on cognitive, behavioral, and emotional development (Lozoff and Georgieff 2006). In fact, prospective studies have shown that infants with ID anemia exhibit socio-emotional and academic impairment (Lozoff et al. 2000; Wachs et al. 2005; Felt et al. 2006b; Lozoff and Georgieff 2006). In addition, studies in patients with ADHD have reported an inverse association between serum ferritin, a measure of body iron, and the severity of inattention, hyperactivity and impulsivity, or sleep disturbances (Konofal et al. 2004; Oner et al. 2008; Cortese et al. 2009).

Moreover, it is particularly disturbing that prospective studies have found persistent cognitive and neurochemical abnormalities, long after the resolution of ID identified postnatally (Lozoff et al. 2000; Burhans et al. 2005; Felt et al. 2006b). For example, >10 years after their iron stores had been replenished, children with a history of ID were more likely to have repeated a grade and/or received educational services, compared to children with a normal iron status (Lozoff et al. 2000). They also exhibited increased behavior problems, including inattention (Lozoff et al. 2000). Further, as noted earlier, these children had altered prolactin response to a clonidine challenge (Felt et al. 2006b). These findings suggest that, at least to some extent, ID in infancy might have a long-lasting effect on brain development, in general, and on the dopaminergic system, in particular. This would also be consistent with studies in ID rats showing only partial recovery of neurotransmission after iron supplementation (Burhans et al. 2005). In fact, the severity of ID and the timing of iron repletion appear crucial in determining the extent to which recovery will be complete (Lozoff and Georgieff 2006).

In sum, preclinical and human research suggests that ID, impaired dopaminergic signaling, and ADHD may be etiologically linked. However, to our knowledge, few have directly investigated the association between iron stores and response to treatment in ADHD. In one study, no difference was found in the clinical response to psychostimulants between children with low (<20 ng/mL) versus high (>60 ng/mL) serum ferritin concentration (Millichap et al. 2006). On the other hand, Turner et al. (2009) found that iron status in toddlerhood was associated with sensitivity to psychostimulants prescribed more that 3 years later. This question is particularly pertinent in light of animal studies showing that ID dampens the inhibitory effect of cocaine on the dopamine transporter (Nelson et al. 1997; Erikson et al. 2000). Such evidence raises the possibility of a modulatory effect of iron status on the efficacy of psychostimulant treatment in patients with ADHD since, as noted earlier, they act by blocking the dopamine transporter (Ford et al. 2003).

Therefore, taking advantage of a clinical trial exploring zinc supplementation in optimizing response to psychostimulants in children, we investigated whether iron status was associated with ADHD symptom severity and with response to psychostimulant treatment. We further explored whether prior psychotropic treatment moderated such associations.

Methods

This study is based on secondary analysis of data collected in a multiphase, parallel-group, randomized, double-blind placebo-controlled pilot trial of zinc supplementation in treating ADHD and optimizing response to psychostimulant treatment (Arnold et al. 2010 in press). Children and adolescents, age 6–14 years inclusive, were recruited from a university child psychiatry clinic, advertisements, flyers, letters to professionals, a Web site, and a waiting list of families interested in participating in ADHD treatment studies. The diagnosis of ADHD was based on the Children's Interview for Psychiatric Symptoms; parent version (P-ChIPS) and clinical *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition, Text Revision (DSM-IV-TR)–based assessment (Fristad et al. 1998; American Psychiatric Association 2000). The eligibility criteria included a primary diagnosis of ADHD, inattentive or combined type, the availability of a reliable primary caretaker and a teacher both willing to complete the clinical ratings, and an item mean of ≥1.5 on a 0–3 metric on parent or teacher ratings of the DSM-IV-TR inattentive or overall ADHD symptoms. Participants with co-morbid psychiatric disorders requiring pharmacotherapy (as judged by the screening clinician), other than catecholaminergic psychostimulants, were excluded. However, patients with milder depression or anxiety not requiring pharmacotherapy were included. Other exclusions included serious medical disorders, current zinc supplementation, contraindications to zinc or amphetamine, current state of infection/inflammation, or plans to move residence.

The trial consisted of three phases: An initial 8-week period comparing zinc monotherapy to placebo, followed by a 2-week period where a standard (weight-based) dose of amphetamine was added, and then a 3-week amphetamine-dose-adjustment period to optimize clinical response. All medicated participants underwent a washout period that lasted at least 1 week. The dose of zinc was 15 ($n = 20$) or 30 mg/day ($n = 8$) in the form of zinc glycinate. Extended-release dextroamphetamine was given in the morning in phase 2 at a dose of 5 mg for those weighing up to 25 kg, 10 mg for those weighing 25–45 kg, and 15 mg for those weighing >45 kg. In phase 3, the dose of amphetamine was titrated, as clinically indicated and tolerated, with the goal to have all 18 DSM-IV-TR ADHD symptoms rated as 0 or 1 (''just a little'').

Parent and teacher ratings of inattention, impulsivity, and hyperactivity were obtained every 2 weeks from baseline through

week 10 (end of phase 2) and weekly during phase 3 (to 13 weeks), using the Swanson, Nolan, and Pelham, Version IV (SNAP-IV) (Swanson 1992). This instrument consists of the 18 DSM-IV-TR ADHD symptoms rated on a 0–3 metric from not at all to very much and constitutes the primary outcome measure. At baseline, the Conners' Parent Rating Scale-Revised (CPRS-R), long version (80-item), was also completed to obtain a broad profile of the subjects (Conners 2001). This was repeated at 8, 10, and 13 weeks as a secondary outcome measure. Serum ferritin concentration was measured at baseline ($n = 46$) and week 8 ($n = 44$) using the Ferritin EIA kit from Alpco Diagnostics (Salem, NH).

Height was measured to the nearest 0.1 cm and weight to the nearest 0.1 kg. Weight, height, and body mass index (kg/m^2) measurements were converted into age- and sex-adjusted z scores using the 2000 Center for Disease Control growth norms (Ogden et al. 2002). Dietary intake during the week before enrollment was estimated using the 2004 Block Kids Food Frequency Questionnaire (Block et al. 2000).

The study was approved by the local Institutional Review Board. Written consents/assents were obtained from the participants and their parents.

Data analysis

This study design allowed us to test two specific hypotheses related to the anticipation that iron status would be associated with ADHD symptomatology and with response to treatment. Thus, using partial Spearman's correlations, we investigated, first, whether serum ferritin concentration at baseline was associated with ADHD symptom severity and, second, whether serum ferritin at baseline and week 8 predicted individual sensitivity to amphetamine, as reflected by the weight-adjusted dose necessary to achieve an optimal clinical response (i.e., week 13 or end of phase 3). These correlations were adjusted for age and sex. To determine whether these correlations varied between previously medicated and psychotropic-naïve participants (i.e., based on prior treatment status), multiple linear regression analysis was used to predict individual ADHD symptom factor scores with baseline serum ferritin concentration, prior psychotropic treatment status, their interaction effect, age, and sex as independent variables. Differences between previously medicated and psychotropic-naïve participants were compared using the Student's *t*-test for continuous variables and the Fisher's Exact test for categorical ones. All the statistical tests were performed using SAS version 9.2 (SAS Institute Inc., Cary, NC).

Results

Fifty-nine children were screened, of whom 52 (83% males) entered treatment (7 screen fails). Their mean age was 10.0 years (standard deviation, SD = 2.6), with the majority having ADHD, combined type. In addition, co-morbidity was common with 34 (65%) participants having oppositional defiant disorder or conduct disorder, 10 (19%) a learning disorder, 9 (17%) an adjustment disorder with depressed mood, 5 (10%) a phonological disorder, and 4 (8%) an elimination disorder. Twenty-two (42%) participants had received trials with psychostimulants, α_2-agonists, and/or atomoxetine (of these 22, three had only received atomoxetine). This left 30 (58%) psychotropic-naïve participants. Table 1 lists the demographic and clinical characteristics of the sample overall and divided based on prior treatment status.

Table 1. Demographic and Clinical Characteristics of the Sample Overall and Split Based on Past Treatment Status with Psychotropics

	Total sample	*Med-naïve participants*	*Medicated participants*	*Statistic*	p-*value*
Male sex, *n* (%)	43 (83)	27 (90)	16 (73)	Fisher's exact	>0.1
Age, years, mean (SD)	10.0 (2.6)	9.2 (2.6)	11.2 (2.2)	$t = 2.82$	<0.007
Race, *n* (%)					
Caucasian	42 (81)	23 (77)	19 (86)	Fisher's exact	>0.3
African American	8 (15)	6 (20)	2 (15)		
Other	2 (4)	1 (3)	1 (5)		
Age- and sex-adjusted measures, mean (SD)					
Weight z score	0.2 (1.0)	0.4 (1.0)	0.0 (1.1)	$t = -1.46$	>0.1
Height z score	0.0 (1.0)	0.1 (0.9)	−0.1 (1.0)	$t = -0.86$	>0.3
BMI z score	0.3 (1.1)	0.5 (1.0)	0.0 (1.1)	$t = -1.63$	>0.1
Attention-deficit/hyperactivity disorder, *n* (%)					
Combined type	38 (73)	24 (80)	14 (64)	Fisher's exact	>0.2
Inattentive type	14 (17)	6 (20)	8 (36)		
Serum ferritin concentration, mean (SD)					
At baseline, ng/mL	18.4 (11.4)	18.7 (12.1)	18.0 (10.6)	$t = -0.20$	>0.8
Week 8, ng/mL	17.1 (11.1)	19.2 (12.6)	14.0 (7.8)	$t = -1.7$	>0.09
Low baseline serum ferritin, *n* (%)					
< 30 ng/mL cutoff	45 (87)	26 (87)	19 (86)	Fisher's exact	1
< 7 ng/mL cutoff	12 (23)	7 (23)	5 (23)	Fisher's exact	1
Dietary intake, mean (SD)					
Total calories, kcal/day	1,721 (609)	1,746 (607)	1,690 (625)	$t = -0.32$	>0.7
Protein, g/day	61.9 (21.5)	62.8 (21.8)	60.8 (21.6)	$t = -0.31$	>0.7
Fat, g/day	63.3 (22.2)	63.2 (22.5)	63.5 (22.4)	$t = 0.05$	>0.9
Carbohydrates, g/day	231.4 (89.3)	236.9 (87.6)	224.3 (93.2)	$t = -0.48$	>0.6
Iron, g/day	12.2 (4.4)	12.7 (4.5)	11.5 (4.3)	$t = -0.96$	>0.3

BMI = body mass index; SD = standard deviation.

Table 2. Means (Standard Deviation) of Clinical Measures in the Sample Overall and Split Based on Past Treatment Status with Psychotropics

Measure	*Group*	*Baseline*	*Week 8*	*Week 10*	*Week 13*
SNAP-IV					
Inattentive symptoms	Med-naïve	2.43 (0.37)	2.16 (0.55)	1.60 (0.68)	1.13 (0.53)
	Medicated	2.20 (0.56)	1.89 (0.64)	1.70 (0.61)	1.39 (0.59)
	Overall	2.34 (0.47)	2.04 (0.60)	1.64 (0.65)	1.23 (0.56)
Hyperactivity-impulsivity symptoms	Med-naïve	2.00 (0.63)	1.80 (0.73)	1.44 (0.52)	0.79 (0.49)
	Medicated	1.66 (0.84)	1.52 (0.83)	1.26 (0.76)	0.94 (0.68)
	Overall	1.86 (0.74)	1.68 (0.78)	1.36 (0.63)	0.85 (0.57)
Total symptoms	Med-naïve	**2.19 (0.45)**[a]	1.96 (0.57)	1.51 (0.49)	0.94 (0.41)
	Medicated	**1.90 (0.59)**	1.69 (0.64)	1.46 (0.60)	1.14 (0.56)
	Overall	2.07 (0.53)	1.92 (0.59)	1.49 (0.53)	1.02 (0.48)
CPRS-R					
Cognitive index	Med-naïve	2.21 (0.42)	***2.05 (0.55)***[b]	1.36 (0.71)	0.98 (0.48)
	Medicated	2.02 (0.54)	***1.75 (0.61)***	1.40 (0.65)	1.13 (0.62)
	Overall	2.13 (0.48)	1.92 (0.59)	1.38 (0.68)	1.04 (0.54)
Hyperactivity index	Med-naïve	***1.71 (0.72)***[b]	1.50 (0.68)	1.05 (0.63)	0.61 (0.45)
	Medicated	***1.34 (0.86)***	1.15 (0.83)	0.93 (0.76)	0.68 (0.67)
	Overall	1.56 (0.79)	1.35 (0.76)	0.99 (0.69)	0.64 (0.54)
Restlessness/impulsivity index	Med-naïve	1.96 (0.61)	1.75 (0.52)	1.22 (0.53)	0.82 (0.47)
	Medicated	1.81 (0.72)	1.61 (0.77)	1.25 (0.74)	0.99 (0.72)
	Overall	1.90 (0.66)	1.69 (0.64)	1.24 (0.62)	0.89 (0.58)
ADHD index	Med-naïve	2.26 (0.42)	***2.13 (0.48)***[b]	1.41 (0.51)	0.99 (0.49)
	Medicated	2.15 (0.53)	***1.83 (0.62)***	1.41 (0.67)	1.20 (0.64)
	Overall	2.21 (0.47)	2.00 (0.56)	1.41 (0.58)	1.08 (0.56)
Oppositionality index	Med-naïve	1.15 (0.68)	1.05 (0.59)	***0.74 (0.53)***[b]	***0.56 (0.35)***[b]
	Medicated	1.31 (0.69)	1.23 (0.72)	***1.07 (0.73)***	***0.82 (0.69)***
	Overall	1.22 (0.68)	1.13 (0.65)	0.89 (0.64)	0.67 (0.53)

Week 8 represented the end of the zinc glycinate versus placebo treatment phase, week 10 represented the end of the augmentation with a fixed dose of amphetamine, and week 13 represented the end of the amphetamine dose-adjustment phase to reach an optimal clinical response. Student's *t*-test compared the ADHD symptom ratings across previously medicated and psychotropic-naïve individuals at each visit. Only statistically significant (bolded) or marginally significant (bolded italics) results are highlighted: [a]$p < 0.05$; [b]$p < 0.1$.

ADHD = attention-deficit/hyperactivity disorder; CPRS-R = Conners' Parent Rating Scale-Revised, long version; SNAP-IV = Swanson, Nolan, and Pelham ADHD rating scale, version IV.

Of the 52 who started treatment, 49 completed all phases of the trial. The average rating per item (±SD) on the SNAP-IV and the CPRS-R at baseline and at the end of each treatment phase are reported in Table 2. As can be seen, the participants exhibited moderately severe ADHD symptoms. Only a few differences were significant between psychotropic-naïve and previously medicated patients (Table 2).

Serum ferritin concentration at baseline and week 8 were highly correlated (Pearson's $r = 0.65$, $p < 0.0001$) and changed little during the course of the study ($p > 0.3$) (Table 1). Using a cutoff of 30 ng/mL, as has been used elsewhere (Konofal et al. 2004), 87% of the sample had a low ferritin concentration at baseline. With a cutoff of 7 ng/mL, the lower limit of normal for this assay, 23% of the sample had low ferritin concentration. No differences in serum ferritin concentration or in the rates of low ferritin between previously treated and medication-naïve participants were statistically significant (Table 1).

There were no differences between the two zinc treatment groups in any of the demographic or clinical characteristics at baseline (all $p > 0.1$). In addition, zinc supplementation was not associated with a significant change in ferritin concentration (Pearson's $r = -0.08$). Because zinc supplementation also had a negligible effect on ADHD symptoms, all participants were pooled for the following analyses.

At baseline, after partialling out the effect of age and sex, serum ferritin concentration was inversely correlated with the SNAP-rated inattention, hyperactivity/impulsivity, and total ADHD symptom scores (Partial Spearman's $r = -0.31$, $p = 0.04$; $r = -0.42$, $p < 0.006$; and $r = -0.43$, $p < 0.004$, respectively). Similarly, ferritin concentration was negatively associated with scores on the CPRS-R-based scales, with the correlation coefficients ranging between −0.18 and −0.35. However, this reached statistical significance only for the restlessness/impulsivity index (Partial Spearman's $r = -0.35$, $p = 0.02$).

After adjusting for age and sex, multiple linear regression analysis predicting SNAP-rated inattention revealed a significant interaction effect of baseline serum ferritin concentration by prior psychotropic treatment status ($\beta = -0.039$, $p = 0.0007$). In other words, as ferritin concentration increased, the severity of inattention symptoms decreased in previously medicated but not in psychotropic-naïve participants (Fig. 1). For example, for a baseline ferritin concentration equal to the sample mean (i.e., 18.4 ng/mL), a psychotropic-naïve child would have a SNAP inattention score of 2.52 compared to a score of 2.19 for a previously treated child ($p = 0.01$). On the other hand, if ferritin was 36.8 ng/mL, the score would remain little changed in a psychotropic-naïve child, at 2.53, but would be much lower in a previously treated one, at 1.48 ($p < 0.0001$). Similarly, the interaction effect significantly predicted SNAP-rated total ADHD symptoms score ($\beta = -0.028$, $p < 0.04$) and the Conners-based cognitive index ($\beta = -0.033$, $p < 0.01$), restlessness/impulsivity index ($\beta = -0.038$, $p = 0.03$), and ADHD index ($\beta = -0.030$, $p < 0.03$). However, although there was a main effect of serum ferritin concentration pre-

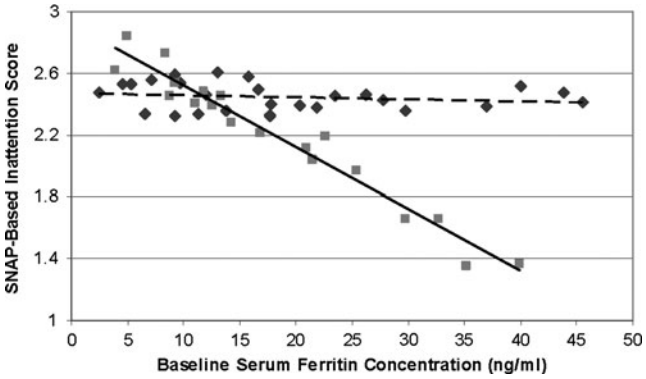

FIG. 1. Association between serum ferritin concentration at baseline and Swanson, Nolan, and Pelham (SNAP)–based inattention symptoms severity in psychotropic-naïve (diamonds and dashed line) and previously medicated (squares and solid line) participants.

dicting the SNAP-based hyperactivity/impulsivity symptoms score, the interaction effect was not significantly associated with it (Fig. 2). In other words, although the severity of the hyperactivity/impulsivity symptoms increased with decreasing serum ferritin, prior psychotropic treatment did not influence this pattern. Similarly, the interaction effect was not significantly associated with the Conners-based hyperactivity index or oppositionality index.

The mean daily dose of amphetamine by the end of the fixed-dose phase, that is, week 10, was 12.3 mg (SD = 4.9) with the mean weight-adjusted dose being 0.34 mg/kg (SD = 0.12). By the end of the dose adjustment phase, they were 13.4 mg (SD = 6.4) and 0.37 mg/kg, respectively. After partialling out the effect of age and sex, both baseline as well as week 8 serum ferritin concentrations were inversely associated with the weight-adjusted dose of amphetamine at week 13 (Partial Spearman's $r = -0.45$, $p < 0.007$ and $r = -0.48$, $p = 0.003$, respectively) (Fig. 3), but this did not reach significance at week 10 (Partial Spearman's $r = -0.27$, $p < 0.1$ and $r = -0.26$, $p = 0.1$, respectively). These associations were no different between psychotropic-naïve and previously treated participants.

At baseline, laboratory tests also included hemoglobin, hematocrit, mean cell volume, and red blood cell distribution width. Interestingly, there was a consistent pattern of nominal inverse association between the first three variables, which decrease in ID,

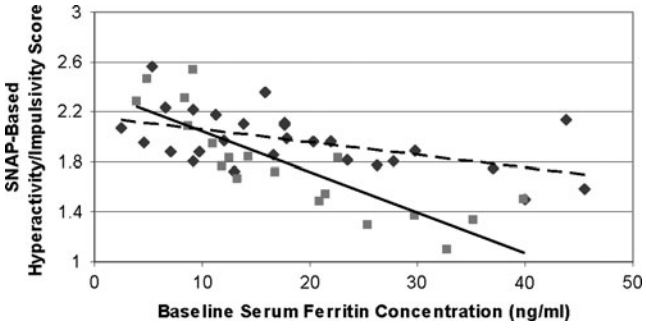

FIG. 2. Association between serum ferritin concentration at baseline and SNAP-based hyperactivity/impulsivity symptoms severity in psychotropic-naïve (diamonds and dashed line) and previously medicated (squares and solid line) participants.

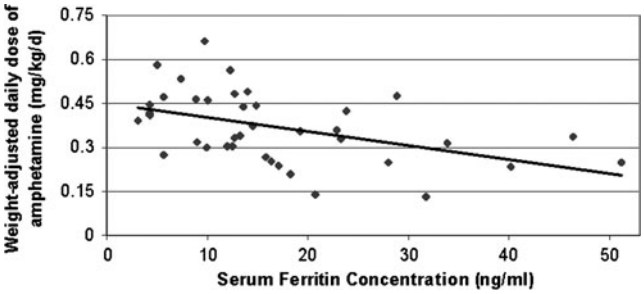

FIG. 3. Scatter plot illustrating the inverse association between serum ferritin and the weight-adjusted dose of amphetamine necessary to reach an optimal clinical response in the overall sample.

and the SNAP-based inattention, hyperactivity/impulsivity, and total ADHD symptom scores. However, in general, these correlations failed to reach statistical significance with the exception of the correlation between mean corpuscular volume and the hyperactivity/impulsivity score (Spearman's $r = -0.29$, $p < 0.05$). On the other hand, red blood cell distribution width, which increases in ID, was positively associated with the weight-adjusted dose of amphetamine used by the end of the dose-adjustment phase (Spearman's $r = 0.36$, $p < 0.03$). Only one 14-year-old participant had frank anemia with a hemoglobin concentration of 11.6 mg/dL and a ferritin concentration of 9.2 ng/mL (Marks et al. 1998). His SNAP average rating per item was 3 for inattentiveness (the highest score one could get) and 2.6 for hyperactivity/impulsivity. By the end of phase 3, his weight-adjusted dose of amphetamine was 0.44 mg/kg/day, which is higher than the group mean of 0.37 mg/kg/day.

Discussion

This report replicates previous findings of an inverse association between serum ferritin concentration and ADHD symptom severity (Konofal et al. 2004; Oner et al. 2008; Cortese et al. 2009). Further, it documents a differential pattern of associations based on prior psychotropic treatment status. Finally, our data show, for the first time, a significant correlation between serum ferritin and sensitivity to psychostimulants, whereby participants with lower ferritin concentrations required higher weight-adjusted doses of amphetamine to reach an optimal clinical response.

Over the last two decades, animal studies have shed light on the critical role iron plays in brain development, in general, and that of the dopaminergic pathways, in particular (Beard and Connor 2003; Lozoff and Georgieff 2006). Thus, it should come as no surprise that ID would predispose to neuropsychiatric dysfunction. In fact, ADHD would be a prime disorder potentially linked to ID for several reasons (Cortese et al. 2008). First, it is a common psychiatric disorder that appears early in childhood (American Psychiatric Association 2000). Second, there is broad agreement that the clinical presentation is heterogenous with both genetic and environmental factors contributing to its genesis (Faraone 2000; Pliszka 2007). Third, a wide range of studies have linked ADHD to dopaminergic dysfunction, with dopaminergic agents being the most efficacious agents in this condition (Pliszka 2007). Fourth, iron is necessary for the production of dopamine, the dopamine transporter, and dopaminergic receptors (Sachdev 1993).

Most phenomenological studies have confirmed that ADHD symptoms segregate in two broad clusters: Inattention and

hyperactivity/impulsivity (American Psychiatric Association 2000). These symptom groups often, but not always, co-occur and are comparably improved by psychostimulants (The MTA Cooperative Group 1999; Pliszka 2007). Thus, one could wonder whether ID is equally likely to precipitate both types of symptoms. In fact, we found serum ferritin concentration to be inversely related to both inattention and hyperactivity/impulsivity using the SNAP-IV though there was an apparent tendency for a stronger association with hyperactivity/impulsivity. This trend is more evident when using the CPRS-R, with the only statistically significant association being between serum ferritin and the restlessness/impulsivity index. The strength of the correlations we found is comparable to those reported by others (Konofal et al. 2004; Oner et al. 2008). Moreover, in those other studies, serum ferritin also tended to be more strongly associated with the hyperactivity subscores (Konofal et al. 2004; Oner et al. 2008). Importantly, however, this quantitative difference was not tested for statistical significance. In addition, observational studies have more frequently linked ID in children to attention problems (Lozoff et al. 2000; Grantham-McGregor and Ani 2001), though one longitudinal study found ID to similarly result in attention and behavior problems, based on parent and teacher report (Lozoff et al. 2000; Grantham-McGregor and Ani 2001). If there is a differential association between serum ferritin and the two ADHD symptom factors, as suggested by our data and others', it is unclear whether biological factors underlie it or whether it is rather due to a differential sensitivity of the rating scales to capture these symptom factors.

Some, but not all, of the associations between serum ferritin concentration and ADHD symptom scores were moderated by whether participants had undergone psychopharmacological treatment before enrollment or not. For example, as illustrated in Figure 1, the association between ferritin and inattention severity was significant in previously treated children but not in psychotropic naïve ones. In fact, some have suggested that psychostimulant-induced anorexia precipitates ID (D'Amato 2005), resulting in the significant effect of ferritin that we and others have found. However, prior treatment status did not significantly influence the association between ferritin and hyperactivity/impulsivity symptoms severity. Moreover, the mean age- and sex-adjusted height, weight, and body mass index measurements in the two groups were not different and those in the previously treated group were essentially average compared with the general population (Table 1). This suggests that anorexia does not account for the low serum ferritin concentration we found. Further, the overall dietary and iron intake was not different between the two groups (Table 1). However, the implications of this finding are limited since, by study design, no one was medicated during the week before the administration of the food frequency questionnaire.

Our study extends the current literature by further showing that ferritin concentration was also correlated with the dose of amphetamine necessary to reach an optimal clinical response. Millichap et al. (2006) failed to find an association between ferritin concentration and response to psychostimulant treatment. However, they had provided iron supplementation to all their participants with low ferritin concentration before initiating psychostimulants. This could have masked a possible link between the two. On the other hand, our findings are consistent with results from another analysis by our group using an unrelated clinical sample (Turner et al. 2009). In this study, we found mean corpuscular volume (which is smaller in ID), obtained at around age 3, to be positively associated with responsiveness to psychostimulant treatment nearly 3 years later. The association between iron status and response to psychostimulants is plausible since ID, in rats, reduces the inhibitory effect of cocaine on the dopamine transporter (Nelson et al. 1997; Erikson et al. 2000). Consequently, ID patients would presumably require the use of higher doses of psychostimulants, which act in part by inhibiting the dopamine transporter, to reach an optimal clinical response.

It also appears that critical time windows determine when iron accumulates in various brain regions (McCann and Ames 2007). In fact, rats exposed to ID in the neonatal period manifest a very different pattern of brain iron distribution compared to those exposed to ID later in life (Pinero et al. 2000). Thus, not only does the severity of ID affect brain structure and function, but its duration and timing are critical determinants as well (Beard and Connor 2003; McCann and Ames 2007). It, then, follows that further longitudinal studies, specifically in children at high risk for ADHD, should be conducted to establish the contribution of ID to the onset of the disorder and response to treatment.

Our findings must be interpreted in light of the study limitations. First, males comprised the majority of the sample, reflecting their higher susceptibility to develop ADHD (American Psychiatric Association 2000). However, further research is needed to replicate the findings in females and in a more racially and ethnically diverse groups. Second, the mean serum ferritin concentration in this sample was fairly low, suggesting that this group of participants was at a particularly elevated risk for ID. Our values are close to those reported in other studies that have found an association between ferritin and ADHD symptom severity but are much lower than those in studies that failed to do so. For example, the mean ferritin concentration was 18 ng/mL in our sample and 23 ng/mL in the positive study by Konofal et al. (2004) compared to around 40 ng/mL in the negative study by Millichap et al. (2006) and around 55 in the negative study by Menegassi et al. (2009). In addition, we did not selectively recruit for ID, especially given that the primary aim of the study was unrelated. Finally, supplementation with zinc glycinate might have precipitated ID. This argument, however, does not hold for two main reasons: First, our findings held true for baseline serum ferritin, collected before zinc supplementation. In addition, zinc appears to interfere with iron absorption primarily within 30 minutes of ingestion and only when the two are given concomitantly as supplements, as opposed to in fortified foods, in high zinc to iron ratios (Rossander-Hulten et al. 1991; Olivares et al. 2007).

In sum, this study offers additional support for the presence of an association between ID, ADHD, and response to psychostimulants. While iron supplementation has been found to attenuate ADHD symptoms in nonanemic children with ID (Konofal et al. 2008), it is unknown whether it can also potentiate response to psychostimulants, thus allowing the use of lower doses. Thus, future research should establish a lower limit for serum ferritin concentration below which iron supplementation might be considered in patients with ADHD and investigate whether such supplementation would, indeed, be beneficial. This is certainly worth pursuing in light of the widespread and increasing use of psychostimulants and the concerns about their cardiovascular and other side effects, generally considered dose dependent.

Disclosures

Drs. Calarge and DiSilvestro and Ms. Farmer report no competing interests.

Dr. Arnold has received research funding from Celgene, Curemark, Lilly, Neuropharm, Novartis, Noven, Shire, Sigma Tau, and

Targacept, the National Institute of Health, and Autism Speaks; has consulted to Abbott, Neuropharm, Novartis, Noven, Organon, Shire, and Sigma Tau; and has been speaker for Abbott, Shire, McNeil, Novartis and Targacept.

Acknowledgment

The authors acknowledge the technical assistance of Elizabeth Joseph, who conducted the ferritin assays.

References

American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, 4th ed., Text Revision (DSM-IV-TR). Washington, DC: American Psychiatric Association; 2000.

Arnold LE, DiSilvestro RA, Bozzolo D, Bozzolo H, Crowl L, Fernandex S, Ramadan Y, Thompson S, Mo X, Abdel-Rasoul M, Joseph E: Zinc for attention-deficit/hyperactivity disorder: Placebo-controlled double-blind pilot trial alone and combined with amphetamine. J Child Adolesc Psychopharm, 2010, in press.

Beard JL, Chen Q, Connor J, Jones BC: Altered monamine metabolism in caudate-putamen of iron-deficient rats. Pharmacol Biochem Behav 48:621–624, 1994.

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

Block G, Murphy M, Roullet JB, Wakimoto P, Crawford PB, Block T: Pilot validation of a FFQ for children 8–10 years (Abstract). Fourth International Conference on Dietary Assessment Methods; 2000.

Burhans MS, Dailey C, Beard Z, Wiesinger J, Murray-Kolb L, Jones BC, Beard JL: Iron deficiency: Differential effects on monoamine transporters. Nutr Neurosci 8:31–38, 2005.

Centers for Disease Control and Prevention: Iron Deficiency—United States, 1999–2000. Morbidity and Mortality Weekly Report. Atlanta, GA, 2002; pp. 897–899.

Conners CK: Conners Rating Scales—Revised. Technical Manual. North Tonawanda, NY: Multi-Health Systems, Inc.; 2001.

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

Cortese S, Konofal E, Bernardina BD, Mouren MC, Lecendreux M: Sleep disturbances and serum ferritin levels in children with attention-deficit/hyperactivity disorder. Eur Child Adolesc Psychiatry 18:393–399, 2009.

Cortese S, Lecendreux M, Bernardina BD, Mouren MC, Sbarbati A, Konofal E: Attention-deficit/hyperactivity disorder, Tourette's syndrome, and restless legs syndrome: The iron hypothesis. Med Hypotheses 70:1128–1132, 2008.

D'Amato TJ: Is iron deficiency causative of attention-deficit/hyperactivity disorder? Arch Pediatr Adolesc Med 159:788; author reply 788, 2005.

Dickstein SG, Bannon K, Castellanos FX, Milham MP: The neural correlates of attention deficit hyperactivity disorder: An ALE meta-analysis. J Child Psychol Psychiatry 47:1051–1062, 2006.

Erikson KM, Jones BC, Beard JL: Iron deficiency alters dopamine transporter functioning in rat striatum. J Nutr 130:2831–2837, 2000.

Erikson KM, Jones BC, Hess EJ, Zhang Q, Beard JL: Iron deficiency decreases dopamine D1 and D2 receptors in rat brain. Pharmacol Biochem Behav 69:409–418, 2001.

Faraone SV: Genetics of childhood disorders: XX. ADHD, Part 4: Is ADHD genetically heterogeneous? J Am Acad Child Adolesc Psychiatry 39:1455–1457, 2000.

Felt B, Jimenez E, Smith J, Calatroni A, Kaciroti N, Wheatcroft G, Lozoff B: Iron deficiency in infancy predicts altered serum prolactin response 10 years later. Pediatr Res 60:513–517, 2006a.

Felt BT, Beard JL, Schallert T, Shao J, Aldridge JW, Connor JR, Georgieff MK, Lozoff B: Persistent neurochemical and behavioral abnormalities in adulthood despite early iron supplementation for perinatal iron deficiency anemia in rats. Behav Brain Res 171:261–270, 2006b.

Ford RE, Greenhill LL, Posner K: Stimulants. In: Pediatric Psychopharmacology, Principles and Practice. Edited by A. Martin, L. Scahill, D.S. Charney, J.F. Leckman. New York, NY: Oxford University Press; 2003; pp. 255–263.

Fristad MA, Teare M, Weller EB, Weller RA, Salmon P: Study III: Development and concurrent validity of the Children's Interview for Psychiatric Syndromes—parent version (P-ChIPS). J Child Adolesc Psychopharmacol 8:221–226, 1998.

Grantham-McGregor S, Ani C: A review of studies on the effect of iron deficiency on cognitive development in children. J Nutr 131:649S–666S; discussion 666S–668S, 2001.

Konofal E, Lecendreux M, Arnulf I, Mouren MC: Iron deficiency in children with attention-deficit/hyperactivity disorder. Arch Pediatr Adolesc Med 158:1113–1115, 2004.

Konofal E, Lecendreux M, Deron J, Marchand M, Cortese S, Zaim M, Mouren MC, Arnulf I: Effects of iron supplementation on attention deficit hyperactivity disorder in children. Pediatr Neurol 38:20–26, 2008.

Lozoff B, Georgieff MK: Iron deficiency and brain development. Semin Pediatr Neurol 13:158–165, 2006.

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

Marks JS, Dietz WH, Holloway BR, Dean AG: Recommendations to Prevent and Control Iron Deficiency in the United States. Morbidity and Mortality Weekly Report Series. Atlanta, GA: CDC; 1998.

McCann JC, Ames BN: An overview of evidence for a causal relation between iron deficiency during development and deficits in cognitive or behavioral function. Am J Clin Nutr 85:931–945, 2007.

Menegassi M, Mello ED, Guimaraes LR, Matte BC, Driemeier F, Pedroso GL, Rohde LA, Schmitz M: Food intake and serum levels of iron in children and adolescents with attention-deficit/hyperactivity disorder. Rev Bras Psiquiatr 32:132–138, 2010.

Millichap JG, Yee MM, Davidson SI: Serum ferritin in children with attention-deficit hyperactivity disorder. Pediatr Neurol 34:200–203, 2006.

Nelson C, Erikson K, Pinero DJ, Beard JL: *In vivo* dopamine metabolism is altered in iron-deficient anemic rats. J Nutr 127:2282–2288, 1997.

Ogden CL, Kuczmarski RJ, Flegal KM, Mei Z, Guo S, Wei R, Grummer-Strawn LM, Curtin LR, Roche AF, Johnson CL: Centers for Disease Control and Prevention 2000 growth charts for the United States: Improvements to the 1977 National Center for Health Statistics version. Pediatrics 109:45–60, 2002.

Olivares M, Pizarro F, Ruz M: New insights about iron bioavailability inhibition by zinc. Nutrition 23:292–295, 2007.

Oner O, Alkar OY, Oner P: Relation of ferritin levels with symptom ratings and cognitive performance in children with attention deficit-hyperactivity disorder. Pediatr Int 50:40–44, 2008.

Pinero DJ, Li NQ, Connor JR, Beard JL: Variations in dietary iron alter brain iron metabolism in developing rats. J Nutr 130:254–263, 2000.

Pliszka S: Practice parameter for the assessment and treatment of children and adolescents with attention-deficit/hyperactivity disorder. J Am Acad Child Adolesc Psychiatry 46:894–921, 2007.

Rossander-Hulten L, Brune M, Sandstrom B, Lonnerdal B, Hallberg L: Competitive inhibition of iron absorption by manganese and zinc in humans. Am J Clin Nutr 54:152–156, 1991.

Sachdev P: The neuropsychiatry of brain iron. J Neuropsychiatry Clin Neurosci 5:18–29, 1993.

Swanson JM: School-Based Assessments and Interventions for ADD Students. Irvine, CA: K.C. Publishing (SNAP also available at www.adhd.net); 1992.

The MTA Cooperative Group: A 14-month randomized clinical trial of treatment strategies for attention-deficit/hyperactivity disorder. Multimodal Treatment Study of Children with ADHD. Arch Gen Psychiatry 56:1073–1086, 1999.

Turner CA, Xie D, Zimmermann B, Calarge CA: Iron Status in Childhood Predicts Response to Psychostimulant Treatment in Children with ADHD. American Academy of Child and Adolescent Psychiatry Annual Meeting, Honolulu, HI; 2009.

Wachs TD, Pollitt E, Cueto S, Jacoby E, Creed-Kanashiro H: Relation of neonatal iron status to individual variability in neonatal temperament. Dev Psychobiol 46:141–153, 2005.

Youdim MB, Yehuda S, Ben-Uriah Y: Iron deficiency-induced circadian rhythm reversal of dopaminergic-mediated behaviours and thermoregulation in rats. Eur J Pharmacol 74:295–301, 1981.

Address correspondence to:
Chadi Calarge, M.D.
Department of Psychiatry
The University of Iowa Carver College of Medicine
500 Newton Road
Iowa City, IA 52242

E-mail: chadi-calarge@uiowa.edu