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### Iron Intake in Children with Attention-Deficit/Hyperactivity Disorder and Restless Legs Syndrome

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IRON INTAKE IN CHILDREN WITH ATTENTION-DEFICIT/HYPERACTIVITY  
DISORDER AND RESTLESS LEGS SYNDROME

A Thesis Presented to  
The Faculty of the School of Medicine  
Yale University

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Master of Medical Science

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## **ABSTRACT**

Growing evidence supports an association between attention-deficit/hyperactivity disorder (ADHD) and restless legs syndrome (RLS) in children. Prior research suggests that in some patients iron deficiency is an underlying cause of pathological changes in the dopaminergic system of the brain that modulates these disorders. However, the underlying cause of such iron deficiency has not yet been identified. We propose to determine the prevalence of low serum ferritin levels, a marker of reduced iron stores, in children with comorbid ADHD and RLS, and if diet is associated with their diagnosis and observed blood iron levels. A case-control study will examine a sample of 245 children with ADHD and 245 children without ADHD. We will use a diagnostic questionnaire to identify RLS in both groups, and dietitians will analyze 3-day food diaries to identify dietary iron intake. Our findings can inform clinicians of the importance of diet in the ADHD and RLS population.

## CHAPTER 1: INTRODUCTION

### 1.1 Background

Attention-deficit/hyperactivity disorder (ADHD) is the most common mental disorder diagnosed in children today.<sup>1</sup> The worldwide pediatric prevalence of ADHD is estimated to be around 5%.<sup>2</sup> However, according to the Centers for Disease Control and Prevention (CDC), approximately 11% of children ages 4-17 in the United States have been diagnosed with ADHD, with the average age of diagnosis at 7 years.<sup>3</sup> ADHD is a neurodevelopmental disorder characterized by a persistent pattern of inattention and/or hyperactivity-impulsivity that interferes with functioning or development.<sup>2</sup> The standard criteria used today to diagnose this disorder in the United States is the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Health Disorders, Fifth Edition (DSM-5), and there are multiple requirements that must be fulfilled in order to give a child this diagnosis.<sup>4</sup> First, six or more symptoms of inattention and/or hyperactivity and impulsivity must be present for at least 6 months, and they must be developmentally inappropriate. Table 1 lists these symptoms.

<b>Symptoms of Inattention</b>	<b>Symptoms of Hyperactivity and Impulsivity</b>
<ul style="list-style-type: none"><li>• Often fails to give close attention to details or makes careless mistakes in schoolwork, at work, or during other activities</li><li>• Often has difficulty sustaining attention in tasks or play activities</li><li>• Often does not seem to listen when spoken to directly</li><li>• Often does not follow through on instructions and fails to finish schoolwork, chores, or duties in the workplace</li></ul>	<ul style="list-style-type: none"><li>• Often fidgets with or taps hands or feet or squirms in seat</li><li>• Often leaves seat in situations when remaining seated is expected</li><li>• Often runs about or climbs in situations where it is inappropriate</li><li>• Often unable to play or engage in leisure activities quietly</li><li>• Is often "on the go," acting as if "driven by a motor"</li><li>• Often talks excessively</li></ul>

<ul style="list-style-type: none"> <li>• Often has difficulty organizing tasks and activities</li> <li>• Often avoids, dislikes, or is reluctant to engage in tasks that require sustained mental effort</li> <li>• Often loses things necessary for tasks or activities</li> <li>• Is often easily distracted by extraneous stimuli</li> <li>• Is often forgetful in daily activities</li> </ul>	<ul style="list-style-type: none"> <li>• Often blurts out an answer before a question has been completed</li> <li>• Often has difficulty waiting his or her turn</li> <li>• Often interrupts or intrudes on others</li> </ul>
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**Table 1.** DSM-5 Symptom Criteria for ADHD.<sup>2</sup>

In addition to this, four other conditions must be met: numerous symptoms of inattentiveness or hyperactivity/impulsivity must be present before the age of 12; they must be present in at least 2 settings (home, school, or work); they must interfere with social, academic, or occupational functioning; and lastly, these symptoms cannot develop exclusively with another psychotic disorder or be better explained by another mental disorder.<sup>2</sup>

Once children are diagnosed with this disorder, health care providers usually recommend stimulant medication or behavior therapy as first line treatment to improve outcomes, depending on the age of the patient.<sup>5</sup> For preschool aged children between 4 and 5 years old, parent and teacher administered behavior therapy is first line treatment, whereas guidelines recommend stimulants for children older than 6 or for preschoolers in whom behavioral interventions do not provide improvement.<sup>6</sup> Current stimulants available include short-, intermediate-, and long-acting methylphenidate or dextroamphetamine. Studies have shown that these treatments reduce core symptoms of inattention, hyperactivity, and impulsivity, as well as improve function in social and classroom domains.<sup>7</sup> However, ADHD should be recognized as a chronic disease as medication is not curative, and it often persists into adulthood.<sup>6</sup>

Along with these daytime symptoms, parents of children with ADHD often report sleep issues in their children. The association of sleep problems and ADHD is a very close one, as 25% to 50% of children and adolescents with ADHD in clinical practice have difficulties initiating and maintaining sleep.<sup>8</sup> Whereas adults usually exhibit excessive daytime sleepiness as a result of a poor night's sleep, children often display the opposite. They show signs of inattention, hyperactivity, and cognitive and behavioral dysfunction—those same symptoms recognized in ADHD.<sup>9</sup> Given this relationship between ADHD and sleep alterations, clinicians began to look for an association between ADHD and restless legs syndrome (RLS), a particular dyssomnia that has been known to cause effects on daytime behavior in children.<sup>9</sup>

RLS is a sensorimotor disorder that causes uncomfortable leg and arm sensations with an irresistible urge to move them.<sup>10</sup> These sensations come at rest, and worsen during the evening or night. In some people, they only occur at night. Those with RLS often have difficulty sleeping due to this unpleasant feeling in their legs. It can delay sleep onset, awaken the individual from sleep, and cause significant sleep fragmentation.<sup>2</sup> This condition was not described until 1945, and the International Restless Legs Syndrome Study Group (IRLSSG) first established four diagnostic criteria for the uniform diagnosis of RLS in adults in 1995.<sup>11</sup> The IRLSSG criteria were developed through a panel of international RLS clinical and research experts, and they have remained the fundamental criteria for diagnosing this disorder.<sup>12</sup> Since 1995, these criteria have undergone two revisions, as RLS has gained more clinical exposure and has been further researched.<sup>12,13</sup> A separate set of criteria was established in 2003 (and revised in 2013) for children aged 2-12 that required a verbal description of their

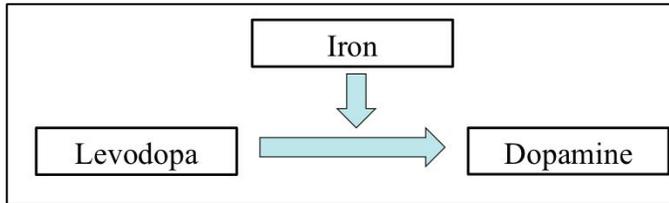
symptoms in their own age appropriate terminology. This was added due to the fact that children often have a difficult time communicating the symptoms that they are feeling with RLS.<sup>14</sup> The pediatric diagnostic criteria are a modified version of the adult criteria, separated into “definite,” “probable,” or “possible” RLS categories for research purposes. Adult criteria are difficult to determine in children who often spend prolonged periods of time seated in school. Table 2 lists the updated criteria.

<b>Diagnostic Criteria for RLS in Adults</b>	<b>Diagnostic Criteria for RLS in Children</b>
<ol style="list-style-type: none"> <li>1. An urge to move the legs, usually but not always accompanied by, or felt to be caused by, uncomfortable and unpleasant sensations in the legs.</li> <li>2. The urge to move the legs and any accompanying unpleasant sensations begin or worsen during periods of rest or inactivity such as lying down or sitting.</li> <li>3. The urge to move the legs and any accompanying unpleasant sensations are partially or totally relieved by movement, such as walking or stretching, at least as long as the activity continues.</li> <li>4. The urge to move the legs and any accompanying unpleasant sensations during rest or inactivity only occur or are worse in the evening or night than during the day.</li> <li>5. The occurrence of the above features is not solely accounted for as symptoms primary to another medical or a behavioral condition (e.g. myalgia, venous stasis, leg edema, arthritis, leg cramps, positional discomfort, habitual foot tapping).</li> </ol> <p><b>Specifiers for clinical course of RLS:</b></p> <ol style="list-style-type: none"> <li>A. Chronic-persistent: symptoms when not treated would occur on average at least twice weekly for the past year.</li> </ol>	<p><b>Criteria for the diagnosis of definite RLS in children</b></p> <ul style="list-style-type: none"> <li>• The child meets all five essential adult criteria for RLS AND</li> <li>• The child relates a description in his or her own words that is consistent with leg discomfort (The child may use terms such as <i>oowies</i>, <i>tickle</i>, <i>spiders</i>, <i>boo-boos</i>, <i>a lot of energy in my legs</i>, <i>need to move</i>, <i>want to move</i>, and <i>got to kick</i> to describe symptoms. Age-appropriate descriptors are encouraged).</li> </ul> <p><b>Criteria for the diagnosis of probable RLS in children</b></p> <ul style="list-style-type: none"> <li>• The child meets all essential adult criteria for RLS, except criterion 4 (occurrence only or worsening in the evening or night).</li> </ul> <p><b>Criteria for the diagnosis of possible RLS in children</b></p> <ul style="list-style-type: none"> <li>• The child is observed to have behavior manifestations of lower extremity discomfort when sitting or lying, accompanied by motor movement of the affected limbs. The discomfort is characterized by RLS criteria 2-5 (is worse during rest and inactivity, relieved by movement, worse in the evening or night, and is not solely accounted for as primary to another medical or a behavioral condition).</li> </ul>

<p>B. Intermittent RLS: symptoms when not treated would occur on average &lt;2/week for the past year, with at least five lifetime events.</p> <p><b>Specifiers for clinical significance of RLS:</b></p> <p>The symptoms cause significant distress or impairment in social, occupational, educational or other important areas of functioning by their impact on sleep, energy/vitality, daily activities, behavior, cognition, or mood.</p>	
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**Table 2.** International RLS Study Group (IRLSSG) Diagnostic Criteria for RLS.<sup>12,14</sup>

RLS is underdiagnosed in both the adult and pediatric populations. In children, it is estimated that the prevalence of RLS is between 2% to 4%.<sup>14</sup> However, studies have shown that up to 44% of the ADHD pediatric population experiences RLS symptoms.<sup>15</sup> Several hypotheses have been proposed to explain this association between RLS and ADHD. Biologically, both disorders have been tied to pathological deficits in the dopaminergic system of the brain.<sup>16</sup> There is evidence for decreased dopaminergic function in the midbrain, frontal, and prefrontal regions of those children with ADHD, and in RLS there seems to be reduced dopamine synthesis.<sup>17,18</sup> It is already well established that iron is a key in the dopamine system as it is needed to produce this neurotransmitter, maintain receptor function in the brain, and to synthesize myelin.<sup>9</sup> Iron is the rate-limiting step in the conversion of tyrosine to levodopa, one of the important precursors to dopamine. When there is less iron available to make levodopa, the production of dopamine is decreased.<sup>19</sup> Figure 1 illustrates this relationship. Because of this common underlying central nervous system pathophysiology, iron status has become a subject of interest when looking at ADHD and RLS both alone and as comorbid phenomena.



**Figure 1.** Conversion of levodopa to dopamine.<sup>19</sup>

Ambiguous data have been found in regard to iron status and ADHD. Reduced brain iron has been hypothesized as an underlying cause of ADHD. Reduced peripheral iron stores—documented by serum ferritin, the most widely accepted and standardized gauge of iron deficiency—have been used most often to assess this relationship.<sup>20,21</sup> Many studies have examined serum ferritin levels in patients with ADHD for a possible peripheral iron deficiency, but the results have been mixed.<sup>17</sup> The majority have found that peripheral serum levels are significantly lower in those with the disorder when compared to a control group. Most have also found that serum ferritin is inversely correlated with ADHD symptom severity.<sup>22-32</sup> However, there have also been studies that have failed to confirm such a relationship or to find significant differences between ADHD patients and healthy controls.<sup>33-36</sup> Overall, differing eligibility criteria and methodology between studies measuring serum ferritin in the ADHD population seem to be the reasons for such variable results.<sup>17</sup>

Similarly, iron deficiency has been considered to be a significant contributing cause of RLS since the disorder was first described.<sup>37,38</sup> Serum ferritin level has been shown to be related to RLS severity<sup>39</sup>, and, because of this, iron supplementation has been used as a therapy to help improve the unpleasant leg symptoms of children with RLS and concurrent iron deficiency.<sup>18,40-45</sup> The high prevalence of RLS in the pediatric ADHD population, the mixed results regarding the relationship between ADHD and serum ferritin levels, and the strong relationship between RLS and iron deficiency

suggests that the subset of children with comorbid ADHD and RLS may be those most at risk for iron deficiency and worsening symptom severity.

## **1.2 Statement of the Problem**

The subset of children with comorbid ADHD and RLS is an important population to study. ADHD is the most commonly diagnosed childhood condition today, and there is ongoing debate, both scientifically and publicly, as to whether ADHD is overdiagnosed in children. Stimulant use has steadily increased over the last 20 years, and as of 2012, about two-thirds of children diagnosed with ADHD were treated pharmacologically.<sup>46</sup> However, up to 75% of ADHD children meet the criteria for another psychiatric diagnosis.<sup>47</sup> RLS produces symptoms that overlap with the criteria for ADHD, while at the same time disrupting sleep patterns and affecting daytime function in children. There is currently limited information regarding the processes underlying their comorbidity and the factors associated with its risk. Further investigation will prove valuable to help adequately treat the ADHD pediatric population.

The association between serum ferritin levels and comorbid ADHD and RLS has not been thoroughly researched. Four major studies have examined this subgroup of the pediatric population. In 2003, Konofal et al. first reported a correlation between serum ferritin levels, ADHD index, and RLS symptom severity in ADHD children. In 2007, this same group of researchers carried out the first study to date assessing iron levels in children with both ADHD and RLS, comparing them to children with ADHD and age- and sex-matched controls.<sup>10</sup> The authors found that mean serum ferritin levels were lowest in the group of children with the comorbid condition, and that such levels were associated with high ADHD index scores. However, a small sample size was used, and

although there was a statistical difference with controls, there was no statistical difference from the group of children with ADHD alone. Oner et al. and Kapisyzi et al. examined this same subgroup of patients and also found that there was a higher prevalence of iron deficiency among this group as compared to a group of children with ADHD alone.<sup>48,49</sup> However, they found no association between iron deficiency and ADHD symptom severity.

A question that all of this research provokes is why children with comorbid ADHD and RLS are more likely to have lower serum ferritin levels than healthy children or children with either disease alone. The most common cause of iron deficiency in children is an iron-poor diet.<sup>50</sup> Diet has often been identified as a culprit for the development of ADHD, and there has been research examining this relationship. However, there has been only a limited amount of research characterizing dietary intake and serum nutrients.<sup>36</sup> Over the past 12 years, 5 studies have measured dietary iron intake in ADHD children.<sup>33,35,36,51,52</sup> The two most recent have shown lower iron intake among ADHD children than healthy controls. Lifestyle factors, such as diet quality, have been proposed to have an effect on the risk or severity of RLS<sup>53</sup>, but the relationship between dietary iron intake in RLS patients has not been investigated thus far. Therefore, our knowledge of dietary iron consumption with the comorbid condition of ADHD and RLS remains limited and incomplete. Given that children with both disorders have shown to be the subset of the ADHD population with the lowest serum ferritin levels, further studies are needed to explore the effect that diet may be having on both their sleep and daytime functioning.

Furthermore, it is important to note that most patients with RLS who have low serum ferritin levels do not actually have anemia.<sup>18,54,55</sup> Low serum ferritin status has been shown to contribute to the development of RLS symptoms. Traditionally, oral iron supplementation has been recommended for serum ferritin levels of <50 ng/ml, which is considered a low-normal value. Practitioners generally target an optimal serum ferritin range of 50 to 100 ng/ml, which reflects a more adequate iron status.<sup>56</sup> However, more recent studies have shown that iron supplementation should begin with serum ferritin levels below 75 ng/ml.<sup>57</sup> Prior research suggests that children with ADHD and RLS are more prone to low iron stores. If this is true, further investigation is needed to determine what guidelines to follow when addressing initiation of iron supplementation in this population.

Lastly, as previously discussed, iron is a critical component of dopamine synthesis in the brain, and low brain iron stores can cause dopaminergic dysfunction. The majority of brain iron is found in ferritin as it is transported from serum to cerebrospinal fluid.<sup>55</sup> It has been hypothesized that in RLS, low brain iron concentration is caused by the dysfunction of iron transport from serum to the central nervous system. However, in those children with comorbid ADHD and RLS, no investigation into such a mechanism has been made. If a low iron diet is associated with low serum ferritin levels, the underlying pathological process in these patients will prove to be multifactorial, not only attributable to the faulty brain iron transport hypothesis.

### **1.3 Goals and Objectives**

The primary goal of this study is to determine the role of diet in the pediatric development of comorbid ADHD and RLS. More specifically, we seek to identify and

analyze the association between mean dietary iron intake and serum ferritin levels in children ages 4-16 with comorbid ADHD and RLS and to compare this to the same associations in healthy children and children with either disorder alone. By completing this study, we hope to identify dietary iron consumption as a modifiable risk for children suffering from both diseases. As a secondary goal, we aim to further confirm the prevalence of low serum ferritin levels in this specific population using a large-scale sample size. Only four prior studies have examined serum ferritin levels in children with ADHD and RLS, and they all have used a relatively small sample. Lastly, we plan to determine if dietary iron intake and serum ferritin levels are correlated with ADHD symptom severity.

#### **1.4 Hypothesis**

Children between the ages of 4 and 16 with comorbid ADHD and RLS are more likely to have both a low dietary iron intake and low serum ferritin level as compared to healthy controls and children with either ADHD or RLS alone.

#### **1.5 Definitions**

- ***Low dietary iron intake*** will be defined from the collection of mean dietary iron intake, measured in mg/day, using a median split technique. Those patients with a mean dietary iron intake less than the median of all sample values will be categorized with a low dietary iron intake.
- ***High dietary iron intake*** will be defined from the collection of mean dietary iron intake, measured in mg/day, using a median split technique. Those patients with a mean dietary iron intake greater than the median of all sample values will be categorized with a high dietary iron intake.

- **Low serum ferritin level** will be defined from the collection of serum ferritin levels, measured in ng/ml, using a median split technique. Those patients with serum ferritin levels less than the median of all sample values will be categorized with a low serum ferritin level.
- **High serum ferritin level** will be defined from the collection of serum ferritin levels, measured in ng/ml, using a median split technique. Those patients with serum ferritin levels greater than the median of all sample values will be categorized with a high serum ferritin level.

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## **CHAPTER 2: LITERATURE REVIEW**

### **2.1 Introduction**

A thorough and systematic review of the evidence regarding the association between serum ferritin levels, dietary iron intake, and RLS in individuals with ADHD was conducted using Ovid MEDLINE, PubMed, Scopus, Embase, PsychINFO, and Web of Science databases. The following keywords were used in various combinations: *ADHD, attention-deficit/hyperactivity disorder, attention deficit disorder, ADD, restless legs syndrome, RLS, iron deficiency, iron, serum ferritin, dietary iron, iron intake, and diet*. All relevant papers were reviewed from 1994, when the American Psychiatric Association published the Diagnostic and Statistical Manual of Mental Disorders, until April 2016 to obtain the most comprehensive and up-to-date information. Because ADHD and RLS exist in both the pediatric and adult populations, papers examining both age groups were included. Case-control, cross-sectional, cohort, and randomized controlled trial studies were included, in addition to systematic reviews. Searches were also performed on these same databases for relevant research on confounders and methodology.

### **2.2 Review of Empirical Studies**

There have been no studies addressing the relationship between dietary iron intake and serum ferritin levels in the population of children with comorbid ADHD and RLS. Thus, this section will review studies that have examined iron status in that specific population, as well as dietary iron intake in both populations separately.

**Serum Ferritin Levels in Comorbid RLS and ADHD.** The first study examining the relationship between serum ferritin and symptom severity in the subset of ADHD children with RLS was in Paris in 2003, when Konofal et al. assessed 43 children with ADHD for RLS according to the IRLSSG criteria. The mean age of children recruited was  $9.2 \pm 2.2$  years, and ADHD was diagnosed using the DSM-IV criteria. Eligibility criteria included being medication free for at least 2 months and no previous iron supplementation. Nineteen of the 43 children (44%) met the guidelines for “definite” or “probable” RLS. The authors also measured serum ferritin levels and found that ADHD symptoms negatively correlated ( $p < 0.0001$ ). Serum ferritin level was also negatively correlated with RLS severity in this population. However, limitations to this study include the small sample size and the absence of any comparison group.<sup>1</sup>

The first study to make such comparisons was a matched, cross-sectional study by Konofal et al. in 2007 of 22 children with ADHD and a group of 10 controls between the ages of 5 and 8. Inclusion criterion for ADHD cases was a diagnosis of ADHD according to the DSM-IV criteria, and exclusion criteria included the presence of additional mild to moderate behavioral, mood, or anxiety disorders, physical diseases, and malnutrition. Controls did not meet diagnostic criteria for ADHD, RLS, or any other psychiatric disorders. They were age- and sex-matched with cases. None of the children involved were on medication for ADHD, RLS, or sleep at the time of assessment. In addition, none were using supplemental iron. RLS was then diagnosed in the ADHD group based on the National Institutes of Health (NIH) consensus criteria for RLS in children. They included children with definite, probable, and possible RLS. ADHD symptom severity was measured using the Conners' Parent Rating Scale (CPRS) and

scored on a four-point Likert-type scale. Twelve of the 22 ADHD cases had comorbid RLS, and analysis of variance (ANOVA) was used for between-group comparison of serum ferritin and symptom severity. There was a statistical difference ( $p=0.001$ ) between the comorbid group and controls for mean serum ferritin, but no statistical difference between the comorbid group and ADHD without RLS group. However, children with a comorbid condition had the lowest mean ferritin levels, followed by those with ADHD only. Children with ADHD and RLS had a higher severity of ADHD symptoms compared with ADHD without RLS and non-ADHD children, but these differences were not significant. Although the study design limits any implication of causality, results would have been stronger and more reliable if the study had been properly powered. Differences between groups were present, yet insignificant given the small sample size. Furthermore, these results are not generalizable to the entire pediatric population, as no participant was over the age of 8 years.<sup>2</sup>

In a cross-sectional study later that year, Oner et al. sampled 87 ADHD subjects from a general outpatient hospital clinic that were diagnosed with the disorder. Unlike the previous study, the age range of these participants was 6-16 years. Children were included if they had an ADHD diagnosis. Exclusion criteria included the presence of primary or secondary sleep disorders, epilepsy, peripheral neuropathy, radiculopathy, psychosis, mental retardation, and any acute medical condition that might lead to alterations in serum ferritin and iron levels. Oner's group measured the frequency of RLS in these children using the IRLSSG criteria for definite RLS. They then compared ADHD symptom severity and iron deficiency, defined as a serum ferritin level  $<12\text{ng/ml}$ , between those children with the comorbid disorders and those with ADHD alone. Using a

Fisher's Exact Test, there were significantly higher rates of iron deficiency in ADHD subjects with RLS than those without ( $p=0.005$ ). There was no statistical difference in ADHD symptom severity, measured using the Child Behavior Checklist, Teacher Report Form, the Conners' Parent Rating Scale, and the Conners' Teacher Rating Form, between the two groups. The results of this study are important because they fall in line with those of Konofal et al. in terms of serum ferritin. However, their definition of iron deficiency as  $<12$  ng/ml is somewhat problematic given that the current, accepted value used for initiation of iron supplementation is  $<50$  ng/ml. Only 6 of the 29 children with the condition were found to have iron deficiency as compared to 1 of the 58 with ADHD alone. Such a narrow definition neglects those children who may be experiencing increased ADHD and RLS symptom severity with low serum ferritin levels, although they may not technically fit the stated definition for iron deficiency.<sup>3</sup>

Kapisyzi et al. performed a cross-sectional study similar to Oner's in 2010 using 43 ADHD subjects, with average age of  $16.3 \pm 2.5$  years, diagnosed by a psychiatrist using the DSM-IV criteria. These children were further evaluated for RLS using the IRLSSG criteria. RLS was found in 32.5% of the subjects, however the severity of ADHD symptoms was not statistically significant between those with and without RLS ( $p > 0.05$ ). These authors also defined iron deficiency as serum ferritin  $<12$  ng/ml, and the rate of iron deficiency was statistically higher in ADHD subjects with RLS when compared to ADHD subjects without RLS ( $p=0.005$ ). This study showed that iron deficiency increases the risk of having RLS in ADHD children. However, the results are not generalizable to the entire pediatric population, as only adolescents between the ages of 13 and 18 were sampled.<sup>4</sup>

All four of the above studies fail to recognize diet's influence on serum ferritin levels. They all suggest that low serum ferritin or iron deficiency increases the risk of having RLS in ADHD children, but offer no information as to why such a deficiency occurs. Furthermore, both Oner et al. and Kapisyzi et al. reported a disproportionate female/male ratio (1 to 9) that may have clouded the results, and they failed to use a healthy control group for comparison. Konofal et al., however, used gender- and age-matched controls in order to take such variables into account in their study design, but did so using only a narrow age range of subjects (5-8 years).

**Dietary Iron Intake, RLS, and ADHD.** Dietary iron intake has not been specifically explored in either adult or pediatric comorbid ADHD and RLS populations. There have been studies looking at dietary iron intake in the ADHD population, and one recent study investigating diet quality in adult RLS patients.

In 2004, Chen et al. conducted a cross-sectional study on the relationships between ADHD and nutritional factors in children in Taiwan. Fifty-eight children diagnosed with ADHD using the DSM-IV criteria and 52 control subjects, between the ages of 4-12, were recruited from local schools to complete a 3-day diet record. Participants were allowed to pick 3 continuous days in which to record their meals. From there, a computer software program was used to analyze the diaries for food components. In addition to the food record, patients provided a fasting blood sample that was used to measure different biochemical data; among them was iron. The authors found statistically significant differences in both iron intake and blood iron content between the ADHD and control groups ( $p < 0.05$ ). The ADHD subjects consumed 43% more iron and had a higher iron content in the blood. The authors concluded that based on these

results, diet failed to explain the relationship between iron deficiency and ADHD. However, this study was subject to study bias due to the fact that children with the disorder were volunteers from the hospital and were not representative of the general population. Also, patients were allowed to choose the days of the week in which they filled out their food diary, not taking into account the variability that this difference could cause. Lastly, this study failed to take socioeconomic status, which could potentially influence both diet and ADHD, into account.<sup>5</sup>

In 2009, Menegassi et al. performed a controlled cross-sectional study that hypothesized that children with ADHD on methylphenidate would have lower iron levels than those patients not on the stimulant due to decreased appetite caused by the medication. Sixty-two children and adolescents between the ages of 6-15 were divided into three groups: patients with ADHD in exclusive and uninterrupted use of immediate-release methylphenidate, patients with ADHD without medication use, and a healthy control group from a primary care center. Children with ADHD were from an ADHD Outpatient Clinic. Nutritional assessment was made with a 24-hour dietary recall, a dietary record over 4 days (two weekdays and a full weekend), and a food-frequency questionnaire. Heme-iron was measured in red and dark meats in mg/day. Additionally, both blood draws and parasitological feces exams were performed on each participant. Serum ferritin was measured in ng/ml and abnormal results were indicated with the following three cut-off scores:  $\leq 15$  ng/ml,  $\leq 30$  ng/ml, and  $\leq 45$  ng/ml. The authors found no significant differences in the values of heme-iron among the groups using the 24-hour recall ( $p=0.62$ ), dietary record ( $p=0.62$ ), or food-frequency questionnaire ( $p=0.81$ ). Likewise, no statistical differences were found in serum ferritin levels for the cut-off

scores of 30 ng/ml ( $p=0.44$ ) and 45 ng/ml ( $p=0.96$ ). The study suggests that serum ferritin levels and iron intake do not differ between children with ADHD (on or off medication) and healthy controls. Furthermore, it suggests that stimulant medication is not associated with decreased dietary intake. A major strength of this study was the stringent eligibility criteria imposed on participants, which helped to restrict confounding. Exclusion criteria included: an IQ below 70, the coexistence of other psychiatric disorders with exception of conduct disorder and oppositional defiant disorder, and the presence of any factor that could interfere with serum iron level including supplemental iron use in the past 3 months, parasitosis, acute or chronic infections, inflammatory processes, blood loss, and chronic diarrhea. However, children with ADHD were selected from outpatient clinics. If this was not done systematically, then there may be some selection bias making the results imprecise. Furthermore, the authors stated that they needed 225 participants in each group in order for the study to be sufficiently powered and to detect a 5% significance level. This further explains their outcomes.<sup>6</sup>

Kiddie et al. (2010) reported findings similar to Menegassi et al. in a case-control study assessing dietary food intake and nutrient status of DSM-IV diagnosed ADHD children ages 6-12 sequentially recruited from an ADHD Program. Unlike Menegassi's study, all participants were on medication for ADHD and there was no control group. All participants had to have been stable on their current medication for at least 6 months and had to be taking it 7 days a week. Participants were excluded if they were on additional medications that altered food intake or had any other medical conditions that could alter nutritional status. Serum ferritin was measured from a nonfasting blood sample, and mean dietary iron intake (mg/day) was calculated from a 24-hour food recall and a 3-day

food record (from two weekdays and one weekend day). Iron intake was then compared to the Estimated Average Requirement (EAR) and U.S. dietary intake data from the second National Health and Nutrition Examination Survey (NHANES II). No significant differences in iron intake (95% CI -4.8052 to 11.0135) or serum ferritin ( $p=0.582$  with 95% CI: -7.2207 to 12.5294 and  $p=0.786$  with 95% CI: -7.9672 to 10.3422 for ages 6-8 and 9-11, respectively) were found, and all participants met the Estimated Average Requirement for iron intake. The results suggest that serum ferritin levels and iron intake do not differ between children with ADHD and national norms. However, a major weakness of this study is that it did not control for iron supplementation. This confounding variable may be masking a real difference in both of these measures. Furthermore, only 44 subjects were examined.

In contrast, two recent studies showed lower iron intake among children with ADHD. Durá Travé et al. used a case-control methodology evaluating the dietary patterns in a group of 100 children with ADHD being treated with methylphenidate extended release medication and 150 healthy controls without ADHD. ADHD children were diagnosed using the DSM-IV criteria and had to be on extended release methylphenidate for at least 12 months. They were grouped into two subtypes: those with predominantly inattention and those with primarily hyperactivity and impulsivity. Exclusion criteria for both groups included any chronic condition known to affect nutritional status, use of vitamin or mineral supplements, or those who had stopped taking medication during summer breaks. Each participant filled out a 3-day food diary, and macronutrient, mineral, and vitamin intake were calculated using a nutritional database. Among other vitamins and minerals, the control group showed significantly higher iron ingestion in mg

( $p < 0.05$ ) compared to the groups with ADHD. Furthermore, all of the children in the control group surpassed the recommended intake for iron in contrast to the groups with ADHD ( $p < 0.05$ ). A major drawback of this study was that any confounding factors, such as socioeconomic status and education level, were overlooked by the researchers.<sup>8</sup>

In 2015 Energin et al. published a case-control study comparing the nutritional status and anthropometric characteristics of children between the ages of 8-11 with and without ADHD. 100 children with ADHD from a child and adolescent psychiatry department in Turkey voluntarily enrolled in the study, while a healthy control group of 100 children was recruited from a school in a part of the city that had similar socioeconomic status with the ADHD group ( $p > 0.05$ ). Inclusion criteria included a DSM-IV diagnosis of ADHD. Exclusion criteria included mental retardation and neurologic or chronic illness. Children in the control group were excluded if they had current or past psychiatric illness or any chronic disease. Like the previous studies discussed, a 3-day food record was given to each patient after parents were trained by a dietician to record dietary intake. Patients were instructed to record a period of three consecutive days (two weekdays and one weekend day). A photographic atlas of portion sizes was used to evaluate portions and amounts. A nutrition information system was used to calculate both the energy and nutrients from the records, and, using the *Dietary Guidelines for Turkey*, deficiencies were defined as intakes less than 67% of the guidelines. Statistical analysis was stratified by gender. Intake levels of all nutrients examined (including iron) were significantly lower in boys with ADHD than in healthy boys ( $p < 0.05$ ). Girls with ADHD showed lower intake levels of all nutrients except for vitamin A and thiamine. However, the difference in iron intake was not statistically

significant ( $p=0.14$ ). Furthermore, all anthropometric measurements of children (weight, height, BMI, waist circumference, hip circumference, mid-upper arm circumference, and hand grip strength) were lower in the children with ADHD (both boys and girls). However, there were only statistical differences between handgrip strength in boys ( $p<0.05$ ) and waist circumference and handgrip strength in girls ( $p=0.04$  and  $p=0.01$ , respectively). This study was important in that it found an association between lower energy and nutrient intake in children with ADHD, and it suggested that their physical condition might be a reflection of this. However, although the authors tried to account for socioeconomic influence on the results, they did not take any other potential confounders into account, particularly medication use.<sup>9</sup>

In terms of RLS, a prospective cohort study published by Batool-Anwar et al. just this year examined the association between modifiable lifestyle factors and the risk of developing RLS in 167,959 adults participating in the Health Professionals' Follow-up Study and the Nurses' Health Study II over 4-6 years of follow-up. RLS was diagnosed based on the IRLSSG criteria, and among other lifestyle factors ascertained, the authors assessed diet quality by the Alternate Healthy Eating Index (AHEI). The conformance to the Dietary Guidelines for Americans for vegetables, fruits, nuts, soy, cereal fiber, white-red meat ratio, polyunsaturated: saturated fat ratio, trans-fat, and multivitamin use was assessed, and participants were given a rating from 0-10 based on their eating behavior. A score of 10 was considered healthy dietary behavior. The authors found no significant association between RLS and diet quality. However, when performing lagged analyses, there was an association between diet and RLS that suggested perhaps patients with the disorder changed their eating habits before they developed symptoms. This study's

strengths included a large sample size and small chance of recall bias given its prospective design. However, iron supplementation was used as a surrogate measure for iron deficiency, which has the potential to cause residual confounding.<sup>10</sup>

### **2.3 Review of Studies Identifying Possible Confounding Variables**

Several studies have investigated the factors that influence dietary intake and absorption of iron by the body, in patients with ADHD and RLS. The findings of these studies will be discussed in order to identify potential confounders that will be accounted for in the proposed study.

**Socioeconomic Status.** Socioeconomic factors—such as race, education, income, and occupation—have been a topic of interest regarding diet and micronutrient intake for many years.<sup>11-18</sup> It has been found that those groups of people with lower incomes and education levels have higher rates of obesity, more diet-related disease, and poor diets.<sup>14</sup> In 2008, Kranz et al. examined sociodemographic, dietary, and anthropometric data from 1,521 children between the ages of 2-5 who participated in the National Health and Examination Survey 1999-2002.<sup>18</sup> The authors found that diet quality decreased with increasing age ( $p < 0.001$ ) and improved with increasing family income ( $p < 0.001$ ). Race also played a role, as Hispanic children's diet quality index scores were significantly better than those of non-Hispanic white children ( $p = 0.033$ ). In another cross-sectional study assessing dietary intake and socioeconomic status, Hulshof et al. analyzed 2-day food diaries from 12,965 adult participants and found that a higher socioeconomic status was associated with a higher intake of micronutrients, including iron ( $p < 0.01$ ).<sup>11</sup>

Socioeconomic status has also been known to influence serum ferritin levels.<sup>19,20</sup> Iron deficiency has been documented to be more common among groups of low socioeconomic status.<sup>21</sup> Gompakis et al. conducted a cross-sectional study exploring the relationship between dietary habits, socioeconomic status, and iron deficiency in children between the ages of 8 months to 15 years.<sup>20</sup> These authors found a higher incidence of iron deficiency (hemoglobin  $\geq 11$  g/dl and ferritin  $< 10$  ng/ml) and iron deficiency anemia (hemoglobin  $< 11$  g/dl and ferritin  $< 10$  ng/ml) in children at any age in semiurban areas that had more citizens of lower socioeconomic status as compared to urban areas ( $p < 0.001$ ). Similarly, Kim et al. performed a cross-sectional study in Korea examining the relationship between household income and the prevalence of iron deficiency, anemia, and iron deficiency anemia.<sup>19</sup> The authors found as income level decreased, the risk for anemia or iron deficiency anemia increased. When compared to the high-income group, the odds ratio for anemia was highest in the lowest income group (OR 7.10, 95% CI 2.49, 20.23).

Socioeconomic status has also been shown to be a risk factor in the development of both ADHD and RLS. In 2014, Russell et al. performed a mediation analysis of data from 8,132 children in a longitudinal cohort study, focusing on socioeconomic factors and the diagnosis of ADHD.<sup>22</sup> The authors measured socioeconomic status using 8 indexes and found that the strongest predictor of ADHD was financial difficulty (OR 2.06, 95% CI 1.44-2.94,  $p < 0.001$ ). Socioeconomic status has also been explored in RLS. Two prospective population-based cohort studies, the Dortmund Health Study and the Study of Health in Pomerania, assessed RLS twice in 5,620 adults according to the IRLSSG criteria and measured sociodemographic factors through interviews.<sup>23</sup> The

authors found that low educational achievement and low income level were significant risk factors for RLS in one study (OR 2.29, 95% CI 1.14-4.59,  $p=0.02$  and OR 1.63, 95% CI 1.05-2.52,  $p=0.03$ , respectively) and that unemployment increased the risk for RLS in both (OR 3.10, 95% CI 1.30-7.42,  $p=0.01$  and OR 1.61, 95% CI 1.03-2.53,  $p=0.04$ ).

**Iron Supplementation.** Studies analyzing dietary iron intake or serum ferritin levels have typically excluded patients on the basis of iron supplementation because supplemental iron may mask the relationship between dietary behavior and serum ferritin level. Children who use supplemental iron may not be getting adequate iron in their diets, or they may alter the foods they eat knowing that they can receive iron through supplements. Supplemental iron will produce normal peripheral serum ferritin levels, even if diet is inadequate. Both Menegassi et al. and Durá Travé et al. excluded those patients who had used iron supplementation.<sup>6,8</sup> Kiddie et al. did not control for nutrient supplementation, making it hard to determine the effects of dietary intake on those variables involved in ADHD.<sup>7</sup>

There have been several studies demonstrating the efficacy of iron supplementation for reduction of both ADHD and RLS symptom severity. In a double-blind, placebo-controlled, randomized trial, Konofal et al. looked at the effect of 12 weeks of ferrous sulfate tablet therapy after randomization of 23 iron deficient (serum ferritin  $<30$  ng/mL) nonanemic children with ADHD.<sup>24</sup> Measuring ADHD symptoms using the ADHD Rating Scale after 12 weeks, the authors found that there was a statistically significant decrease ( $p<0.008$ ) in severity in the treatment group but not in the placebo group ( $p=0.308$ ). Iron supplementation has been even more researched and supported in patients with RLS.<sup>25-34</sup> In 2009, Wang et al. performed the first double-

blind, placebo-controlled study looking at oral iron supplementation in symptomatic patients with RLS and low-normal serum ferritin levels (15-75 ng/ml).<sup>25</sup> Eleven adults received 12 weeks of ferrous sulfate and the authors found that there was a statistically significant decrease in their RLS symptom severity as compared to the placebo group (p=0.01).

**Stimulant Use.** Stimulants are the treatment of choice for ADHD patients.

However one of their side effects includes decreased appetite.<sup>35,36</sup> Low dietary iron intake could therefore be due to a reduction in intake of iron-rich foods of those children who are being medicated with stimulants. Related to this, Calarge et al. performed a cross-sectional study in which they measured serum ferritin levels of 52 children with ADHD undergoing a trial of amphetamine medication.<sup>37</sup> The authors found children with lower serum ferritin concentrations required higher doses of medication (p=0.03), concluding that stimulant medication is related to serum ferritin levels. A recent study done by Percinel et al. found that when observing a group of stimulant-naïve children with ADHD, iron deficiency parameters, symptom severity, and serum ferritin levels were not significantly different between different ADHD presentation types.<sup>38</sup> This suggested that psychostimulants may play a role in the previously documented relationships between these factors. They also cited lack of information regarding nutrition and dietary intake as a limiting factor in their study.

**Age and Gender.** Age and gender are also other potential confounders to the proposed study. It is well known that the growth spurt in adolescence affects iron metabolism and iron requirements.<sup>39</sup> The increase in mean total iron requirements in teenage boys more than doubles the requirements of preadolescent males due to the

expansion of the total blood volume and the increase in lean body mass.<sup>40</sup> In addition to these same growth requirements, adolescent females begin to lose iron after the initiation of their menstrual cycles.<sup>41</sup> To balance these losses, daily iron requirements nearly double for teenage girls as compared to preadolescent females.<sup>40</sup> Because of their rapid growth, menstrual blood losses, and poor dietary intake, adolescent females are the most susceptible to dietary iron deficiency.<sup>42</sup> Furthermore, as children age, they begin to show more undesirable eating behaviors, such as skipping breakfast and family dinners.<sup>43</sup> These tendencies are associated with decreased diet quality and failure to meet dietary recommendations.<sup>44</sup> A longitudinal study by Mannino et al. tracked dietary intake of girls at ages 5, 7, and 9, and they found a statistically significant decrease in the nutrient density of iron in the diets from age 5 to 9 ( $p < 0.0001$ ).<sup>45</sup>

Age and gender are also risk factors in the development of ADHD and RLS. In a longitudinal cross-sectional study performed by Döpfner in 2015, the ADHD symptoms of inattention, hyperactivity-impulsivity, and both combined were measured in children and adolescents between the ages of 7-19.<sup>46</sup> The authors then separated patients into subgroups (high, moderate, and low) according to different developmental trajectories and found a decrease in symptoms with age in almost all severity groups ( $p < 0.01$ ). Furthermore, researchers from the Centers for Disease Control and Prevention have found that boys (13.2%) are more likely than girls (5.6%) to have ever been diagnosed with ADHD.<sup>47</sup> Energin et al.'s study previously discussed also showed that differences exist between boys and girls with ADHD and their dietary patterns.<sup>9</sup>

Age and gender have also been known to be risk factors for RLS. The separate diagnostic criteria first established by the IRLSSG in 2003 for children under the age of

12 recognized the fact that RLS diagnosis is difficult in the younger populations.<sup>48</sup> Berger et al. performed a study in 2004 and found that the prevalence of RLS increased with age in both males and females.<sup>49</sup> They also found that women were twice as often affected with RLS than men (OR 2.64, 95% CI 1.92-3.39,  $p < 0.001$ ). This is consistent with other research citing evidence for a female preponderance in the prevalence of RLS.<sup>50,51</sup>

**BMI.** Elevated BMI has been associated with low serum ferritin levels, as well as the development of ADHD and RLS. In a study performed by Pinhas-Hamiel et al. in 2003, the authors measured BMI and mean iron level in 321 children and adolescents.<sup>52</sup> They found that iron levels below 8  $\mu\text{mol/l}$  were noted in 38.8% of obese patients (BMI > 97<sup>th</sup> percentile), 12.1% of overweight patients (85<sup>th</sup> percentile < BMI < 97<sup>th</sup> percentile), and only 4.4% of the normal-weight patients (BMI < 85<sup>th</sup> percentile) with  $p < 0.001$ . Research regarding the association between BMI and ADHD has been increasing recently, and multiple studies have shown that they could be related.<sup>53-55</sup> A prospective cohort study done by Anderson et al. found that children with ADHD, female or male, had higher BMI z scores at all ages compared with subjects without a disruptive disorder. Likewise, in Batool-Anwar et al.'s prospective cohort study assessing risk factors for RLS, obesity was associated with an increased risk of having RLS, with the odds ratio for women with a BMI > 30  $\text{kg/m}^2$  (categorized as obese) versus < 25  $\text{kg/m}^2$  was 1.64 (95% CI 1.37-1.96,  $p < 0.0001$ ). The corresponding odds ratio for men was 1.46 (95% CI 0.96-2.2,  $p < 0.005$ ).

**Malabsorption.** Intestinal malabsorption of micronutrients has been cited to be a cause of low serum ferritin levels in RLS patients.<sup>56,57</sup> Celiac disease, a chronic immune-mediated disorder in which villi of the small intestine are destroyed and unable to

properly absorb nutrients, has been associated with RLS. In a consecutive case series done by Manchanda et al., the authors found four patients with RLS and serum ferritin below 25 ng/ml who had a positive serological screening for celiac disease.<sup>56</sup>

**Infection.** Lastly, infection and inflammation have the potential to raise serum ferritin levels.<sup>58-60</sup> In a longitudinal study done by Birgegård et al., serum ferritin samples of 18 patients hospitalized for acute infections, both bacterial and viral, were taken during their time in the hospital and afterwards at follow-up.<sup>59</sup> The authors found that serum ferritin rose within a couple days of onset of infectious symptoms and remained elevated past the acute phase and recovery. The difference in mean ferritin level during days 1-3 after the onset of symptoms and the level after 35 days was statistically significant ( $p < 0.05$ ).

## **2.4 Review of Relevant Methodology**

**Study Design.** The proposed study will be a matched, case-control study exploring the association between dietary iron intake and serum ferritin levels in children with ADHD, with and without comorbid RLS. As previously discussed, there are a number of confounding variables that have the potential to distort our comparisons. Age, gender, and socioeconomic status are such variables that have been shown to have strong influences on serum ferritin, dietary iron intake, and ADHD and RLS development. Konofal et al. matched for age and sex, and Energin et al. accounted for socioeconomic status.<sup>2,9</sup> The proposed study will follow both methods. Socioeconomic status will be based on the income-to-poverty ratio, the ratio of household income and the poverty line published by the Census Bureau for a certain family size in that calendar year.<sup>61</sup> Kranz et al. and Wang et al. both used this method in their study assessing diet quality and

socioeconomic status in data from the National Health and Nutrition Examination Surveys.<sup>18,61</sup> A low socioeconomic status will be defined as an income-to-poverty ratio less than 1, and a high socioeconomic status will be defined as an income-to-poverty ratio greater than 3. A middle socioeconomic status will be any values between those just mentioned. Individual matching on these three variables will allow the proposed study to start with subjects that have similar characteristics and to gain precision in the estimate of the effects.

**Patient Recruitment and Selection.** Because the proposed study will be case-controlled and will select for patients with ADHD, the recruitment process must be designed in an unbiased manner. Kiddie et al. performed a case-control study to measure dietary iron intake, and the subjects were recruited in a sequential manner.<sup>7</sup> The proposed study will use this technique as well.

Furthermore, as discussed in Chapter 1, the DSM-5 and IRLSSG criteria are currently the most rigorous criteria used to diagnose ADHD and RLS, respectively. All studies previously cited have used the DSM criteria, and the proposed study will also use it. The Kiddie-SADS-Lifetime Version (K-SADS-PL) is a semi-structured interview that has been validated to assess current and past psychiatric history in children according to the DSM-IV criteria, and it will be used by clinicians in the proposed study.<sup>62</sup> Currently, there is no validated, widely accepted RLS screening questionnaires for children, and so the proposed study will use versions developed by Mindell and Owens.<sup>63</sup> Inclusion criteria have been consistent throughout the reviewed studies, however exclusion criteria have varied in each study. Potential confounders have been identified, and, through matching, will be somewhat controlled for. However, exclusion criteria will be used to

further remove any bias from the study. Exclusion criteria will include: major comorbid medical problems, iron supplementation, RLS therapy, current or recent infection, children under the age of 4 or over the age of 16, and children under the age of 9 whose parents cannot read or write English.

Because we are interested in two specific disorders, we want to recruit relatively healthy children with ADHD only. Any children with major medical problems, other developmental disorders, cognitive impairment, eating disorders, or substance use disorders will be excluded from the proposed study. Each of these exclusion criteria were used in the previously described studies, and each has the ability to distort the association between dietary iron intake, serum ferritin levels, and ADHD.<sup>2,3,6,8,9</sup> Iron supplementation was part of the exclusion criteria used in Menegassi and Durá Travé et al.'s studies, and Kiddie et al. failed to account for its use in their study.<sup>6-8</sup> As previously mentioned, iron supplementation can cause discrepancies in dietary iron intake measurements, and influence serum ferritin level and the development of RLS and ADHD. Birgegård et al. found that infection can cause an increase in serum ferritin levels for up to a month after the infection is cleared, and so those children who have a current or recent infection will be restricted from the proposed study.<sup>59</sup> Furthermore, RLS therapy will be part of the exclusion criteria. The proposed study is interested in ADHD children with undiagnosed RLS. Children receiving RLS therapy will already be diagnosed.

Lastly, the age group of interest is children between 4-16 years of age. According to the American Academy of Pediatrics' Clinical Practice Guideline for the Diagnosis, Evaluation, and Treatment of ADHD in Children and Adolescents, the diagnosis for ADHD can be made at the age of 4.<sup>64</sup> Egger et al. performed a study in 2006 measuring

ADHD symptoms in preschool children, and found that the majority of ADHD symptoms occurred in less than 10% of the children assessed. The most common symptom, interruption/intrusion, was endorsed by 44.7% of parents of children without ADHD, while 100% of parents of children with ADHD endorsed the symptom ( $p < 0.001$ ).<sup>65</sup>

**3-Day Food Diary.** Dietary iron intake will be measured using a 3-day food diary, with recording done on two weekdays and one weekend day. All previous research measuring iron intake in the ADHD population that have been discussed thus far has used some kind of food diary to record iron intake. More specifically, Chen, Kiddie, Durá Travé, and Energin et al. all employed a 3-day food diary as the duration for measurement.<sup>7-9,66</sup> The 3-day food record has been validated for use in children. Crawford et al. compared it to both a 24-hour recall, done through interview, and a 5-day food frequency questionnaire in girls between the ages of 9-10. For the 3-day food record, children were educated on its use and portion sizes. Observers were assigned to watch each child at lunch, and the reporting of their meals was compared to what was seen by observers. The 3-day food record had the highest food agreement score (65% agreement) out of all methods.<sup>67</sup> In the proposed study, one weekend day will be included for meal measurement to get an appropriate balance between days that are likely to differ with respect to food intake. Furthermore, Singer et al. used 3-day food diaries to record nutrient intake in children between the ages of 3-8.<sup>68</sup> By the age of 8, children were able to fill out their own food diaries. Given these data, the proposed study will exclude any children under the age of 8 whose parents cannot read or write in English in order to prepare a food diary for them.

Dietary iron intake will be assessed by trained dieticians using the Nutrition Data System for Research (NDSR) program. The NDSR was developed by the Nutrition Coordinating Center at the University of Minnesota and has an extensive food list that allows products to be added to the database.<sup>69-71</sup> The program imputes nutrient content from labels so that total nutrient intake is not underestimated. Hyman et al. used this software to measure nutrient intake data from 3-day food records, and it has been licensed to the Yale University School of Medicine.<sup>70</sup>

**ADHD Symptom Severity.** The Conners' Parent Rating Scale (CPRS) will be used to assess ADHD symptom severity. The Conners' scale is a widely used and validated instrument for screening and assessing behavior problems.<sup>72</sup> The most updated, computerized version has strong DSM-5 connections.<sup>73</sup> Both Konofal and Oner et al. used this scale when measuring symptom severity in their sample of children with comorbid ADHD and RLS.<sup>2,3</sup> This form contains 48 items, which evaluate behavior of children assessed by their parents.<sup>74</sup> It includes oppositional behavior, inattentiveness, hyperactivity, psychosomatic, and irritability domains.<sup>3,75</sup> The form uses a four-point Likert scale, and a total score is generated.

## **2.5 Conclusion**

Several studies have shown statistically significant differences in serum ferritin levels among children with comorbid ADHD and RLS and controls.<sup>2-4</sup> However, no studies have compared these children's dietary iron intake to their serum ferritin levels. Although the literature examining dietary iron intake's relationship with ADHD suggests that an association exists between the two variables in the population of children with both disorders, there is a gap in knowledge that warrants investigation. It can be

concluded from the literature that a case-control study exploring this relationship while accounting for the potential confounders of age, gender, and socioeconomic status would be beneficial in advancing our knowledge of these two very common pediatric disorders.

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## **CHAPTER 3: STUDY METHODS**

### **3.1 Study Design**

We propose a matched case-control study to evaluate dietary iron intake among cases of children with ADHD and controls with no ADHD diagnosis, with or without comorbid RLS. Cases and controls will be matched by age, gender, and socioeconomic status. To determine if there is an association between dietary iron intake and serum ferritin levels in children with comorbid ADHD and RLS, we will identify those children in both the case and control groups who have RLS based on the “definite” or “probable” IRLSSG criteria.

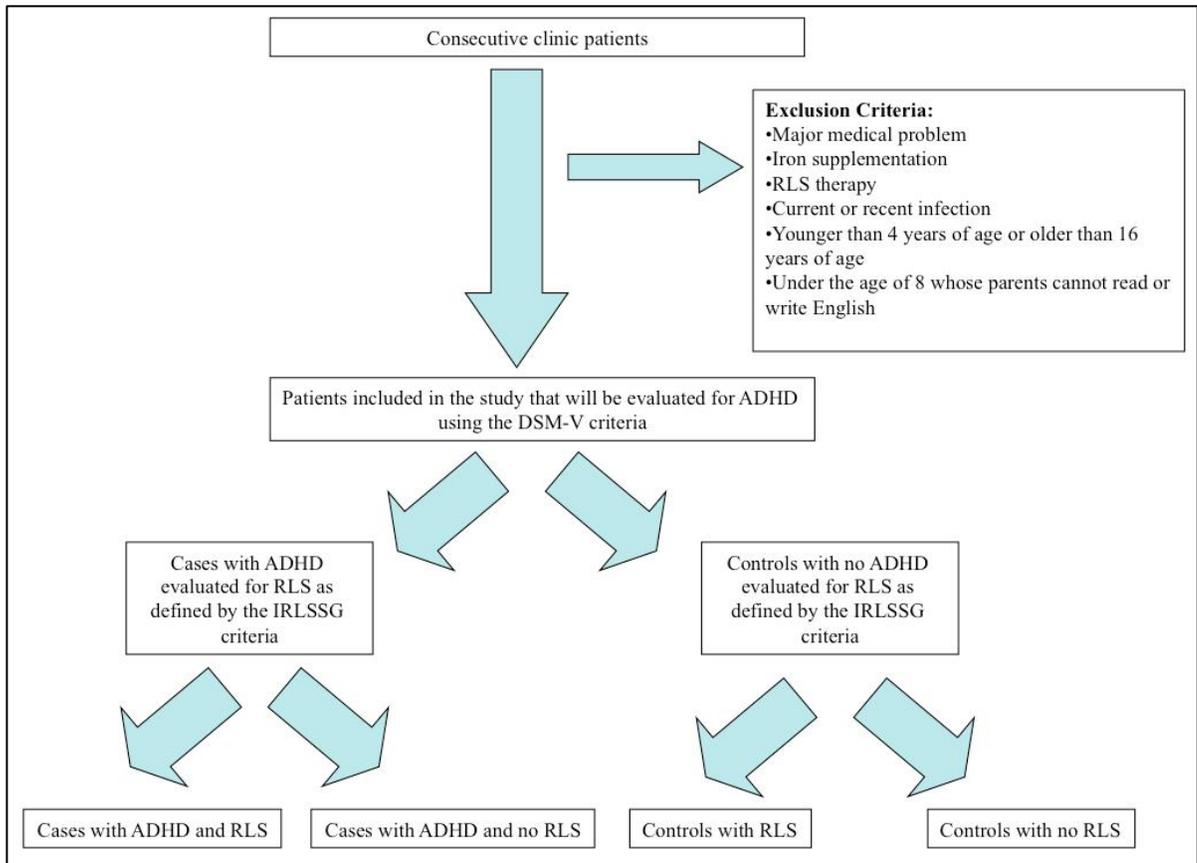
### **3.2 Study Population and Sampling**

The study population will be drawn from patients at the Yale Child Study Center Outpatient Psychiatric Clinic for Children and the University of Connecticut Child and Adolescent Psychiatric Outpatient Clinic. Consecutive patients with a diagnosis of ADHD will be recruited from children coming in for initial or follow-up assessment at these clinics from August 2016 to June 2018. Yale-New Haven Hospital and the University of Connecticut Health Center serve the same local populations in the state of Connecticut. Personal communication with staff at these sites indicated that close to 600 children with a diagnosis of ADHD receive care from providers at these two outpatient clinics. Their patient populations range from 60-67% Caucasian, 11-20% African American, and 20% Hispanic. The majority of patients are in the lower middle class or receive Medicaid. The large population of children with ADHD seen between these two

clinics, along with their very similar socioeconomic makeup, will help facilitate the matching process with controls.

Prior to enrollment, consecutive clinic patients with a diagnosis of ADHD will be screened on specific inclusion and exclusion criteria. Patients who meet eligibility will be included as participants in the study. Eligible ADHD subjects will be children aged 4-16 and will have a history of ADHD. A consultant child psychiatrist will confirm this diagnosis using the DSM-5 criteria by means of a semi-structured psychiatric interview that uses the validated Schedule for Affective Disorders and Schizophrenia for School Age Children-Present and Lifetime Version (K-SADS-PL). Children with significant health problems other than ADHD, including pervasive developmental disorders, cognitive impairment, eating disorders, or substance use disorders will be excluded. Other exclusion criteria are: use of iron supplementation, current RLS therapy, current or recent infection (within 1 month), or being under the age of 8 with parents or guardians who cannot read or write English.

The non-ADHD controls will be selected sequentially from the same clinics as the ADHD cases. They will be given the same full diagnostic assessment as those children with ADHD and will be screened for the absence of ADHD. They will be subject to the same exclusion criteria as the cases. Figure 1 illustrates the eligibility criteria and patient selection process. Because we are using a matched design, each case and control set will be the same age (difference between birthdays within 6 months), same gender, and similar level of socioeconomic status. Socioeconomic status will be assessed using the income-to-poverty ratio.



**Figure 2.** Flow diagram of patient selection.

### 3.3 Subject Protection and Confidentiality

Before we begin recruiting participants for our study, we will first obtain approval from the Yale University Institutional Review Board, the Human Investigation Committee (HIC), as part of their Human Research Protection Program (HRPP) protocol. Because our subjects are children, the first draft of the proposal will be submitted to an oversight committee, the Pediatric Protocol Review Committee (PPRC). They will review the proposal, application, and consent documents prior to submission to the HIC. This process will begin eight weeks prior to our anticipated start date of August 2016. All research personnel will complete the required Human Subjects Protection Training and Health Insurance Portability and Accountability Act (HIPAA) training prior to submission of our proposal to the review committee. They will also sign confidentiality

forms. Furthermore, because we will be working with clinicians, staff, and patients at the University of Connecticut Health Center, approval from their Institutional Review Board will be obtained as well. The Principal Investigator will submit this to the HIC.

We will provide an informed consent form to the parents or guardians of the children, along with a child assent form for patients under the age of 13 or an adolescent assent form for patients between the ages of 13-17. These can be found in Appendices A, B, and C. Each of these documents will outline a description of the study, procedures, risks and inconveniences, benefits, confidentiality under the HIPAA privacy regulations, and the voluntary nature of the study. This information will also be verbalized to the patient and his/her parents or guardians. Eligible patients, their guardians, and the Principal Investigator must sign these documents before enrollment in the study.

All information collected during the study will be de-identified following HRPP policy. Each participant will receive a study number that will be connected to their study data. Any paper documents will be catalogued by subject number and will be locked in a file system. Electronic information and records will be entered into password-protected computers that are on secure servers. Only the Principal Investigators, research assistants, and involved medical professionals will have access to the locked file system and computers. All participants will be given the research team's contact information, and will receive copies of the consent and assent forms. They will be informed that they have the opportunity to leave the study at any time they wish.

### **3.4 Recruitment**

All psychiatrists, physician assistants, and nurse practitioners involved in patient care at the Yale Child Study Center Outpatient Psychiatric Clinic and the University of Connecticut Child and Adolescent Psychiatric Outpatient Clinic will be informed about the study and its eligibility criteria. As noted above, they will approach consecutive patients about participation in our research study. If the patient agrees to a referral, the clinician will note this in the patient's chart and send their information to us to initiate participation. We will contact the patient's parents or guardians in order to set up a screening appointment. During this visit, we will obtain consent from parents and assent from children so that the patients do not feel pressured by their healthcare provider to join the study. The consent process will follow the order of referrals sequentially until the desired number of participants is achieved.

### **3.5 Study Variables and Measures**

Given that we will be implementing a case-control study, we will begin with cases of the disease. In our study, the outcome variable we will select for, as explained above, is ADHD diagnosed using the DSM-5 criteria. The primary predictor variable of interest is mean daily iron intake (measured in mg/day) recorded by patients and/or parents using a 3-day food diary. All patients will record their data in the span of 3 consecutive days, 2 of which will be weekdays and 1 of which will be a weekend day.

Secondary variables include RLS status, serum ferritin levels, and ADHD symptom severity. RLS status will be determined by a screening questionnaire that will be completed by parents or guardians for children under the age of 13 or independently by children over the age of 13. Those children whose screening questionnaire is positive

for RLS will receive a formal diagnosis from a clinician using the IRLSSG criteria for definite or probable RLS. One time fasting serum ferritin levels will be measured in ng/mL prior to initiation of the 3-day food diary. ADHD symptom severity will be measured using the Conners' Parent Rating Scale. A total scale score will be generated for each subject.

Confounding variables must also be identified and addressed in this study. Potential confounders include body mass index (BMI), race, parent education level, history of blood loss or heavy menses, stimulant use, and gastrointestinal absorption issues. These factors have the capability to influence the variables of this study, and therefore this information must be collected from each participant. We will use the Intake Questionnaire as a way to measure these confounders.

### **3.6 Data Collection**

Study enrollment, data collection, and data analysis will begin in August 2016. Once we receive referrals from clinicians, potential participants will be contacted by phone in order to set up a screening appointment at their respective outpatient clinic. During this appointment, we will meet with patients to get the consent and assent forms signed. Research assistants will record demographic information and take anthropometric measurements of weight and height at this time. The Intake Questionnaire can be found in Appendix D. Patients will then meet with a psychiatrist for a structured interview and verification of ADHD status as determined by the DSM-5 criteria. Patients and their parents will fill out the RLS questionnaire. Parents of ADHD patients will also complete the Conners' Parent Rating Scale. Lastly, blood collection will be obtained using venipuncture on the arms or hands of the patients. In addition to measuring serum ferritin,

a complete blood count (for hemoglobin, hematocrit, mean corpuscular volume, and red cell distribution width) and a full iron panel (for serum iron, transferrin, and total iron-binding capacity) will also be obtained for possible use in future analyses. 6.5 ml of blood, 4 ml for a standard biochemistry tube and 2.5 ml for an EDTA tube, will be drawn. This sample will be taken after 8 hours of fasting.

At the end of the visit, a dietician will educate the patients and their parents or guardians on how to complete the 3-day food diary. The dietician will give instruction on describing, measuring, and recording food intake. This will include the use of common household measures and food models to estimate portion sizes. As previously mentioned, participants will be asked to record in their diary for 3 consecutive days, 2 weekdays and 1 weekend day. Children older than 8 will be able to fill out the diary on their own, but those younger will require the assistance of a parent or guardian. A pre-paid, addressed envelope will be provided so that the diaries can be easily returned when the three-day period is over. A dietician will analyze the data for daily iron intake using the Nutrition Data System for Research (NDSR) program. Any additional debriefing or clarification needed will be done through a phone interview between the research assistant and the patient and/or parents.

### **3.7 Sample Size Calculation**

Our primary study hypothesis is that children with both ADHD and RLS are more likely to have a low serum ferritin and low daily dietary iron intake as compared to controls and those children with either disorder alone. Given an expected prevalence of RLS of 44%, in a fixed sample size of 200 children with ADHD, around 88 of them should fit the criteria for RLS and 112 should not. We expect the difference in iron intake

between groups to be normally distributed with a standard deviation of  $\pm 5.8$  mg. Using these values and Power and Precision V4 Software's calculator (Biostat Inc., 2000, Englewood, NJ), a sensitivity analysis for a t-test of two independent samples with common variance showed that for a fixed sample size of 200 cases, we will be able to detect a mean difference in daily dietary iron intake of  $\pm 2.5$  mg with at least 80% power and a two-tailed alpha level of 0.05. We will require an equal number of controls given our matched study design, providing us with 400 subjects. After accounting for confounders and an expected attrition rate of 7.5%, we will need a total of 490 participants for our proposed study, 245 cases and 245 controls. As stated above, around 600 children with an ADHD diagnosis attend the two designated outpatient clinics. Assuming that 50% of these patients meet the eligibility criteria and are interested in participating, we will be able to recruit our desired number of subjects. Please see Appendix H for additional information regarding the sample size calculation.

### **3.8 Analysis**

Data will be analyzed using SPSS for Windows statistical package version 19 (SPSS Inc., Armonk, NY). Numerical data will be expressed as means and standard deviations. Categorical data will be expressed as frequencies and percentages. Baseline characteristics and demographics will be obtained from the Intake Questionnaire given at the initial visit for descriptive analysis and to identify any possible confounders. Gender (male or female), stimulant medication ('yes' or 'no'), family history of RLS ('yes' or 'no'), and menstruation status ('yes' or 'no') will be described as dichotomous variables and will be analyzed with a chi-square test. Race (White, Black or African American, Hispanic or Latino, Native American, Asian or Pacific Islander, Other) and parent

education level (less than high school, high school, beyond high school) will be described as nominal variables and will be analyzed with a Fisher's Exact test. The income-to-poverty ratio (<1, between 1 and 3, or >3) will be described as an ordinal variable and will be analyzed with a Kruskal-Wallis test. Age (in years) will undergo analysis of covariance (ANCOVA). Lastly, the research assistant will collect the height and weight of each subject at the intake appointment and will calculate BMI as weight (in kg) divided by height (in meters). BMI will be expressed as a percentile relative to standardized American growth charts. It will be described as an ordinal variable (<5% is underweight, 5-84% is healthy, 85-95% is overweight, and  $\geq 95\%$  is obese). BMI will be analyzed with a Kruskal-Wallis test. If any differences are found at baseline for these variables, they will be considered covariates and will be accounted for in later analyses.

The primary independent variable, ADHD, and the secondary variable of RLS will both be treated as dichotomous variables ('yes' or 'no'). As stated above, the screening of RLS in the cases and controls will create four groups. The primary predictor variable is mean daily iron intake measured in mg/day. This is a continuous variable that will be transformed into a dichotomous variable (low mean daily iron intake or high mean daily iron intake) using a median split. Those patients whose mean daily iron intake is below the median for the distribution will be labeled with a diet low in iron and those above the median will be labeled with a diet high in iron. An odds ratio with 95% confidence intervals will be calculated for each of the 3 groups. Chi-square tests will be used to analyze the association between disease status and iron intake, and any significant differences will warrant further Chi-square testing between groups. A Chi-square test will also be used to look at this association across all groups. The secondary variable of serum

ferritin level, measured in ng/mL, will also be described as a continuous variable that will be transformed into a dichotomous variable (low serum ferritin level or high serum ferritin level). It will follow the same statistical procedure as outlined above for iron intake in order to examine the association between disease status and ferritin levels. ADHD symptom severity will be represented by the total score generated from the computerized Conners' Scale. It will be treated as a continuous variable.

To test the main hypothesis and look at the association between mean daily iron intake and serum ferritin level, Chi-square tests will again be performed within groups, between groups, and across all groups as part of the multivariate analysis. We will then test for trend between the four groups. Conditional logistic regression will be performed with a  $p < 0.05$  in order to account for possible confounding variables in the analysis. This will be done between the ADHD groups and controls, and then with a subgroup analysis between the two groups with ADHD. The final selection of confounding variables will be determined by the distribution of the study sample characteristics. Pearson correlation will be used to test correlation between mean dietary iron intake and ADHD symptom severity in each group.

### **3.9 Timeline and Resources**

Our case-control study will occur within a 2-year period. The proposal for the study will be submitted to the Pediatric Protocol Review Committee on June 1, 2016 and, after their authorization, to the HIC. We will allow 8 weeks for approval from the IRB. Consecutive enrollment of eligible participants will begin in August 2016 and will continue throughout the rest of this 2-year period. Dieticians will continuously analyze

the food diaries as they are received for mean iron intake so that these data are ready for statistical testing when the study concludes in June 2018.

In order to complete this study in the desired timeframe, we will assemble a research team that will work together at both site locations. The Principal Investigator, Dr. Meir Kryger, and Co-Principal Investigator, Olivia Rojas, will oversee the study, manage the project's finances and personnel, and ensure that it is conducted in compliance with laws and regulations. We will hire two dietitians to educate patients on the correct use of the food diary and to analyze the diaries using the NDSR software. We will also need a research assistant at each site to perform data collection, anthropometric measurements, and to assist the dietitians with follow-up of the food diaries. Two phlebotomists will be hired to draw the blood specimens, and a statistician will help us to analyze the data.

Each clinic site will provide an exam room with a computer for the initial intake visit and their respective laboratories will be used for specimen analysis. Our office will be located in the Yale Child Study Center, and it will be equipped with a password-protected computer on a secure server that will contain statistical software for data entry and analysis. We will also have a locked cabinet for all paper records collected throughout the study. All patients will receive compensation for parking at their intake visit and a pre-paid envelope in which to return their food diaries.

## CHAPTER 4: CONCLUSION

### 4.1 Advantages and Disadvantages

Some of the major advantages of our study lie in its case-control design. We will be able to select for a large group of children with ADHD at the study outset without having to sample a large pediatric population.<sup>1</sup> Although it is the most common psychiatric disorder in children, performing random sampling in a public school, for example, in order to find an adequate number of children with the disorder would require a much larger population size and much more recruitment time. The use of the two outpatient clinics will allow us to quickly identify those children meeting inclusion criteria. Also, the sample of cases will not be exposed to considerable selection bias.<sup>2</sup> We will recruit consecutive patients from a general ADHD visit, and we will not be selecting for sleep problems. Therefore, our sample group will be representative of those children seen in a general pediatric psychiatric clinic.

Secondly, we will be taking multiple steps to control for confounding variables. We have proposed a matched design based on age, gender, and socioeconomic status in order to reduce variability and allow for comparability of dietary iron intake between the cases and controls.<sup>3</sup> We expect age, gender, and socioeconomic status to be considerable confounding variables in this study, as all of these factors have been shown to influence the diet of children. We also will adhere to the strict inclusion and exclusion criteria previously presented so that any association we find between diet and serum ferritin levels will not be distorted. We will further account for confounding variables specific to

the study sample at the end of data gathering. This includes statistical techniques previously mentioned that will be used for analysis.

Next, we are using cost-effective tools to assist us in completing the study. Subjects will complete questionnaires in order to evaluate ADHD symptom severity and to screen for RLS. Dietary iron intake will be measured by a 3-day food diary that patients can easily return when complete. These different measures allow for quick and efficient participation from each subject. Furthermore, they are only expected to make one office visit for an initial assessment. Because of the minimal time requirements, we expect a small attrition rate. This will allow for more reliable and accurate results.

Lastly, the proposed study will include a large sample size and a wide age range. Studies examining the association between serum ferritin levels, ADHD, and RLS mentioned in previous chapters often found differences that were not statistically significant.<sup>4-6</sup> These studies were not fully powered, and therefore, skewed the result. Furthermore, many of the studies that laid the foundation for ours chose very narrow age ranges for inclusion of subjects. Because we are including children from preschool through high school, our results will be more generalizable.

One of the main disadvantages of this study is the potential for sampling bias when choosing controls. We are proposing a convenience sample, as controls will be sampled in the same way as cases and from the same outpatient psychiatric clinics. While this is advantageous, it may reduce the external validity of the study.<sup>1</sup> The children being considered the healthy control group may not be representative of healthy children out in the community. This may likewise be true of the children with ADHD being seen in the

clinic as compared to those children with ADHD being treated by a primary care physician.<sup>3</sup>

Another limitation of this study involves the chance of misreporting due to the use of the 3-day food diary. This is a self-reported method of measuring dietary intake, and, for this reason, there is a chance that participants could underestimate their portion sizes or energy intake. This trend has been noted to increase with the age of the child.<sup>7</sup> However, such errors in estimation have been documented to occur in other methods of dietary measure as well, including food frequency questionnaires, 24-hour recalls, and food diaries for other specified periods of time.<sup>8</sup> In addition, we anticipate that errors will be evenly distributed in the four groups. Misreporting is something that is unavoidable when looking at diet and should not bias our result.

Furthermore, it is possible that, given our calculated sample size, we will not have a large enough group of controls with RLS to make any statistically significant comparisons. The prevalence of RLS in the general pediatric population is small, around 2-4%, and so we expect a group of controls with RLS to be around 4 to 9 patients.<sup>9</sup> Our proposed study may be underpowered to detect any significant difference between this group and another. However, given that our primary hypothesis is most concerned with the group of children with comorbid ADHD and RLS, the lack of a group with RLS only will not skew our results regarding dietary iron intake.

Finally, we will not be able to detect causality between dietary iron intake and low serum ferritin levels.<sup>5</sup> For this reason, our results must be interpreted with caution. We are interested in assessing the strength of the relationship between an exposure,

dietary iron intake, and disease, the disorders of ADHD and RLS.<sup>3</sup> We will be able to calculate an odds ratio to determine the relative importance of this predictor variable in relation to the presence or absence of RLS and ADHD.<sup>1</sup>

#### **4.2 Clinical and Public Health Significance**

We expect to find an association between ADHD and RLS symptoms and the way in which children with both disorders eat. If those children with the comorbid condition tend to have dietary behaviors that favor low iron foods, this information would have valuable implications for those providing care and possibly even prevention or progression in vulnerable populations. As previously stated, overdiagnosis of ADHD has recently become a large concern in American society, and stimulant use has been on the rise. Knowing the dietary factors putting children at risk for aggravation of their diseases would help health care providers give appropriate nutritional counseling to their pediatric patients. It would also help providers to alleviate some of the symptoms that interfere with healthy daily functioning without—or with less—use of stimulant medication or iron supplementation. This would simplify care for both the patients and their parents or guardians, while at the same time saving children from exposure to unnecessary medication. In addition, it will further support the idea of routine serum ferritin level checks and RLS screening in the pediatric ADHD population.<sup>4,10,11</sup>

Furthermore, by examining the serum ferritin levels of a large group of children with both ADHD and RLS, we will get a better sense of the role of iron supplementation in this population. To the best of our knowledge, there have been no studies attempting to identify at what cut-off iron supplementation should begin in this population.

Currently, studies suggest that children with serum ferritin below 75 ng/ml require iron

supplementation. If dietary iron intake and serum ferritin levels prove to be normal, this would suggest that there is a problem with transportation of iron from the periphery to the brain. If the diets of these children are adequately enriched with iron yet they still exhibit low serum ferritin levels, then this will suggest other pathophysiology occurring in the body, such as problems with gastrointestinal absorption. Advances in the understanding of how ADHD and RLS manifest will help to improve the quality of life of children suffering from these disorders.

### 4.3 References

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**APPENDIX A: PARENT CONSENT FORM**  
**PARENTAL PERMISSION FOR PARTICIPATION IN A RESEARCH PROJECT**  
**310 FR. 2 (2016-1)**  
**YALE UNIVERSITY SCHOOL OF MEDICINE – YALE-NEW HAVEN**  
**HOSPITAL**  
**UNIVERSITY OF CONNECTICUT HEALTH CENTER**

**Study Title:** Iron Intake in Children with Attention-Deficit/Hyperactivity Disorder and Restless Legs Syndrome

**Principal Investigators:** Meir Kryger, MD, FRCPC and Olivia Rojas, PA-SII

**Funding Source:** Yale Physician Associate Program

**Invitation to Participate and Description of Project**

We are inviting your child to participate in a research study designed to look at the relationship between dietary iron intake, serum ferritin levels, and restless legs syndrome (RLS) in children with attention-deficit/hyperactivity disorder (ADHD). Your child has been asked to participate because he/she is between the ages of 4-16 or may meet the criteria of ADHD. Approximately 490 other children in New Haven and the surrounding area will be participating in the study.

In order to decide whether or not you wish your child to be a part of this research study you should know enough about its risks and benefits to make an informed decision. This permission form gives you detailed information about the research study, which a member of the research team will discuss with you. This discussion should go over all aspects of this research: its purpose, the procedures that will be performed, any risks of the procedures, and possible benefits. Once you understand the study, you will be asked if you wish your child to participate; if so, you will be asked to sign this form.

**Description of Procedures**

If you agree to your child participating in this study, your child will be asked to meet with a clinician in order to be weighed, measured, and evaluated for ADHD by a child psychiatrist through a short interview. While in the office, you and your child will be asked to fill out two short questionnaires, one regarding your child's demographics and medical history, and the other regarding symptoms of RLS. You will be asked to provide your child's ethnicity, your household income, and both parents' education level. Additionally, your child will be asked to supply approximately one 6.5 ml fasting blood sample for analysis of serum ferritin level, which is a marker for blood iron stores, blood iron levels, and whether your child has anemia. Lastly, you and your child will be asked to complete a 3-day food journal at home that you may send back through U.S. mail in order for us to determine how much iron your child consumes daily.

You will be told of any significant new findings that are developed during the course of your child's participation in this study that may affect your willingness to continue to participate.

### **Risks and Inconveniences**

Blood drawing is a relatively safe and short procedure, however there are risks. When drawing blood from the arm or hand, there is a possibility of temporary discomfort from the needle stick, bruising, redness, bleeding, or minor swelling at the site of the injection. An infection or blood clot can develop at the site, however this is rare. Some patients experience dizziness or lightheadedness when they give blood. Experienced nurses will be drawing your child's blood so that these issues are avoided.

### **Benefits**

We may identify symptoms of ADHD or RLS in your child that you weren't previously aware of but could be treated. Furthermore, what we learn about diet's influence on ADHD and RLS may help improve the treatment regimen for other children suffering from these disorders in the future.

### **Confidentiality**

Any identifiable information that is obtained in connection with this study will remain confidential and will be disclosed only with your permission or as required by U.S. or State law. Examples of information that we are legally required to disclose include abuse of a child or elderly person, or certain reportable diseases. All information collected during the study will be de-identified. A code number will be assigned to each participant that will be connected to their study data. Any paper documents will be locked in a file system, and any electronic information will be entered into password-protected computers on secure servers. When the results of the research are published or discussed in conferences, no information will be included that would reveal your child's identity unless your specific permission for this activity is obtained.

Representatives from Yale University, the Yale Human Research Protection Program and the Yale Human Investigation Committee (the committee that reviews, approves, and monitors research on human subjects) may inspect study records during internal auditing procedures. However, these individuals are required to keep all information confidential.

### **In Case of Injury**

If your child is injured while on study, seek treatment and contact the study doctor as soon as you are able. Yale School of Medicine, Yale-New Haven Hospital, and the University of Connecticut Health Center do not provide funds for the treatment of research-related injury. If your child is injured as a result of his or her participation in this study, treatment will be provided. You or your insurance carrier will be expected to

pay the costs of this treatment. No additional financial compensation for injury or lost wages is available. You do not give up any of your legal rights by signing this form.

**Voluntary Participation and Withdrawal**

You are free to choose not to have your child participate and if you do decide to have your child become a subject you are free to withdraw him/her from this study at any time during its course. Refusing to participate or withdrawing from the study will involve no penalty or loss of benefits to which your child is otherwise entitled. If your child decides not to participate or if you withdraw him/her, it will not harm you or your child's relationship with your own doctors or with Yale-New Haven Hospital or the University of Connecticut Health Center. You can call the Co-Principal Investigator at (313) 310-1320 and tell her that you no longer want to participate.

The researchers may withdraw your child from participating in the research if necessary. This includes if your child is diagnosed with a comorbid condition or if your child exhibits non-compliance to study guidelines.

**Questions**

We have used some technical terms in this form. Please feel free to ask about anything you don't understand and to consider this research and the permission form carefully – as long as you feel is necessary – before you make a decision.

**Authorization and Permission**

I have read (or someone has read to me) this form and have decided to allow my child to participate in the project described above. Its general purposes, the particulars of my child's involvement and possible hazards and inconveniences have been explained to my satisfaction. My signature also indicates that I have received a copy of this permission form.

By signing this form, I give permission to the researchers to use and give out information about my child for the purposes described in this form. By refusing to give permission, I understand that my child will not be able to be in this research.

Name of Child: \_\_\_\_\_

Parent 1 Signature: \_\_\_\_\_

Parent 2 Signature (*if applicable*): \_\_\_\_\_

Date: \_\_\_\_\_

Date: \_\_\_\_\_

\_\_\_\_\_  
Signature of Person Obtaining Permission

\_\_\_\_\_  
Date

If you have further questions about this project or if you have a research-related problem, you may contact the Co-Principal Investigator Olivia Rojas, PA-SII at (313) 310-1320. If after you have signed this form you have any questions about your privacy rights, please contact the Yale Privacy Officer at (203) 432-5919.

If you would like to talk with someone other than the researchers to discuss problems, concerns, and questions you may have concerning this research, or to discuss your rights as a research subject, you may contact the Yale Human Investigation Committee at (203) 785-4688.

**THIS FORM IS NOT VALID UNLESS THE FOLLOWING BOX HAS BEEN COMPLETED IN THE HIC OFFICE.**

THIS FORM IS VALID ONLY THROUGH:

\_\_\_\_\_

HIC PROTOCOL #: \_\_\_\_\_

INITIALED: \_\_\_\_\_

**APPENDIX B: PATIENT ASSENT FORM**  
**310 FR.1**  
**(2016-1)**  
**Child's Assent for Being in a Research Study**  
**Yale-New Haven Hospital/Yale University School of Medicine**  
**University of Connecticut Health Center**

Title: Iron Intake in Children with Attention-Deficit/Hyperactivity Disorder and Restless Legs Syndrome

Why am I here?

We are asking you to take part in a research study because we are trying to learn more about how a certain part of your food, a nutrient called iron, affects the amounts that are in your blood. We also want to know how it affects your behavior. We are inviting you to be in the study because you are between the ages of 4-16 or may have a condition called attention-deficit/hyperactivity disorder, or ADHD. If you have ADHD, you might find it hard to focus and pay attention.

Why are they doing this study?

We want to measure how much iron you are eating each day and see if this is similar to the amount that is found in your body. We also want to know if these amounts are related to certain behaviors that children show when they have ADHD. Another disease, called restless legs syndrome, can also be affected by iron. We want to see how that illness is related, too.

What will happen to me?

If you agree to be a part of this study, you will come to the office with your parents to meet with a doctor. The doctor will weigh and measure you, and also interview you to see if you have the disease called ADHD. While you are in the office, you and your parents will fill out two forms about your family and your behavior. We will also collect 6.5 ml, or two small tubes, of blood so we can measure the amount of iron in it. Once you go home, you and your parents will keep a journal of all of the foods you eat for 3 days. You will send it back to us in the mail when you finish it.

Will the study hurt?

We will be using a small needle to remove some of the blood from your arm or hand. This will be a small amount that fits into a small tube. You may feel a pinch when we first begin taking your blood, and there may be some redness or bruising on your arm or hand afterwards. The nurses drawing your blood have done this many times before, and they will do their best to make you feel as little pain as possible.

Will the study help me?

We may find that what you are eating everyday has something to do with the amounts of iron in your body. This may be causing you to have difficulty focusing or may cause you to have trouble falling asleep at night. If we find this out, we can help you with these problems. Also, you will be helping other children who may get ADHD in the future.

What if I have any questions?

You can ask any questions that you have about the study. If you have a question later that you didn't think of now, you can call me ((313) 310-1320) or ask me next time.

Do my parents know about this?

This study was explained to your parents and they said that you could be in it. You can talk this over with them before you decide.

Do I have to be in the study?

You do not have to be in the study. No one will be upset if you don't want to do this. If you don't want to be in this study, you just have to tell them. You can say yes now and change your mind later. It's up to you.

Writing your name on this page means that you agree to be in the study, and know what will happen to you. If you decide to quit the study all you have to do is tell the person in charge.

\_\_\_\_\_  
Signature of Child

\_\_\_\_\_  
Date

\_\_\_\_\_  
Signature of Researcher

\_\_\_\_\_  
Date

**APPENDIX C: ADOLESCENT ASSENT FORM**  
**ADOLESCENT ASSENT FOR PARTICIPATION IN A RESEARCH PROJECT**  
**YALE UNIVERSITY SCHOOL OF MEDICINE – YALE-NEW HAVEN**  
**HOSPITAL**  
**UNIVERSITY OF CONNECTICUT HEALTH CENTER**  
**310 FR 3 (2016-1)**

**Study Title:** Iron Intake in Children with Attention-Deficit/Hyperactivity Disorder and Restless Legs Syndrome

**Principal Investigators:** Meir Kryger, MD, FRCPC and Olivia Rojas, PA-SII

**Funding Source:** Yale Physician Associate Program

**Invitation to Participate and Description of Project**

You are invited to take part in a research study that will look at the relationship between dietary iron intake, serum ferritin levels, and restless legs syndrome (RLS) in children with attention-deficit/hyperactivity disorder (ADHD). You have been asked to take part because you are between the ages of 4-16 or may meet the criteria of ADHD. Approximately 490 other children in New Haven and the surrounding area will be participating in the study.

In order to decide whether or not you wish to be a part of this research study, you should know enough about its risks and benefits to make an informed decision. This assent form gives you detailed information about the study, which a member of the research team will discuss with you and your parents. This discussion should go over all aspects of this research: its purpose, the procedures that will be performed, any risks of the procedures, and possible benefits. Once you understand the study, you will be asked if you wish to be in the study; if you do, you will be asked to sign this form. Your parents will also be asked to allow you to be in the study.

**Description of Procedures**

If you agree to be in this study, you will be asked to meet with a clinician in order to be weighed, measured, and evaluated for ADHD by a child psychiatrist through a short interview. While in the office, you and your parents will be asked to fill out two short questionnaires, one regarding your demographics and medical history, and the other regarding symptoms of restless legs syndrome. You will be asked to provide your ethnicity, your household income, and both of your parents' education level. Additionally, you will be asked to supply approximately one 6.5 ml fasting blood sample for analysis of serum ferritin level, which is a marker for blood iron stores, blood iron levels, and whether you have anemia. Lastly, you will be asked to complete a 3-day food journal at home that you may send back through U.S. mail in order for us to determine how much iron you consume daily.

You and your parents will be told of any important new findings that are found while you are in this study that may change your decision about participating.

### **Risks and Inconveniences**

Blood drawing is a relatively safe and short procedure, however there are risks. When drawing blood from the arm or hand, there is a possibility of temporary discomfort from the needle stick, bruising, redness, bleeding, or minor swelling at the site of the injection. An infection or blood clot can develop at the site, however this is rare. Some patients experience dizziness or lightheadedness when they give blood. Experienced nurses will be drawing your blood so that these issues are avoided.

### **Benefits**

We may identify symptoms of ADHD or RLS that you weren't previously aware of but could be treated. Furthermore, the things we learn about diet's influence on ADHD and RLS may help improve the treatment regimen for other children suffering from these disorders in the future.

### **Confidentiality and Privacy**

Any identifiable information that we obtain about you during this study will stay confidential and will be shared only if you agree to it. There are also situations where we would have to release your identifiable information (your name for example) according to the U.S. or State law. Examples of information that we have to report to authorities include abuse of a child, certain reportable diseases (such as being HIV positive or having Hepatitis B), or when we believe you may harm yourself or someone else. When the results of the research are published or discussed in conferences, no information will be included that would reveal your identity unless you allow us to do so.

We understand that information about your health is personal, and we are committed to protecting the privacy of that information. If you decide to be in this study, the researchers will get information that identifies you and your personal health information. This may include information that might directly identify you, such as your name, date of birth, race, information from the intake questionnaire, and laboratory results from your blood sample. This information will be de-identified as soon as possible, which means that we will replace your identifying information with a code. The principal investigator (the person who is responsible for this research) will keep a link that matches you to your coded information, and this link will be kept safe and available only to a few people on this research team.

The information about your health that will be collected in this study includes:

- *Research study records*
- *Medical and laboratory records of only those services provided in connection with this Study.*

- *The following information: age, gender, ethnic background, religion, parent information, past medical history, and medication history.*

Information about you and your health may be used by or given to:

- *The U.S. Department of Health and Human Services (DHHS) agencies.*
- *Representatives from Yale Human Research Protection Program and the Yale Human Investigation Committee (the committee that reviews, approves, and monitors research on human subjects), who are responsible for ensuring research compliance. These individuals are required to keep all information confidential.*
- *Those providers who are participants in the Electronic Medical Record (EMR) system.*
- *Those individuals at Yale who are responsible for the financial oversight of research including billings and payments.*
- *The Principal Investigator: Meir Kryger, MD, FRCPC*

By signing this form, you let us use the information in the way we described above for this research study.

The research staff at the Yale School of Medicine and the University of Connecticut Health Center have to obey the privacy laws and make sure that your information stays confidential. Some of the people or agencies listed above may not have to obey those laws, which means that they do not have to protect the data in the same way we do. They could use or share your information in ways not mentioned in this form. However, to better protect your health information, agreements are in place with these individuals and/or companies that require that they keep your information confidential.

### **In Case of Injury**

If you are injured while on study, seek treatment and contact the study doctor as soon as you are able. Yale School of Medicine and the University of Connecticut Health Center do not provide funds for the treatment of research-related injury. If you are injured as a result of your participation in this study, treatment will be provided. Your parents or your insurance carrier will be expected to pay the costs of this treatment. No additional financial compensation for injury or lost wages is available. You do not give up any of your legal rights by signing this form.

### **Voluntary Participation and Withdrawal**

You do not have to take part in this study. Refusing to participate will involve no penalty or loss of benefits to which you are otherwise entitled (such as your health care outside the study, the payment for your health care, and your health care benefits). Your health care outside the study will not change if you do not agree to participate. However, you will not be able to enroll in this research study and will not receive study procedures as a study participant if you do not allow use of your information as part of this study. If you decide to take part in this study, and then change your mind, you can always stop and withdraw from this study at any time during its course. Withdrawing from the study will

involve no penalty or loss of benefits to which you are otherwise entitled. It will not harm your relationship with your own doctors or with Yale-New Haven Hospital or the University of Connecticut Health Center.

To stop your participation in the study, you can call a member of the research team at any time and tell them that you no longer want to take part. If you stop participating in the study, the researchers will still be able to use the information that has already been collected about you. That information could also be given to others until the end of the research study, to make sure that the study produces valid results.

The researchers may withdraw you from participating in the research if necessary. This includes if you are diagnosed with a comorbid condition or if you exhibit non-compliance to study guidelines.

**Questions**

We have used some complicated terms in this form. Please feel free to ask about anything you don't understand and to think about this research and the assent form carefully – as long as you need to – before you make a decision. We encourage that you talk to your family about your decision as well. If you come up with questions after reading this form, you can call me at (313) 310-1320.

**Authorization and Permission**

I have read (or someone has read to me) this form and have decided to participate in the project described above. Its general purposes, the things I will do in the study and possible risks and inconveniences have been explained to my satisfaction. My signature also shows that I have been given a copy of this assent form.

By signing this form, I give permission to the researchers to use and give out information about me for the reasons described in this form. If I decide not to give permission, I understand that I will not be able to be in this research.

Name of Subject: \_\_\_\_\_

Signature: \_\_\_\_\_

Date: \_\_\_\_\_

\_\_\_\_\_  
Signature of Person Obtaining Assent

\_\_\_\_\_  
Date

If you have further questions about this project or if you have a research-related problem, you may contact the Co- Principal Investigator Olivia Rojas, PA-SII at (313) 310-1320.

If you would like to talk with someone other than the researchers to discuss problems, concerns, and questions you may have concerning this research, or to discuss your rights as a research subject, you may contact the Yale Human Investigation Committee at (203) 785-4688. If after you have signed this form you have any questions about your privacy rights, please contact the Yale Privacy Officer at (203) 432-5919.

**APPENDIX D: PATIENT INTAKE QUESTIONNAIRE**

Name: \_\_\_\_\_

Age: \_\_\_\_\_

Gender (Circle One): Male or Female

Ethnicity:  White  Black or African American  
 Hispanic or Latino  Asian or Pacific Islander  
 Native American  Other

Total Household Income: \_\_\_\_\_

Parent Education Level: Father:  Less than high school  High school  Beyond high school  
Mother:  Less than high school  High school  Beyond high school

Have you ever been diagnosed with RLS?  Yes  No

If yes, and you are currently being treated, please provide the name, dose, and frequency of medication: \_\_\_\_\_

Is there a family history of RLS?  Yes  No If yes, who? \_\_\_\_\_

Do you currently have an infection:  Yes  No

Have you had an infection in the past month?  Yes  No

Do you have a history of psychiatric issues?  Yes  No

If yes, explain: \_\_\_\_\_

Do you currently use cigarettes, alcohol, or any illicit drugs?  Yes  No

If yes, please describe what you use, how much, and how frequently:  
\_\_\_\_\_

Do you have a history of gastrointestinal disorders, such as celiac disease, Crohn's disease, ulcerative colitis, or chronic diarrhea?  Yes  No

If yes, explain: \_\_\_\_\_

If you are female, have you begun menstruating?  Yes  No

If yes, when was your last menstrual period? \_\_\_\_\_

Are you currently using medication for your ADHD?  Yes  No  N/A

If yes, please provide the name, dose, and frequency of medication:

\_\_\_\_\_

Are you currently using iron supplementation?  Yes  No

Have you used iron supplementation within the last month?  Yes  No

**APPENDIX E: RLS QUESTIONNAIRE FOR PARENTS**

Screening Questionnaire:  
Restless Legs Syndrome  
(Parent Version)

Child's Name: \_\_\_\_\_

Person filling out form: \_\_\_\_\_

1. Does your child ever have "growing pains"? (Check One)

\_\_\_\_\_ never \_\_\_\_\_ occasionally \_\_\_\_\_ sometimes \_\_\_\_\_ frequently  
(less than 1x/month) (1-2x/month) (1-2x/wk to daily)

2. Does your child complain of uncomfortable or funny feelings (creeping, crawling, tingling) in his/her legs? (Check One)

\_\_\_\_\_ never \_\_\_\_\_ occasionally \_\_\_\_\_ sometimes \_\_\_\_\_ frequently  
(less than 1x/month) (1-2x/month) (1-2x/wk to daily)

	<u>YES</u>	<u>NO</u>	<u>DON'T KNOW</u>
3. Does your child:			
A. Notice funny feelings in his/her legs (or do they seem worse) when lying down or sitting?	_____	_____	_____
B. Have partial relief with movement (wiggling feet, toes, or walking?)	_____	_____	_____
C. Complain that the feelings are worse at night?	_____	_____	_____
D. Have a lot of fidgeting or wiggling of the toes or legs or the whole body while sleeping?	_____	_____	_____

4. Does your child appear restless while sleeping (thrashing around, banging feet against wall, twisting covers, or falling out of bed)? (Check One)

\_\_\_\_\_ never \_\_\_\_\_ occasionally \_\_\_\_\_ sometimes \_\_\_\_\_ frequently

(less than 1x/month)      (1-2x/month)      (1-2x/wk to daily)

5. Does your child seem more restless, fidgety or hyperactive than most children his/her age?

\_\_\_\_\_ never      \_\_\_\_\_ occasionally      \_\_\_\_\_ sometimes      \_\_\_\_\_ frequently  
(less than 1x/month)      (1-2x/month)      (1-2x/wk to daily)

6a. Has anyone in the family (including grandparents, aunts/uncles) been diagnosed with restless legs or periodic leg movements during sleep?      \_\_\_\_\_ Yes      \_\_\_\_\_ No

If so, who: \_\_\_\_\_

6b. Does anyone in the family have several problems falling or staying asleep?

If so, who: \_\_\_\_\_. Type of problem, if known: \_\_\_\_\_

7. How often, on average, does your child consume caffeine-containing beverages or food? (coffee, tea, cola beverages, chocolate)

\_\_\_\_\_ never      \_\_\_\_\_ occasionally      \_\_\_\_\_ sometimes      \_\_\_\_\_ frequently  
(less than 1x/month)      (1-2x/month)      (1-2x/wk to daily)

8. Has your child ever been diagnosed and/or treated for anemia?

Yes \_\_\_\_\_ No \_\_\_\_\_ Don't Know \_\_\_\_\_

Date, type of anemia, and treatment, if known: \_\_\_\_\_

**APPENDIX F: RLS QUESTIONNAIRE FOR ADOLESCENTS**

Screening Questionnaire:  
Restless Legs Syndrome  
(Adolescent Self-Report Version)

Your name: \_\_\_\_\_

1. Have you ever had “growing pains”? (Check one)

\_\_\_\_\_ never \_\_\_\_\_ occasionally \_\_\_\_\_ sometimes \_\_\_\_\_ frequently \_\_\_\_\_ only in the past  
(less than 1x/month) (1-2x/month) (1-2x/wk to daily)

2. Do you have uncomfortable or funny feelings (creeping, crawling, tingling) in your legs? (Check one)

\_\_\_\_\_ never \_\_\_\_\_ occasionally \_\_\_\_\_ sometimes \_\_\_\_\_ frequently \_\_\_\_\_ only in the past  
(less than 1x/month) (1-2x/month) (1-2x/wk to daily)

3. Do you ever:

	<u>YES</u>	<u>NO</u>	<u>DON'T KNOW</u>
A. Notice funny feelings in your legs (or do <input type="checkbox"/> they seem worse) when lying down or sitting?	<input type="checkbox"/>		<input type="checkbox"/>
B. Have partial relief with movement (wiggling <input type="checkbox"/> feet, toes or walking?)	<input type="checkbox"/>		<input type="checkbox"/>
C. Notice that the feeling is worse at night? <input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>
D. Have a lot of fidgeting or wiggling of your feet <input type="checkbox"/> or toes when sitting or lying down?	<input type="checkbox"/>		<input type="checkbox"/>

E. Have repeated jerking movements in toes or    
  
 legs or the whole body while sleeping?

Mindell JA & Owens JA. *A Clinical Guide to Pediatric Sleep: Diagnosis and Management of Sleep Problems*. Philadelphia: Lippincott Williams & Wilkins. 2015.

**APPENDIX G: SAMPLE PAGE FROM 3-DAY FOOD DIARY**

**Directions:** Please complete this diary for 3 consecutive days, including one weekend day. Record all food and drinks you consume as soon as possible after eating. For each item you consume, indicate the serving size, the method of preparation, and any added condiments you may use. List brands and restaurant names when applicable.

**Date:** \_\_\_\_\_

SELECT ONE: WEEKDAY or WEEKEND DAY

<b>Breakfast</b>		<b>Time:</b>
Food/Drinks		Amount
<b>Lunch</b>		<b>Time:</b>
Food/Drinks		Amount
<b>Dinner</b>		<b>Time:</b>

Food/Drinks		Amount
<b>Snacks</b>		
Times	Food/Drinks	Amount

#### **APPENDIX H: SAMPLE SIZE CALCULATION**

The sample size for the proposed study will be based off of the results of the studies done by Konofal et al. and Durá Travé et al.<sup>1,2</sup> Because our study will be case-controlled, a fixed sample size will be used. In order to determine a sufficient size, the expected prevalence of RLS will be 44%. We will include those with either a “definite” or “probable” RLS diagnosis. Multiple studies have explored the prevalence of RLS in the ADHD population, however each study used different criteria when diagnosing RLS and RLS symptoms. Because the IRLSSG criteria are the standard and most rigorous, the two studies that used IRLSSG guidelines were considered to determine sample size. As previously discussed the study done in 2003 by Konofal et al. found a 44% prevalence for “definite” or “probable” RLS in a group of children with ADHD.<sup>1</sup> In 2014, Kwon et al. conducted a cross-sectional study to determine RLS prevalence in children with ADHD, and 7.2% of subjects were diagnosed with “definite” or “probable” RLS.<sup>3</sup> However, this study had a small sample size, no control group, and did not exclude the influence of drug

therapy as Konofal et al. did. In a fixed sample of 200 children with ADHD, 88 should fit the criteria for RLS.

We will also use the study done by Durá Travé to determine standard deviation and effect size of the proposed study.<sup>2</sup> This study assessed dietary iron intake in children with ADHD and found results similar to what we are hypothesizing. Energin et al. also found results fitting our hypothesis, but stratified their results by gender.<sup>4</sup> Furthermore, there were no studies examining dietary iron intake in RLS patients, and for this reason intake as related to RLS cannot be taken into account when determining sample size. Using Durá Travé's study, we expect each group's iron intake to be normally distributed with a standard deviation of  $\pm 5.8$  mg.<sup>2</sup> They were able to detect an effect size of 3.8 mg between ADHD and a healthy control group. Because the proposed study is comparing iron intake of children with the comorbid condition with that of children with each disease alone, a smaller effect size is expected and will be estimated in order to power the study adequately. Therefore, with 200 ADHD patients, we will be able to detect a difference in daily iron ingestion of 2.5 mg with a power of 80%.

Lastly, we will gather an equal number of controls without ADHD, or 200 children, and adjust for losses to follow-up and for confounding variables. In Donfrancesco et al.'s case control study examining the relationship between serum ferritin and ADHD, an attrition rate of 7.5% was determined.<sup>5</sup> To correct for confounding, we will add 10 people to each group for every secondary variable that we are comparing. Accounting for all of this will bring us to a total of 490 participants (245 with ADHD and 245 controls without ADHD).

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