Serum Ferritin, Weight Gain, Disruptive Behavior, and Extrapyramidal Symptoms in Risperidone-Treated Youth

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Abstract

Background: Iron deficiency disrupts dopaminergic signaling in rodents, resulting in cognitive deficits that may be reversed with psychostimulants. In humans, iron deficiency with or without anemia has similarly been found to cause neuropsychological and behavioral impairments. However, the clinical effects of low body iron stores in antipsychotic-treated children have not been examined.

Methods: Medically healthy, 5- to 17-year-old boys treated with risperidone for at least 1 year were enrolled between February 2009 and November 2013 in a multiphase study, examining the skeletal effects of calcium and vitamin D supplementation in risperidone-induced hyperprolactinemia. Anthropometric measures were collected and medical and pharmacy records were reviewed to obtain treatment history. Psychiatric diagnoses were based on clinical interviews, structured interviews, rating scales, and a review of their medical records. Extrapyramidal symptoms were assessed, and a food frequency questionnaire was completed in a subsample. Laboratory tests, including ferritin concentration (a marker of body iron status), were obtained upon study entry.

Results: A total of 114 participants (mean age: 11.0 ± 2.6 years) were included, the vast majority (>90%) having attention-deficit/hyperactivity disorder and/or disruptive behavior disorder. They had taken risperidone for an average 3.1 ± 2.0 years. Their serum ferritin concentration was 37.3 ± 25.6 $\mu\text{g/L}$ with 21% of the sample having a level <20 $\mu\text{g/L}$, despite appropriate daily dietary iron intake. Ferritin concentration was inversely associated with weight gain following risperidone treatment onset but was not significantly associated with prolactin. After adjusting for the weight-adjusted dose of psychostimulants and risperidone and the daily dose of selective serotonin reuptake inhibitors, ferritin was inversely associated with the severity of disruptive behavior and positively associated (albeit marginally) with prosocial behavior. No association was found between ferritin concentration and extrapyramidal symptoms.

Conclusions: Body iron stores are inversely related to risperidone-induced weight gain, even after extended treatment and despite adequate iron intake. Low iron stores are associated with poorer treatment response. Future research should examine iron absorption during antipsychotic treatment and whether repleting iron stores would facilitate clinical response.

Introduction

IRON IS INCORPORATED in various structural and transport proteins in the brain and is a cofactor of key enzymes, including tyrosine hydroxylase, the rate-limiting enzyme for catecholamine synthesis (Sachdev 1993; Beard and Connor 2003; Lozoff and Georgieff 2006). In rats, iron deficiency reduces the density of the dopamine transporter and the dopamine D₁ and D₂ receptors in the basal ganglia (Beard et al. 1994; Nelson et al. 1997; Erikson et al. 2000, 2001; Burhans et al. 2005). In children, iron deficiency has been associated with motor, attentional, and memory dysfunction as well as internalizing symptoms (Lozoff et al. 2000; Grantham-McGregor and Ani 2001; McCann and Ames 2007). Moreover, low serum ferritin concentration (a marker of body iron stores) in children with attention-deficit/hyperactivity disorder (ADHD) has

been associated with more severe symptoms and poorer response to psychostimulants (Konofal et al. 2004, 2008; Oner et al. 2008; Calarge et al. 2010; Scassellati et al. 2012).

Antipsychotic medications, particularly second-generation antipsychotics (SGAs), are widely used in children and adolescents (Olfson et al. 2015). They can cause significant weight gain (Calarge et al. 2012). Much has been written about the long-term cardiovascular sequelae of excessive SGA-induced weight gain, particularly when it starts in childhood. In contrast, far less has been discussed about the more immediate effects of SGAs. Specifically, rapid weight gain may promote the development of iron deficiency. This is reminiscent of infants and peripubertal children outgrowing their iron stores due to inadequate iron intake relative to the growth-induced need (Georgieff et al. 1990, 2002; Yang et al. 2009).

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Previously, we have found that pretreatment serum ferritin did not predict risperidone-induced weight gain in youth with autism spectrum disorder, but the change in ferritin concentration was inversely related to the magnitude of weight gain following 18 weeks of treatment (Calarge et al. 2015b). Depletion of body iron stores in association with risperidone-induced weight gain was also found in a longer term study, where we also reported that weight loss following the discontinuation of risperidone was associated with an improvement in serum ferritin (Calarge and Ziegler 2013; Calarge et al. 2015b). Finally, preliminary evidence suggested that risperidone treatment might inhibit iron repletion because ferritin concentration remained low despite normal dietary iron intake and because ferritin concentration failed to increase in those who lost weight but continued on risperidone (Calarge and Ziegler 2013; Calarge et al. 2015b). Of interest, the typical antipsychotic chlorpromazine has the potential to chelate iron and chronic chlorpromazine or haloperidol treatment has been associated with reduced hepatic nonheme iron in rats (Rajan et al. 1974; Weiner et al. 1977; Ben-Shachar and Youdim 1990; Ben-Shachar et al. 1991). Whether risperidone has similar effects is not known.

Due to iron's potential to affect dopaminergic signaling and because of its key role in oxidative stress, possibly implicated in the development of tardive dyskinesia, body iron stores have also been examined in patients receiving typical antipsychotics in relation to the development of extrapyramidal side effects (EPS). Low ferritin has been associated with akathisia and high ferritin with tardive dyskinesia, although these findings have not been consistent (O'Loughlin et al. 1991; Gold and Lenox 1995; Wirshing et al. 1998; Hofmann et al. 2000; Kuloglu et al. 2003; Chong et al. 2004).

Building on this evidence and using data from a new sample, we here examine the clinical effects of low body iron stores. Based on our earlier findings in ADHD (Calarge et al. 2010), we hypothesized that serum ferritin concentration will be inversely related to externalizing symptom severity. We further explored whether the ferritin concentration would be associated with EPS severity.

Materials and Methods

Participants

Boys, 5–17 years old, treated with risperidone for at least 1 year, regardless of clinical diagnosis, were screened between February 2009 and November 2013 to establish eligibility for a randomized placebo-controlled trial examining the skeletal effects of calcium and vitamin D supplementation (ClinicalTrials.gov Identifier: NCT00799383). Exclusion criteria included the presence of chronic diseases affecting a vital organ or the skeleton, malnutrition conditions, hypothyroidism, a history of renal calculi, a fasting random urine calcium/creatinine ratio >0.2 , other medical disorders that contraindicate calcium and vitamin D supplementation, bilateral wrist or forearm fractures, lead poisoning, concurrent treatment with antipsychotics other than risperidone, chronic use of drugs that might affect bone metabolism, calcium and vitamin D supplementation in the 3 months before study entry, moderate to profound intellectual disability (IQ <40), inability to cooperate with study procedures, nonadherence with risperidone, or plans to move out of state within the next 9 months.

Procedures

The study was approved by the University of Iowa Institutional Review Board. Assent was obtained from children and written consent from parents or legal guardians.

A best-estimate diagnosis, following the *Diagnostic and Statistical Manual of Mental Disorders Fourth Edition–Text Revised (DSM-IV-TR*, American Psychiatric Association 2000), was generated based on a review of the psychiatric record supplemented by a clinical interview (by C.A.C.), and the Child Behavior Checklist (CBCL, Achenbach and Rescorla 2001). The parent also completed the Nisonger Child Behavior Rating Form (NCBRF, Aman et al. 1996) for all participants and the National Institute of Mental Health Diagnostic Interview Schedule for Children (DISC-IV, Shaffer et al. 2000) on a subgroup. The presence of tardive dyskinesia followed the Schooler and Kane criteria (i.e., presence of at least one rating of moderate severity or two or more ratings of mild severity on the Abnormal Involuntary Movement Scale, Schooler and Kane 1982; Munetz and Benjamin 1988). Akathisia was based on item 4 from the Barnes Akathisia Rating Scale (Barnes 1989). Finally, the parkinsonian symptom severity rating was based on the Simpson–Angus Rating Scale (Simpson and Angus 1970). All EPS scales were completed by C.A.C.

The medical and pharmacy records were reviewed to document all psychotropic treatments, including the start and stop date of each drug as well as its dosage (Calarge et al. 2015a). All dosages of psychostimulants were expressed in methylphenidate equivalents. At study entry, weight was measured following standard procedures (Calarge et al. 2014). In addition, weight measurements were extracted from the medical record when available.

Participants with hyperprolactinemia at the initial screening visit were invited to return a week later to recheck their prolactin concentration and determine eligibility for the next study phase. At that time, they completed the 2004 Block Kids Food Frequency Questionnaire to determine iron intake and multivitamin use, among other things (Block et al. 2000).

At study entry, a morning fasting blood sample was obtained to measure serum ferritin, trough risperidone and 9-hydroxy-risperidone, and prolactin concentrations, and a complete blood count. The latter was available on 72% of the participants as we added it only after finding an elevated rate of low ferritin concentration in risperidone-treated children (Calarge and Ziegler 2013). In prior work (Calarge et al. 2010, 2015b; Calarge and Ziegler 2013), we had measured ferritin through the core laboratory at the University of Iowa Hospitals and Clinics, using an Enzyme-linked Immunosorbent Assay Kit manufactured by ALPCO Diagnostics (Salem, NH). However, for the present study, a coauthor (E.E.Z.) measured ferritin using a kit manufactured by Ramco Laboratories, Inc. (Stafford, TX). Inadvertently, one sample was measured using both kits and yielded very discrepant values. This prompted us to examine the precision and bias of each of the kits to determine the best one to use. Thus, we selected 32 samples covering a wide range of values and assayed them using both kits to examine the agreement between the two kits (Bland and Altman 1999). The Ramco kit yielded higher results in every case (mean difference: 35 $\mu\text{g/L}$, median difference: 26.7 $\mu\text{g/L}$, range: 3–160.5 $\mu\text{g/L}$). Figure 1 displays the limits of agreement of the two kits. We further assayed a subgroup of samples through the pathology laboratory at the University of Iowa Hospitals and Clinics, using an electrochemiluminescence immunoassay kit manufactured by Roche Diagnostics (Indianapolis, IN). We considered the latter assay the gold standard as it is used in clinical settings. The limits of agreement with the Roche kit were better for the Ramco compared with the ALPCO kit, and there was minimal bias.

FIG. 1. Agreement between the Ramco and ALPCO kits to measure serum ferritin concentration. The former test provided higher ferritin results in all samples assayed ($N=32$, mean difference: $35 \mu\text{g/L}$, median difference: $26.7 \mu\text{g/L}$, range: $3\text{--}160.5 \mu\text{g/L}$). The solid line denotes the mean association. Dotted lines represent the 95% Confidence Interval.

Data analysis

Weight measurements were converted into age- and sex-specific Z-scores (Ogden et al. 2002). To examine the association between ferritin concentration and change in weight after starting risperidone, we subtracted weight Z-score at study entry from the one extracted from the medical records, as long as it fell within 30 days before or 3 days after risperidone initiation (i.e., baseline weight). The rationale was as follows: (1) clinicians do not always measure weight at the onset of risperidone treatment, (2) using the suggested range would optimize the sample size without leading to significant error in estimating baseline weight, and (3) our previous research has shown a good agreement between measurements obtained in clinical versus research settings (Calarge et al. 2012).

Pearson's correlations examined the zero-order associations between variables of interest. Multivariable linear regression analysis examined the association between ferritin concentration and (1) prolactin concentration, (2) the CBCL factor T scores or the NCBRF factors, (3) medication dosages, and (4) the EPS scales scores, while accounting for relevant confounding variables.

All the statistical tests performed were two tailed, using SAS version 9.3 for Windows (SAS Institute, Inc., Cary, NC), with statistical significance set at $\alpha=0.05$. No corrections for multiple comparisons were made in this exploratory analysis.

Results

Subject characteristics

Tables 1–3 describe the demographic and clinical characteristics of the 114 boys who contributed data to this analysis. There was a significant increase in weight Z-score after starting risperidone treatment a mean 3.1 years earlier. The ferritin concentration was below $20 \mu\text{g/L}$ in 21% of the participants, but only one child (1%) had iron deficiency anemia (age: 8 years, serum ferritin concentration of $10.5 \mu\text{g/L}$, and hemoglobin concentration of 11.7 g/dL).

Ferritin concentration (natural log transformed) was inversely associated with change in age- and sex-specific weight Z-score between the onset of risperidone treatment and study entry (Pearson's $r=-0.29$, $p<0.01$, $N=84$), but not with weight Z-score at study entry

TABLE 1. DEMOGRAPHIC, ANTHROPOMETRIC, AND LABORATORY CHARACTERISTICS OF THE PARTICIPANTS

Age, years	11.0 $\pm$ 2.6
Race/ethnicity, n (%)	
White	96 (85)
African American	15 (13)
Other	1 (1)
Hispanic	2 (2)
Tanner stage I/II/III/IV/V, %	41/19/9/16/15
Overweight/obese (BMI Z-score)	53 (46)
Percentile ≥ 85 , n (%)	
Increase in weight Z-score ^a	0.53 $\pm$ 0.84
Dietary iron intake, mg/day ($N=42$)	13.2 $\pm$ 5.4
Multivitamin use, n (%)	3 (3)
Ferritin concentration, $\mu\text{g/L}$	37.3 $\pm$ 25.6
Ferritin concentration $<20 \mu\text{g/L}$, n (%)	24 (21)
Ferritin concentration $<12 \mu\text{g/L}$, n (%)	5 (4)
Hemoglobin, g/dL ($N=82$)	13.7 $\pm$ 1.0
Red blood cell count, $\times 10^6/\text{mm}^3$ ($N=82$)	4.92 $\pm$ 0.90
Mean cell volume, fL ($N=82$)	83.3 $\pm$ 3.9
Prolactin concentration, ng/mL	20.7 $\pm$ 13.1
Risperidone concentration, ng/mL ^b	14.2 $\pm$ 12.6

$N=114$; mean $\pm$ standard deviation unless otherwise noted.

^aRepresents the change in weight Z-score between the time risperidone was started (within -30 to $+3$ days) and study entry ($N=84$).

^bRepresents the combination of risperidone and 9-OH-risperidone concentration.

TABLE 2. PSYCHIATRIC CHARACTERISTICS OF THE PARTICIPANTS

Psychopathology, n (%)	
Attention-deficit/hyperactivity disorder	109 (96)
Disruptive behavior disorder	107 (94)
Anxiety disorder	42 (37)
Tic disorder	14 (12)
Autism spectrum disorder	27 (24)
Bipolar disorder	2 (2)
Psychopharmacology	
Risperidone treatment duration, years	3.1 $\pm$ 2.0
Risperidone dose, mg/kg/day	0.04 $\pm$ 0.02
Psychostimulants, n (%)	89 (78)
Psychostimulant treatment duration, years	4.5 $\pm$ 2.3
Psychostimulant dose, mg/kg/day	1.3 $\pm$ 0.7
SSRIs, n (%)	51 (45)
SSRI treatment duration, years	2.5 $\pm$ 2.0
SSRI unit per day ^a	1.1 $\pm$ 0.8
Extrapyramidal symptoms	
Simpson–Angus Scale mean ($N=88$) ^b	0.1 $\pm$ 0.2
Parkinsonism present, n (%) ^b	6 (7)
Barnes Akathisia Rating Scale ($N=92$)	0.8 $\pm$ 0.8
Akathisia present, n (%) ^c	22 (24)
AIMS ($N=87$) ^d	1.1 $\pm$ 1.5
Tardive dyskinesia present, n (%) ^d	2 (2)

$N=114$; mean $\pm$ standard deviation unless otherwise noted.

^aAn SSRI unit was defined as equivalent to a daily dose of 20 mg of fluoxetine ($n=24$) or citalopram ($n=9$), or 50 mg of sertraline ($n=8$), or 10 mg of escitalopram ($n=10$).

^bThe threshold for the presence of parkinsonism was a score of ≥ 0.3 on the Simpson–Angus Scale (Simpson and Angus 1970).

^cThe threshold for the presence of akathisia was a score of ≥ 2 on the Barnes Akathisia Rating Scale (Barnes 1989), see text for more details.

^dFor the AIMS, participants with a tic disorder were excluded. The Schooler and Kane criteria were used to determine the presence of tardive dyskinesia (Schooler and Kane 1982).

AIMS, Abnormal Involuntary Movements Scale; SSRIs, selective serotonin reuptake inhibitors.

TABLE 3. FACTOR T SCORES ON THE CHILD BEHAVIOR CHECKLIST AND FACTOR SCORES ON THE NISONGER CHILD BEHAVIOR RATING FORM

Child Behavior Checklist T scores	
Attention problems	68.5 ± 9.0
Rule breaking behavior	64.6 ± 8.0
Aggressive behavior	71.2 ± 11.5
Anxious depressed	64.2 ± 10.0
Withdrawn depressed	62.5 ± 8.2
Somatic problems	60.5 ± 8.4
Social problems	66.0 ± 8.9
Thought disorder	69.4 ± 8.1
Internalizing problems	64.0 ± 9.1
Externalizing problems	68.3 ± 8.4
Total problems	69.2 ± 8.0
Nisonger Child Behavior Rating Form factor scores	
Compliant/calm	6.8 ± 2.4
Adaptive/social	5.0 ± 1.8
Positive social total score	11.8 ± 3.8
Conduct problem	22.2 ± 10.9
Insecure/anxious	15.6 ± 8.1
Hyperactive	14.2 ± 6.2
Self-injury/stereotypic	2.8 ± 3.3
Self-isolated/ritualistic	5.3 ± 3.8
Overly sensitive	6.7 ± 3.7
Behavior problems total score	72.6 ± 30.2

N = 114; mean ± standard deviation.

($r = 0.03$, $p > 0.70$, $N = 114$). Adjusting for age and duration of risperidone treatment did not appreciably alter the findings.

Ferritin concentration (natural log transformed) was also significantly correlated with hemoglobin (Pearson's $r = 0.30$, $p < 0.006$, $N = 82$), hematocrit (Pearson's $r = 0.25$, $p < 0.03$, $N = 82$), red blood cell count (Pearson's $r = 0.24$, $p < 0.04$, $N = 82$), and mean cell volume (Pearson's $r = 0.31$, $p < 0.005$, $N = 82$).

Ferritin concentration and treatment response

Table 4 lists the significant results of the multivariable linear regression analyses examining the association between ferritin concentration and the CBCL factor T scores or the NCBRF factor scores,

while adjusting for the weight-adjusted dose of psychostimulants and risperidone as well as the daily dose of selective serotonin reuptake inhibitors (SSRIs). As can be seen, ferritin concentration was inversely associated with the severity of externalizing symptoms on both scales, with a trend for a positive association with prosocial skills on the NCBRF. While the association between ferritin concentration and the attention problems T score on the CBCL was not significant in the overall sample (Table 4), there was a significant inverse association in prepubertal children (β estimate = -0.15 ± 0.06 , $p < 0.02$), after adjusting for the weight-adjusted dose of psychostimulants and risperidone and SSRI dose. No significant associations were found with the other CBCL or NCBRF factors.

Of note, after adjusting for age (all p -values < 0.006) and the CBCL externalizing problems T score (all p -values < 0.09), there was no significant association between ferritin concentration and the weight-adjusted dose of psychostimulants or risperidone. In contrast, after adjusting for age (β estimate = 0.11 ± 0.02 , $p < 0.0001$) and the CBCL withdrawn/depressed problems T score (β estimate = 0.02 ± 0.01 , $p = 0.0004$), the ferritin concentration was inversely correlated with the daily dose of SSRIs (β estimate = -0.005 ± 0.003 , $p < 0.05$).

Ferritin concentration and EPS

After excluding participants with tic disorders, the ferritin concentration (natural log transformed) was not correlated with the Abnormal Involuntary Movements Scale (AIMS) total score. Adjusting for age (β estimate = -0.22 ± 0.06 , $p = 0.0005$) and the duration of risperidone treatment ($p > 0.60$) did not alter the results. Similarly, ferritin concentration (natural log transformed) was not correlated with the Simpson–Angus total score. Adjusting for age (β estimate = 0.013 ± 0.007 , $p < 0.07$) and the duration of risperidone treatment (β estimate = 0.03 ± 0.01 , $p < 0.005$) did not alter the findings. Finally, there was a trend for an inverse association between ferritin concentration (natural log transformed) and the akathisia global assessment score (Spearman's $r = -0.18$, $p < 0.10$, $N = 92$). However, adjusting for age ($p > 0.20$), duration of risperidone treatment ($p > 0.50$), and the attention problems T score on the CBCL ($p > 0.20$) rendered the association nonsignificant ($p > 0.20$). We adjusted for attention problems severity as many of our participants endorsed “non-specific inner tension and fidgety movements” (wording from the global assessment item of the

TABLE 4. RESULTS OF THE MULTIVARIABLE LINEAR REGRESSION ANALYSES EXAMINING THE ASSOCIATION BETWEEN FERRITIN CONCENTRATION AND SYMPTOM SEVERITY

	Psychostimulant dose, ^a β estimate ± SE	Risperidone dose, ^a β estimate ± SE	SSRI dose, ^b β estimate ± SE	Ferritin concentration, β estimate ± SE
Child Behavior Checklist factors				
Attention	0.7 ± 1.0	41.4 ± 35.2	-0.7 ± 1.1	-0.04 ± 0.03
Rule breaking	1.6 ± 0.9	22.5 ± 29.9	-1.7 ± 1.0	-0.07 ± 0.03**
Aggressive	1.9 ± 1.2	121.6 ± 42.2**	1.6 ± 1.4	-0.09 ± 0.4*
Externalizing	1.4 ± 0.9	80.2 ± 31.3*	0.5 ± 1.0	-0.07 ± 0.03*
Total problems	1.0 ± 0.9	63.2 ± 30.5*	1.3 ± 1.0	-0.03 ± 0.03
Nisonger Child Behavior Rating Form factors				
Social total	-0.3 ± 0.4	-5.6 ± 15.0	0.3 ± 0.5	0.03 ± 0.01
Compliant	-0.1 ± 0.3	-6.3 ± 0.3	0.3 ± 0.3	0.02 ± 0.01
Conduct	1.9 ± 1.2	122.1 ± 40.5**	1.5 ± 1.3	-0.07 ± 0.04

Only significant or marginally significant findings are reported here. Bolded results are significant (* $p < 0.05$ and ** $p < 0.01$) and those bolded and italicized, without asterisk, are at a trend level ($p < 0.10$).

^aWeight-adjusted daily dose.

^bSSRI unit, defined as equivalent to a daily dose of 20 mg of fluoxetine ($n = 24$) or citalopram ($n = 9$), or 50 mg of sertraline ($n = 8$), or 10 mg of escitalopram ($n = 10$).

SSRI, selective serotonin reuptake inhibitor.

Barnes Akathisia Rating Scale), scoring at least 1 on the scale. However, these symptoms are likely better accounted for by ADHD, present in 96% of our participants. In fact, if we use a more liberal threshold of a global assessment score ≥ 1 , as opposed to ≥ 2 as in Table 2, the prevalence of akathisia increases from 24% to 59%.

Ferritin concentration and prolactin concentration

Finally, in contrast to our earlier finding (Calarge and Ziegler 2013), after adjusting for age ($p > 0.80$), weight Z-score ($p > 0.10$), the weight-adjusted dose of psychostimulants ($p > 0.80$), SSRI dose ($p > 0.60$), and the combined risperidone and 9-hydroxy-risperidone concentration ($p < 0.0001$), ferritin concentration (natural log transformed) was not significantly associated with prolactin concentration ($p > 0.30$).

Discussion

Previously, we found risperidone-induced weight gain to be inversely associated with ferritin concentration following acute and chronic treatment (Calarge and Ziegler 2013; Calarge et al. 2015b). In this study, we replicate this finding in a new group of participants and extend it by reporting an inverse association between ferritin concentration and externalizing symptom severity. However, no association with EPS was found.

Studies in rodents have shown that iron deficiency disrupts dopaminergic signaling (Beard et al. 1994; Nelson et al. 1997; Erikson et al. 2000, 2001; Burhans et al. 2005). This is associated with cognitive impairment potentially alleviated by psychostimulants (Mohamed et al. 2011). In humans, iron deficiency with and without anemia has been associated with deficits on a range of neuropsychological and behavioral measures (Lozoff et al. 2000; Grantham-McGregor and Ani 2001; McCann and Ames 2007). In patients with ADHD, body iron stores have been linked to ADHD symptom severity as well as response to psychostimulants, although the findings have been inconsistent (Calarge et al. 2010; Cortese et al. 2012; Scassellati et al. 2012).

In contrast, the effect of SGA-induced weight gain on body iron stores has not been widely investigated. In prior work, we have found that up to 59% of children and adolescents chronically treated with risperidone exhibited iron depletion/deficiency (Calarge and Ziegler 2013). Findings reported here reveal that the ALPCO kit we had used before suffers from low sensitivity, thus underestimating serum ferritin concentration. Nonetheless, using the more sensitive Ramco kit, 21% of the present sample still exhibited a ferritin concentration below 20 $\mu\text{g/L}$. Moreover, we again found that ferritin concentration was inversely related to the magnitude of weight gain observed with risperidone treatment. This is despite the fact that (1) risperidone had been started more than 3 years earlier, (2) much of the excess weight is gained early in treatment, and (3) the daily iron dietary intake fell within the recommended range (Trumbo et al. 2002). Again, this raises the possibility that risperidone may be inhibiting iron absorption, consequently impeding iron repletion. As noted earlier, some typical antipsychotics can chelate metals, including iron (Rajan et al. 1974; Weiner et al. 1977). Whether this is the case with risperidone has, to our knowledge, not been examined.

An alternative explanation is that obesity, following risperidone initiation, induces subclinical inflammation, promoting the release of hepcidin, the primary regulator (inhibitor) of iron absorption (Nemeth and Ganz 2009; Cepeda-Lopez et al. 2010). In fact, we have previously found an inverse association between interleukin-

6, a potent inducer of hepcidin release, and body iron stores in risperidone-treated participants (Calarge and Ziegler 2013). However, while we did not measure markers of inflammation in the present study, we failed to find an association between serum ferritin concentration and concurrent weight Z-score. This suggests that excessive weight *per se* does not account for our finding, at least not fully.

The human body has evolved to prioritize the use of iron for hemoglobin synthesis, given that oxygen transport is the most vital function for life. Therefore, significant iron deficiency may be present without anemia. As noted earlier, such nutritional deficiency has been associated with neuropsychological impairment, which may be reversed with iron supplementation (Falkingham et al. 2010). We did not include any neuropsychological measures in this study but did find an inverse association between ferritin concentration and disruptive behavior severity. Similarly, there was an inverse association with attention problems, although only in prepubertal children. Conversely, ferritin concentration was positively associated (marginally) with prosocial behavior. Importantly, this was true after accounting for the weight-adjusted dose of stimulants and risperidone and the dose of SSRIs. Previously, we failed to find an association between ferritin concentration and symptom severity in youth with autism spectrum disorder enrolled in an acute clinical trial (Calarge et al. 2015b). This may have been due to the small sample size (only a subgroup of the participants had data available for analysis) or the fact that symptoms were assessed only 18 weeks after starting risperidone, which is different from the current study where the average risperidone exposure exceeded 3 years. Both the duration and severity of iron deficiency are likely to influence the emergence of neuropsychiatric sequelae.

Iron deficiency has been also hypothesized to increase the risk for akathisia, akin to its association with restless leg syndrome (Gold and Lenox 1995). Conversely, excess body iron stores have been associated with tardive dyskinesia due to iron's role in catalyzing the formation of free radicals, contributing to neurotoxicity (Sachdev 1994; Wirshing et al. 1998; Chong et al. 2004). However, clinical studies have been inconsistent in confirming these associations which, to our knowledge, have not been investigated in children and adolescents in long-term treatment with risperidone. Remarkably, only a few participants had parkinsonism or tardive dyskinesia, even though none was prescribed an anticholinergic medication. This may be due to the characteristics of individuals who remain on the same antipsychotic for more than 3 years. Specifically, these patients have likely exhibited a relatively good response and tolerability. In contrast, the rate of akathisia was significant, probably driven by the high prevalence of ADHD. Of note, ferritin concentration did not significantly correlate with severity on any of the three EPS scales.

In a landmark longitudinal study, Lozoff et al. (2000) found that children who were anemic in infancy exhibited more anxiety and depressive symptoms in early adolescence (McCann and Ames 2007). In our participants, ferritin concentration failed to be significantly associated with any of the internalizing symptom scale scores on the CBCL but it was inversely associated with the daily dose of SSRIs. This finding, which requires replication, suggests that optimizing body iron stores may improve treatment response. This would be particularly relevant to investigate in postmenarcheal females, who are at an increased risk for both internalizing disorders and iron deficiency.

Despite yielding novel findings, this study suffers several limitations. First, participants had already been taking risperidone for

years before study entry. Thus, no baseline information was directly collected at the time of risperidone initiation, whether anthropometric, neuromotor, or laboratory, including ferritin concentration. In fact, baseline weight was extracted from the medical records and was missing for some participants. Previously, however, we have reported similar findings for weight and ferritin in a clinical trial with rigorously collected baseline and follow-up data (Calarge et al. 2015b). Second, no neuropsychological assessment was conducted. Such testing would have strengthened the findings and allowed a comparison with published data in non-psychiatric populations. Third, only ferritin was used as an index of body iron storage. However, ferritin is an acute-phase protein that increases with inflammation, potentially masking the presence of more severe iron deficiency. Nonetheless, a low ferritin is still a valid measure of iron deficiency, regardless of inflammation. Moreover, our participants were medically healthy. Finally, future studies should enroll larger and more diverse groups of children to investigate the potential moderating effects of sex, racial/ethnic backgrounds, and pubertal development. They should also examine whether our findings extend to patients treated with antipsychotics other than risperidone.

Conclusion and Clinical Significance

In sum, this study found a substantial minority of boys treated with risperidone chronically to have iron deficiency despite adequate dietary iron intake. The degree of low body iron stores was inversely associated with weight gain following the initiation of risperidone and with the severity of externalizing problems but not with the severity of EPS. Future research should examine iron absorption during antipsychotic treatment and whether repleting iron stores would potentiate treatment response. Meanwhile, at the present state of knowledge, it seems prudent to monitor ferritin concentration in antipsychotic-treated children, at least those gaining excessive weight.

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