Food intake and serum levels of iron in children and adolescents with attention-deficit/hyperactivity disorder

Ingestão alimentar e níveis séricos de ferro em crianças e adolescentes com transtorno de déficit de atenção/hiperatividade

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Abstract
Objective: To investigate hematologic variables related to iron deficiency and food intake in attention-deficit/hyperactivity disorder. Method: The sample comprised 62 children and adolescents (6-15 years old) divided into three groups: Group 1: 19 (30.6%) patients with attention-deficit/hyperactivity disorder using methylphenidate for 3 months; Group 2: 22 (35.5%) patients with attention-deficit/hyperactivity disorder who were methylphenidate naïve and Group 3: 21 (33.9%) patients without attention-deficit/hyperactivity disorder. Serum iron, ferritin, transferrin, hemoglobin, mean corpuscular volume, red cell distribution width, mean corpuscular hemoglobin concentration, nutritional diagnostic parameters - Body Mass Index Coefficient, food surveys were evaluated among the groups. Results: The attention-deficit/hyperactivity disorder group drug naïve for methylphenidate presented the highest red cell distribution width among the three groups (p = 0.03). For all other hematologic and food survey variables, no significant differences were found among the groups. No significant correlation between dimensional measures of attention-deficit/hyperactivity disorder symptoms and ferritin levels was found in any of the three groups. Conclusion: Peripheral markers of iron status and food intake of iron do not seem to be modified in children with attention-deficit/hyperactivity disorder, but further studies assessing brain iron levels are needed to fully understand the role of iron in attention-deficit/hyperactivity disorder pathophysiology.

Descriptors: Nutritional status; Iron deficiency; Diet surveys; Attention-deficit/hyperactivity disorder; Methylphenidate

Resumo
Objetivo: Investigar as variáveis hematológicas relacionadas à deficiência de ferro e à ingestão alimentar no transtorno de déficit de atenção/hiperatividade. Método: São sessenta e duas crianças e adolescentes (6-15 anos) divididos em três grupos: Grupo 1: 19 (30,6%) pacientes com transtorno de déficit de atenção/hiperatividade com uso de metilfenidato durante três meses; Grupo 2: 22 (35,5%) pacientes com transtorno de déficit de atenção/hiperatividade sem uso de medicamento; e Grupo 3: 21 (33,9%) pacientes sem transtorno de déficit de atenção/hiperatividade. Ferro sérico, ferritina, transferrina, hemoglobina, volume corpuscular médio, amplitude de distribuição dos eritrócitos, concentração da hemoglobina corpuscular média, parâmetros de diagnóstico nutricional – Coeficiente de Índice de Massa Corporal, inquérito alimentar e a correlação entre os sintomas do transtorno e os níveis de ferritina foram avaliados. Resultados: O grupo com transtorno de déficit de atenção/hiperatividade não medicado com metilfenidato apresentou maior amplitude de distribuição dos eritrócitos dentre os três grupos (p = 0.03). Nas outras variáveis hematológicas e inquéritos alimentares não encontramos diferença significativa entre os grupos. Não observamos correlação entre os sintomas do transtorno de déficit de atenção/hiperatividade e ferritina. Conclusão: Marcadores periféricos do estado nutricional de ferro e a ingestão alimentar de ferro não parecem estar modificados em crianças com transtorno de déficit de atenção/hiperatividade, mas mais estudos avaliando os níveis de ferro no cérebro são necessários para compreensão plena do papel do ferro na fisiopatologia do transtorno de déficit de atenção/hiperatividade.

Descritores: Estado nutricional; Deficiência de ferro; Inquéritos alimentares; Transtorno de déficit de atenção/hiperatividade; Metilfenidato

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Introduction

Several recent investigations on the neurobiology and treatment of Attention-Deficit/Hyperactivity Disorder (ADHD) have suggested that possible deficiencies of nutrients (i.e. iron, zinc, magnesium, polyunsaturated fatty acids) and sensitivity to some foods and food additives could be involved with the pathophysiology of the disorder.\(^1\)\(^3\)

The iron is distributed heterogeneously in different regions and brain cells. The concentrations are not static, they increase with age and with some diseases, and decrease when the diet does not provide the required amount of nutrition.\(^4\)

The brain iron homeostasis is required for its normal function; a decrease in iron concentration is accompanied by changes in the conduction of cortical fibers, changes in serotonergic and dopaminergic systems, as well as in the formation of myelin.\(^5\)\(^-\)\(^7\)

In children, there is an association between anemia caused by iron deficiency or iron deficiency without anemia and poor cognitive and motor development, and behavioral problems,\(^4\)\(^,\)\(^8\)\(^,\)\(^9\) indicating that iron plays an important role in normal functioning and neurological development.\(^9\) Significantly low levels of ferritin have been observed in children with ADHD, correlating with the severity of the symptoms. Since iron has a role as a cofactor of tyrosine hydroxylase, its decrease limits enzyme involvement in the synthesis of dopamine, and its deficiency may change the receptor density and the dopamine activity.\(^10\)\(^-\)\(^12\) The benefits of iron supplementation on attention function in children have been assessed through the use of neurophysiologic measures. The findings suggest that iron supplementation could improve attention in children with iron deficiency.\(^13\)

However, other studies did not confirm the finding that iron deficiency plays a role in the pathophysiology of ADHD and, if so, whether oral administration of iron for affected children is recommended.\(^14\) Moreover, Milichap et al. found a proportion of children with ADHD with relatively low levels of ferritin suggestive of iron deficiency, but without a specific relationship to the symptoms and causes of ADHD.\(^15\)

Due to the evidence of the involvement of iron in the dopamine metabolism, the relevance of dopaminergic systems in the pathophysiology of ADHD and the controversial findings regarding the role of iron deficiency in ADHD etiology, our study sought to investigate levels of iron in the disorder, as well as undertake food surveys. Our hypothesis was that patients with ADHD show iron levels significantly lower than the control group and that this difference would be even greater in the group of patients with ADHD treated with MPH, since findings in the literature suggest that low iron stores in children with ADHD who are taking medication\(^15\)\(^,\)\(^16\) might be due to a decreased appetite caused by these medications.\(^17\)

Method

This was a controlled cross-sectional study. The sample was enrolled from the ADHD Outpatient Clinic at the Child and Adolescent Psychiatric Division of Hospital de Clínicas de Porto Alegre (PRODAH) from March 2006 to December 2008. The project was approved by the Ethical Committee of Hospital de Clínicas de Porto Alegre, RS, Brazil (project number 05532). Written informed consent from parents or a legal guardian and assent from the child were obtained.

1. Subjects

Children and adolescents from the ADHD Outpatient Program were allocated into 2 groups: 1) group 1 - patients with ADHD, in exclusive and uninterrupted use of immediate-release MPH for 3 months; and 2) group 2 - patients with ADHD without medication use. A third group (control group) was also recruited from a primary care center linked with the University Hospital: 3) group 3 – control group, without ADHD or use of medication. For each patient diagnosed with ADHD (MPH naïve) a new patient with ADHD using MPH and a control subject were enrolled. All control patients were evaluated prior to the study by a pediatrician who ruled out the presence of infections and inflammatory processes and the use of drugs that could interfere in the serum iron levels.

Subjects were excluded if they had an IQ below 70, the coexistence of other psychiatric disorders with exception of Conduct Disorder (CD) and Oppositional Defiant Disorder (ODD), as well as any factor that could interfere in the serum iron level, including the use of supplemental iron in the past 3 months, parasitosis, acute or chronic infections, inflammatory processes, blood loss and chronic diarrhea. Illiterate patients were also excluded, due to the impossibility of completing the food survey.

2. Diagnostic procedures

The diagnostic process of ADHD and comorbid disorders for children and adolescents in our unit has been extensively described.\(^18\) Briefly, the diagnosis of ADHD was obtained from the use of a semi-structured interview, Schedule for Affective Disorders and Schizophrenia for School-Age Children, Epidemiological Version (K-SADS-E)\(^19\) applied by trained research assistants and clinical evaluation of ADHD and comorbid conditions using DSM-IV\(^20\) criteria by child psychiatrists in interviews with the child and parents. For dimensional analyses of ADHD symptoms, we employed the Swanson, Nolan and Pelham – IV Questionnaire (SNAP-IV).\(^21\) Cognitive evaluation relied on the vocabulary and block design sub-tests of the Wechsler Intelligence Scale – Third Edition (WISC-III)\(^22\) administered by a trained psychologist to estimate the children’s overall IQ.

In addition, socioeconomic status (SES) was systematically collected from parents using the socioeconomic scale from Associação Brasileira de Institutos de Pesquisa de Mercado.\(^23\)

3. Laboratory measures

For the parasitosis control, Parasitological Feces Exams were performed with three samples from each patient using the Hoffman, Pons and Janer method.\(^24\) The hematological exams were collected in the laboratory of the HCPA with minimum 4-hour fast for the serum iron assessment (Ferrozine colorimetric method), ferritin (electrochemiluminescence method), transferrin (immunoturbidimetry method) and

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hemoglobin, mean corpuscular volume (MCV), red cell distribution width (RDW), mean corpuscular hemoglobin concentration (MCHC) through absorbance of light/impedance/flow cytometry method.

The following cut-off scores were used to indicate abnormal results: 1) Hemoglobin: 5 to 11 years old (< 11.5g/dl), 12 to 14 years old (< 12g/dl), over 15 years old (< 13g/dl); 2) Ferritin: 12 to 15ng/mL and the cut-off used by Konofal et al. of ≤ 30ng/mL and by Cortese et al. of ≤ 45ng/mL; 3) RDW: > 14.5%; 4) VCM: 5 to 8 years old (77 to 95fL), 13 to 18 years old (78 to 96fL); 5) iron serum: 55-120µg/dL; 6) CHCM: 31 to 37%; and 7) Transferrin: 170 to 250mg/dL.

4. Nutritional assessment

After the selection of patients, a nutritional history and the measurement of weight and height were performed by a nutritionist. Three food surveys were applied to evaluate the dietary iron and caloric intake: 1) 24-Hour Dietary Recall; 2) Dietary record over 4 days (i.e. two weekdays and full weekend); 3) Food–Frequency Questionnaires.

The iron intake was measured from the amount of heme-iron found in high levels in red or dark meats. Heme-iron has an important nutritional benefit because the bioavailability of this form of dietary iron is high and generally it is not affected by important iron absorption inhibitors in the diet, such as polyphenols and phytate.

NutWin software was used to calculate the food surveys and Epi Info™ Version 3.5.1 (2008) to measure the nutritional indicator. The body mass index coefficient, BMI (weight in kilos divided by the square of height in meters) was used as the nutritional diagnosis parameter. Denoting the standard deviation as SD, the following cut-offs (z-score) were used: 1) severe thinness if BMI < -3SD; 2) thinness if -3SD < BMI < -2SD; 3) eutrophic if -2SD < BMI < SD; 4) overweight if SD < BMI < 2SD; and 5) obesity if BMI > 2SD. At the end of this study, all participants received nutritional instructions comprised of a standard diet for their age, according to their food habits, and a food reeducation program was proposed to those who presented inadequate food intake.

5. Statistical analysis

The results were analyzed by Statistical Package for the Social Sciences Version 16.0 (SPSS). Continuous variables (age, schooling, IQ, hematologic variables, food surveys) were described through mean and standard deviation (symmetrical distribution) or median and interquartile range (asymmetrical distribution). Continuous data were presented as mean ± standard deviation (SD) or median ± interquartile range. Categorical data (gender, SES, ADHD subtypes, ODD, CD, cut-offs for iron deficient, nutritional assessment-BMI) were described as absolute and relative frequencies and p-values < 0.05 were considered significant. For continuous variables, the differences among groups were assessed using One-Way Analysis of Variance (ANOVA) in case of a symmetrical distribution, or Kruskal-Wallis for asymmetrical distribution. Duncan test was applied to locate the statistical significance among groups. The Pearson’s Chi-Square test was applied to compare the categorical variables among the groups. The relationship between SNAP and ferritin was assessed with Spearman’s rho coefficient (r). The effect size assessment for clinical relevance was also calculated using the Effect Size Statistics (ES).

Results

The sample was composed of 62 children and adolescents between 6 and 15 years old (average of 8.9 [sd = 2.5] years), of which 46 (74.2%) were male. The sample was divided into three groups: Group 1 - 19 (30.6%), Group 2 - 22 (35.5%) and Group 3 - 21 (33.9%). Table 1 shows the socio-demographic and clinical characteristics of the subjects.

A significant difference (p = 0.03) among the groups was found only for RDW, with the highest detected levels in ADHD patients who were MPH naïve. However, we did not find significant differences among the groups (p = 0.13) in the analyses based on the dichotomic RDW scores (≥ 14.5%). In addition, only four patients had the RDW ≥ 14.5% associated with other hematological alterations (MCV ≤ 77fL and/or ferritin ≤ 30ng/mL).

Table 1 - Socio-demographic and clinical characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group 1 (n = 19)</th>
<th>Group 2 (n = 22)</th>
<th>Group 3 (n = 21)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs) – (mean [sd])</td>
<td>8.8 [2.4]</td>
<td>9.0 [2.6]</td>
<td>8.9 [2.7]</td>
<td>0.99</td>
</tr>
<tr>
<td>Male – n (%)</td>
<td>15 (78.9%)</td>
<td>16 (72.7%)</td>
<td>15 (71.4%)</td>
<td>0.85</td>
</tr>
<tr>
<td>IQ – (mean [sd])</td>
<td>99.2 [16.9]</td>
<td>96.5 [12.5]</td>
<td>97.6 ± 10.5</td>
<td>0.81</td>
</tr>
<tr>
<td>ADHD subtypes - n (%):</td>
<td>13 (68.4%)</td>
<td>14 (63.6%)</td>
<td>-</td>
<td>0.3</td>
</tr>
<tr>
<td>Combined</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inattentive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperactive/Impulsive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ODD-n (%)</td>
<td>3 (15.8%)</td>
<td>7 (31.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD-n (%)</td>
<td>1 (5.3%)</td>
<td>1 (4.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SES-n (%): B – C</td>
<td>18 (94.7%)</td>
<td>22 (100%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schooling – (median ± iq/2)</td>
<td>1 (0 to 3)</td>
<td>1.5 (0 to 3.25)</td>
<td>2 (0 to 3.5)</td>
<td></td>
</tr>
</tbody>
</table>

IQ: Intelligence quotient; ODD: Oppositional defiant disorder; CD: Conduct disorder; SES: Socioeconomic status; ADHD: Attention-Deficit/Hyperactive Disorder.
For the other hematologic variables evaluated in this study, there were no significant differences among the groups, as indicated in Table 2.

We also assessed all hematologic variables for iron deficiency or anaemia using designated cut-off scores (see Table 3). Anaemia was detected in all groups: 4 (21.1%) patients in group 1, 2 (9.1%) patients in group 2 and 3 (14.3%) subjects in the control group. However, no significant differences were found among the groups (p = 0.55). For ferritin, the minimum and maximum values observed were 15.5ng/mL and 139.6ng/mL, and no significant difference was verified for the cut-off scores of 30ng/mL (p = 0.44) and 45ng/mL (p = 0.96). Furthermore, no significant correlations were observed between the symptoms (measured by SNAP – inattentiveness, hyperactivity, ODD and total scores) and ferritin levels in all groups (see Table 4).

From these data, 8.1% of subjects had levels of ferritin ≤ 30ng/mL, with more than half (60%) from the control group. Values of ≤ 45ng/mL were found in 24.2% of the sample, equally distributed in 5 patients of each group. No patient presented ferritin values ≤ 15ng/mL and transferrin values ≤ 170mg/dL.

Ten subjects (16.1%) presented serum iron below 55g/dL, 5 (8.1%) < VCM 77fL and 2 (3.2%) had values of MCHC < 31%, and again no significant difference (p = 0.53, p = 0.08 and p = 0.56, respectively) was verified among the groups.

The values of heme-iron from food surveys (Table 5) were not significantly different among the groups in the 24-hour recall (p = 0.62), dietary record (p = 0.39) and food–frequency questionnaire (p = 0.81).

The food intake (Table 5) measured by total intake of calories also showed no significant difference among the groups, both for intake measured using the 24-hour recall (p = 0.54) and dietary record (p = 0.39).

In all groups, a predominance of the eutrophic nutritional status according to BMI was observed: 14 (73.7%) subjects in the MPH use group, 17 (77.3%) individuals in the group with ADHD who were MPH naïve and 16 (76.2%) subjects in the control group (Figure 1). No significant difference was found among the groups (p = 0.88). Only one patient (5.3%) in group 1 had thinness according to BMI (z-score < -2).

**Discussion**

To our knowledge, this is the first report investigating the association between serum levels of iron and ADHD, controlling for several potential confounders and including the use of methylphenidate. In addition, we are not aware of other studies comparing food surveys of patients with ADHD to controls. Our results suggest a lack of significant difference in the hematologic variables and food intake among patients with ADHD using medication, ADHD not medicated and non-ADHD patients.

Although a significant difference in RDW was found, the literature indicates that the RDW for the diagnosis of iron deficiency should be considered with MCV and ferritin.34-36 Due to the performance limitation of each isolated biochemical test, the literature suggests that joint analyses should be used to increase the sensitivity and specificity to the diagnosis of iron deficiency.35,37 In our study, only four subjects had other hematologic changes.
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associated with abnormal RDW values, which would be suggestive of iron deficiency.

Some reports suggested that iron (usually assessed by ferritin levels) is closely associated with the severity of ADHD and comorbid symptoms. However, no correlations between ADHD symptoms and ferritin levels were observed in any groups of our study. Moreover, the frequency of iron deficiency was considerably higher in the studies performed by Konofal et al. and in the study of Cortese et al., where 60% of the patients presented serum ferritin levels ≤ 45ng/dL. However, our results demonstrated lower prevalence of ferritin values ≤ 30ng/dL and ≤ 45ng/dL (8.1% and 24.2%, respectively). These results raise some doubts about whether iron deficiency might have physiological significance in ADHD and if the ferritin levels used as reference in other studies could be falsely altered due to acute or chronic inflammation or the presence of infection.

To assess food behavior with higher precision, we quantified the food intake by associating different types of surveys, since no method is available for the food consumption assessment that is free of drawbacks and biases. Moore et al. showed that the items associated with an unhealthy diet may be omitted from the questionnaires, and factors such as lower socio-economic class and school performance may be associated with bias in reporting food consumption.

According to D’Amato, low iron stores in children with ADHD who are taking medication, as reported in the literature, might be due to decreased appetite associated with these medications and additionally that those with ADHD (whether they are taking medications or not) might have reduced ability to sit still for a meal and therefore decreased nutritional intake of iron. However, we did not observe suppression of appetite caused by medication, or any effect on feeding behavior caused by the mechanisms intrinsic

![Figure 1 - Nutritional Assessment (%): Body Mass Index (BMI) z-score; p = 0.88](image)

### Table 4 - Correlation ($r_s$) between ADHD symptoms and ferritin levels ($p < 0.05$)

<table>
<thead>
<tr>
<th></th>
<th>SNAP</th>
<th>Group 1 (n = 19)</th>
<th>Group 2 (n = 22)</th>
<th>Group 3 (n = 21)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>-0.05</td>
<td>-0.08</td>
<td>0.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inattentive</td>
<td>0.08</td>
<td>0.06</td>
<td>0.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperactivity</td>
<td>0.06</td>
<td>-0.32</td>
<td>0.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oppositional</td>
<td>0.13</td>
<td>0.03</td>
<td>0.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.60</td>
<td>0.90</td>
<td>0.32</td>
<td></td>
</tr>
</tbody>
</table>

$r_s$ = Spearman-rank correlation coefficient

### Table 5 - Food surveys

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group 1 (n = 19)</th>
<th>Group 2 (n = 22)</th>
<th>Group 3 (n = 21)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-hour dietary recall-kcal</td>
<td>2.025 ± 1.012</td>
<td>1.836 ± 691</td>
<td>2.113 ± 809</td>
<td>0.55</td>
</tr>
<tr>
<td>Record dietary-kcal (mean ± sd)</td>
<td>2.021 ± 815</td>
<td>1.942 ± 544</td>
<td>2.147 ± 591</td>
<td>0.60</td>
</tr>
<tr>
<td>Heme iron* (median ± iq/2)</td>
<td>1.5 (1.1-5.7)</td>
<td>2.6 (1.1-3.8)</td>
<td>2.4 (1.5-6.7)</td>
<td>0.62</td>
</tr>
<tr>
<td>Heme iron** (median ± iq/2)</td>
<td>3.5 (2.5-8.1)</td>
<td>2.8 (2.5-5.1)</td>
<td>4.3 (3.1-5.3)</td>
<td>0.62</td>
</tr>
<tr>
<td>Heme iron*** (median ± iq/2)</td>
<td>3.0 (1.4-4.8)</td>
<td>3.0 (2.1-4.8)</td>
<td>3.6 (2.1-5.2)</td>
<td>0.81</td>
</tr>
</tbody>
</table>

* Obtained with the 24-hour Dietary Recall; ** Obtained with the Dietary Record for 4 days; *** Obtained with Food – Frequency Questionnaires.
to ADHD in the food intake of heme-iron and energy among the groups of ADHD children medicated, not medicated or the control group.

The attention to the length of time on stimulants or on other medications, as well as the withdrawal of medication 2 to 3 months before the study, were mentioned in some investigations, but it is not clear either whether this prior period of medication suspension would be sufficient to normalize possible nutritional deficiencies, or which drugs were used and for how long they were used before the interruption in most studies. These factors might justify the deficiencies of iron reported by these authors.

Although the most adverse effects of medications are mild and transient, there is a concern about the effect of these drugs on weight and growth. In our study, patients using methylphenidate exclusively and continuously for three months showed no differences in the nutritional food intake compared to the group without medication and the control group. Furthermore, no difference was observed in the anthropometric measurements among the groups.

Our findings should be considered in the light of the following limitations. First, the three-month period of medication use might not have been sufficient to change the hematological variables or the patient’s growth. Also, we might not have been able to detect significant differences in most variables because of our limited sample size. However, taking the ferritin variable as an example, establishing the means and SD of the groups as found and accepting a study power of 90%, we would need at least 225 in each group to detect a difference at a 5% significance level. Moreover, effect sizes found were small among the groups (medicated ADHD vs. drug naive ADHD, ES: 0.27; medicated ADHD vs. control, ES: 0.02; drug naive ADHD vs. control, ES: 0.19), documenting that between-group differences were of low clinical relevance. In addition, our sample size was similar to that of other studies on this subject in the literature.

Conclusion

We believe that the results of our study contribute to the understanding of the role of iron in the disorder, adding to the findings in the literature and suggesting that peripheral iron levels and food intake of iron did not differ between patients with ADHD and controls. Therefore, we suggest that future studies evaluating the levels of iron in the brain should be performed to confirm whether this mineral is involved in the pathophysiology of ADHD.

Acknowledgements

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References