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Brain iron concentrations in the pathophysiology of children with attention deficit/hyperactivity disorder: a systematic review

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1 List of Abbreviations

- 2 ADHD, Attention Deficit/Hyperactivity Disorder
- 3 CRS, Conners Rating Scale
- 4 CTRS, Conners Teacher Rating Scale
- 5 CPRS, Conners Parent Rating Scale
- 6 DNA, deoxyribonucleic acid
- 7 DSM-IV/5, Diagnostic and Statistical Manual of Mental Disorders 4th/5th revision
- 8 ECL, Electrochemoluminescence
- 9 ELISA, enzyme-linked immunosorbent assay
- 10 HC, healthy controls
- 11 ICD-10, International Classification of Diseases 10th Revision
- 12 ID, iron deficiency
- 13 MFC, magnetic field correlation
- 14 MRI, magnetic resonance imaging
- 15 NIH, National Institutes of Health
- 16 NOS, Newcastle-Ottawa Scale
- 17 PICOS, Population, Intervention, Comparison, Outcome, Study Design
- 18 PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses
- 19 ROI, region of interest
- 20 RCT, randomized controlled trial
- 21 SD, standard deviation
- 22 SES, socioeconomic status

Abstract

Context: Attention deficit/hyperactivity disorder (ADHD) is a neurological disorder associated with iron dysregulation in children. While previous focus was on examining systemic iron status, brain iron content may be a more reliable biomarker of the disorder.

Objective: The present systematic review aimed to examine whether children with ADHD have lower serum as well as brain iron concentration, compared to healthy controls.

Data sources: A systematic literature search was conducted in Medline via PubMed, the Cochrane Library, Web of Science, Embase and Ovid for papers between 2000 to June 7th 2019.

Data extraction: Studies were included if the mean difference of iron concentration, measured as serum iron, serum ferritin or brain iron, between children with ADHD and healthy controls was an outcome measure.

Data analysis: Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were followed. Risks of bias within and between studies were assessed using the quality assessment tools of the National Institutes of Health.

Out of 599 records screened, 20 case-control studies met the inclusion criteria. In 10 out of 18 studies assessing serum ferritin and 2 out of 10 studies assessing serum iron, a significant difference between children with ADHD and healthy controls was observed, respectively. Results of systemic iron levels were inconsistent. In 3 studies where brain iron was assessed, a statistically significantly lower thalamic iron concentration was found in children with ADHD compared to healthy controls.

Conclusion: The evidence, though limited, reveals that brain iron rather than systemic iron levels may be more associated with the pathophysiology of ADHD in children. Larger longitudinal magnetic resonance imaging studies are needed in order to examine any correlations of iron deficiency in specific brain regions and symptoms of ADHD.

Keywords: children, attention deficit/hyperactivity disorder, iron status, brain iron, serum ferritin, serum iron.

1 **Introduction**

2 Attention Deficit/Hyperactivity Disorder (ADHD), a neurodevelopmental disorder, is characterised by
3 difficulties in attention, impulsivity and hyperactivity and reflected by various structural and functional
4 neural abnormalities, resulting in diverse cognitive and behavioural sub-types.¹ In 2015, it was
5 estimated that 7.2% of children worldwide were affected by ADHD,² with a higher prevalence in
6 boys.³ While ADHD generally develops early in childhood, 30-60% of patients persist to have
7 symptoms into adulthood with negative consequences on professional and personal life, as well as
8 associated co-morbidities such as depression, anxiety and bipolar disorder and drug use.⁴

9 The aetiology of ADHD is not fully understood, although a strong hereditary component has been
10 supported by family, adoption and twin studies.⁵⁻⁸ Most of the genes associated with ADHD are related
11 to the dopaminergic transmitter system (e.g. the dopamine transporter gene (DAT1) and dopamine
12 receptor type 4 (DRD4)).⁹ In fact, numerous studies reveal the essential role of the neurotransmitter
13 dopamine in ADHD pathophysiology determining its major clinical characteristics; executive
14 functions and psychomotor activity.¹⁰ Moreover, pre- and perinatal factors such as environmental lead
15 and maternal tobacco smoking increase the risk of ADHD.¹¹ Crucially, optimal maternal and infant
16 nutrition is fundamental to normal neurodevelopment^{12,13} and iron has been highlighted as one of the
17 key micronutrients for neurocognitive activity and development.

18 Indeed, iron has a central role in the developing brain, where it supports myelination, synaptogenesis,
19 neurotransmitter function and epigenetic regulations.¹³ Early-life iron deficiency (ID), however,
20 results in cognitive function, affective and social behaviour deficiency that persist beyond the period
21 of ID despite rapid iron supplementation.¹⁴ There appears to be a critical period, between 6 and 24
22 months of age, where ID most drastically and irreversibly affects neurocognitive and behavioural
23 development¹³, highlighting the importance of maternal and infant nutrition.

Furthermore, epidemiological evidence supports that ID in infants, even in the absence of anaemia, is strongly associated with neurocognitive and behavioural dysfunctions¹⁵ similar to those of ADHD. Moreover, ID is frequently displayed by children with ADHD¹⁶, hence, many studies have investigated systemic iron status and ADHD symptoms.¹⁷⁻²⁰ To date, four meta-analyses have been conducted, which all considered serum ferritin and all found a significant difference between children with ADHD compared to healthy controls.²¹⁻²⁴ The two latest meta-analyses, which also considered serum iron status in ADHD found conflicting results when comparing serum iron status in ADHD and healthy controls, thus emphasizing the need for further investigation.^{23,24}

Importantly, while the neurodevelopmental trajectory is most vulnerable to ID during the critical period of infancy,¹³ it has been proposed that a brain iron set point is established at the same timepoint.⁹ Thereafter, brain iron homeostasis seems to be tightly regulated. Evidence on diet modifying brain iron later in life is inconsistent; a study has reported that iron-restricted mice during weaning were not able to replete their stores at a later stage,²⁵ whereas isotopically enriched iron supplementation was found, in one study, to increase brain iron concentrations.²⁶ Serum ferritin and iron levels are more dependent on iron intake through diet compared to brain iron concentration. Hence, brain iron status, measured using magnetic resonance imaging (MRI), a non-invasive, highly specific and sensitive method, in children with ADHD might be a more accurate and reliable index of brain iron status. Brain iron homeostasis may be important in the pathophysiology of ADHD because iron is a cofactor of the rate-limiting enzyme tyrosine hydroxylase, that is required for dopamine synthesis in the metabolism of dopamine,²⁷ and the hypo-dopaminergic state of the brain in ADHD results in the symptoms of the disorder.¹ Moreover, iron has been found to be co-localized with dopamine in the brain of children with ADHD.²⁸

To date, however, there is no systematic review investigating brain iron status in children with ADHD. The present systematic review, therefore, aimed to update Tseng et al.'s²⁴ meta-analysis on serum

iron status in ADHD and to examine whether children with ADHD have lower brain iron concentrations compared to healthy controls. It is hypothesized that brain iron concentrations rather than systemic iron levels may contribute to the pathophysiology of ADHD in children.

Methods

The current study followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines²⁹ and was based on the protocol (cf. supplementary material).

Search strategy and study selection

Two independent authors (DA, JR) conducted a systematic literature search from 2000 to June 7th 2019, in Medline via PubMed, the Cochrane Library, Web of Science and EMBASE and Ovid. The following MESH headings, EMBASE thesaurus and keywords were searched: ADHD **or** attention deficit hyperactivity disorder **or** hyperactivity **and** iron status **or** iron deficiency **or** iron-deficient anaemia **or** anaemia **or** ferritin **or** brain iron. Additionally, the references of included studies were screened to identify further relevant published manuscripts.

Study selection was also conducted by the two authors independently. First, clearly irrelevant articles were excluded after screening titles and abstracts. Thereafter, full texts were considered against eligibility criteria. If required, a third author (EP or GOLD) resolved any disagreement about the inclusion or exclusion of an article.

Eligibility criteria

The PICOS criteria for inclusion and exclusion are described in **Table 1**. The inclusion criteria were a) observational studies (cohort, cross-sectional and case-control studies) examining the association of iron status via the following biomarkers: serum ferritin, serum iron and/or brain iron, and ADHD prevalence (and severity), b) ADHD diagnosis and symptom severity defined by a validated method, c) studies done in children (0-18 years) and d) studies published in English. Narrative and systematic reviews, meta-analyses, case studies, surveys, abstracts and conference reports were excluded, as well as original articles conducted in adults, animals and *in vitro* studies. Studies investigating iron status via biomarkers other than the ones stated above, as well as studies in medicated and iron-supplemented children with ADHD, were also excluded. However, when studies included both medicated and non-medicated children with ADHD, data from medicated children were disregarded. Lastly, studies with insufficient information to evaluate the association between ADHD prevalence (and severity) and iron status were excluded.

Primary outcome

The primary outcome measure was the mean difference of iron concentration (serum or central) between children with ADHD and healthy controls.

Study design and methods used

All studies were case-control studies, which identified ADHD patients, measured their iron concentration – either serum iron, serum ferritin or brain iron – and compared their iron status to healthy controls. The methods used for iron status measurements were different. For analysing serum iron; 9 studies used enzyme-linked immunosorbent assay, 1 study used the Ferrozine method, 1 study used the Tosho AIA360, 2 studies used the elecsys enzymun-test, 1 study used the multichannel atomic absorption spectrophotometer, 1 study necessitated an automatic autoanalyzer and 3 studies did not

specify their method. For assaying serum ferritin; 2 studies used enzyme-linked immunosorbent assay, 2 studies used the Ferrozine method, 1 study used the multichannel atomic absorption spectrophotometer, 2 studies necessitated an automatic autoanalyzer and 2 studies did not specify their method.

Data extraction

Data were extracted by one author (AD) using a pre-determined list of variables of interest, which included: name of the authors, publication date, title, study design, sample size, sex (% female), mean age, sample completing the study, percentage that dropped out and reasons, method of ADHD diagnosis, method of recruitment, inclusion and exclusion criteria, description of controls, primary and other aims of the study, biomarker used for iron status, biomarker concentration, p-value for difference in mean serum iron/serum ferritin/brain iron concentrations, diet assessment (if applicable) and authors' conclusion. The second author (RJ) reviewed the extracted data and both authors came to an agreement about individual items that were unclear.

Assessment of risk of bias

The quality assessment tools of the National Heart, Lung and Blood Institute of the National Institutes of Health³⁰ for Case-Control Studies and for Observational Cohort and Cross-Sectional Studies were used to assess the risk of bias of the selected studies. Risk of bias assessment was performed by two reviewers (AD, RJ) independently. If ratings differed, a consensus was reached between the reviewers following discussion and a third author (EP or GOLD) was involved when consensus was not achieved. Risk of bias assessment of individual studies was done at the outcome level (i.e. not the study level).

Analysis

Serum ferritin and serum iron concentrations were converted to conventional units, ng/ml and ug/dl, in order to reflect the same units across all studies.

Serum ferritin/iron and brain iron concentration data in children with ADHD compared to controls were analysed.

Results

Study Selection

Figure 1 summarizes the study selection process. The search of the databases identified a total of 599 records. Duplicates across the different databases were removed using RefWorks, leaving 243 unique records. After screening titles and abstracts, 206 records were excluded due to various reasons. Thirty-eight (38) full-text records were assessed for eligibility, 18 studies were excluded for the reasons provided in Figure 1. Twenty (20) case-control studies met the inclusion criteria for the present review.

Participants

The 20 studies included in the present review (**Table 2**) contained data on a total of 5191 participants (2209 children with ADHD and 2982 healthy controls). Most included studies involved less than 100 participants in each group. Four (4) studies had more than 100 participants.^{20,31-33} Most studies included non-medicated children with ADHD, but two studies also included medicated children.^{34,35} All studies contained data on both females and males. The mean age of ADHD patients and healthy controls ranged from 4 to 12.6 years. With regard to geographic distribution, most studies were conducted in the Middle East (Egypt, n=4; Turkey; n=2, Qatar; n=1), 6 studies were conducted in Asia

(Taiwan, n=1; India, n=2; South Korea, n=1; China, n=2), 4 studies in Europe (France, n=3; Italy, n=1), two studies were conducted in the USA and another one in Brazil.

Outcome

Due to the inclusion criteria, at least one of the outcomes of every study was to compare systemic or brain iron concentration between children with ADHD and healthy controls.

Other outcomes included assessment of the relationship between iron concentration and ADHD symptom severity, analysing of dietary intake or other iron metabolism markers such as hepcidin or correlation of brain and systemic iron concentrations.

Results of individual studies

Results of individual studies assessing serum ferritin/iron and brain iron concentrations are presented in **Table 2** and **Table 3**, respectively.

Serum Ferritin

There was great variability in the measured serum ferritin concentrations. The mean values of serum ferritin ranged from 12.9 ± 1.6 ng/ml⁵³ to 134.2 ± 14.8 ng/ml³⁶ in children with ADHD and from 12.7 ± 1.1 ng/ml⁵³ to 110.3 ± 6.5 ng/ml in healthy controls.³⁶ Ten out of 18 studies assessing serum ferritin concentrations found a statistically significantly higher serum ferritin concentration in patients with ADHD compared to healthy controls.

Serum iron

Out of 10 studies assessing serum iron, only two studies found statistically lower serum iron concentrations in children with ADHD compared to healthy controls ^{31,37} and one study found a statistically higher serum iron concentration in children with ADHD compared to healthy controls. ³⁸ Serum iron concentrations ranged from 45.5 ± 4.5 ug/dl ²⁰ to 110.1 ± 35.8 ug/dl ³⁸ in children with ADHD and 46.0 ± 5.0 ug/dl ²⁰ to 103.7 ± 122.7 ug/dl ³⁹ in healthy controls.

Brain iron

Overall, the three studies that assessed brain iron found a statistically significantly lower iron concentration in children with ADHD compared to healthy controls.^{34,40,41} Regions of interest were the globus pallidus, caudate nucleus, thalamus and putamen.

The three studies that investigated brain iron used relaxation rates (R2, R2* or R2') to measure brain iron concentrations. Both R2 and R2* were reported to represent a valid account of iron concentration *in vivo* ⁴²⁻⁴⁴. MRI relaxometry is the quantification of the intensity of darkening that is generated when protons that are excited by radio-waves relax to their equilibrium magnetic state. ⁴⁵ The exponential decay of magnetic relaxation is denoted as T1, T2, and T2*, time constants or their mathematical inverses, R1, R2 and R2* relaxation rates. These rates correlate directly with tissue iron concentration, of which R2 and R2* are the more sensitive and robust. ⁴⁶

Adisetyio et al. ³⁴ found no significant difference between groups using this method whereas Cortese et al. ⁴¹ and Hasaneen et al. ⁴⁰ found statistically significantly lower iron concentrations in the thalamus of children with ADHD compared to healthy controls. With the use of magnetic field correlation (MFC), Adisetyio et al. ³⁴ found a significant decrease in iron concentration of the caudate nucleus, putamen and thalamus of children with ADHD and healthy controls.

Risk of Bias within Studies

In regard to the population studied, sample size was justified and reported in one study.³¹ Direct recruitment of patients referred to clinics due to ADHD suspicion was mostly used. Referral to clinics necessitates recognition of an issue by parents or teachers and a level of education. Therefore, selection bias could have led participants with higher socioeconomic status (SES) to be included. All studies clearly defined the study population and different groups; i.e. ADHD patients and healthy controls. Most of the studies selected controls by drawing volunteers from the primary schools, healthcare professional's families or the communities that patients were also drawn from. One study⁴⁷ compared serum ferritin of participants with ADHD to a larger national control sample derived from a previous study. Hence, controls were not matched to patients and representativeness of the sample is questioned. Concurrent controls were used only in one study.³¹ The use of concurrent controls was judged non-essential since serum/brain iron or ferritin concentrations were only measured at one single time point. Iron status and ADHD presence were investigated within a very short time frame in all studies. It is therefore impossible to confirm that the exposure/risk occurred prior the condition and blinding is unnecessary.

Methods used

Different techniques were employed to assess serum ferritin/iron, which were all valid and reliable.⁴⁸ In two studies, the authors did not specify the method used for iron assay,^{39,47} which could increase measurement bias. Blinding was not reported in most studies. The concentration of iron can be measured using this technique since iron affects the relaxation rate of tissue water surrounding ferritin. While studies assessing brain iron concentration used MRI and relaxation rates as parameters, Adisetyio et al.³⁴ also used magnetic field correlation to determine brain iron concentration. The validity of magnetic field correlation has been supported with results from phantom experiments.^{49,50} Moreover, it is specific because it is insensitive to non-iron mechanisms.^{51,52}

Adjusting analysis for confounders

Only 4 studies measured and controlled for confounding variables, such as age, gender, body mass index, SES and dietary intake, in their analyses.^{31,34,38,53} This may reduce internal validity since matching controls to participants is not sufficient to eliminate confounders and the latter have to be adjusted for in the study analyses. In the remaining studies, controlling was neither clearly reported nor conducted. The overall methodological quality of studies was adjudged fair, and the lack of sample size justification and control of potential confounders in analyses were the major culprits of included studies.

Risk of bias across studies

The outcome of investigation for risk of bias evaluation was the difference in serum ferritin, serum iron or brain iron concentration between children with ADHD and healthy controls. Based on the NIH risk assessment (NIH, 2019), the majority of the studies had “fair” risk of bias, while two studies had “high” risk of bias,^{39,47} and four studies had “low” risk of bias.^{31,34,38,53} Selective reporting was noticed in one study; where serum iron was measured but concentrations were not reported.¹⁷

A meta-analysis was not conducted since the study methods and participants were very heterogeneous.

Discussion

This systematic review highlights the inconsistency in results in systemic iron status in children with ADHD and the promising, yet still insufficient, evidence for brain iron content as contributing to ADHD. With regard to serum ferritin, results proved to be inconsistent since only half of the assessment studies found a significantly lower serum ferritin concentration in children with ADHD compared to healthy controls. Differences in results between studies may be due to the differences in

the medication history of patients. Most studies often reported the exclusion of children with ADHD that were being treated with psychostimulants. However, the history of treatment or the wash-out period, except for 2 months in the study by Konofal et al.,⁵⁴ before participating in the studies were not reported. Psychostimulants are known to affect appetite, which in turn impacts food and thereby iron intake.⁵⁵ It is unknown whether different techniques to measure serum ferritin are equivalent. In their subgroup meta-analysis, Wang et al.²³ found that serum ferritin was lower if assessed by enzyme-linked immunosorbent assay compared to other techniques in patients with ADHD. Nonetheless, geographical differences among studies, as well as the variety of techniques used for measuring serum ferritin, are unlikely to affect patients with ADHD or healthy controls separately. Instead, in previous meta-analyses,^{24,56} which also only considered studies of strictly non-medicated children, a significant difference in serum ferritin between groups was found. Although the current review evaluates most of the studies in the meta-analyses of Wang et al.⁵⁶ and Tseng et al.²⁴, a few different but stringent inclusion criteria were employed and newer publications^{20,37,57,58} were also included in addition. Nevertheless, inconsistency in these findings does not confirm the observation of reduced serum ferritin in ADHD pathophysiology.

With regard to serum iron concentration, the majority of studies found no significant difference in serum iron between children with ADHD and healthy controls, which agrees with both recent meta-analyses.^{24,56} However, after sensitivity analysis, and by removing the results by Chen et al.³⁸ because dietary assessment revealed higher iron and vitamin C intake of children with ADHD, Tseng et al.²⁴ reported a statistically significantly lower serum iron in children with ADHD compared to healthy controls. Two studies in this review,^{31,37} one of which had “low” risk of bias, also found lower serum iron in children with ADHD compared to healthy controls. Taken together, there is tentative evidence for reduced systemic iron (serum iron/ferritin) concentration in ADHD.

1 The three studies ^{34,53,59} that examined brain iron status found reduced iron concentration in the
2 bilateral thalamus in children with ADHD compared to healthy controls. These results were consistent
3 irrespective of the MRI method used, the geographical location of the study and sample size.
4 Moreover, two of these studies were judged to have a “low” risk of bias.^{34,53} Therefore, the current,
5 though restricted, evidence strongly suggests a difference in thalamic iron concentration between the
6 groups. This finding is significant because ADHD pathophysiology is characterised by iron-deficient
7 hypo-dopaminergic state of the thalamus and a lack of arousal and by sleep disturbances.^{10,60}
8 However, the results of Adisetyio et al.³⁴ and Cortese et al.⁴¹ on brain iron concentration in putamen
9 and caudate nucleus are conflicting, and this may be explained by the different MRI techniques
10 employed. In fact, magnetic field correlation (MFC) is solely sensitive to iron whereas relaxation rates
11 (R_2 , R_2^* , R_2') are also impacted by non-iron mechanisms. MFC iron concentrations were reported to
12 correlate with post-mortem iron concentrations ⁵⁰ and MFC can detect grey matter interregional
13 differences due to pathological conditions.^{61,62} Importantly, in a recent randomized controlled trial in
14 mice,⁶³ it was reported that mice fed an iron-restricted diet for one month had increased brain ferritin
15 in the striatum and reduced brain ferritin in the hippocampus compared to normal diet-fed mice. This
16 suggests that iron intake, at least in mice, has a differential effect on various brain regions. Measuring
17 iron content in different brain regions is crucial since dysregulations in various areas lead to different
18 behavioural, social and emotional symptoms.⁶⁴ For example, restless leg syndrome was associated
19 with decreased iron concentration in the substantia nigra ⁶⁵ while increased iron concentration in the
20 substantia nigra was observed in Parkinson disease patients. ⁶⁶ It is hypothesized that a brain iron set
21 point is established during infancy. Thus, infancy provides a window of opportunity ¹³ for building an
22 optimal brain iron concentration in order to support normal neurodevelopment. While regulatory
23 mechanisms ensure homeostasis of brain iron later in life, ⁹ the correlation between serum ferritin and
24 brain iron is still unclear ⁶⁷ and chronic, severe iron deficiency also impacts brain iron status. ⁶³ Dietary
25 assessment and measurement of iron intake were not performed in most of the included studies. Hence,

1 it is unknown whether there is an association of low iron intake or low serum iron status with low brain
2 iron status in children with ADHD or whether low brain iron status in ADHD is independent of serum
3 iron and iron intake. In the latter case, dysregulations of iron metabolism in the brain would be the
4 main cause of ADHD pathophysiology. Mechanisms by which low brain iron status disrupts cognitive
5 function may be cerebral hypoxia ⁶⁸ and poor myelin integrity besides reduced neurotransmitter
6 function. ⁶⁹

7
8 A body of evidence suggests that iron deficiency is associated with neurocognitive impairments and
9 several mechanisms have been proposed. First, iron depletion may dysregulate dopaminergic neurons
10 and it is therefore critical to ADHD pathophysiology.⁶³ There is a need for MRI and functional MRI
11 studies investigating both brain iron status and dopamine activity in ADHD. Second, iron deficiency
12 is related to reduced dopamine transporter density and activity and reduced dopamine receptors in the
13 striatum.^{70,71} Third, restless leg syndrome, also associated with ID, is a common comorbidity of
14 ADHD, and therefore, a mutual pathophysiological pathway may be present.⁷² Fourth, it has also been
15 suggested that low serum ferritin concentration may indicate a certain level of oxidative stress, ⁷³ which
16 possibly leads to impairment of neurodevelopmental trajectories as well as gene functions.

17
18 As an association between ID and ADHD was demonstrated, ⁵⁴ it is reasonable to assume that iron
19 supplementation would benefit children with ADHD. Very few studies have investigated the effect of
20 iron supplementation in ADHD patients. Sever et al. ⁷⁴ gave 14 children with ADHD iron supplements
21 over 30 days and found an improvement in Connors Parent's Rating scales, a subjective measure of
22 behavioural symptom severity. Furthermore, a randomized controlled trial by Konofal et al.⁷⁵
23 randomly assigned oral iron supplements or placebo to 23 children with ADHD and observed a
24 significant improvement of ADHD symptoms using the Clinical Global Impression-Severity, but not
25 the Connors Parent's/Teacher's Rating scales, after 12 weeks. Hence, to date, evidence suggests that

iron supplementation reduces the severity of ADHD symptoms. Longer and larger trials are needed, since psychostimulants, currently used for treating ADHD symptoms, have side effects that include appetite suppression, headaches, and delay in sleep onset.⁷⁶ Furthermore, the question of whether iron supplementation during infancy would prevent or reduce ADHD symptoms remains to be examined.

Several limitations to this review should be taken into consideration. All the included studies were case-control studies, in which iron status was measured and ADHD diagnosis was performed at the same time. Thus, no cause and effect relationship can be drawn (a low iron status could be due to ADHD and vice versa), which is further highlighted by the lack of control of confounders in most of the studies that were included. Possible confounders are dietary iron intake, as well as intake of iron absorption-enhancing nutrients such as Vitamin C,⁷⁷ psychological⁷⁸ and oxidative stress, as well as medication, which are all known to affect serum ferritin concentrations. Moreover, serum ferritin is an acute phase protein that can be elevated by inflammation and only a small number of studies on brain iron concentrations met the selection criteria of the current systematic review. These are points for considerations for future study designs on ADHD, an important childhood disorder.

This review also has a number of strengths. This is the first systematic review conducted to assess the association between brain iron concentrations, as well as serum ferritin/iron concentrations, and ADHD. Furthermore, the Study Quality Assessment tool by the NIH was used instead of the Newcastle-Ottawa Scale (NOS) for risk of bias assessment. While the latter was used in two recent meta-analyses,^{23,24} it presents several limitations.⁷⁹ For example, one of the items in the NOS assesses identical response proportions of the case and control group; i.e. if the same number of cases and controls have chosen to respond or partake in the experiment. Due to the recruitment method and the fact that the case-control studies were not longitudinal, identical response rate is not a safeguard against selection bias. Hence, the risk of bias was more accurately appreciated in the present systematic review.

Conclusion

The current systematic review adds to the literature in suggesting that brain iron concentrations, specifically in the thalamus, are lower in children with ADHD compared to healthy controls. It highlights the need for larger MRI studies using MFC to measure brain iron concentration in different brain regions. Furthermore, because a brain iron set point was hypothesized to be determined in infancy, it is proposed that longitudinal studies that measure brain iron concentrations over longer periods (i.e. from early childhood to adolescence), be conducted in order to examine the role of iron depletion in ADHD pathophysiology in specific brain regions and at critical time points.

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Author contributions:

The authors' responsibilities were as follows—AD, EP and GOL-D conceived the idea and designed the research; AD and RJ performed the literature and screened the articles under EP and GOL-D supervision, EP and GOL-D validated the screened articles, AD and RJ extracted all data with the input of EP and GOL-D where necessary; AD wrote the original review, EP and GOL-D reviewed and edited the manuscript and all authors read and approved the final paper. No funding was received.

Declaration of Interest

None

1 **Table 1.** PICOS criteria for inclusion and exclusion of studies

PICOS criteria	Inclusion	Exclusion
Population	Children (<18 years old) diagnosed with ADHD, non-medicated	Adults (>18 years old), medicated children with ADHD, nonhuman studies
Intervention/Exposure	Measure of serum iron, serum ferritin or brain iron concentrations	Articles not evaluating serum iron, serum ferritin or brain iron concentrations
Comparator	Healthy controls, non-medicated	Children diagnosed with chronic, genetic or psychological disorders
Outcome	Presence of ADHD, diagnosed according to formal criteria (e.g. DSM-IV)	
Study design	Case-control studies, cross-sectional studies, cohort studies	Intervention studies, narrative and systematic reviews and meta-analyses, abstracts, reports, case reports, surveys

- 2 ADHD, Attention Deficit/Hyperactivity Disorder; DSM-IV, Diagnostic and Statistical Manual of Mental
- 3 Disorders 4th revision

4 **Table 2.** Characteristics of case-control studies comparing serum ferritin followed by serum iron concentrations between children with ADHD and healthy
5 controls

Biomarker	References	Diagnosis Criteria	Groups	Sample size in subgroup	Mean age in subgroup	Female (%) in subgroup	Serum ferritin/Iron	Mean $\pm$ SD (ng/ml)
<i>Ferritin</i>								
	Abd El Naby et al. (2018) ³⁶	DSM-IV	HC	25	5.7 $\pm$ 3.9	48	ELISA	134.2 $\pm$ 14.8 vs.
			ADHD	25	4 $\pm$ 2.5	24		110.3 $\pm$ 6.5*
	Adisetiyo et al. (2014) ³⁴	DSM-IV	HC	27			ECL	38.2 $\pm$ 22.8 vs.
			ADHD	12	12.6 $\pm$ 2.8	32		51.3 $\pm$ 24.1
	Bener et al. (2014) ³¹	DSM-IV (&CRS)	HC	630	11.5 $\pm$ 3.6	50.3	Competitive binding	38.2 $\pm$ 5.6 vs.
			ADHD	630	11.5 $\pm$ 3.8	50	radio-immunoassay techniques	36.3 $\pm$ 5.9*
	Cortese et al. (2013) ⁴¹	DSM-IV	HC	14	10 $\pm$ 2.2	44.4	Ferrozine method	12.9 $\pm$ 1.6 vs.
			ADHD	28	9.9 $\pm$ 1.5	11		12.7 $\pm$ 1.1*
	Donfrancesco et al. (2013) ⁸⁰	DSM-IV	HC	93	9.2 $\pm$ 3.1	13.4	ELISA	33.1 $\pm$ 18.7 vs.
			ADHD	101	8.9 $\pm$ 2.5	9.8		33.0 $\pm$ 17.8

Islam et al. (2018) ³⁷	DSM-5 (&CRS)	HC	119	11.2 ± 3.8	28.6	Tosho AIA 360	43.8 ± 6.1 vs.
		ADHD	119	11 ± 3.7	29.4		35.2 ± 4.7*
Juneja et al. (2010) ⁸¹	DSM-IV	HC	25	8.0 ± 1.5	16	ELISA	49.0 ± 41.6 vs.
		ADHD	25	8.4 ± 1.7			6.0 ± 3.85**
Konofal et al. (2005) ⁸²	DSM-IV (&interview)	HC	27	9.5 ± 2.8	26	Elecsys Enzymun-Test	44.0 ± 22.0 vs.
		ADHD	53	9.2 ± 2.2	15		23.0 ± 13.0**
Konofal et al. (2007) ⁸³	DSM-IV	HC	10	7.0 ± 0.9	30	Elecsys Enzymun-Test	46.0 ± 18.0 vs.
		ADHD	10	6.7 ± 0.9	10		25.0 ± 15.0*
Kwon et al. (2011) ⁸⁴	DSM-IV	HC	48	(Combined)	(Combined)	<i>Not specified</i>	38.2 ± 5.6 vs.
		ADHD	48	6.9 ± 0.4	41		36.3 ± 5.9
Mahmound et al. (2011) ⁸⁵	DSM-IV (&CRS)	HC	25	8.3 ± 1.8		ELISA	32.6 ± 18.7 vs.
		ADHD	58	8.3 ± 1.8			24.8 ± 14.1*

Menegassi et al. (2010) ⁸⁶	Schedule for Affective Disorders and Schizophrenia for School-Age Children (K- SADS-E), DSM- IV	HC ADHD	21 22	8.9 ± 2.7 9.0 ± 2.6	28.6 27.3	ECL	58.8 ± 28.9 vs. 54.2 ± 17.2
Millichap et al. (2006) ⁴⁷	<i>Not specified,</i> CRS?	HC ADHD	1053 68	<i>Not</i> <i>specified</i>	20.5	<i>Not specified</i>	37.5 ± 49.0 vs. 39.9 ± 40.6
Percinel et al. (2016) ⁸⁷	DSM-IV	HC ADHD	100 200	11.0 ± 3.0 11.0 ± 2.4	40 36.5	ECL	30.8 ± 17.5 vs. 27.6 ± 15.3
Seleem et al. (2015) ³⁹	DSM-IV (&CRS, ICD-10)	HC ADHD	15 30	9.7 ± 2.8 8.6 ± 2.5	26.7 20	<i>Not specified</i>	30.7 ± 21.7 vs. $16.4 \pm 15.0^*$
Wang et al. (2019) ⁵⁸	DSM-IV	HC ADHD	216 216	9.2 ± 1.7 9.2 ± 1.8	14 14	Chemiluminescent immunoassay	44.7 ± 18.8 vs. $39.9 \pm 17.2^*$
Yang et al. (2019) ²⁰	DSM-5	HC	395	8.9 ± 1.7	8.9	Multichannel	46.0 ± 5.0 vs.

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			ADHD	419	8.8 ± 2.1	7.9	atomic absorption spectrophotometer	45.5 ± 4.5
	Yazici et al. (2015) ⁸⁸	DSM-IV and DSM-5	HC ADHD	69 70	10.6 ± 2.7 10.9 ± 2.8	29 35.7	Automatic autoanalyzer (Siemens)	28.0 ± 18.1 vs. 27.1 ± 17.1
<hr/>								
	<i>Serum Iron</i>							(<i>ug/dl</i>)
	Bener et al. (2014) ³¹	DSM-IV (&CRS)	HC ADHD	630 630	11.5 ± 3.6 11.5 ± 3.8	50.3 50	Competitive binding radio-immunoassay techniques	85.6 ± 12.5 vs. $82.1 \pm 13.6^*$
	Chen et al. (2004) ³⁸	DSM-IV	HC ADHD	52 58	8.5 ± 2.2 7.9 ± 2.0	28.3 30	Auto-analyser	92.7 ± 38.0 vs. $110.1 \pm 35.8^*$
	Islam et al. (2018) ³⁷	DSM-5 (&CRS)	HC ADHD	119 119	11.2 ± 3.8 11 ± 3.7	28.6 29.4	Ferrozine method	92.5 ± 7.8 vs. $81.6 \pm 6.6^{**}$
	Konofal et al. (2005) ⁸²	DSM-IV (&interview)	HC ADHD	27 53	9.5 ± 2.8 9.2 ± 2.2	26 15	<i>Not specified</i>	

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Kwon et al. (2011) ⁸⁴	DSM-IV	HC	48	All	41	<i>Not specified</i>	85.6 ± 12.5 vs.
		ADHD	48		6.9 ± 0.4		82.1 ± 13.6
Menegassi et al. (2010) ⁸⁶	Schedule for Affective Disorders and Schizophrenia for School-Age Children (K- SADS-E), DSM- IV	HC	21	8.9 ± 2.7	28.6	Ferrozine method	92 ± 31.4 vs.
		ADHD- nonM	22	9.0 ± 2.6	27.3		78.6 ± 24.0
Percinel et al. (2016) ⁸⁷	DSM-IV	HC	100	11.0 ± 3.0	40	Automatic autoanalyzer	77.9 ± 30.6 vs.
		ADHD	200	11.0 ± 2.4	36.5	(Siemens)	71.9 ± 31.0
Seleem et al. (2015) ³⁹	DSM-IV (&CRS, ICD-10)	HC	15	9.7 ± 2.8	26.7	<i>Not specified</i>	103.7 ± 122.7 vs.
		ADHD	30	8.6 ± 2.5	20		61.4 ± 94.6
Yang et al. (2019) ²⁰	DSM-5	HC	395	8.9 ± 1.7	8.9	Multichannel	46.0 ± 5.0 vs.
		ADHD	419	8.8 ± 2.1	7.9	atomic absorption spectrophotometer	45.5 ± 4.5

Yazici et al.	DSM-IV and	HC	69	10.6 ± 2.7	29	Chemiluminescent	74.6 ± 33.9 vs.
(2019) ⁸⁹	DSM-5	ADHD	70	10.9 ± 2.8	35.7	immunoassay	72.9 ± 34.4

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- 10 DSM-IV/5, Diagnostic and Statistical Manual of Mental Disorders 4th/5th revision; CRS, Conners Rating Scale; CTRS, ConnersTeacher Rating Scale; CPRS,
- 11 Conners Parent Rating Scale; ICD-10, International Classification of Diseases 10th Revision; HC, healthy controls; ADHD, children with attention deficit
- 12 hyperactivity disorder; ECL, Electrochemoluminescence; ELISA, enzyme-linked immunosorbent assay, SD, standard deviation; * significance $p < 0.05$; n.s.,
- 13 non-significant
- 14 Noted and corrected mistakes for serum iron ⁸⁶; reported g/dl instead of $\mu\text{g/dl}$ and ²⁰ reported $\mu\text{mol/dl}$ instead of $\mu\text{g/dl}$
- 15

16 **Table 3.** Results of case-control studies comparing brain iron concentrations between children with ADHD and HC

Biomarker	References	Diagnosis Criteria	Groups	Sample size in subgroup	Mean age in subgroup	Female (%) in subgroup	Iron assay/MRI method (unit)	ROI	Mean $\pm$ SD
<i>Brain Iron</i>									
	Adisetiyo et al. (2014) ³⁴	DSM-IV	HC	27			MCF (<i>s-2</i>)	Globus Pallidus	468 $\pm$ 157 vs.
			ADHD	12	12.6 $\pm$ 2.8	32			354 $\pm$ 96
								Putamen	205 $\pm$ 68 vs. 151 $\pm$ 41*
								Caudate Nucleus	248 $\pm$ 76 vs. 182 $\pm$ 49*
								Thalamus	169 $\pm$ 50 vs. 136 $\pm$ 28*
							R2* (<i>s-1</i>)		Raw data not accessible

Cortese et al. (2013) ⁴¹	DSM-IV	HC	9	10 ± 2.2	44.4	R2* (<i>S-I</i>)	Right Globus Pallidus	18.33 ± 2.82
		ADHD	18	9.9 ± 1.5	11			vs.
								17.49 ± 1.91
							Left Globus Pallidus	17.68 ± 2.84
								vs.
								17.44 ± 1.87
							Right putamen	16.39 ± 2.25
								vs.
								15.01 ± 1.14
							Left putamen	15.54 ± 3.30
								vs.
	15.00 ± 1.15							
						Right caudate nucleus	16.01 ± 2.40	
							vs.	
							14.79 ± 1.42	

								Left caudate nucleus	15.33 ± 2.82
								vs.	
									14.64 ± 1.08
								Right thalamus	17.71 ± 4.21
								vs.	
									14.61 ± 1.16*
								Left thalamus	17.71 ± 3.10
								vs.	
									14.80 ± 1.30*
	Hasaneen et al.	DSM-IV	HC	18	8.5 ± 1.7	38.9	R* (<i>s-I</i>)	Thalamus	16.6 ± 0.9 vs.
	(2017) ⁴⁰		ADHD	17	8.4 ± 0.8	29.4			14.9 ± 1.3**

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- 18 ROI, region of interest; MRI, magnetic resonance imaging; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders 4th/5th revision; HC,
- 19 healthy controls; ADHD, children with attention deficit hyperactivity disorder; MCF, magnetic field correlation; R2*, relaxation rate (R2=1/T2*);R*,
- 20 relaxation rate; SD, standard deviation; * significance p <0.05; ** significance p < 0.001; n.s., non-significant

Figure 1. Flow diagram of selection process

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Figure Legend

Figure 1. Flow diagram of selection process

Table Legends

Table 1. PICOS criteria for inclusion and exclusion of studies

Table 2. Characteristics of case-control studies comparing serum ferritin followed by serum iron concentrations between children with ADHD and healthy

Table 3. Results of case-control studies comparing brain iron concentrations between children with ADHD and HC