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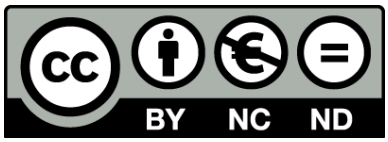
Diet and sleep in children and adolescents with Attention-Deficit Hyperactivity Disorder

Alejandra Margarita Ríos Hernández



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DIET AND SLEEP IN CHILDREN AND ADOLESCENTS WITH ATTENTION-DEFICIT HYPERACTIVITY DISORDER

Memoria presentada para optar al titulo de Doctor

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A mi familia y Eliseo Velázquez

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List of Abbreviations

AA	Araquidonic acid
ADHD	Attention-deficit/hyperactivity disorder
AFCE	Artificial food colors elimination
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
BMI	Body Mass Index
BMI-Z score	Body Mass Index Z-score
DHA	Docosahexanoic acid
DSM-IV-TR	Diagnostic and Statistical Manual of Mental Disorders
EPA	Eicosapentaenoic acid
EFSA	European Food Safety Agency
FFQ	Food frequency questionnaire
FSA	Food Safety Agency
IgG	Immunoglobulin G
IQ	Intelligence quotient
KIDMED	Mediterranean diet quality
K-SADS-PL	Kiddie Schedule for Affective Disorders and Schizophrenia-Present and Lifetime
Mg	Magnesium
PA	Physical activity
PFC	Prefrontal cortex
PUFA	Polyunsaturated fatty acids
RED	Restricted elimination diets
SPSS	Statistical package for the Social Sciences
TBHQ	Tertiary butylhydroquinone

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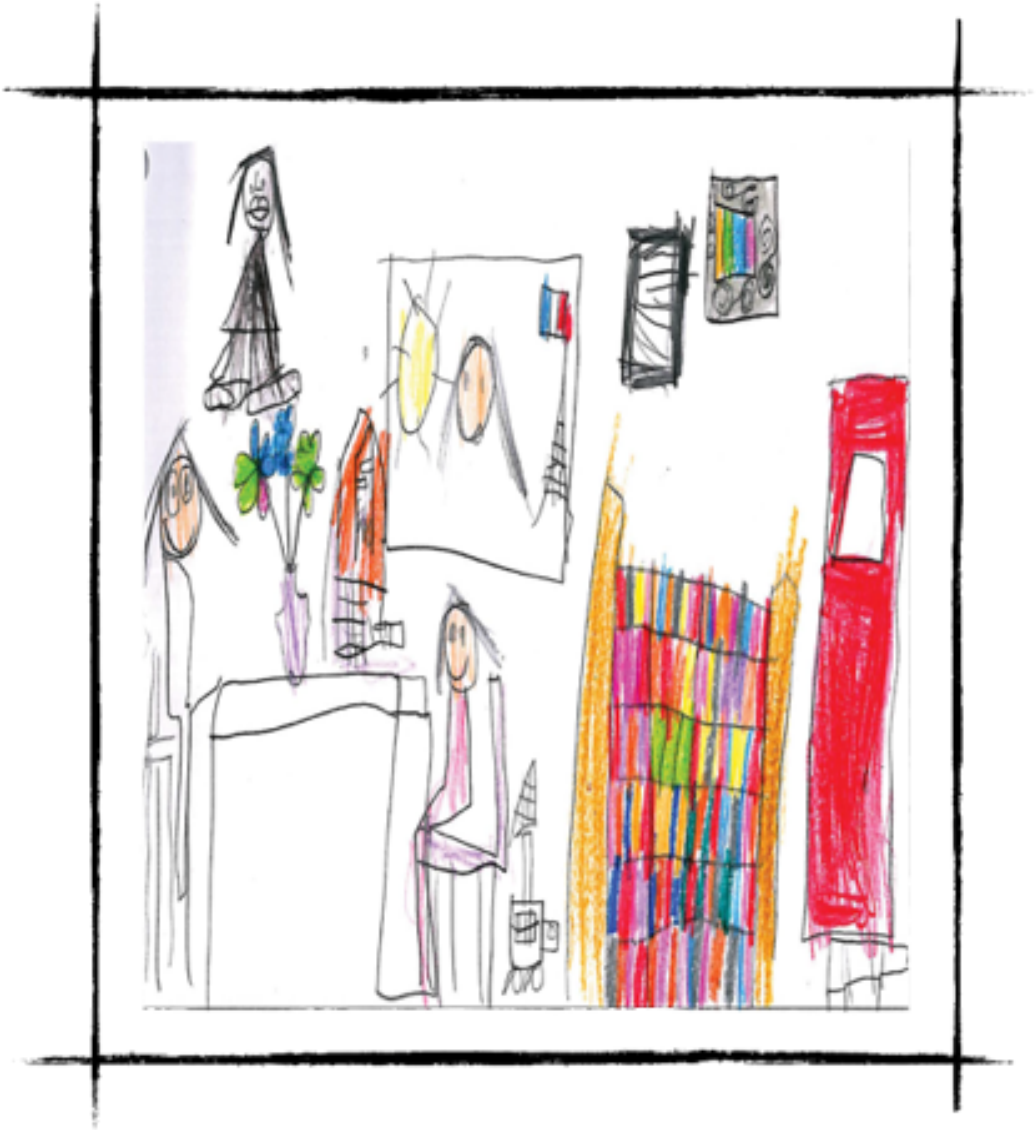
Presentation



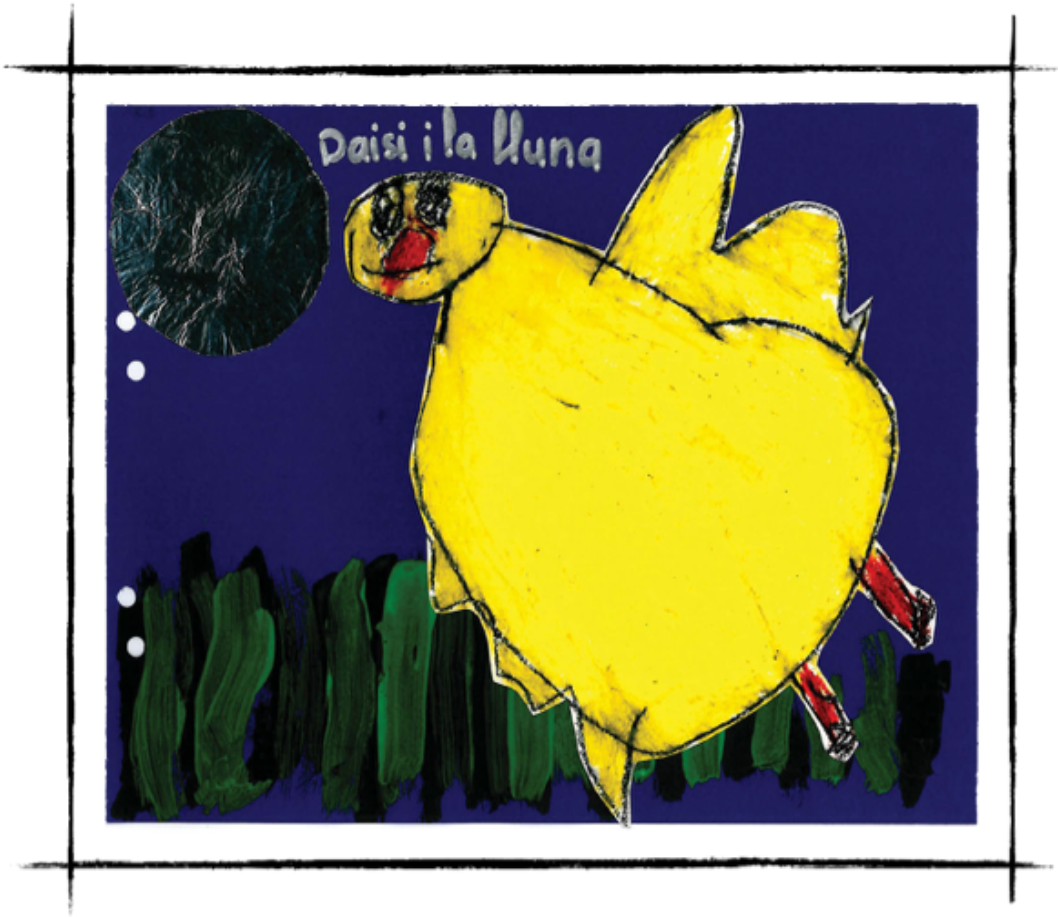
Attention-deficit hyperactivity disorder (ADHD) is one of the most common psychiatric disorders in early childhood and adolescence and its consequences can persist through adulthood. It is characterized by continuous inattention and/or hyperactivity- impulsivity. Cognitive impairments are also associated with this disorder. The etiology of ADHD continues to be debated, although several contributing factors have been acknowledged, including diet and sleep.

Several studies have analyzed the association between **dietary patterns** and ADHD. The common result is that unhealthy dietary patterns (i.e. high in saturated fat, refined sugars and low in fruits and vegetables) are associated with ADHD. Although the mechanisms linking low quality diet and ADHD are still unknown, an unbalanced diet, can lead to deficiencies in essential nutrients or higher intakes of certain food components. For instance, low levels of serum iron, ferritin, and long-chain omega-3 seem to have a relationship with ADHD. Specifically, **iron deficiency** has been reported to be a possible risk factor in the pathophysiology of ADHD. However, to date, the results regarding the potential relationship between iron deficiency and ADHD have shown some inconsistency.

On the other hand, **sleep disturbances** are particularly widespread in ADHD and they contribute to ADHD symptomatology. Moreover, there is evidence that suggest an additional and independent relationship between diet and sleep. The data on this topic are really scarce but associations between more sleep disturbance and higher intakes of carbohydrates, fat, and, most particularly, sugar have been reported. Serum iron levels and long-chain polyunsaturated fatty acid also seem to play a role in the sleep quality of children and adolescents with ADHD.

Therefore, the aim of the present thesis project is to assess the possible relationship between our characteristic and healthy dietary pattern, the **Mediterranean diet**, and the incidence of ADHD. Moreover, the influence of the dietary intake and nutrient deficiencies in children and adolescents with ADHD on their behavioral symptoms and their cognitive abilities as well as their sleep quality will be also analyzed.

1. Introduction



INTRODUCTION

1.1 Attention-Deficit and Hyperactive Disorder

1.1.1 Definition: Prevalence, characteristics and diagnostic

Attention deficit and hyperactivity disorder (**ADHD**) is one of the most common psychiatric disorders in early childhood and adolescence and its consequences can persist through adulthood. The ADHD worldwide-pooled prevalence is reported to be 3.4 % (CI 95% 2.6-4.5) (Polanczyk et al., 2015) in children and adolescents, while the prevalence in Spain is reported to be 5-8% in children and 2.5-4.0% in adolescents (González et al., 2015).

ADHD is characterized by a level of impulsivity, activity and inadequate attention, reason why many children and adolescents with ADHD have difficulty regulating their behavior and adjust to the expected standards for their age (*American Psychiatric Association,* 2013). As a consequence, they have difficulties in adjusting to their family and school environment. Some behaviors can be failure to pay close attention to details, excessive talking and difficulty organizing tasks and activities.

According to the DSM-5 the diagnostic criteria for ADHD is the following:

A. Persistent pattern of inattention and/or hyperactivity-impulsivity that is more frequently displayed and is more severe than is typically observed in individuals at comparable level of development. Individual must meet criteria for either (1) or (2):

(1) *Symptoms of Inattention,* when six or more symptoms of inattention for children up to age 16, or five or more for adolescents age 17 and older and adults are present, such as:

(a) Often fails to give close attention to details or makes careless mistakes in school work, work, or other activities

(b) Often has difficulty sustaining attention in tasks or play activities

(c) Often does not seem to listen when spoken to directly

(d) Often does not follow through on instructions and fails to finish schoolwork, chores, or duties in the workplace (not due to oppositional behavior or failure to understand instructions)

(e) Often has difficulty organizing tasks and activities

(f) Often avoids, dislikes, or is reluctant to engage in tasks that require sustained mental effort

(g) Often loses things necessary for tasks or activities

(h) Is often easily distracted by extraneous stimuli

(i) Is often forgetful in daily activities

Those symptoms of inattention have been present for at least 6 months and they are inappropriate for developmental level.

(2) *Symptoms of Hyperactivity-Impulsivity, when* six or more symptoms of hyperactivity-impulsivity for children up to age 16, or five or more for adolescents 17 and older and adults:

(a) Often fidgets with hands or feet or squirms in seat

(b) Often leaves seat in classroom or in other situations in which remaining seated is expected

c) Often runs about or climbs excessively in situations in which it is inappropriate (in adolescents or adults, may be limited to subjective feelings of restlessness)

(d) Often has difficulty playing or engaging in leisure activities quietly

(e) Is often "on the go" or often acts as if "driven by a motor"

(f) Often talks excessively

Symptoms of impulsivity

(g) Often blurts out answers before questions have been completed

(h) Often has difficulty awaiting turn

(i) Often interrupts or intrudes on others

The symptoms of hyperactivity-impulsivity have been present for at least 6 months to an extent that is disruptive and inappropriate for the person's developmental level.

B. Some symptoms of hyperactivity/impulsivity or inattention that caused impairment were present before age 12 years.

C. Some impairment from the symptoms is present in two or more settings (e.g., at school or work and at home; with friends or relatives).

D. There must be **clear evidence that symptoms interfere** with, or reduce the quality of, social, academic or occupational functioning.

E. The **symptoms are not better explained by another mental disorder** (such as a mood disorder, anxiety disorder, dissociative disorder, or a personality disorder). The symptoms do not happen only during the course of schizophrenia or another psychotic disorder.

Moreover, and according to the Diagnostic and Statistical Manual of Mental Disorders 5th edition (DSM-5) (*Diagnostic and Statistical Manual of Mental Disorders: Dsm-5*, 2013) there are three subtypes of ADHD, depending on the level of the symptoms presented:

1. ADHD predominantly *Inattentive Type* if only 6 or more symptoms of inattention symptoms have been present for the past 6 months;
2. ADHD predominantly *Hyperactive-Impulsive Type* if only 6 or more symptoms of hyperactive/impulsive have been present for the past 6 months;
3. ADHD *Combined Type* if only 6 or more symptoms of both inattention and hyperactivity symptoms have been present for the past 6 months.

1.1.2 Etiology of ADHD and pharmacological treatment.

Etiology of ADHD remains unknown, although several studies recognize the important influence of variety of factors (Barkley, 2006). The factors that have been received more attention are genetic factors, neurobiological factors and environmental factors, among them the diet. Regarding genetic risk factors (Faraone et al., 2005; Mick & Faraone, 2008), children born from parents with ADHD show a seven-fold increased risk for developing the disorder than children born from non-affected parents (Faraone & Biederman, 2000). A large longitudinal study of children aged 3–12 year estimated heritability for attention problems at 75% at all ages (Rietveld et al., 2004), while in adults heritability of ADHD has been reported to be lower percentage than children, with an estimation of 30 % in men and women (Boomsma et al., 2010).

Most of the research on understanding the neurobiology that contributes to the symptoms of ADHD has focused on an hypothesized mechanism of deregulated dopamine in the prefrontal cortex (PFC) (Arnsten, 2009b). The PFC is critical for regulating attention, memory, behavior, emotions, thoughts and conflict resolution. These regulatory abilities are often referred to as executive functions (Siddiqui et al., 2008). A growing body of evidence indicates that patients with ADHD have shown reduced size and functional activity on the right PFC (Arnsten & Rubia, 2012; Arnsten, 2009a). The prefrontal cortex is highly interconnected with much of the brain regions, and its function is particularly dependent on its neurochemical environment, which is mediated by the proper catecholamine transmission (Arnsten, 2007). Further studies in this area have suggested that the symptoms associated with ADHD are caused by a reduction in the production of dopamine and norepinephrine (Patte et al., 2016; Wu et al., 2012); neurotransmitters that play an essential role in the neurocognitive, behavioral and physical growth of the children.

Pharmacological treatment is a crucial part of ADHD management. The number of children and adolescents who receive medication treatment has increased markedly over the last two decades (Castle et al., 2007; Visser et al., 2014). The most frequently medicaments used are the stimulant drugs which include in Spain: Lisdexamfetamine (Elvanse®) and Methylphenidate (Concerta®, Ritalin®, Equasym®, Medikinet®) among others. In cases where stimulants do not work or cause unpleasant effects, non-stimulants might help. Non-stimulants medication includes: Atomoxetine (Strattera®) and Guanfacine (Intuniv®).

The primary pharmacological treatment today for ADHD is the methylphenidate (De Sousa & Kalra, 2012), from the group of stimulant medication. The drug acts by increasing activity in the brain, particularly in areas that play a part in controlling attention and behavior. The benefits of this drug often include: Improve concentration, increase attention while performing a task, less impatience and impulsiveness, increase organizing task at classroom, and improve listening skills. However, methylphenidate shows also many side effects like: Small increase in blood pressure and heart rate, loss of appetite, trouble sleeping (insomnia), headaches and stomachaches, and mood swings.

Unfortunately, stimulant medications do not cure ADHD and cannot be used to treat all cases of ADHD; neither helps the patient to have a good functioning of academic, social and cognitive difficulties that ADHD individual's experience and for last the frequently side effects mentioned before.

1.1.3 Non-pharmacological treatments

A variety of non-pharmacological treatments have become more popular as a complementary or alternative therapy to treat ADHD (Arnold et al., 2013; Bader & Adesman, 2012; Sonuga-Barke et al., 2013), some of the reasons for this popularity are due to the side effects of medication given to children. However, evidence supporting their efficacy on ADHD symptomatology remains limited or uncertain. Some authors have been analyzing the positive effect of different types of intervention such as psychological (cognitive and behavioral), physical activity, herbal supplementations and different dietary interventions. Nevertheless, the dietary interventions will be explained in detail in the following section. Regarding psychological interventions, their main goal is to improve the daily functioning of the children with ADHD by improving their behavior and family peer relationships. Psychological interventions for ADHD include cognitive and behavioral therapies. The cognitive therapy will help the children to develop a more planned and reflective way of thinking and behaving. The behavior therapy involves the parents and teachers to implement contingency management programs with children. A recent study with children with ADHD (ages 5-12) conclude that beginning with behavior modification therapy reduced significantly the classroom rule violations and indiscipline events relative to beginning with a low dose of medication (Pelham et al., 2016).

Herbal supplements are becoming more popular as they appeal to be a natural treatment. However, most of the supplements tested in children are either ineffective or represent a risk for the health. One of the herbs which has been studied for treatment is the Ginko biloba that was claim to improve cerebrovascular blood flow and to reduce hyperactivity (Ponto & Schultz, 2003). Nevertheless, the action of Ginko biloba was studied in a recent double blind, randomized, parallel group comparison of Ginko biloba and methylphenidate 50 outpatients with a diagnosis of ADHD. It was conclude that the application of Ginko biloba in the treatment of ADHD is not recommended (Salehi et

al., 2010). Another herbal supplement is the pycnogenol, which is a potent antioxidant derived from maritime pine bark. It contains concentrated polyphenolic compounds, especially procyanidins and phenolic acids. It has been found that pycnogenol improves blood circulation, therefore it may help with the cerebral blood flow, which is thought to be impaired in ADHD. However, conflicting results have been found in controlled studies (Tenenbaum et al., 2002; Trebatická et al., 2006). Another compounds used in the ADHD treatment are: melatonin, a natural hormone that regulates sleep, which has been found to improve sleep in ADHD children (Bendz & Scates, 2010; Van der Heijden, 2007), but not to improve ADHD symptoms and L-carnitine, which has demonstrated to improve symptoms of inattention but not symptoms of hyperactivity-impulsivity (Arnold et al., 2007; Van Oudheusden & Scholte, 2002).

Finally, it is worthy to point out that in the last decade, several studies have been addressed to study the potential benefits of physical exercise in children diagnosed with ADHD. The evidence suggests that physical exercise may have benefits in behavioral, neurocognitive, and scholastic performance (Gapin & Etnier, 2010; Pontifex et al., 2013; Smith et al., 2013) and in inhibitory control (Hillman et al., 2014). The putative mechanisms by which physical activity (PA) impacts cognitive performance suggest that PA might be particularly beneficial for ADHD individuals (Gapin & Etnier, 2010).

Studies carried out in ADHD children have found positive influence on the motor, cognitive, social and behavioral functioning of the children after moderate PA (Smith et al., 2013; Verret et al., 2010; Verret et al., 2012). Children with ADHD might have lower participation in sport activities, because of their mood liability, disciplinary problems, poor self-esteem, anxiety and inattention. However, research evidence has showed that ADHD children who participated in more than three sports (basketball, soccer, swimming) present fewer anxiety or depression symptoms (Kiluk et al., 2009).

Pontifex *et al.* (2013) concluded that moderately intense aerobic exercise might have positive implications on neurocognitive function and inhibitory control in children with ADHD, improving their school performance. The children could better focus and less distracted after a quick workout. It seems that this type of exercise produces enhancements in reading and in mathematics (Hillman et al., 2014). This is very relevant because children with ADHD have usually more learning problems in these two areas.

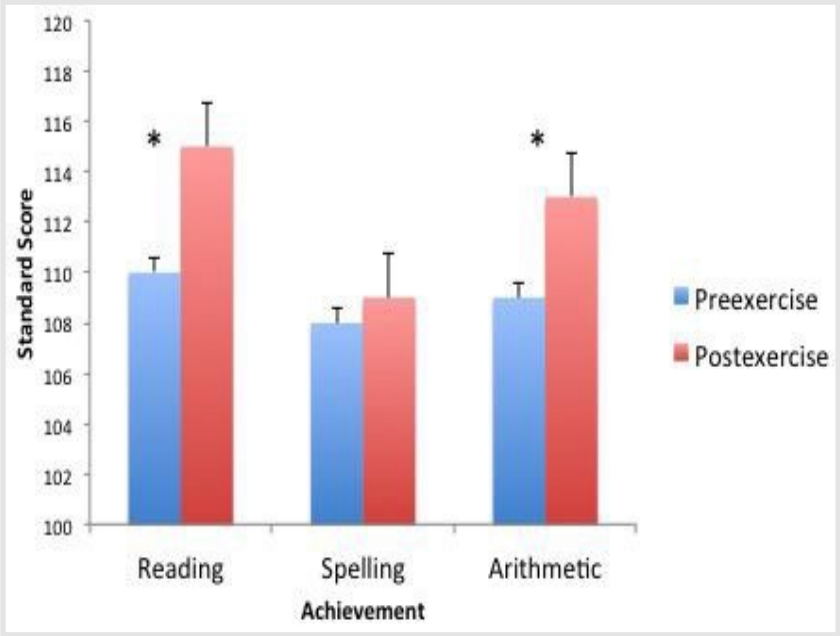


Figure 1. Mean (+SE) standard score for each session on each of the three academic performance tests done. Bars with * are statistically different (adapted from Pontifex et al., 2013).

1.2 Role of diet on ADHD

1.2.1 Dietary patterns and ADHD

Associations between different dietary patterns and ADHD have been recently examined in several cross-sectional studies **(Table 1).** This new approach is of great interest since nutrients are nearly always consumed together, and they are highly interrelated in the food matrix. The study of dietary patterns is really useful for understanding much better the role of diet in ADHD. Assessing the whole diet instead of the effects of a single nutrient on the relation between diet and ADHD may contribute even more to understand this complex relationship. The majority of studies on diet and ADHD conclude that ADHD patients have a tendency to have a poor quality diet, which could cause certain nutrient deficiencies. Those deficiencies might affect the neurocognitive, behavioral and physical development at this important stage of life.

Park *et al.* (2012) found that higher intakes of sweetened desserts, fried food, and salt were associated with more learning, attention, and behavioral problems. On the other side, a balanced diet, regular meals, and a high intake of dairy products and vegetables were associated with less learning, attention, and behavioral problems. Howard *et al.* (2011) suggested that children eating a "Western" diet, high in fried food, sweetened desserts and unbalanced, are also likely to have micronutrient and/or PUFA deficiencies. Iron, zinc or magnesium deficiencies and lower circulating levels of omega-3, higher levels of omega-6, and a lower omega-3 *versus* omega-6 ratio has been reported in children and adolescents with ADHD. An inadequate micronutrient intake, coming from an unbalanced dietary pattern, could result in suboptimal brain function in children and adolescents (Azadbakht & Esmaillzadeh, 2012). Furthermore, Van Egmond-Fröhlich *et al.* (2012) pointed out that ADHD symptoms might be associated with poor food selection rather than overeating in terms of volume.

Table 1**. S**tudies on the influence of the diet on children and adolescents with ADHD.

Reference	Design	N; age	Country	Main Findings
Ghanizadeh and Haddad (2015)	Randomized controlled clinical trial	106; 5-14y	Iran	Encouraging the children with ADHD to increase their intake of recommended diet markedly improves their attention.
Liu et al. (2014)	Cross-sectional study	417; 6-11y	China	Positive correlation between diet intake (processed meat, salty snacks) and hyperactivity index. Children's diet pattern is an important environmental impact factor for ADHD.
Woo et al. (2014)	Case- Control study	192; 7-12y	Korea	The traditional-healthy Korean[d] dietary pattern was associated with lower odds having ADHD
Van Egmond-Fröhlich et al. (2012)	Cross-sectional study	9,428; 6-17y	Germany	Poor nutrition quality and high-energy intake appear to be independently associated with ADHD.
Park et al. (2012)	Cross-sectional study	986; 8-11y	Korea	High intake of sweetened desserts, fried food, and salt is associated with more learning, attention, and behavioral problems, whereas a balanced diet, regular meals, high intake of dairy products and vegetables is associated with fewer problems.
Azadbakht & Esmaillzadeh (2012)	Cross-sectional study	375; 6-11y	Iran	Significant independent associations between the sweet[b] and fast-food[c] dietary patterns and the prevalence of ADHD.
Howard et al. (2011)	Cross-sectional study	115; 14y follow-up	Australia	A Western-style diet[a] may be associated with ADHD.

The "unhealthy" dietary patterns identified in the different studies (such as "Western", "fast food" or "sweet" patterns) were generally high in total fat, saturated fat, refined sugars, and sodium. The relationship observed between higher scores for the "unhealthy" dietary pattern and increased odds for ADHD supports the hypothesis that highly processed and energy-dense foods are linked with ADHD symptomatology (Howard et al., 2011; Park et al., 2012; Van Egmond-Fröhlich et al., 2012).

"Unhealthy" or "junk foods" besides being usually high in fat and sugars may be rich also in artificial food colorings and preservatives, which could negatively affect ADHD symptoms (Sonuga-Barke et al., 2013). It has been suggested, as it will be discussed below, that certain food additives may lead to hyperactivity or changes in neurotransmitter function (McCann et al., 2007). An interesting point is that the relationship observed between poor dietary choices and ADHD may be bidirectional (Howard et al., 2011). The results observed could be explained, especially for adolescents, by the tendency of them to experience emotional distress to crave fat-rich snack foods as a self-soothing strategy. Therefore, the results found could be more reflective of adolescent dietary preferences and cravings rather than nutritional factors alone. Also, it has been observed that a healthy diet is related to better family functioning (Ambrosini et al., 2009) and given that families of children and adolescents with ADHD are more likely to face parenting challenges, it is possible that the relationship between a "unhealthy" dietary pattern and ADHD diagnosis is mediated by poor family functioning (Howard et al., 2011).

Despite the fact that conclusions of these studies are challenging, we cannot justify that a poor dietary choice is the responsible for ADHD. The idea that dietary factors are the exclusive and sufficient explanation for childhood behavioral problems may place a barrier in the way of access to appropriate evidence-based assessment and treatment and it may place the child at unnecessary risk (Sonuga-Barke., 2015). Further studies are necessary to understand the role that the dietary pattern has in this disorder and to know which dietary approaches can benefit the ADHD symptomatology.

1.2.1.1 Restrictive dietary treatments

There are mainly two dietary treatments for ADHD, which have been tested in repeated, randomized controlled trials: the artificial food colors elimination (AFCE) and the restricted elimination diets (RED).

Artificial food colorants elimination (AFCE): The research within artificial food colorants and other additives began in the 1970s. Dr. Benjamin Feingold proposed a new diet called the "Kaiser Permanente diet" also known as the "K-P diet" or the

Feingold diet. It was hypothesized that the hyperactivity and learning problems observed in certain school children were due to the ingestion of certain foods and food additives (Stevens et al., 2013).

The K-P diet removed all foods containing artificial food colors and flavorings and certain preservatives and also food, which naturally contain salicylates (**Table 2**). It was very popular during the 70s and 80s, although it received repeated criticism because solid scientific studies demonstrating its efficacy were very scarce (Stevens et al., 2013) and subsequently support from professionals waned. The "K-P diet" is not longer used, but some of the recommendations, including the elimination of artificial colors, are still being applied. Indeed, two recent meta-analyses carried out concluded that artificial food colorants have small, but statistically significant adverse effect on ADHD symptoms in some children (Nigg et al., 2012; Sonuga-Barke et al., 2013), even though the conclusions were based on studies of limited quality, as the authors themselves pointed out.

Table 2. Dietary guidelines of the "Kaiser Permanente diet" (Izquierdo-Pulido et al.2015).

Kaiser Permanente diet	To avoid all food, medications, and cosmetic which may contain artificial colors and flavors.
	To avoid foods that naturally contain salicylates: almonds, apples, peaches, apricots, nectarines, cherries, grapes, raisins, oranges, plums, tomatoes, cucumber, coffee and tea.
	To avoid all food that may contain preservatives such as BHA, BHT, TBHQ, and sodium benzoate[a]

[a] Those preservatives were later added to the list.

In the same direction, Stevenson *et al* (2014) concluded that the artificial food color elimination is a potentially valuable treatment for ADHD but its effect size remains uncertain, as does the type of child for whom it is likely to be efficacious. The authors added the urgent need for studies using more redefined methodologies with blind evaluation to unselected samples of children with ADHD and also the concern that some studies of food colorings and additives were undertaken some time ago, so the findings could be no clear as diet and food products have changed markedly.

The possible mechanisms by which the food colorants and other additives may trigger symptoms are not well understood (Rowe & Rowe, 1994). Therefore, the controversy about the hypothesis that certain food colorants and additives mainly may cause hyperactivity and inattention in children both ADHD diagnosed or without this disorder is still open. Some authors strongly affirm that these additives do not cause ADHD (Biederman & Faraone, 2005; Pelsser et al., 2010), relaying in the fact that the symptomatology of ADHD is different from those induced by artificial coloring (McCann et al., 2007; Rowe & Rowe, 1994). The last ones have been associated with more irritability and insomnia than restlessness and inattention.

In 2007, a study funded by the Food Safety Agency (FSA) from UK and conducted by McCann *et al.* (2007) had a high impact on the public opinion. The authors provided statistically evidence on the relationship between the consumption of certain mixtures of artificial food colorings (tartrazine, quinoline yellow, sunset yellow, azorubine, cochineal red and allura red) and an artificial preservative (sodium benzoate) and the increase of the hyperactivity in children of 3 years and also in children from 8 to 9 years. In view of these results, the FSA recommended to parents with hyperactive children to consider limiting the intake of these colorants and preservatives. The study, however, has certain methodology weaknesses, as the authors themselves recognized in their publication. The changes observed in the hyperactivity children were very small relative to the inter- individual variation, while the changes in behavior were not evident in all the studied children. Furthermore, it was not possible to extrapolate the study findings to each single additive, which was in the mixture assayed. Moreover, information about the possible biological mechanisms was not provided.

While neither the EFSA (European Food Safety Agency) nor the European Commission have issued any cautious recommendation, now days, in the European Union, is required on the food packaging the following warning *"This product may have adverse effect on activity and attention in children"* when sunset yellow (E110), quinoline yellow (E104), azorubine/carmoisine (E122), allura red AC (E129), tartrazine (E102) and cochineal natural red (E124) are employed in foods and beverages.

There is a consensus in the scientific community about the need for more studies on the association between artificial colorings and hyperactivity and ADHD. It is required some caution before advising a complete restriction of foods containing these colorings. The imposition of a diet completely free of artificial colorings should not be done until a reliable methodology is developed to identify which colorant or colorants may be responsible, and who is really sensitive to these compounds, given the inter- individual variation observed.

Restricted elimination diets (RED) or few foods diets: A restricted elimination diet (also called oligoantigenic) removes most foods that may have antigenic or allergenic potential, such as milk and dairy products, eggs, nuts and some fruits, among others. It is thought that ADHD may be, in some children, a hypersensitivity reaction to certain foods (Millichap & Yee, 2012; Pelsser et al., 2010; Stevens et al., 2013). Therefore, according to this allergic hypothesis, there would be foods that induce high levels of IgG, leading to a relapse in ADHD child behavior, while the intake of those that does not induce IgG or very low levels of them, would not cause a recurrence in ADHD symptoms (National Institute of Mental Health). While interesting the hypothesis, it has not yet been fully demonstrated. Pelsser *et al.* (2010) carried out a study about restricted elimination diets with uncertain results. They did conclude that the children who responded to the dietary intervention, independently of whether IgG levels were high or low, showed a decrease of 20.8 points on the ADHD rating scale (ADHD-RS) and 11.6 points on the Conners Scale (Conner's Score). However, the determination of IgG levels was not useful, since the levels of IgG and symptoms of ADHD were totally independent.

More recently a meta-analysis on ADHD, restriction diet and food color additives has been published (Nigg et al., 2012), concluding that a restriction diet benefits some children with ADHD since it reduces ADHD symptoms; however, the authors themselves strongly recommended a renewed investigation of diet and ADHD. From a practical point of view, the restricted elimination diets are very difficult to follow, both for ADHD patients and for the families. Moreover, children and adolescents who are prescribed to follow a different diet than their friends may influence in their behavior, creating unnecessary stress situations (Kanarek, 2011;

Stevens et al., 2013). To summarize, restricted elimination diets may be beneficial, but large- scale studies are needed, using blind assessment, and including assessment of long-term outcome. On the other hand, artificial food color elimination is a potentially valuable treatment but its effect size remains uncertain, as does the type of child for whom it is likely to be efficacious. Three recommendations have been suggested for the design of future studies: 1) To have a sample of children with ADHD who have not been selected on the basis of previous responses to food constituents, 2) To include observations of the children´s behavior by a reporter who is truly blind as to dietary treatment, and 3) To control for nonspecific treatment effects (Sonuga-Barke et al., 2013; Stevenson et al., 2014).

1.2.2 Simple sugars

Diet rich in simple sugars or certain food additives like artificial dyes have been also associated with increased hyperactivity in both healthy children and children diagnosed with a behavioral disorder. However, this relationship has not been confirmed with strong and scientific evidence on the impact of simple sugars or artificial colors on the symptoms of ADHD. Wolraich et al. (1995) concluded that the sugar does not have affect, cognitive performance or behavior in healthy children, although minor effects are discarded in certain subgroups, characterized by an increase in the duration of episodes of aggression or an increase the lack of attention (Wender & Solanto., 1991). However, more studies are need to be done, as another study by Kim and Chang (2011) suggested that increased consumption of sugar is positively correlated with higher levels of hyperactivity and attention deficit. It has pointed out that this possible cognitive impairment resulting from the intake of simple sugars would be caused by the reactive hypoglycemia that occurs after the increment of the glucose consequence of the ingestion of simple sugar, to which are more sensitive children than adults. For this reason, it has been recommended to avoid abuse of foods high in sugars of rapid absorption (i.e. high glycemic index) in children with ADHD. This could help to prevent the exacerbation of some symptoms of this disorder (Newmark, 2009; Millichap & Yee, 2012). In relation to the possible effects of carbohydrate intake on sleep in children with ADHD, a recent study by Blunden et al. (2011) has shown a relationship between diet and sleep in a group of 88 children aged 6-13 years with ADHD. In this group, 30% of children had sleep disturbances and, among them, those

who showed greater sleep disturbance also had a higher intake of carbohydrates (especially simple sugars) and fats. Other studies have confirmed that skipping breakfast or substituting it for a sugary drink impairs attention and episodic memory in children (Wesnes et al., 2003).

1.2.3 Essential fatty acids (omega-3 and omega-6)

The intake of fatty acids, especially the omega-3 fatty acids, has received special attention in the treatment of ADHD. Many authors have found lower plasma levels of these fatty acids in children with ADHD when compared with controls (Colter et al., 2008; Hawkey & Nigg, 2014). The omega-3 fatty acids are essential and fundamental to the successful development and brain function. Some authors suggest that this deficiency could result in altered levels of serotonin and dopamine (Young & Conquer, 2005; Chalon, 2006) neurotransmitters implicated in the pathophysiology of ADHD. LaChance et al. (2016) recently suggested that the omega-6/omega-3 ratio might be more important than absolute levels of them separately. They found that children and youth with ADHD have elevated ratios of omega-6/omega-3 compared to controls without ADHD. High omega-6/omega-3 ratio can alter cell membrane properties and increase the production of inflammatory mediators. Omega-3 fatty acids are anti-inflammatory and therefore, a high dietary omega-6/omega-3 ratio could promote neuro-inflammation (Young & Conquer, 2005). The western dietary pattern is rich in omega-6 fatty acids and poor in omega-3 fatty acids. It has been reported, as it has been pointed out above, that children and adolescents who adhere to a western dietary pattern may have an increased risk of ADHD (Howard et al., 2011).

In some studies, the supplementation with omega-3 has showed to improve the ADHD symptomatology, but in other cases the results of various controlled trials have been contradictory. That could be due to the fact that the studies have involved different populations, treatment formulations and doses, as it is showed in Table 3. Recently, two meta-analyses of randomized placebo-controlled trials have reported a small but beneficial effect of omega-3 supplementation on reducing symptoms of ADHD (Bloch & Qawasmi, 2011; Sonuga-Barke et al., 2013).

Table 3. Research studies on omega-3 and omega-6 fatty acids in ADHD children and adolescents.

Study	Study design	Main Findings
Laura LaChance et al. (2016)	Meta-analysis	Children and adolescents with ADHD have elevated ratios of both blood omega-6/omega-3 and AA/EPA fatty acids compared to controls.
Cooper et al. (2016)	Systematic review and meta-analysis	Omega-3 PUFA reduces emotional labiality and oppositional behavior in children with ADHD.
Parletta N et al. (2016)	Case-control	Children with ADHD had low levels of EPA, DHA and AA and high ratio of omega-6/omega-3 and these correlated significantly with symptoms.
Widenhorn-Müller et al. (2014)	Randomized placebo-controlled intervention trial	Supplementation improves working memory function in ADHD children.
Gillies et al. (2012)	Systematic review	A combination of omega-3 and -6 is not statistically significant in the treatment of ADHD.
Bloch & Qawasmi (2011)	Systematic review and meta-analysis	Supplementation was statistically significant beneficial for children with ADHD. EPA's effect was bigger than DHA' effect.
Transler et al. (2010)	Placebo-controlled studies	A daily supplementation (for 4 months) with a mixture of omega-3 and -6 decreased frequency and severity of symptoms.
Colter et al. (2008)	Case-control	Children with ADHD have lower omega 3 and -6 plasma levels than healthy children.

1.2.4 The effect of micronutrient deficiencies on ADHD symptoms

1.2.4.1 Iron

Iron is an essential element that participates in a wide variety of metabolic process. It also has an important function in the central nervous system, playing a number of roles in the neurotransmission. Iron is primarily storaged in the liver, spleen and bone marrow and the majority of iron is bound to ferritin. Therefore, serum ferritin is the most convenient laboratory test to estimate iron stores (Moran & Lowe, 2016).

Significantly low levels of ferritin have been observed in children with ADHD, correlating with the severity of the symptoms. Konofal et al. (2004) reported, in a case-control study, lower serum ferritin levels in children with ADHD when comparing with controls. Moreover, the lower levels of ferritin were correlated with more severe general ADHD symptoms. Later on, Oner et al. (2012) suggested that hyperactivity was significantly associated with ferritin levels but cognitive measures were not associated.

The role of iron deficiency in the physiopathology of ADHD is not well known, but is supported by several hypotheses:

1. Iron acts as an essential coenzyme for synthesis and catabolism of monoamine neurotransmitters, which are involved in the pathophysiology of ADHD (Cortese et al., 2008).
2. Iron deficiency is associated with decreased expression of dopamine transporter. They have been linked genetic variations of the dopamine transporter gene with a certain genetic vulnerability to ADHD (Wigglesworth., 1998).
3. Iron deficit has been reported in children with cognitive and behavioral impairments that normally include inattention and hyperactivity. However, other studies did not confirm these findings. Several authors have pointed out that a possible reason is that there is no universal threshold ferritin to estimate iron deficiency with physiological effects, without reaching a state of anemia. The studies published to date have used different levels of ferritin as a threshold value for deficiency, which in some cases could justify the variability in the conclusions (Cortese et al., 2009; Oner et al., 2012; Donfrancesco et al., 2013).

Several clinical trials have been carried out about the effectiveness of iron supplementation but the results obtained are not entirely conclusive (Konofal et al., 2008; Bloch & Mulqueen, 2014). The first study done with iron supplementation was in 1970s by Sever et al. (1997) who observed a significant decrease of the Conners' Parent Rating Scale scores after iron supplementation in ADHD children without iron deficiency Later on, Konofal et al. (2008) in a double blind, placebo-controlled, randomized trial, found that the iron supplementation (80 mg/day) improved ADHD symptoms in children with low serum ferritin levels.

More studies have been carried out, as it is summarized in Table 4, but findings are still controversial. As it has been pointed out before, the main problem it is the lack of a global threshold ferritin value for estimating the iron deficiency showing physiological effects, but without reaching an anemic state.

Table 4. Research studies on iron in ADHD children and adolescents.

Study	Study design	Main Findings
Song li Ahn et al.(2016)	Case-control	Lower levels of blood iron were associated with ADHD symptom severity, IQ, and frontal lobe-mediated neurocognitive function.
Bener (2015)	Case-control	Deficiency of serum iron, ferritin, calcium and vitamin D were associated with ADHD.
Donfrancesco et al. (2013)	Case-control	No significant relationship between serum ferritin levels and ADHD.
Menegassi Márcia et al. (2010)	Cross-sectional	Peripheral markers of iron status and food intake of iron do not seem to be modified in ADHD children
Konofal et al. (2008)	Double-blind, placebo-controlled, randomized trial	Supplementation (80 mg/day) appeared to improve ADHD symptoms in children with low serum ferritin levels.
Oner et al. (2010)	Cross-sectional	Hyperactivity was significantly associated with ferritin levels but not with cognitive measures.
Konofal et al. (2004)	Case- control	Serum ferritin levels were lower in children with ADHD than controls. Lower ferritin levels were correlated with more severe ADHD symptoms.

1.2.4.2 Zinc

Zinc plays a key role in the immune function, growth and development and reproduction, and is also necessary for the brain development. It is an essential cofactor for more than 100 enzymes and is crucial for the conversion of the pyridoxine (B6) to its active form. This mineral is also necessary for the conversion of tryptophan to

serotonin. Additionally, zinc is involved in the production and regulation of melatonin, which is involved in the metabolism of dopamine, and is also cofactor of delta-6 desaturase, a key enzyme in fatty acid synthesis (Dodig-Curković et al., 2009).

Several studies published to date have shown low concentrations of zinc (plasma, erythrocytes, hair, urine) in children diagnosed with ADHD compared with controls (Arnold et al., 2005; Kiddie et al., 2010). However, some of these studies were conducted in countries such as Turkey and Iran in which there may be endemic deficiencies of this mineral. The zinc deficiency could occur for multiple reasons such as: a lower consumption of zinc food sources (Kiddie et al., 2010), a lower micronutrient absorption, an increased excretion of the mineral and/or the result of the interactions between zinc and certain drugs or food additives (Arnold et al., 2005). Regarding zinc supplementation, two placebo-controlled double-blind studies have found positive results about supplementing this mineral. In the first study, Bilici et al. (2004) conclude that supplementation was significantly better to placebo in reducing the symptoms of hyperactivity and impaired socialization in patients with ADHD while, the second done by Arnold et al. (2011), concluded that zinc supplementation alone for 8 weeks did not improve inattention, but when combined with pharmacological treatment the optimal dose of amphetamine was reduced by 37%. On the other hand, Ward et al. (2009) studied the influence of tartrazine (artificial coloring) over the levels of zinc in hyperactive children. They observed that tartrazine caused a decrease in the concentrations of zinc in serum and saliva and an increase of zinc in urine. Additionally it was observed deterioration in the emotional responses and behavior of the hyperactive children of the study.

However, the data available so far do not justify that low levels of zinc cause ADHD, neither to recommend a generalized supplementation of zinc to all the children with the disorder (Table 5). Future research that include different aspects that might have influence (relationship of zinc with other nutrients, possible interactions with food components or drugs, etc.) are needed to determine the actual role of this micronutrient in this psychiatric disorder.

Table 5. Research studies on zinc in ADHD children and adolescents.

Study	Study design	Main Findings
Farida Elbaz et al. (2016)	Case-control	Children with ADHD have lower levels of zinc, cooper and magnesium compared to both laboratory references range and to normal controls in both hair and serum. Zinc was correlated with hyperactivity, inattention and impulsivity.
Salehi et al. (2016)	Double blind and randomized trial	Zinc supplementation accompanied by the pharmacological treatment improves symptoms of ADHD.
Arnold et al. (2011)	Placebo-controlled double-blind	Zinc supplementation alone (8 weeks) did not improve inattention, but when combined with pharmacological treatment, the optimal dose of the drug was reduced by 37%.
Bilici et al. (2004)	Placebo-controlled double-blind study	Supplementation was significantly better to placebo in reducing symptoms of hyperactivity, impulsivity and impaired socialization in patients with ADHD.
Akhondzadeh et al. (2004)	Double blind and randomized trial	Supplementation might be beneficial in the treatment of children with ADHD

1.2.4.3 Magnesium

Similar to what occurs with iron and zinc, several studies have also noted a relationship between low serum magnesium levels and ADHD. In a case-control study conducted by Kozielec and Starobrat-Hermelin (1997) with Polish children with ADHD, it was observed that 95% of these children showed deficiency in magnesium compared with controls. The authors observed that hyperactivity was reduced in ADHD children after 6 months of magnesium supplementation (200 mg/day). Similar results were observed by Mousain-Bosc et al. (2004) who found lower levels of magnesium in 30 of 52 hyperactive children. They also showed that the symptomatology (hyperactive, sleep problems, and poor school performance) was improved after magnesium supplementation (100 mg/day) combined with vitamin B6 for 6 months (Mousain-Bosc et al., 2004). In a study done by German researchers (Huss & Stauss-Grabo, 2010), after a supplementation (omega-3, omega-6, magnesium and zinc) for 12 weeks to children diagnosed with ADHD (n=810), it was observed that most of the individuals reduced their symptomatology of attention deficit and hyperactivity. The authors concluded that

considering the benefits observed and the low associated risk, the supplementation with omega-3 in combination with zinc and magnesium could be recommended.

In the same line with other micronutrients, routine magnesium supplementation in children with ADHD is not justified, since more studies are needed to determine whether supplementation with magnesium or with other micronutrients such as iron or zinc, can provide an improvement of the quality of life of the patients with ADHD.

Table 6. Research studies on magnesium in ADHD children and adolescents

Study	**Study design**	**Main Findings**
Elbaz et al. (2016)	Case-control	Children with ADHD have lower levels of magnesium a compared to both laboratory references range and to normal controls in both hair and serum. Also, lower levels of magnesium were correlated with hyperactivity, inattention and impulsivity.
Huss et al. (2010)	Observational study	Hyperactivity and inattention of most of patients was reduced after supplementation for 12 weeks with a combination of omega-3 and 6, magnesium and zinc.
Mousain-Bosc et al. (2004)	Placebo-controlled double-blind	Magnesium supplementation (100 mg/day) combined with vitamin B6 for 6 months improved symptomatology.
Kozielec and Starobrat-Hermelin (1997)	Case-control	95% of ADHD children showed deficiency in magnesium comparing to controls. After 6 months of magnesium supplementation (200mg/day) hyperactivity was reduced.

In summary, the use of supplements of iron, omega-3 or zinc is not entirely supported by the current clinical evidence as primary treatment for ADHD or related conditions, so further research in this area is clearly justified. However, and given their relative safety and overall health benefits, they can offer a promising approach complementing the usual treatments.

1.3 Role of the diet on sleep in ADHD patients

1.3.1 Sleep disorders in children with ADHD

A growing number of literature reports an association between ADHD and sleep disturbances (Owens et al., 2000; Cortese et al., 2009; Yoon et al., 2012; Hvolby, 2014). According to the literature, the prevalence of sleep disturbances in ADHD individuals is reported to be between 25 and 55% (Sung et al., 2008). The most common sleep disorders in children with ADHD are: resistance to bedtime, difficulty-maintaining sleep, sleep breathing disorder, increase movements during sleep, and daylight sleepiness (Owens et al., 2000; Golan et al., 2004; LeBourgeois et al., 2004) compared with children without ADHD. Diagnosis of sleep disorders can be analyzed by a subjective criteria (e.g., sleep diaries or questionnaires) and objective criteria (e.g., polysomnography (PSG), actigraphy). The PSG is the "gold standard" test to confirm the diagnosis; however, the advantage of actigraphy above PSG is that it allows collecting data for a longer period of time in the subject's home and it has a lower cost.

The association of sleep problems with ADHD is complex as it could be that the symptoms of the disorder exacerbate the sleep disturbances or the other way that the sleep problems may lead to the development of ADHD. There is evidence that chronic disruption of nighttime sleep has social, emotional, and cognitive implications in the quality of life of the children with ADHD and their families (Yürümez & Kılıç, 2016). It is also known that the use of psychostimulant medication to treat the ADHD may impair sleep (Lim et al., 2008). On the other hand, it has also been reported that a higher intake of carbohydrates, fats and sugars may lead to more sleep problems in ADHD children (Blunden et al., 2011). Taking into consideration all this factors, a multimodal treatment is recommended to improve the quality of life of the child and family and to decrease the severity of the ADHD symptoms (Hvolby, 2014).

1.3.1.1 Association between sleep disorders and nutrients

Sleep and diet are factors highly interrelated in the symptomatology of ADHD. Some evidences have been found about the relationship between diet and sleep, showing that sleep has an influence on dietary choices (Blunden et al., 2011; Beebe et

al., 2013; Hogenkamp et al., 2013) but also that diet may influence sleep (Afaghi et al., 2007; Peuhkuri et al., 2012a; Grandner et al., 2013;). In recent studies, some authors have reported that children with sleep disturbances showed a relative high intake of carbohydrates, sugar, fat, polyunsaturated fat, cholesterol and a low intake of dietary fiber (Blunden et al., 2011; Grandner et al., 2013) This unhealthy pattern diet could lead to deficiencies of certain nutrients that may disrupt sleep by altering neural responses. Therefore and having evidence that children with ADHD tend to have a poor quality diet and more sleep problems, is interesting to explore if there is an association between sleep and nutrient intake. It has been pointed out those deficiencies in certain nutrients, such as group B vitamins and minerals, may disrupt sleep since their influence on the secretion of melatonin (Peuhkuri et al., 2012a). However, studies done specifically on the field of sleep disorders and nutrients in ADHD patients are really scarce.

1.3.1.1.1 Energy and macronutrients

Only a few observational studies have examined whether there is an association between habitual sleep patterns and diet, mainly focused on sleep duration (Grandner et al., 2010; Grandner et al., 2013). In children with ADHD, Blunden et al. (2011) reported that children who had a higher intake of carbohydrate, fats, and, most particularly, sugar showed more sleep problems. However, clinical trials assessing the impact of single macronutrients, such as carbohydrates, fats or proteins are scarce. Some studies have supported that protein fragments or amino acids, such as tryptophan, when administered in pharmacologic doses, may have sleep-promotion influence, even though the clinical evidence is still challenged (Grandner et al., 2010; Grandner et al., 2013).

According to cross-sectional studies, the influence of dietary fat on sleep duration is conflicting, with contradictory results, since in some studies a trend toward longer sleep duration is observed in subjects consuming more energy from fat, whereas in other studies, short sleep duration was correlated with increased fat intake (Peuhkuri et al., 2012a). In a large study, with 4,552 individuals, it was found that difficulty falling asleep was associated with greater intake of hexadecanoic acid, a saturated fat, whereas it was associated with less intake of dodecanoic acid, a saturated fatty acid, (Grandner et al., 2013). However, the physiological mechanisms of those associations are still unknown. Regarding very long-chain polyunsaturated fatty acids, it is known

that they play an important role in the pineal gland and in the production of melatonin (Catalá, 2010). Despite this clear biological connection to sleep, no improvement either in subjective or objective parameters of sleep or secretion of melatonin was noticed in more than 100 adults with chronic insomnia after supplementation with polyunsaturated fatty acid capsules (Peuhkuri et al., 2012a)

1.3.1.1.2 Vitamins and minerals

Vitamins B are needed for the syntheses and release of certain neurotransmitters and neuro-hormones that are involved in the regulation of sleep and of the circadian cycle, such as melatonin (Partinen & Westermarck, 2014). B vitamins have been advanced as a preventive for insomnia based on research that suggests deficiencies in vitamin B6 promote psychological distress and ensuing sleep disturbances. Less intake of vitamin B6 was associated with greater excessive daytime sleepiness. In the brain, vitamin B6 is needed in the synthesis of serotonin from tryptophan, which is converted to 5-Hydroxytryptophan (5-HTP) and consequently to serotonin by the enzyme called aromatic L-amino acid decarboxylase (AADC). In a study done with elderly woman with poor sleep, it was observed a had significantly lower vegetable consumption and vitamin B6 intakes compared to good sleepers (Huang et al., 2013). On the other hand, folic intake was independent inverse associated with sleep disturbance (Beydoun et al., 2014) and it was found a lower intake of carbohydrates, folic acid and B12 in insomniacs comparing to normal sleepers (Zadeh & Begum, 2011). It is known that vitamin B12 contributes to melatonin secretion (Peuhkuri et al., 2012a) and it has been observed that treatment with different doses of vitamin B12 has a potentially beneficial effect on the sleep-wake rhythm and in delayed sleep-phase syndrome in healthy subjects measured by actigraphy equipment (Peuhkuri et al., 2012a).

Reduced **vitamin D** intake has been also associated with later sleep timing and increased subjective napping in postmenopausal women (Grandner et al., 2010) and also with less difficulty maintaining and falling sleep (Grandner et al., 2013). In another study, low levels of total serum of 25-dihydroxyvitamin D are associated with poorer sleep including short sleep duration and lower sleep efficiency (Massa et al., 2015). Now, it is well known that several nuclei in the hypothalamus and brainstem which are involved in sleep have high levels of vitamin D receptors (Gominak & Stumpf, 2012).

It is worthy also to point out that there is a relationship between vitamin D and mood. Serum 1,25-dihydroxyvitamin D levels might affect the levels of serotonin in the hypothalamus and thereby enhance the synthesis and transmission of serotonin, leading to improvement in mood (Partinen & Westermarck, 2014).

Even though **iron** has been one of the most studied micronutrient involved in the pathophysiology of ADHD, only a few studies have been explored the association between iron and sleep disturbances in patients with ADHD. Studies have found associations between low ferritin levels and both restless legs syndrome and sleep wake transition disorders in ADHD children (Oner et al., 2007). On the other hand, Cortese et al. (2009) published a study in which they evaluated motor activity during sleep in children with ADHD, in which concluded that ferritin levels below 45 mg/l might indicate a risk for disruption of normal sleep patterns (abnormal movements and small awakenings) in children with ADHD. This abnormal sleep pattern it can be a cause of stress for both parents and affected children, which could aggravate the symptoms characteristic of ADHD (Cortese et al., 2009).

A relationship between **magnesium** blood levels and sleep has been suggested (Peuhkuri et al., 2012a). Although clinical research on sleep effects and magnesium is scare, there are a few studies which conclude that optimal peripheral and central magnesium levels are needed for sleep consolidation, especially after sleep deprivation (Chollet et al., 2001). Also, it has been observed that the administration of nightly melatonin, magnesium, and zinc appears to improve the quality of sleep and the quality of life in long-term care facility residents with primary insomnia (Rondanelli et al., 2011). Magnesium is part of many key steps in the nervous system and a its deficiency could affect mechanisms involved in the influence of promoting sleep.

Zinc like magnesium may play a role in facilitating sleep. Zinc exhibits an antidepressant-like activity, as stated in a preclinical model of depression and in some clinical trials. Significant clinical correlates were shown related to its action as an antagonist of the glutamate/N-methyl-D-aspartate receptor (Rondanelli et al., 2011). The *N*-methyl-d-aspartate glutamate (NMDA) receptor in the central nervous system, which mediates mood, cognition, pain perception, and sleep (Song et al., 2012).

Zinc is also an important cofactor for metabolism relevant to neurotransmitters, prostaglandins, and melatonin, and indirectly affects dopamine metabolism (Partinen & Westermarck, 2014). Regarding ADHD, in a study with 810 children from 5 to 12 years of age after 12 weeks of intake of a combination of omega-3 and omega-6 fatty acids, magnesium and zinc, a considerable reduction in symptoms of attention deficit and hyperactivity/impulsivity and sleep-related problems was shown. Problems to fall sleep, to sleep through the night and low sleep quality were reduced significantly by more than 40% children (Huss et al., 2010).

2. Hypothesis and Aims

HYPOTHESIS AND AIMS

2.1 Hypothesis

In spite that several studies have demonstrated that there is a relationship between nutrient deficiencies and "unhealthy diets" and ADHD, to our knowledge no research has been done to know the possible relationship between our characteristic dietary pattern, the Mediterranean diet, and the incidence of ADHD. Therefore, we **hypothesize** that a low adherence to the Mediterranean diet would be positively associated with an increase of ADHD diagnosis. On the other hand, and on the basis of the background that it has been developed in the introduction, we also **hypothesize** that dietary intake and nutrient deficiencies in children and adolescents with ADHD could affect their behavioral symptoms and their cognitive abilities as well as their sleep quality and other sleep patterns.

2.2 Objectives

In order to test our hypothesis, three specific **objectives** were proposed:

OBJECTIVE 1. To evaluate the adherence to the Mediterranean diet in children and adolescents newly diagnosed with ADHD, in comparison to a control group. The results obtained from this objective are part of the following publication:

- ***The Mediterranean Diet and ADHD in Children and Adolescents.*** Alejandra Rios-Hernández[a], RD, José A. Alda[b], MD, PhD, Andreu Farran-Codina[a], PhD, Estrella Ferreira-García [b,c] MSc, PhD; Maria Izquierdo-Pulido[a,d], PharmD, PhD.
  ***Pediatrics*, 2017**; **doi: 10.1542/peds.2008-1536**; Impact Factor: 5.8

OBJECTIVE 2. To investigate the influence of diet and nutrient deficiencies on the sleep quality of children and adolescents newly diagnosed with ADHD in comparison to a control group. The results obtained from this objective are part of the following manuscript:

- ***Sleep quality and sleep disturbances associate with diet in children and adolescents with ADHD: a control-case study.***
  Alejandra Rios-Hernández[a], Andreu Farran-Codina[a], Estrella Ferreira-García [b,c], Oscar Sans[d], José A. Alda[b#] and Maria Izquierdo-Pulido[a,e*]

OBJECTIVE 3. To study the relationships between nutrients and ADHD symptomatology and comorbidities as well as cognitive abilities in children and adolescents newly diagnosed with ADHD, in comparison to a control group. The results obtained from this objective are part of the following manuscript:

- ***Iron and ferritin serum levels associates with behavioral symptoms and cognitive abilities in children and adolescents with ADHD***
  Rios-Hernández A[1]; Farran-Codina, A[1]; Ferreira-García [2,3]; Alda JA[2*]; Izquierdo-Pulido, M [1,4*.]

3. Subjects and Methods

SUBJECTS AND METHODS

3.1 Subjects

From 130 referrals, 60 children and adolescents (ages 6-16 years) newly diagnosed with ADHD (naïve) and 60 sex- and age- matched controls participated **(Figure 1)**. Cases were recruited at the ADHD Unit of the Department of Child and Adolescent Psychiatry and Psychology of the Hospital of *Sant Joan de Deu* in Barcelona (Spain). The ADHD diagnosis was performed according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) (Association AP., 2000). The ADHD RS-IV-parents was used as screening for the diagnosis of ADHD (DuPaul et al., 1998). The Kiddie Schedule for Affective Disorders and Schizophrenia-Present and Lifetime version (K-SADS-PL) was also used to confirm the ADHD diagnosis and other comorbidities (Kaufman et al., 1997) while the Wechsler Intelligence Scale for Children-IV (WISC-IV) (Wechsler, 2005) was applied to generate the intelligence quotient. Experienced psychiatrists conducted ADHD diagnosis.

Exclusion criteria were: IQ<70, autism spectrum disorder, psychosis, developmental disorders, any ADHD drug treatment or nutrient (mineral/vitamin) complement. Subjects in which the severity of symptoms was significant and a symptomatic treatment needed urgently (anxiolytic, antipsychotic, etc.) before completing the whole evaluation process were also excluded.

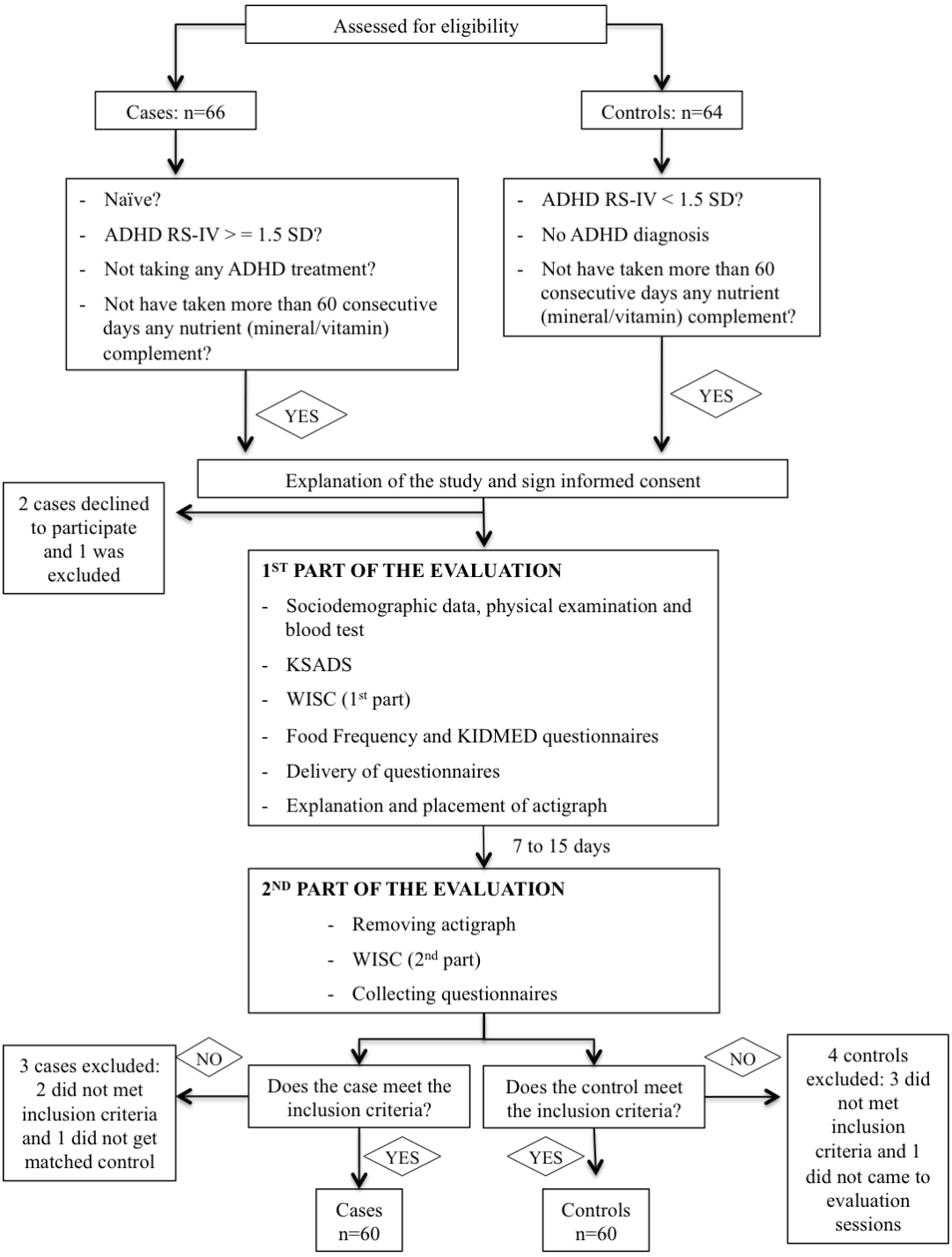
Controls were recruited from the ADHD patients' classmates (40%) and from patients attending other hospital services (60%) (i.e. minor surgery ambulatory, etc.). Controls were screened for the absence of ADHD symptoms and the same exclusion criteria were applied also to controls.

Procedure

The study was approved by the Ethical Committee of the Hospital of *Sant Joan de Déu* (Barcelona, Spain). Written informed consent was obtained from participant's

parents, and verbal assent from the participants. Demographic and clinical data were obtained from both subjects and parents. Participants underwent a physical examination, including height and weight. BMI was calculated as weight (kilograms) divided by height (meters) squared and BMI was standardized to BMI Z-score using age and gender. The whole evaluation, from the first to the last visit spread at most 3 weeks.

Figure 2. Participant flow diagram



3.2 Methods

3.2.1 Assessment of dietary intake

The estimation of dietary intake was performed through a clinical interview using a food frequency questionnaire (FFQ) (See appendix A2) specially designed and validated for the estimation of micronutrients intake (Rodríguez et al., 2008). Containing 12 categorized food groups for use in the dietary pattern analysis. They are dairy products (milk, cheese, yogurt, milk ice-cream, milk desserts), cereals (pasta, rice, potatoes, breakfast cereal, cookies), bakery (muffins, donuts, croissant), vegetables (raw and cooked), citric fruits (orange, mandarin kiwi), other fruits (apple, banana, pear and natural juices), meat (pork, chicken, lamb), blue fish (tuna, salmon, sardines), white fish (hake, codfish), artificial sweetened juices, cola beverages sugar and candies. For each food item, participants were asked to record their usual consumption for the nine frequency categories of the FFQ, ranging from never or less than once per month to six or more times per day. In addition, a 24-hr recall interview was conducted by telephone by a trained interviewer. Total energy intake as well as nutrient composition were analyzed using the nutritional evaluation software program PCN Pro v1.32 (Cantós et al., 2004), on the basis of Spanish food composition tables (Farran et al., 2004). According to Willett et al. (1997) an adjustment of total energy was made for independent variables using the nutrient residual model (adjusted for energy). Taking into consideration that most nutrients are positively correlated with energy intake and this could introduce a confounding factor. The Willett's methodology allows the calculation of the effect of a specific nutrient beyond any effect due to the energy intake.

3.2.2 Assessment of adherence to the Mediterranean diet

The KIDMED test (Mariscal-Arcas et al., 2009) (see appendix A1) was used to evaluate adherence to the Mediterranean diet by the participants and was administered by a trained interviewer. KIDMED is based on the principles that sustain Mediterranean dietary patterns and those that undermine it. Items denoting lower adherence were assigned a value of −1 (4 items), and those related to higher adherence were scored +1 (12 items). Scores range from −4 to 12 with higher scores indicating greater adherence

to the Mediterranean diet. The KIDMED index is calculated from the sum of the values of the questionnaire, which is classified into three categories: Good adherence (8-12 points), average adherence (4-7 points) and poor adherence (less than 3 points).

3.2.3 Measurement of anthropometric

Height and weight were measured by trained medical stuff. Children wearing minimal clothing and without shoes using standardize methods. Children weight status was classified based on the International Obesity Task Force definition (IOTF) (Cole & Lobstein, 2012) for girls and boys according to age- and sex- specific cut-off points for BMI. BMI was categorized into 4 groups: underweight (<18.5 kg/m^2), normal weight (18.5-25 kg/m^2), overweight (25-30kg/m^2) and obese (>30 kg/m^2). For the BMI Z-score was obtained based on the Center for Disease Control (CDC) growth charts (Centers for Disease Control and prevention (CDC, 2009).

3.2.4 Blood parameters measurement

Fasting blood samples were obtaining by venipuncture in the forearm of each participant. Nutrient in blood and plasma levels were determined in the Laboratory of Biochemistry of the Hospital *Sant Joan de Déu* (Barcelona, Spain).

3.2.5 Measurement of sleep and physical activity

Sleep time and quality was measured with the actigraph ActiSleep (ActiGraph, Pensacola, FL, USA) that monitors the body movements by analyzing movement patterns, it can differentiate between sleep and wakefulness. We obtained information on the quality and quantity of sleep, such sleep latency (amount of time it takes to fall asleep), sleep efficiency (time that actually sleeps), total sleep time, the difference between total time in bed and asleep, awakenings and mean duration of awakenings, among other variables, which have a high correspondence with those obtained by polysomnography. The subjects in the study wear continuously the actigraph in their no dominant wrist for 7 days to compute the sleep parameters.

Parents also were trained to complete a sleep diary during two weeks, writing down the time the children went to sleep, the time they woke up, sleep latency, efficiency, total time in bed, awakenings, perception of the mood when they wake up, total sleep time, perturbations of awakenings, caffeine consumption, activities previous going to bed, food consumption before going to bed and medication that could affect the quality of sleep.

Sleep disturbances were measure with the Sleep Disturbance Scale for Children Bruni (Bruni et al., 1996) which is a validated tool in evaluating the sleep disturbances of school-age children in clinical and non-clinical populations, designed to detect sleep disorders (apnea-hypopnea syndrome, obstructive sleep [OSA], restless legs, parasomnias, etc.) in the last 6 months. Parents answered to 26 questions grouped into six sleep factors: Disorder of Initiating and Maintaining Sleep (DIMS), Sleep Breathing Disorder (SBD), Disorder of Arousal (DA), Sleep Wake Transition Disorder (SWTD), Disorder of Excessive Somnolence (DOES), Sleep Hyperhidrosis (SHY) and for last Total Sleep Problems (TSP-sum of all individual questions scores).

The actigraph also was used to measure physical activity by monitoring the body movements and registering the total activity kilocalories, average of hourly kilocalories, steps, sedentary time, light movements, lifestyle, moderate, vigorous, and very vigorous activity per day.

3.2.6 *Diagnostic procedures*

Kiddie Schedule for Affective Disorders and Schizophrenia for School-Age Children- Present and Lifetime (K-SADS-PL): The K-SADS-PL (Kaufman et al., 1997) is a semi-structured diagnostic interview designed to assess current and past episodes of psychopathology in children and adolescents according DSM-IV criteria and it is the "gold" standard diagnostic tool. Administered by interviewing the parents, the child, and finally achieving summary ratings. The main diagnoses evaluated with the K-SADS-PL include affective disorders, psychotic disorders, behavioral disorders and substance abuse. The majority of the items are scored using a 0-3 point rating scale. Scores of 0 indicate no information is available; scores of 1 suggest the symptom is not present; scores of 2 indicate sub-threshold levels of symptomatology, and scores of 3

represent threshold criteria. The remaining items are rated on a 0-2 point rating scale on which 0 implies no information; 1 implies the symptom is not present; and 2 implies the symptom is present.

ADHD Rating Scale IV (ADHD-RS-IV): ADHD-RS-IV (DuPaul et al., 1998) is an 18-item scale, based in the Diagnostic and Statistical Manual of Mental Disorders (DSM), divided into 2 scales: hyperactivity/impulsivity (9 items) and inattentive (9 items). The answers to each question vary from never or rarely (0 points), sometimes (1 point), often (2 points) to very often (3 points). The total score is computed as the sum of the scores on each of the 18 items. The inattention subscale score is the sum of the odd-numbered items and the hyperactivity/impulsivity subscale is the sum of the scores on the even-numbered items. For the case group, it was a requisite to score greater than 1.5 standard deviation from the normal age for the diagnosis subtype.

The Conners' Parent Rating Scale (CPRS): The CPRS (Conners et al., 1998) consists in a questionnaire of 27 items that aims to evaluate the children between the ages of 3 and 17 years who might suffer from attention- deficit/hyperactivity disorder according to parental reports. It provides index score of oppositional, inattention, hyperactivity behaviors and also an ADHD index score, which besides the previous symptoms, also covers conduct problems, learning problems, psychosomatic problems, impulsive-hyperactive behaviors and anxiety. This is the best index to identify children/adolescents “at risk” for ADHD. Questions scored on a 4-point scale (0-3), where a higher score indicates more severe symptoms.

Child Behavior Checklist of Achenbach for parents (CBCL): The CBCL (Mazefsky et al., 2011) is used as a diagnostic tool for a variety of behavioral and emotional problems such as ADHD, oppositional defiant disorder, conduct disorder, childhood depression, separation anxiety, childhood phobia, social phobia, specific phobia and a number of other childhood and adolescent issues. The checklist is created on 113 questions in which the items scored on a 3 point scale from 0=no true to 2= very true or often true. Questions are associated with problems on a syndrome scale in eight different categories: anxious/depressed, withdrawn/depressed, somatic complaints, social problems, thought problems, attention problems, rule breaking, and aggressive

behavior.

Wechsler Intelligence Scale for Children 4th Edition (WISC-IV): WISC (Wechsler, 2005) is a test that evaluates the intellectual abilities of children and adolescents, between 6 and 16 years. It consists of 15 tests, 5 of them are optional, organized into four indexes, which are: verbal comprehension, perceptual reasoning, working memory and processing speed. Also, it generates a total intellectual coefficient. The score of each of the 4 indexes is obtained by adding the scores of the tests that form them (mean=10, standard deviation=3) and subsequently transforming them into the intellectual coefficient scores (mean=100, standard deviation=15). The total intellectual coefficient is obtained by adding the scores of the four indexes.

3.2.7 Statistics

Continuous variables were expressed as mean ± standard deviation (SD) or median and interquartile range, whereas categorical variables were expressed as percentage. Differences in normally and non-normally distributed continuous variables were compared using the Student's T test and the Mann-Whitney U test, respectively. Categorical variables were compared using the χ^2 test. Logistic regression and multivariate regression were used to examine possible associations between the different parameters. Analyses were performed using the SPSS 21.0 statistical software package (SPSS Inc., Chicago, Ill., USA) and the R package *mmeta*. A p value$\leq$0.05 was considered statistically significant.

4. Results

4.1 The Mediterranean diet and ADHD in children and adolescents

The Mediterranean Diet and ADHD in Children and Adolescents

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Type: Regular Article

Dear Prof. Izquierdo-Pulido and colleagues:

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Once again, we are very pleased to feature your article in an upcoming issue of Pediatrics and look forward to seeing it published.

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ABSTRACT

Objectives: Although attention-deficit/hyperactivity disorder (ADHD) has been related with nutrient deficiencies and "unhealthy" diets, to date there are no studies that examine the relationship between the Mediterranean diet and ADHD. We hypothesized that a low adherence to Mediterranean diet would be positively associated with an increase in ADHD diagnosis.

Methods: 120 children and adolescents (60 with ADHD newly diagnosed and 60 controls) were studied in a sex- and age-matched case-control study. ADHD diagnosis was performed according to the DSM-IV-TR. Energy, dietary intake, adherence to Mediterranean diet, iron and zinc plasma levels, and familial background were objectively measured. Logistic regression was used to determine associations between the adherence to Mediterranean diet and ADHD diagnosis.

Results: Lower adherence to Mediterranean diet was associated with ADHD diagnosis (odds ratio, OR= 7.07, 95% confidence interval= 2.65; 18.84; relative risk, RR = 2.80, 95% CI=1.54; 5.25). Both remained significant after adjusting for potential confounders. Lower frequency of consuming fruit, vegetables, pasta and rice and higher frequency of skipping breakfast and going to fast-food restaurants were associated with ADHD diagnosis ($p<0.05$). High consumption of sugar and candy, cola beverages, and non-cola soft drinks ($p<0.01$) and low consumption of blue fish ($p<0.05$) were also associated with higher prevalence of ADHD diagnosis.

Conclusions: Although these cross-sectional associations do not establish causality, they raise a question whether low adherence to Mediterranean diet might play a role in ADHD development. Our data supports the notion that not only "specific nutrients" but also the "whole diet" should be considered in ADHD.

Introduction

The etiology of attention-deficit/hyperactivity disorder (ADHD) continues to be debated, although several contributing factors have been acknowledged, including diet.[53] However, research on the relationship between ADHD, nutrients and food components thus far has yielded inconsistent results.[3,4] Therefore, a dietary approach to ADHD treatment is still regarded as controversial without a comprehensive evidence base.[5] Some studies have analyzed the association between dietary patterns and ADHD. The common result is that unhealthy dietary patterns (i.e. high in saturated fat, refined sugars and low in fruits and vegetables) are associated with ADHD.[6-9]

The Mediterranean diet is a healthy well-balanced diet which provides most of the nutrients in their right proportions.[10] However, the Spanish population is moving away from this traditional pattern by increasing their consumption of processed foods and refined sugars, while decreasing of vegetable and fruit intakes; consequently losing the richness and balance of the Mediterranean diet.[11] This eating pattern change will affect children and adolescents the greatest as they have the highest nutritional needs for optimal growth and development.[8] To our knowledge, there are no studies that examine the potential relationship between adherence to the Mediterranean diet and ADHD.

The ADHD worldwide-pooled prevalence is reported to be 3.4% (CI 95% 2.6-4.5) in children and adolescents,[12] while in Spain it is reported to be 5-8% in children and 2.5-4% in adolescents.[13] The aim of this study was to compare the dietary intake and adherence to Mediterranean diet in Spanish children and adolescents newly diagnosed with ADHD with healthy subjects. We hypothesized that a low adherence to the Mediterranean diet would be associated with an increase in the prevalence of ADHD diagnosis.

Methods

Subjects

From 130 referrals, 60 children and adolescents (ages 6-16 years) newly diagnosed with ADHD (naïve) and 60 sex- and age- matched controls participated **(Figure 1)**. Cases were recruited at the ADHD Unit of the Department of Child and Adolescent Psychiatry and Psychology of the Hospital of *Sant Joan de Deu* in Barcelona (Spain). The ADHD

diagnosis was performed according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR).[14] The ADHD RS-IV-parents was used as screening for the diagnosis of ADHD.[15] The Kiddie Schedule for Affective Disorders and Schizophrenia-Present and Lifetime version (K-SADS-PL) was also used to confirm the ADHD diagnosis and other comorbidities[16] while the Wechsler Intelligence Scale for Children-IV (WISC-IV)[17] was applied to generate the intelligence quotient. ADHD diagnosis was conducted by experienced psychiatrists.

Exclusion criteria were: IQ<70, autism spectrum disorder, psychosis, developmental disorders, any ADHD drug treatment or nutrient (mineral/vitamin) complement. Subjects in which the severity of symptoms was significant and a symptomatic treatment needed urgently (anxiolytic, antipsychotic, etc.) before completing the whole evaluation process were also excluded.

Controls were recruited from the ADHD patients' classmates (40%) and from patients attending other hospital services (60%) (i.e. minor surgery ambulatory, etc.). Controls were screened for the absence of ADHD symptoms and the same exclusion criteria were applied also to controls.

Procedure

The study was approved by the Ethical Committee of the Hospital of *Sant Joan de Déu* (Barcelona, Spain). Written informed consent was obtained from participant's parents, and verbal assent from the participants. Demographic and clinical data were obtained from both subjects and parents. Participants underwent a physical examination, including height and weight. BMI was calculated as weight (kilograms) divided by height (meters) squared and BMI was standardized to BMI Z-score using age and gender. The whole evaluation, from the first to the last visit spread at most 3 weeks **(Figure 1)**.

Assessment of dietary intake

Food consumption and nutrient intake were measured by a validated food frequency questionnaire (FFQ)[18] administered by a trained interviewer. The FFQ comprised 45 items, including foods and beverages. For each food item participants were asked to record their usual consumption, ranging from never or less than once per month to six

or more times per day. In addition, a 24-hr recall interview was conducted by telephone. Total energy and nutrient intake were analyzed using the nutritional evaluation software program PCN Pro v1.32[19, 20]. According to Willett et al. [21] an adjustment of total energy was made for independent variables using the nutrient residual model (adjusted for energy). Taking into consideration that most nutrients are positively correlated with energy intake and this could introduce a confounding factor. The Willett's methodology allows the calculation of the effect of a specific nutrient beyond any effect due to the energy intake.

Assessment of adherence to the Mediterranean diet

The KIDMED test[22] was used to evaluate the adherence to the Mediterranean diet. KIDMED is based on the principles that sustain Mediterranean dietary patterns and those that undermine it. Items denoting lower adherence were assigned a value of −1 and those related to higher adherence were scored +1. Scores range from −4 to 12 with higher scores indicating greater adherence to the Mediterranean diet.

Physical activity

Participants used the actigraph ActiSleep (ActiGraph, Pensacola, FL, USA), on their non-dominant wrist continuously for 7 days to measure rates of physical activity.

Biochemical parameters

Fasting blood samples were obtained by venipuncture in the forearm of each participant. Plasma iron, ferritin, transferrin, and zinc were determined in the Laboratory of Biochemistry of the Hospital *Sant Joan de Déu* (Barcelona).

Statistical analyses

Continuous variables were expressed as mean ± standard deviation (SD) or median and interquartile range, whereas categorical variables were expressed as percentage. Differences in normally and non-normally distributed continuous variables were compared using the Student's T test and the Mann-Whitney U test, respectively. Categorical variables were compared using the χ^2 test. Logistic regression was used to examine associations between the score (after calculating tertiles) of the adherence to the Mediterranean diet and the odds of ADHD diagnosis. In addition, the odds of

ADHD diagnosis were estimated according to tertiles of the intake of food groups of which consumption was different between cases and controls, to determine whether any key food group could explain any significant relationship with ADHD. The relative risk was also compute as the rate for the lowest adherence divided by that with the highest adherence to the Mediterranean diet. Analyses were performed using the SPSS 21.0 statistical software package (SPSS Inc., Chicago, Ill., USA) and the R package *mmeta*. A p value≤0.05 was considered statistically significant.

Results

General characteristics

The baseline characteristics of cases and controls are shown in **Table 1.** There were statistically significant differences associated with ADHD for BMI (and its Z-Score) and physical activity but not for body weight at birth. On the other hand, the percentage of subjects with ADHD who were breastfed was lower than in subjects without ADHD, however the length for those who were breastfed was the same in both groups. Significant differences were also observed for variables related to familial background. Regarding nutrient plasma levels, no statistically significant differences were found, even though cases showed slightly lower plasma concentrations of iron and ferritin. On the other hand, the cases showed an average value of ADHD-RS (DMS-IV) of 34.2 (9.7) and the K-SADS confirmed the diagnosis of all the cases. In regard to comorbid diagnoses, 33.3% of ADHD patients met cut-off criteria for oppositional defiant disorder (ODD), 23.3% for anxiety, 3.3% for conduct disorder (CD) and 1.7% for depression.

ADHD is associated with a lower adherence to the Mediterranean diet.

Children and adolescents with ADHD showed statistically significant lower score of adherence to Mediterranean diet than controls (**Table 2**). Within the ADHD subjects, no significant differences in KIDMED score were observed in those patients with comorbidities such as anxiety (ADHD without anxiety: 6.5(2.0) vs. ADHD with anxiety 5.5(2.0), p=0.096) or ODD (ADHD without ODD: 6.0(2.1) vs. ADHD with ODD 6.5(1.7), p=0.132). When compared to controls, the percentages of ADHD subjects who consume a second serving of fruit every day, daily or more than once a day fresh or cooked vegetables, and pasta or rice almost every day, were significantly lower (**Table 2**). In addition, the percentage of ADHD subjects who went more frequently to a fast-

food restaurant was higher than controls and the percentage of subjects who skipped breakfast was significantly higher for the cases. However, no statistically significant differences were observed with respect to the consumption of fish, dairy and cereals products or bakery goods.

Statistical differences were found between children and adolescents with or without ADHD regarding with some food group intakes (**Table 3**). Cases consumed lesser amounts of vegetables, citrus fruits and blue fish, but larger amounts of non-cola soft drinks, cola beverages, sugar and candy than controls. Moreover, individuals with ADHD consumed statistically higher amounts of simple sugars and caffeine and lower amounts of total protein than controls. On the other hand, no significant differences were found for total daily energy intake or other nutrient intakes, including iron and zinc commonly related to ADHD.

We further compared low, medium and high adherence to the Mediterranean diet with ADHD diagnoses (**Table 4**). Children and adolescents with a low adherence to the Mediterranean diet were more likely to be associated with ADHD diagnosis in the crude model ($p<0.001$). The relative risk (RR) was also significant: RR= 2.80, 95% CI=1.54-5.25. Both OR and RR remained significant after adjusting for potential confounding variables, such as: BMI, level of activity, breastfeeding, maternal smoking during pregnancy, maternal educational level, paternal educational level, biological father living with family and parents divorced. When examining the consumption of specific food groups and ADHD diagnosis, an intake in the low tertile of vegetables, citrus fruit, and blue fish was associated with ADHD diagnosis in the crude model (**Table 4**). Further adjusting for potential confounding variables attenuated the association with the exception of blue fish consumption, which remains statistically significant. On the other hand, subjects with an intake in the highest tertile for sugar and candy, cola beverages, and non-cola soft drinks were associated with higher prevalence of ADHD diagnosis (**Table 4**). The association remained significant after adjusting for confounding variables.

Discussion

This is the first study to show that low adherence to the Mediterranean diet is associated with the odds of ADHD diagnosis in children and adolescents. This association remained significant after adjusting for confounding variables. Among the habits that characterize a Mediterranean dietary pattern, individuals with ADHD more often missed having a second serving of fruit daily, showed reduced intake of vegetables, pasta and rice almost every day when compared to controls. Moreover, subjects with ADHD went to fast-food restaurants and skipped breakfast more often than controls. In addition, a high consumption of sugar and candy, cola beverages, and non-cola soft drinks and a low consumption of blue fish were also associated with a higher prevalence of ADHD diagnosis.

Several advantages supporting the study of dietary pattern versus single nutrients in health promotion, including mental health, have previously been discussed. [2,23] It seems that besides analyzing the impact that a single food component may have on ADHD, the role of dietary patterns as a whole can be more interesting. Some studies have been carried out analyzing different types of dietary patterns, but not one specifically on Mediterranean diet. In a cohort of Australian adolescents,[6] a dietary pattern identified as "Western" type, was significantly associated with ADHD diagnosis. Comparably, in a cross-sectional study of Iranian children, a greater adherence to fast-food and sweet dietary patterns was associated with higher prevalence of ADHD.[7] Recently, in a case-control study of Korean children, the traditional-healthy dietary pattern, characterized by high intakes of kimchi, grains, bonefish, and low intakes of fast foods and beverages, was associated with a lower probability of ADHD diagnosis.[9] Moreover, other studies have confirmed that skipping breakfast or substituting it for a sugary drink impairs attention and episodic memory in children.[24] Therefore, low quality diets are persistently associated to a higher risk of ADHD. Consistent with this, in a clinical trial examining the effect of overall dietary characteristics in medicated children with ADHD, found that a balanced diet, regular meals, and a high intake of dairy products and vegetables were associated with less attention and behavioral problems.[25]

Although the mechanisms linking low quality diet and ADHD are still unknown, an unbalanced diet, can lead to deficiencies in essential nutrients or higher intakes of certain food components (i.e. food additives).[26] There are numerous potential biological

pathways by which diet quality may have an impact on mental health.[2,27] For instance, iron and zinc, which contribute to a healthy neurocognitive and physical growth, are cofactors for dopamine and norepinephrine production, which both play an essential role in ADHD's etiology. Low plasma levels of iron, ferritin and zinc[28-30] have been found in children with ADHD. However, in our study, no significant differences regarding the intakes of those nutrients or their plasma levels were observed between cases and controls. Donfrancesco et al.[31] concluded that normal ferritin levels should not suggest that iron deficiency is not involved in the pathophysiology of ADHD. Indeed, serum ferritin is a marker of peripheral but not of the brain iron status, where iron is necessary as a cofactor. The extent to which serum ferritin correlates with iron levels in the brain remains unclear.[32] Omega-3 fatty acids seem also to have a relationship with ADHD. Two recent meta-analyses report a small but beneficial effect of omega-3 supplementation on reducing symptoms of ADHD. [33-35] However, is not entirely supported by the current evidence as primary treatment for ADHD.[36] In this study, the omega-3 intake estimation among controls and cases was not possible, since the Spanish food composition tables[25] do not contain information about omega-3. Nonetheless, we found that blue fish intake, which is the main source of long chain polyunsaturated omega-3 in the Spanish diet, was significantly lower in cases than in controls.

Our observed relationship between intakes in the highest tertile of sugary products and increased odds for ADHD is in line with the findings of other studies.[6,9] It might occur that a high intake of these low nutrient products could be indicating that what really exists is a poor micronutrient intake. Two recent studies have found that a vitamin and mineral supplementation resulted in significant reductions in ADHD symptoms in both children and adults with ADHD.[37,38] In the case of children, these reductions were reversed when the treatment was withdrawn. This kind of approach makes physiological sense considering that nutrients are required for many critical biochemical reactions to occur and also because it is unlikely that one nutrient by itself would resolve all vulnerabilities present in a complex disorder such as ADHD[39].

We cannot overlook that the relationship found between diet and ADHD could be both ways.[6] Individuals with ADHD are often characterized by impulsivity traits and emotional distress[40] that may lead to poor dietary choices (i.e. fat-rich or sugar-rich

snack foods) to compensate their emotions, as a form of self-medication.[27,41] In our population, we found that the intake of sugary beverages and foods was significantly higher in cases than in controls, and those higher intakes were reflected in a larger amount of sugar intake. On the other hand, the role of the family cannot be dismissed considering that a healthy diet is related to a better functioning family.[42,43] Parents of individuals with ADHD often report a more dysfunctional family environment, [40,44,45] so it is plausible that the relationship between low adherence to a healthy diet and ADHD diagnosis may be orchestrated by a dysfunctional family environment.[6] All of these factors could support a vicious cycle: impulsiveness and family dysfunction could lead to a worse choice of foods, lowering the diet quality, which eventually could provoke a low intake of certain nutrients. This may induce certain nutritional subclinical deficiencies and hence, worsening ADHD symptomatology.

The statistical differences of the BMI and z-score found between cases and controls deserve discussion, even though the physical activity was higher in cases than in controls while the energy intake was similar in both groups. Our findings are in line with a recent study, which provided meta-analytic evidence for a significant association between ADHD and obesity/overweight postulating that the impulsivity and inattention that characterize ADHD might lead to deregulated eating patterns and consequently, weight gain.[46] Other possible explanation is the *thrifty* phenotype theory, which proposes a mechanism of early programming where a wide range of environmental conditions before and during pregnancy determine susceptibility to disease later.[47] Several studies have shown that children whose mothers smoke during pregnancy were at elevated risk of being overweight.[47] In our work, we observed higher percentage of maternal smoking during pregnancy in cases compared to controls.

Finally, the significant differences observed for other indicators of certain social disadvantages (i.e. maternal and/or paternal education and lone parenthood) or maternal prenatal smoking or insufficient breastfeeding are in accordance with data previously published.[43,48-50] Regarding the association between insufficient breastfeeding and ADHD development, it provides the typical chicken-and-the-egg question of which came first, the disorder or the inability to be breastfeed, since infants who appear to reject the breast might show the early manifestation of a neuropsychiatric disorder, such as ADHD.[42]

Some limitations of our design and methods should be acknowledged, such as the case-control study design, which prevents our ability to assess cause-and-effect associations. Furthermore, all dietary instruments such as food frequency questionnaires measuring past food intake are vulnerable both to random and systematic measurement errors. Nonetheless, this study has several important strengths, including the fact that all the cases included were naïve, taking no medication. The use of certain drugs might affect the food choices and provoke changes to the child and adolescent's diet. Also, well-trained, experienced psychiatrists and psychologists performed the evaluation.

In summary, we demonstrated a positive relationship between a lower adherence to the Mediterranean diet and ADHD diagnoses. The current findings suggest that certain dietary habits may play a role in ADHD development, even though further work is required to investigate causality and also to figure out if diet manipulation could reverse the symptoms of ADHD taking in consideration all potential factors. Therefore, our main recommendation is that clinicians focus on diet not with the expectation of dietary changes improving the behavior, but with the concern that children with ADHD are more likely to be eating unhealthy diets and that it should be a part of the evaluation to improve their health.

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References

1. Stevenson J, Buitelaar J, Cortese S. Research review: the role of diet in the treatment of attention-deficit/hyperactivity disorder--an appraisal of the evidence on efficacy and recommendations on the design of future studies. *J Child Psychol Psychiatry*. 2014;55(5):416–427
2. Sarris J, Logan AC, Akbaraly TN. Nutritional medicine as mainstream in psychiatry. *Lancet Psychiatry*. 2015;2(3):271-274

3. Rucklidge JJ, Johnstone J, Kaplan BJ. Nutrient supplementation approaches in the treatment of ADHD. *Expert rev neurother*. 2009;9(4) 461-476
4. Arnold LE, Hurt E, & Lofthouse N. Attention-deficit/hyperactivity disorder: dietary and nutritional treatments. *Child Adolesc Psychiatri Clin N Am*. 2013;22(3):381–402
5. Sonuga-Barke EJS, Brandeis D, Cortese S, et al. Nonpharmacological interventions for ADHD: systematic review and meta-analyses of randomized controlled trials of dietary and psychological treatments. *Am J Psychiatry*. 2013;170(3):275–289
6. Howard AL, Robinson M, Smith GJ, et al. ADHD is associated with a "Western" dietary pattern in adolescents. *J Atten Disord*. 2011;15(5):403–411
7. Azadbakht L, & Esmaillzadeh A. Dietary patterns and attention deficit hyperactivity disorder among Iranian children. *Nutrition*. 2012;28(3):242–249.
8. Park S, Cho SC, Hong YC, et al. Association between dietary behaviors and attention-deficit/hyperactivity disorder and learning disabilities in school-aged children. *Psychiatry Res*. 2012;198(3):468–476
9. Woo HD, Kim DW, Hong YS. Dietary patterns in children with attention deficit/hyperactivity disorder (ADHD). *Nutrients*. 2014;6(4):1539–1553
10. Donini L, Serra-Majem L, Bulló M, Gil Á, & Salas-Salvadó J. The Mediterranean diet: culture, health and science. *Br J Nutr*. 2015;113 Suppl(S2): S1–3
11. Grosso G, & Galvano F. Mediterranean diet adherence in children and adolescents in southern European countries. *NFS Journal*. 2016;3:13–19.
12. Polanczyk GV, Salum GA, Sugaya LS, Caye A, Rohde LA. Annual research review: A meta-analysis of the worldwide prevalence of mental disorders in children and adolescents. *J Child Psychol Psychiatry.* 2015; 56(3):345–365
13. Gonzalez-Collantes R, Rodríguez-Sacristán A, Sánchez-García. Epidemiología del TDAH. [Epidemiology of ADHD]. *Rev Esp de Pediatr*. 2015;71(2):58-61
14. American Psychiatric Association . Diagnostic and statistical manual of mental disorders. 4th ed. Washington, DC; 2000.
15. DuPaul GJ, Power TJ, Anastopoulos AD, Reid R. ADHD Rating Scale-IV: Checklists, Norms and Clinical Interpretation. New York: The Guilford Press; 1998
16. Ulloa RE, Ortiz S, Higuera F, et al. Interrater reliability of the Spanish version of Schedule for Affective Disorders and Schizophrenia for School-Age Children--Present and Lifetime version (K-SADS-PL). *Actas Esp Psiquiatr*. 2006;34(1):36–40
17. Wechsler D. Escala de Inteligencia de Wechsler Para Niños – IV (WISC-IV). Madrid, Spain: *TEA Edicions*. 2005.
18. Rodríguez IT, Ballart JF, Pastor GC, Jordà EB, Val VA. Validation of a short questionarire on frecuency of dietary intake: reproducibility and validity. *Nutr Hosp. 2008;*23(3):242–252

19. Cantós D, Farran A, & Palma I. PCN Pro. Barcelona: Universitat de Barcelona. 2004
20. Farran A, Zamora R, & Cervera P. Tablas de composición de alimentos del CESNID [CESNID Food composition tables]. Barcelona: *McGraw-Hill/Interamericana de España - Edicions UB;2004*
21. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. *Am J Clin Nutr*. 1997; 65(4 Suppl):1220S–1228S; discussion 1229S–1231S
22. Serra-Majem L, Ribas L, Ngo J, et al. Food, youth and the Mediterranean diet in Spain. Development of KIDMED, Mediterranean Diet Quality Index in children and adolescents. *Public Health Nutr*. 2007;7(7):931–935
23. Tucker KL. Dietary patterns, approaches, and multicultural perspective. *Appl Physiol Nutr Metab*. 2010;35(2):211–218
24. Wesnes KA, Pincock C, Richardson D, Helm G, Hails S. Breakfast reduces declines in attention and memory over the morning in schoolchildren. *Appetite*. 2003;41(3):329-331
25. Ghanizadeh A, & Haddad B. The effect of dietary education on ADHD, a randomized controlled clinical trial. *Ann Gen Psychiatry*. 2015;1*;14:12*
26. Izquierdo-Pulido M, Ríos-Hernández A, Farran A, & Alda JÁ. The role of diet and physical activity in children and adolescents with ADHD. *Recent Advances in Pharmaceutical Sciences V*. Research Signpost. 2015;Chapter 4:51-64
27. O'Neil A, Quirk SE, Housden S, et al. Relationship between diet and mental health in children and adolescents: A Systematic Review. *American Journal of Public Health*. 2014;104(10):e31-e42
28. Kiddie JY, Weiss MD, Kitts DD, Levy-Milne R, & Wasdell MB. Nutritional status of children with attention deficit hyperactivity disorder: a pilot study. *Int J Pediatr*. *2010*, doi:10.1155/2010/767318
29. Arnold LE, Disilvestro RA, Bozzolo D, et al. Zinc for attention-deficit/hyperactivity disorder: placebo-controlled double-blind pilot trial alone and combined with amphetamine. *J Child Adolesc Psychopharmacol*. 2011; 21(1):1–19
30. Cortese S, Angriman M, Lecendreux M, & Konofal E. Iron and attention deficit/hyperactivity disorder: What is the empirical evidence so far? A systematic review of the literature. *Expert Rev Neurother*. 2012;12(10):1227–1240
31. Donfrancesco R, Parisi P, Vanacore N, et al. Iron and ADHD: time to move beyond serum ferritin levels. *J Atten Disord*. 2013;17(4):347–357
32. Cortese S, Azoulay R, Castellanos FX, et al. Brain iron levels in attention-deficit/hyperactivity disorder: a pilot MRI study. *World J Biol Psychiatry. 2012;* 13(3):223–231

33. Bloch MH, Qawasmi A. Omega-3 fatty acid supplementation for the treatment of children with attention-deficti/Hyperactivity disorder symptomatology: systematic review and meta-analysis. *J Am Acad Child Adolesc Psychiatry*. 2011;50(10):991-1000
34. Sonuga-Barke EJS, Brandeis D, Cortese S, et al. Nonpharmacological Interventions for ADHD: Systematic Review and Meta-Analyses of Randomized Controlled Trials of Dietary and Psychological Treatments. *Am J Psychiatry*. 2013;170(3):275-289
35. Hawkey E & Nigg JT. Omega−3 fatty acid and ADHD: Blood level analysis and meta-analytic extension of supplementation trials. *Clin Psychol Rev*. 2014; 34(6): 496–505
36. National Collaborating Centre for Mental Health. *Attention Deficit Hyperactivity Disorder*. London, UK: The British Psychological Society and The Royal College of Psychiatrists; 2009.
37. Rucklidge JJ, Frampton CM, Gorman B, Boggis, A.Vitamin-mineral treatment of attention-deficit hyperactivity disorder in adults: double-blind randomised placebo-controlled trial. *Br J Psychiatry*. 2014;204:306-315
38. Gordon HA, Rucklidge JJ, Blampied NM, Johnstone JM. Clinically Significant Symptom Reduction in Children with Attention-Deficit/Hyperactivity Disorder Treated with Micronutrients: An Open-Label Reversal Design Study. *J Child Adolesc Psychopharmaco*. 2015;25(10):783-798
39. Rucklidge JJ, Kaplan BJ. Broad-spectrum micronutrient treatment for attention-deficit/hyperactivity disorder: Rationale and evidence to date. *CNS Drugs*. 2014;28(9):775-785
40. Wehmeier PM, Schacht A, Barkley RA. Social and emotional impairment in children and adolescents with ADHD and the impact on quality of life. *J Adolesc Health.* 2010;46(3):209–217
41. Barkley RA. ADHD, Obesity, and Eating Pathology. *The ADHD Report*. 2014;22*(5)*:1–6
42. Ambrosini GL, Oddy WH, Robinson M, et al. Adolescent dietary patterns are associated with lifestyle and family psycho-social factors. *Public Health Nutr.* 2009;12(10):1807–1815
43. Russell G, Ford T, Rosenberg R, Kelly, S. The association of attention deficit hyperactivity disorder with socioeconomic disadvantage: Alternative explanations and evidence. *J Child Psychol Psychiatry*. 2014;55(5):436-445
44. Foley M. A comparison of family adversity and family dysfunction in families of children with attention deficit hyperactivity disorder (ADHD) and families of children without ADHD. *J Spec Pediatr Nurs*. 2011;16(1):39–49
45. Singer-Leshinsky S. Attention-deficit/hyperactivity disorder: helping families to achieve success. *JAAPA*. 2011;24(3):52–57

46. Cortese S, Moreira-Maia CR, St Fleur D, et al. Association Between ADHD and Obesity: A Systematic Review and Meta-Analysis. *Am J Psychiatry*. 2016;173(1): 34-43
47. Gillman MW. Developmental origins of health and disease. *N Engl J Med*. 2005;353(17):1848-1850
48. Arnold LE, Elliott M, Lindsay RL, et al. Gestational and postnatal tobacco smoke exposure as predictor of ADHD, comorbid ODD/CD, and treatment response in the MTA. *Clin Neurosci Res*. 2005;5(5-6):295-306
49. Melchior M, Hersi R, Van der Waerden J, et al. Maternal tobacco smoking in pregnancy and children's socio-emotional development at age 5: The EDEN mother-child birth cohort study. *Eur Psychiatry*. 2015;30(5):562–568
50. Say GN, Babadağı Z, Karabekiroğlu K. Breastfeeding history in children with autism and attention deficit hyperactivity disorder. *Breastfeed Medicine*. 2015; 10(5):283-284

Figure 1. Participant flow diagram

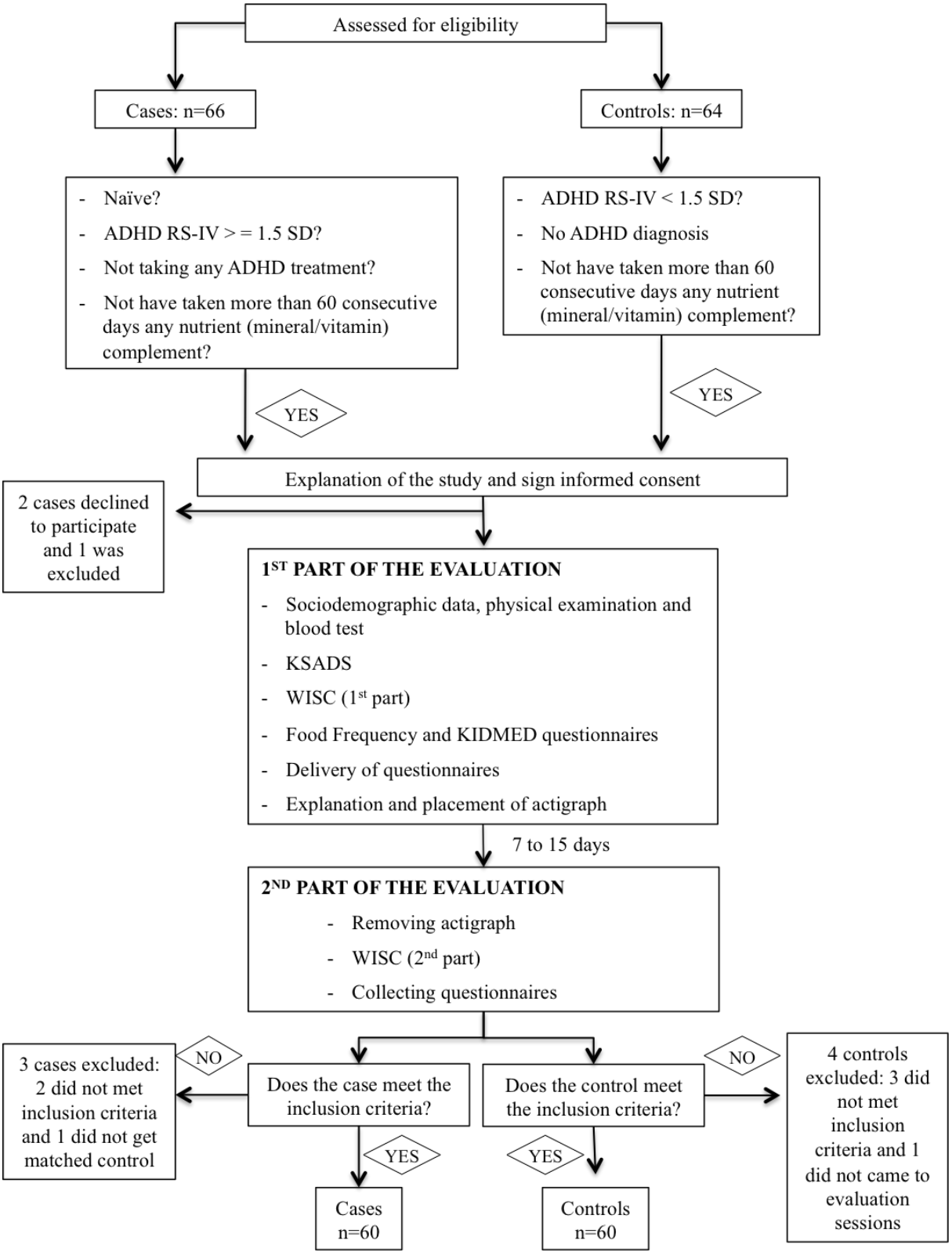

Table 1. General characteristics and blood micronutrient levels in subjects with attention-deficit hyperactivity disorder (ADHD) and in control subjects.[a]

	Cases (ADHD) n=60	Controls n=60	P-value
Gender, %male (n)	56.7 (34)	56.7 (34)	---
Age, years	9.3 (2.8)	9.3 (2.8)	---
Height, cm	136.5 (16.8)	138.6 (17.3)	0.496
Weight, kg	38.1 (16.2)	36.4 (14.5)	0.536
BMI[b], kg/m^2	19.6 (4.3)	18 (3.3)	**0.042**
BMI Z-Score	0.69 (1.1)	0.25 (1.1)	**0.027**
Body weight at birth			
Lower than 2.5kg, %	13.3	5.0	0.114
Higher than 2.5kg, %	86.7	95.0	
Physical activity, kcal/day[c]	1248.3 (824.6)	861.4 (483.3)	**0.013**
Maternal smoking pregnancy, %	22.0	8.3	**0.037**
Breastfeeding, %	66.7	83.3	**0.035**
Breastfeeding length, months[d]	5.8 (5.7)	5.7 (5.9)	0.931
Maternal education level, %[e]	63.3	88.3	**<0.001**
Paternal education level, %[f]	55.0	76.7	**0.012**
Biological father living with family, %	81.7	96.7	**0.008**
Marital status of parents, %[g]	18.3	5.0	**0.023**
Nutrient plasma levels			
Iron, ug/dl	80.1 (32.5)	85.4 (32.1)	0.366
Transferrin, mg/dl	266.2 (29.9)	265.7 (27.9)	0.922
Ferritin, ug/L	36.2 (21.0)	42.2 (36.9)	0.273
Zinc, ug/L	988.6 (182.0)	975.5 (162.0)	0.680

[a]Data are shown mean (SD) except for gender that is expressed as % of male participants (number) and body weight at birth; [b]Body mass index; [c] Measured with the actigraphy accelerometer ActiSleep; [d]Calculated for those who have breastfeeding; [e]More than primary school; [f]More than primary school; [g]Separated/divorced; Bold face representing statistical differences with $p<0.05$.

Table 2. Mediterranean diet quality (KIDMED score and index) and frequencies of response to each item of the KIDMED test in in subjects with attention-deficit hyperactivity disorder and in control subjects.[a]

	Cases (ADHD) n=60	Controls n=60	P-value
Score total KIDMED test	6.2 (2.0)	8.1 (1.8)	**<0.001**
KIDMED index[b]			
Poor (≤3)	11.7	0.0	
Average (4-7)	58.3	36.7	**<0.001**
Good (≥ 8)	30.0	63.3	
KIDMED test	**% yes**	**% yes**	
- Fruit or fruit juice daily	71.7	78.3	0.399
- Second serving fruit daily	20.0	38.3	**0.027**
- Fresh or cooked vegetables daily	35.0	58.3	**0.010**
- Fresh or cooked vegetables more than once a day	31.7	61.7	**<0.001**
- Regular fish consumption (at least 2–3/ week)	81.7	86.7	0.453
- >1/week fast-food (hamburger) restaurant	20.0	1.7	**<0.001**
- Pulses more than once a week	68.3	81.7	0.092
- Pasta or rice almost every day (≥5/week)	55.0	85.0	**<0.001**
- Cereals or cereal product (bread) for breakfast	91.7	98.3	0.094
- Regular nut consumption (at least 2–3/week)	33.3	26.7	0.426
- Use of olive oil at home	98.3	98.3	1.000
- No breakfast	6.7	0.0	**0.042**
- Dairy product for breakfast (yoghurt, milk, etc.)	98.3	98.3	1.000
- Commercially baked goods or pastries for breakfast	61.7	61.7	1.000
- Two yoghurts and/or some cheese (40 g) daily	81.7	90.0	0.119
- Sweets and candy several times every day	48.3	35.0	0.139

[a] Data are given in percentage except for the score total KIDMED test that is mean (SD). Bold face representing statistical differences with $p<0.05$ [b] Good adherence range from: 8 to12 points; average adherence range from: 4-7 points and poor adherence: less than 3 points.

Table 3. Food group, energy and nutrient daily intake in subjects with attention-deficit hyperactivity disorder and in control subjects.[a]

	Cases (ADHD) n=60	Controls n=60	P-value
Food group intake (in grams)[b]			
Dairy products	296.0 (207.8; 331.2)	272.3 (210.3; 322.4)	0.773
Cereals	161.5 (143.6; 188.0)	168.1 (142.9; 215.9)	0.289
Bakery	6.6 (3.0; 10.0)	5.0 (2.9; 9.6)	0.715
Vegetables	59.6 (25.1; 91.0)	81.6 (45.4; 110.9)	**0.018**
Citric Fruits	17.8 (3.2; 34.5)	26.0 (13.4; 53.0)	**0.031**
Other fruits	53.4 (29.7; 95.3)	60.1 (29.9; 115.8)	0.299
Meat	53.9 (40.4; 67.6)	53.3 (40.9; 73.2)	0.735
Blue fish	7.5 (0.0; 11.9)	8.8 (3.9; 18.0)	**0.045**
White fish	12.7 (7.5; 18.0)	12.4 (8.8; 16.3)	0.592
Sugar and candy	4.7 (1.5; 11.1)	2.4 (0.6; 5.3)	**0.007**
Non-cola soft drinks[c]	41.1 (6.0; 98.0)	22.0 (0.0; 65.5)	**0.017**
Cola beverages	17.2 (0.0; 50.3)	9.2 (0.0; 24.2)	**0.041**
Energy, nutrient intake (per 1000 kcal) and caffeine intake (in mg)			
Energy, kcal	1609.9 (375.3)	1626.9 (382.8)	0.806
Carbohydrates, g	122.7 (10.9)	119.6 (11.0)	0.114
Simple sugars, g	60.4 (16.7)	54.5 (10.5)	**0.022**
Protein, g	47.3 (7.3)	50.1 (7.0)	**0.031**
Animal protein, g	34.5 (7.1)	36.6 (7.8)	0.129
Vegetal protein, g	12.8 (2.2)	13.5 (2.6)	0.087
Fat, g	35.2 (3.9)	35.4 (4.1)	0.780
Saturated fatty acids, g	14.0 (2.1)	14.2 (2.0)	0.448
Monounsaturated fat, g	11.5 (1.3)	11.5 (1.5)	0.793
Polyunsaturated fat, g	6.1 (1.7)	5.9 (1.6)	0.441
Dietary fiber, g	9.4 (2.1)	10.2 (2.5)	0.057
Iron, mg	6.1 (1.0)	6.4 (1.1)	0.129
Zinc, mg	5.1 (0.8)	5.4 (0.7)	0.066
Caffeine, mg	3.8 (5.3)	1.5 (1.9)	**0.003**

[a]Data are given either in median and 25th and 75th percentiles or in mean and standard deviation, as appropriated. [b]Dairy products: milk, cheese, yogurt, milk ice-cream, milk desserts; Cereals: pasta, rice, potatoes, breakfast cereals; Bakery: cookies, muffins, donuts, croissants, etc.; Vegetables (raw and cooked): salads, tomatoes, spinach, broccoli, green beans, etc.; Citrus fruits: orange, mandarin, kiwi; Other fruits: apple, banana, pear, etc.; Meat: pork, chicken, beef, lamb, sausages, etc.; Blue fish: such as sardines, anchovies, tuna, mackerel, salmon etc.; White fish: hake, codfish, etc.; Non-cola soft drinks: lemon, orange, other flavors soda drinks (all of them caffeine-free); Cola drinks: regular and diet cola drinks. [c] 72% of no-cola soft drinkers also drank cola in the cases and 80% in the controls.; [d] 80% of cola drinkers also drank no-cola soft drinks in the cases and 70% in the controls. Bold face representing statistical differences with $p<0.05$.

Table 4. Odds ratio (OR) with 95% confidence intervals (CI) for ADHD by tertile categories of Mediterranean diet score and other food group consumption.

		Crude OR (95% CI)
Mediterranean diet score	n	
High adherence	36	1(reference)
Medium adherence	37	2.84 (1.05; 7.67)
Low adherence	47	7.07 (2.65; 18.84)
P for linear trend		**<0.001**
Vegetable consumption		
High consumption	40	1(reference)
Medium consumption	40	1.60 (0.68; 4.13)
Low consumption	40	3.85 (1.53; 9.75)
P for linear trend		**0.004**
Citric fruit consumption		
High consumption	40	1(reference)
Medium consumption	40	1.36 (0.55; 3.29)
Low consumption	40	2.68 (1.08; 6.65)
P for linear trend		**0.034**
Blue fish consumption		
High consumption	40	1(reference)
Medium consumption	40	1.84 (0.75; 4.49)
Low consumption	40	2.50 (1.02; 6.15)
P for linear trend		**0.046**
Sugar and candy consumption		
Low consumption	40	1(reference)
Medium consumption	42	1.11 (0.45; 2.70)
High consumption	38	3.25 (1.28; 8.25)
P for linear trend		**0.014**
Cola beverage consumption		
Low consumption	40	1(reference)
Medium consumption	40	0.73 (0.29; 1.80)
High consumption	40	3.55 (1.40; 9.01)
P for linear trend		**0.008**
Soft drinks consumption		
Low consumption	40	1(reference)
Medium consumption	40	1.23 (0.50; 3.02)
High consumption	40	3.89 (1.53; 9.87)
P for linear trend		**0.004**

4.2. Sleep quality and sleep disturbances associate with diet in children with ADHD: a control-case study

Sleep quality and sleep disturbances associate with diet in children and adolescents with ADHD: a control-case study

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Abbreviations: ADHD: attention-deficit/hyperactivity disorder; Sleep quality; Diet; Nutrients.

Introduction

Sleep disturbances are particularly widespread in one of the most common neurodevelopmental disorders in childhood and adolescence– attention deficit hyperactivity disorder (ADHD) (Cohen-Zion and Ancoli-Israel, 2004; Miano et al., 2016; Vélez-Galarraga et al., 2016). Although rates estimated have varied over the years, it has been reported that up to 55% of children with ADHD may have sleep problems (Corkum et al., 1998). In general, sleep disturbances have negative consequences in different areas of a child's life. For instance, sleep problems have a negative impact on attention and/or behavior and on daytime sleepiness levels of children (Fallone et al., 2005) which can have repercussions on academic or cognitive performance, which may be already affected in children with ADHD (Díaz-Roman et al., 2016).

Another factor that has been shown to contribute to ADHD symptomatology is diet (Bellisle, 2004), with some studies reporting manifestation of ADHD like behavior after consumption of certain foods (Pelsser et al., 2009). Therefore, both sleep disturbances and diet may be contributing factors to ADHD symptomatology. Furthermore, there is evidence to suggest that there is an additional and independent relationship between diet and sleep (Peukuri et al., 2012a and 2012b; Grandner et al., 2014; St-Onge et al., 2016). Several studies have shown that diets high in carbohydrate, particularly those with a high glycemic index and low in fats, promote increased sleep duration and reduce sleep-onset latency (Afaghi et al., 2007; St-Onge et al., 2016) while high-fat and simple sugar intakes can affect negatively sleep quality (St-Onge et al., 2016). On the other hand, some foods, such as milk products, fish, fruit, and vegetables, might also have sleep-promoting effects (Peuhkuri et al., 2012a; St-Onge et al., 2016).

Given that both sleep disturbance and diet are contributing factors to ADHD symptomology, and diet has been shown to affect sleep, we aimed to investigate relationships between sleep and dietary intake in children and adolescents with ADHD in comparison with subjects without ADHD. Only a preliminary study has been previously published (Blunden et al., 2010) which found that total sleep disturbances were related to higher intake of sugars and fats in children with ADHD.

Methods

Subjects

From 130 referrals, 60 children and adolescents (ages 6-16 years) newly diagnosed with ADHD (naïve) and 60 sex- and age- matched controls participated. Cases were recruited at the ADHD Unit of the Department of Child and Adolescent Psychiatry and Psychology of the Hospital of *Sant Joan de Deu* in Barcelona (Spain). The ADHD diagnosis was performed according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) [129] The ADHD RS-IV-parents was used as screening for the diagnosis of ADHD [143]. The Kiddie Schedule for Affective Disorders and Schizophrenia-Present and Lifetime version (K-SADS-PL) (Ulloa RE, 2006) was also used to confirm the ADHD diagnosis and other comorbidities while the Wechsler Intelligence Scale for Children-IV (WISC-IV) [132] was applied to generate the intelligence quotient. ADHD diagnosis was conducted by experienced psychiatrists. Exclusion criteria were: IQ<70, autism spectrum disorder, psychosis, developmental disorders, any ADHD drug treatment or nutrient (mineral/vitamin) complement.

Controls were recruited from the ADHD patients' classmates (40%) and from patients attending other hospital services (60%) (i.e. minor surgery ambulatory, etc.). Controls were screened for the absence of ADHD symptoms and the same exclusion criteria were applied also to controls.

Procedure

The study was approved by the Ethical Committee of the Hospital of *Sant Joan de Déu* (Barcelona, Spain). Written informed consent was obtained from participant's parents, and verbal assent from the participants. Demographic and clinical data were obtained from both subjects and parents. Participants underwent a physical examination, including height and weight. BMI was calculated as weight (kilograms) divided by height (meters) squared and BMI was standardized to BMI Z-score using age and gender. The whole evaluation, from the first to the last visit spread at most 3 weeks.

Assessment of dietary intake

Food consumption and nutrient intake were measured by a validated food frequency questionnaire (FFQ) [133]¹³³ administered by a trained interviewer. The FFQ comprised 45 items, including foods and beverages. For each food item participants were asked to record their usual consumption, ranging from never or less

than once per month to six or more times per day. In addition, a 24-hr recall interview was conducted by telephone. Total energy and nutrient intake were analyzed using the nutritional evaluation software program PCN Pro v1.32 (Cantós D et al., 2004). According to Willett et al. (1997) an adjustment of total energy was made for independent variables using the nutrient residual model (adjusted for energy). Taking into consideration that most nutrients are positively correlated with energy intake and this could introduce a confounding factor. The Willett's methodology allows the calculation of the effect of a specific nutrient beyond any effect due to the energy intake.

Assessment of actigraphy sleep parameters

Actigraphy was used to estimate sleep parameters objectively (Ancoli-Israel et al., 2003) All the participants wore an actigraph around the non-dominant wrist (ActiSleep, ActiGraph, Pensacola, FL, USA) continuously for 7 consecutive days and nights, and the actigraph was only to be removed while bathing. A validated algorithm was used to calculate: time in bed (hh:mm:ss), time out bed (hh:mm:ss), total sleep time (min), total time in bed (min), latency (min), efficiency (%), wake after sleep onset (WASO, min), number of awakenings and time average of awakenings (min). The energy expenditure by physical activity of participants was obtained also throughout the actigraph (kcal/day).

Assessment of sleep disturbances

Sleep disturbances were measured with the Sleep Disturbance Scale for Children (SDSC, Bruni et al., 1996), a well-validated, parental report instrument. Parents were asked to answer 26 questions which are grouped into six sleep factors: Behavioral Sleep problems of Initiating and Maintaining Sleep (BSP); Sleep Breathing Disorder (SBD); Parasomnias-Arousal Disorders (AD) such as nightmares and night terrors, and Sleep-Wake Transition Disorders (SWTD), such as sleep walking, sleep talking and bruxism; Excessive Daytime Somnolence (EDS); Sleep Hyperhidrosis – night sweating (HYH); and Total Sleep Problems (TSP) which results of the sum of all individual questions scores.

Biochemical parameters

Morning fasting blood samples were obtained by venipuncture in the forearm of each participant. Serum levels of iron, ferritin, transferrin, zinc, omega-6 and omega-3 polyunsaturated acids were determined in the Laboratory of Biochemistry of the Hospital *Sant Joan de Déu* (Barcelona).

Statistical analyses

Continuous variables were expressed as mean ± standard deviation (SD) or median and interquartile range, whereas categorical variables were expressed as percentage. Differences in normally and non-normally distributed continuous variables were compared using the Student's T test and the Mann-Whitney U test, respectively. Categorical variables were compared using the χ^2 test. We assessed the associations between actigraphic sleep parameters or subjective sleep disturbances (SDSC scale) on dietary intake or biochemical parameters using Pearson correlations. Any significant relationships between variables were further investigated with multivariable linear regression. Analyses were performed using the SPSS 21.0 statistical software package (SPSS Inc., Chicago, Ill., USA) and a p value$\leq$0.05 was considered statistically significant.

Results

General characteristics

The baseline characteristics of cases and controls are shown in **Table 1.** There were statistically significant differences associated with ADHD for BMI (and its Z-Score) and physical activity. Regarding nutrient plasma levels, no statistically significant differences were found, even though cases showed slightly lower plasma concentrations of iron and ferritin and slightly higher levels of omega-6. The cases showed an average value of ADHD-RS (DMS-IV) of 34.2 (9.7) and the K-SADS confirmed the diagnosis of all the cases. In regard to comorbid diagnoses, 33.3% of ADHD patients met cut-off criteria for oppositional defiant disorder (ODD), 23.3% for anxiety, 3.3% for conduct disorder (CD) and 1.7% for depression.

Regarding dietary intakes, individuals with ADHD consumed statistically higher amounts of simple sugars and caffeine and lower amounts of total protein than controls

(**Table 2**). Moreover, no significant differences were found for total daily energy intake or other nutrient intakes, besides vitamin A and B12, both higher in cases than in controls.

ADHD is associated with a higher incidence of sleep disturbances but not actigraphy sleep parameters.

No statistically significant differences were observed with respect to actigraphy sleep parameters among cases and controls, even though cases seem to be in bed and to get out bed later and showed slightly less total sleep time (**Table 3**). On the contrary, children and adolescents with ADHD showed statistically significant higher score of sleep total problems (STP) than controls and the percentage of the ADHD patients who meet cut-off criteria for STP was larger when compare with controls (**Table 3**). In addition, when compare to controls, cases showed more behavioral sleep problems of initiating and maintaining sleep and more sleep-wake transition disorders and a higher excessive daytime somnolence. The percentage of ADHD subjects who met the cut-off criteria was also higher than in controls for those sleep disturbances.

Sleep duration and efficiency associations with dietary intake and plasma levels of nutrients

No associations were evident between actigraphy parameters and dietary intake, except for caffeine intake which was associated with the "time in bed", both in cases and controls: [ß (95%CI) = 0.06(0.02-0.10), p=0.006] and [ß (95%CI) = 0.12(0.004-0.229), p=0.043], respectively, after adjusting for gender, age and BMI.

On the other hand, a positive association was evident between sleep efficiency and iron serum levels (p=0.038), after adjusting for gender, age and BMI (**Figure 1**). The association was only found in subjects with ADHD and not in controls. No further associations were found among sleep actigraphy parameters and the other nutrient serum values.

Sleep disturbances associations with dietary intake in ADHD individuals

Associations between sleep disturbances and nutrient intake in children and adolescent with AHDH are presented in **Table 4**. No correlation between sleep disturbances and dietary intake was identified in controls. Positive associations were identified between

sleep breathing disorders (SBD) and protein intake and between excessive daytime somnolence (EDS) and fat intake. Intake of dietary fiber was negatively associated with both sleep total problems (STP) and excessive daytime somnolence (EDS). Regarding micronutrient intakes (**Table 4**), negative associations were identified between excessive daytime somnolence (EDS) and the intake of certain vitamins and minerals such as: thiamin, vitamin, B6, acid folic, iron and magnesium. Intake of folic acid and iron was also negatively associated with sleep total problems (STP). All of these associations were maintained after adjusting for gender, age and BMI. Lastly, no associations were identified between sleep disturbances and serum nutrient levels, with the exception of a relationship between the ratio of AA/DHA and Behavioral Sleep Problems of Initiating and Maintaining Sleep [ß=1.4 95%IC 0.1-2.8; p=0.034], after adjusting for gender, age and BMI.

Discussion

To the authors' knowledge this is the first study investigating the relationships between dietary intake, nutrient serum levels, sleep duration, sleep quality and sleep disturbances in children and adolescents with ADHD. We are aware of one former similar study but only the relationship between dietary intake and sleep disturbances in children with ADHD was investigated (Blunden et al., 2010). Our main finding was that iron serum levels were associated to sleep quality parameters, specifically sleep efficiency. This relationship was not found in controls. Moreover, children and adolescents with ADHD who had a higher intake of dietary fiber and of certain vitamins and minerals showed less total sleep problems and less excessive daytime somnolence while ADHD subjects with a higher ratio of AA/DHA had more difficulty in initiating and maintaining sleep. In contrast to the findings of Blunden et al (2010), we did not find any significant relationship among sleep disturbances and higher intakes of energy, fat, carbohydrates and sugar with the exception of saturated fat intake, which was correlated with excessive daytime somnolence.

Although iron has been one of the most studied micronutrient involved in the pathophysiology of ADHD, only few works have explored the association between iron and sleep disturbances in patients with ADHD. Recent studies have found associations between low serum of ferritin and restless legs syndrome in ADHD children (Oner et al., 2007) and sleep wake transition disorders, which includes items of abnormal

movements and small awakenings during sleep (Cortese et al., 2009). Consistent with that previous study, we found that lower iron serum levels were associated with lower sleep quality (measured as sleep efficiency). Cortese et al. (2009) published a study in which they evaluated motor activity during sleep in children with ADHD. They concluded that ferritin levels below 45 μg/l might indicate a risk for disruption of normal sleep patterns (abnormal movements and small awakenings) in children with ADHD. However, in our study, no significant differences regarding ferritin levels and disruption of normal sleep patters were found.

The importance of long-chain polyunsaturated fatty acids (LC-PUFA) for sleep initiation and maintenance has long been known. Evidence suggests that the balance of DHA and AA in the pineal gland regulates melatonin production, with higher levels of DHA relating to increased levels of melatonin (Montgomery et al., 2014). DHA seems needed for one of the enzymes, which transforms serotonin into melatonin (Peuhkuri et al., 2012b). Epidemiological studies find higher levels of omega-3 fatty acids associated with fewer sleep problems in infants (Cheruku et al., 2002) and children with ADHD (Burgess et al., 2000). However, no improvement either in subjective or objective parameters of sleep or secretion of melatonin was notices in more than 100 adults with chronic insomnia after supplementation with polyunsaturated fatty acid capsules (Cornu et al., 2010). Consistent with the fact that DHA seems to be needed for the synthesis of melatonin, we did find a positive relationship between the ratio of AA/DHA and difficulties in initiating and maintaining sleep. Thus, an increase in DHA levels would decrease the ratio AA/DHA and eventually the difficulties would decrease.

The fact that caffeine intake was related with the time to go to bed in both controls and cases can be more related to certain behaviors rather than a cause-consequence. The interesting fact was that caffeine intake did not correlated with other sleep quality parameters neither with total sleep time. Caffeine has both the stimulatory and inhibitory mechanisms affecting the levels of melatonin (Peuhkuri et al., 2012b). Which of these dominate in normal healthy subjects is not clear. Caffeine can decrease the synthesis of melatonin but caffeine and melatonin compete for the same metabolizing liver cytochrome P450 enzymes, resulting in higher serum levels of melatonin after large doses of caffeine (Ursing et al., 2003)

There is a growing body of evidence showing that ingested food can affect sleep (Peuhkuri et al., 2012). Cow's milk, certain fruits (cherries and kiwifruits) and herbal products, such as chamomile tea are considered sleep-promoting foods. Here again, robust scientific evidence supporting this is, in many cases, nonexistent (Peuhkuri et al., 2012; St-Onge et al., 2016). Similarly, the evidence supporting a beneficial influence of macronutrient composition of the diet on sleep parameters is weak. Of proteins, amino acid tryptophan is the most promising candidate as a sleep-promoting nutrient since is a precursor to the neurotransmitter serotonin and the neuro-secretory hormone melatonin, both of which are linked to sleep and alertness (Peuhkuri et al., 2012; Kaput et al., 2014). In our study, we were not able to calculate the average intake of tryptophan of cases and controls. We found that a higher intake of saturated fatty acids was associated with excessive daytime sleepiness. Grandner et al. (2014) found in a large sample of children and adults that high intakes of palmitic acid, a saturated fatty acid, was related to sleep problems, such as difficulty in falling sleep. Interestingly, the same authors showed that reduced intake of lauric acid, another saturated fatty acid, was associated with both difficulties falling asleep and maintaining sleep. Future studies examining the roles of diets containing saturated fatty acids are need and the possible mechanisms, which could explain these relationships.

Additionally, deficiencies of group B vitamins and minerals (e.g. magnesium) may disrupt sleep, and their effect seems to be based on their influence on the secretion of melatonin (Peuhkuri et al., 2012a). In our study, lower intakes of vitamin B6 and folic acid were associated with greater excessive daytime sleepiness, and with higher total sleep problems specifically in the case of folic acid. Some clinical evidence substantiates the influence of B vitamins on sleep (Peukuri et al., 2012a,b; St-Onge et al., 2016). In the brain, vitamin B6 is needed in the synthesis of serotonin from tryptophan. Huang et al. (2013) found significantly lower vegetable consumption and vitamin B6 intakes in a group of elderly woman with poor sleep compared to good sleepers. In the same line, a placebo, double-blind study suggested that vitamin B6 was found to increase cortical arousal during REM sleep and to increase the vividness of the dreams in a group of college students, thus demonstrating some influence on sleep (Ebben et al., 2002). Folic acid intake was independent inverse associated with sleep disturbance (Beydoun et al., 2014) and a lower intake of carbohydrates, folic acid and B_{12} was found in insomniacs comparing to normal sleepers (Zadeh et al., 2011). Folate,

vitamin B6, magnesium and zinc deficiencies have been linked with lower melatonin levels in rodents (Peuhkuri et al., 2012b). Folate and B6 vitamin are also supposed to boost the formation of serotonin and tryptophan as coenzymes. Zinc and magnesium instead, are supposed to enhance the formation of melatonin from serotonin by binding to arylalkylamone-N-acetyltransferase enzyme (AANAT), thus activating it and increasing the affinity of serotonin for binding to AANAT (Peuhkuri et al., 2012b). In humans, the role of these vitamins and minerals is less well studied in this connection.
Although clinical evidence in humans on sleep effects and magnesium is scarce, a relationship between the concentration of magnesium in the blood and sleep has been suggested (Chollet et al., 2001). Nonetheless, oral magnesium supplementation has improved sleep quality and total sleep time in subjects with low magnesium (Peuhkuri et al., 2012a). Rondanelli et al (2011) found that the administration of melatonin, magnesium, and zinc appears to improve the quality of sleep and the quality of life in long-term care facility residents with primary insomnia compare with a placebo capsule. This is believed to be based on magnesium enhancing the secretion of melatonin from the pineal gland by stimulating serotonin N-acetyltransferase activity, the key enzyme in melatonin synthesis (Peuhkuri et al., 2012a).

Some limitations of our design and methods should be acknowledged, such as the case-control study design, which prevents our ability to assess cause-and-effect associations. Furthermore, all dietary instruments such as food frequency questionnaires measuring past food intake are vulnerable both to random and systematic measurement errors. Neither the timing of food consumption nor the food additive content were measured in this study and these reportedly affect sleep (Harada et al., 2007) and behavior (Bateman et al., 2004). Nonetheless, this study has several important strengths, including the fact that all the cases included were naïve, taking no medication. The use of certain drugs might affect the food choices and provoke changes to the child and adolescent's diet and sleep. Also, well-trained, experienced psychiatrists and psychologists performed the evaluation.

In summary, our findings suggest that the interrelationship between diet and sleep in children wit ADHD may be of consequence and warrants further investigation. Given the importance of treating ADHD from a holistic and multidisciplinary perspective and the fact that both sleep and dietary intake are potentially modifiable behaviors, our

findings suggest the need for future studies to further understand and enable a more holistic approach to the treatment of children and adolescent with ADHD symptoms.

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REFERENCES

- American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 4th ed. Washington, DC; 2000.
- Afaghi A, O'Connor H, Chow CM. High-glycemic-index carbohydrate meals shorten sleep onset. Am J Clin Nutr 2007; 85:426-30.
- Ancoli-Israel S, Cole R, Alessi C et al. The role of actigraphy in the study of sleep and circadian rhythms. Sleep 2003; 26: 342-92.
- Bateman B, Warner JO, Hutchinson E et al. The effects of a double blind, placebo controlled, artificial food colourings and benzoate preservative challenge on hyperactivity in a general population sample of preschool children. Arch Dis Childhood 2004; 89: 506-511.
- Beydoun MA, Gamaldo AA, Canas JA, Beydoun HA, Shah MT, McNeely JM, Nakagomi M. Serum Nutritional Biomarkers and Their Associations with Sleep among US Adults in Recent National Surveys. *PLoS ONE 2014; 9*:e103490.
- Bellsile F. Effects of diet on behavior and cognition in children. Br J Nutr 2004; 92:S227-32.
- Blunden SL, Milte CM, Sinn N. Diet and sleep in children with attention deficit hyperactivity disorder: Preliminary data in Australian children. J Child Health Care 2010; 14-24.
- Burgess JR, Stevens L, Zhang W, Peck L. Long-chain polyunsaturated fatty acids in children with attention-deficit hyperactivity disorder. Am J Clin Nutr 2000; 50:991-1000.
- Bruni O, Ottaviano S, Guidetti V et al., The Sleep Distubarnce Scale for Children (SDSC): construction and validations of an instrument to evaluate sleep disturbances in childhood and adolescence. J Sleep Res 1996; 5:251-61.
- Cantós D, Farran A, & Palma I. PCN Pro. Barcelona: Universitat de Barcelona. 2004
- Chaput JP. Sleep patterns, diet quality and energy balance. Physiol Behav 2014; 134: 86-91.
- Cheruku SR, Montgomery-Downs HE, Farkas SL et al. Higher maternal plasma docosahexaenoic acid during pregnancy is associated with more mature neonatal sleep-sate patterning. Am J Clin Nutr 2002; 76: 608-13.

- Chollet D, Franken P, Raffin Y et al. Magnesium involvement in sleep: genetic and nutritional models. Behav Genet 2001; 31: 413-25.

- Cohen-Zion M, Ancoli-Israel S. Sleep in children with attention deficit hyperactivity disorder (ADHD): A review of naturalistic and stimulant intervention studies. Sleep Med Rev 2004; 8: 379-402.

- Corkum P, Tannock R, Moldofsky H. Sleep disturbances in children with attention-deficit/hyperactivity disorder. J Am Acad Child Adolesc Psychiatry 1998; 74:444-5.

- Cornu C, Remontet L, Noel-Baron F et al. A dietary supplement to improve the quality of sleep: a randomized placebo controlled trial. BMC Complement Alternativ Med 2010; 10: 29-32.

- Cortese S, Konofal E , Bernardina BD, Mouren M-C, & Lecendreux M. Sleep disturbances and serum ferritin levels in children with attention-deficit/hyperactivity disorder. Eur Child & Adolesc Psychiatry 2009; 18; 393–9.

- Dashti HS, Zuurbier LA, de Jonge E. et al. Actigraphic sleep fragmentation, efficieny and duration associate with dietary intake in the Rotterdam Study. J Sleep Res 2016; 25: 404-11.

- Diaz-Román A, Hita-Yáñez E, Guela-Casal G. Sleep characteristics in children with attention deficit hyperactivity disorder: Systematic review and meta-analysis. J Clin Sleep Med 2016; 12: 747-56.
- DuPaul GJ, Power TJ, Anastopoulos AD, Reid R. ADHD Rating Scale-IV: Checklists, Norms and Clinical Interpretation. New York: The Guilford Press; 1998

- Ebben M, Lequerica A, Spielman A. Effects of pyridoxine on dreaming: a preliminary study. Percept Mot Skills 2002; 94: 135-40.

- Fallone G, Acebo C, Seifer R, Carskadon MA. Experimental restriction of sleep opportunity in children: effects on teacher ratings. Sleep 2005; 28: 1561-7.

- Grandner MA, Jackson N, Gerstner JR, Knutson KL. Sleep symptoms associated with intake of specific dietary nutrients. J Sleep Res 2014; 23: 22-34.

- Harada T, Hiortani M, Maeda M et al. Correlation between breakfast tryptophan content and morning-evening in Japanese infants and students aged 0-15 years. J Physiological Anthropol 2007; 26: 201-7.

- Huang, Y-C, Wahlqvist ML & Lee M-S. Sleep Quality in the Survival of Elderly Taiwanese: Roles for Dietary Diversity and Pyridoxine in Men and Women. J Am Coll Nut 2013; 32: 417–427.

- Montgomery P, Burton JR, Sewell RP et al. Fatty acids and sleep in UK children: subjective and pilot objective sleep results form the DOLAB study – a randomized controlled trial. J Sleep Res 2014; 23: 364-88.

- Oner P, Dirik EB, Taner Y, Caykoylu A, & Anlar O. Association between low serum ferritin and restless legs syndrome in patients with attention deficit hyperactivity disorder. Tohoku J Ex Med 2007; 213: 269–276.

- Pelssner L, Frankena K, Toorman J et al. A randomized controlled trial into the effects of food on ADHD. Eur Child Adolesc Psyc 2009; 18: 12-29.

- Peuhkuri K, Sihvola N, Korpela R. Diet promotes sleep duration and quality. Nutr Res 2012a; 32:309-19.

- Peuhkuri K, Sihvola N, Korpela R. Dietary factors and fluctuating levels of melatonin. Food Nutr Res 2012b; 56: 17252.

- Rodríguez IT, Ballart JF, Pastor GC, Jordà EB, Val VA. Validation of a short questionarire on frecuency of dietary intake: reproducibility and validity. Nutr Hosp. 2008;23:242–252

- Rondanelli M, Opizzi A, Monteferrario F et al., The effect of melatonin, magnesium, and zinc on primary insomnia in long-term residents in Italy: a double-blind, placebo-controlled clinical trial. J Am Geriatr Soc 2011; 59: 82-90.

- St-Onge MP, Mikic A, Pietrolungo CE. Effects of diet on sleep quality. Adv Nutr 2016; 7: 938-49.

- Ulloa RE, Ortiz S, Higuera F, et al. Interrater reliability of the Spanish version of Schedule for Affective Disorders and Schizophrenia for School-Age Children--Present and Lifetime version (K-SADS-PL). Actas Esp Psiquiatr 2006;34:36–40

- Ursing C, Wikner J, Brismar K, Röjdmark S. Caffeine raises the serum melatonin levels in healthy subjects: an indication of melatonin metabolism by cytochrome P450 (CYP)1A2. J endocrinol Invest 2003; 26:403-6.

- Vélez-Galarraga R, Guillén-Grima F, Crespo-Eguilaz N, Sánchez-Carpintero R. Prevalence of sleep disorders and their relationship with core symptoms of inattention and hyperactivity in children with attention-deficit/hyperactivity disorder. Eur J Paediatric Neurol 2016; http://dx.doi.org/10.1016/j.ejpn.2016.07.004.

- Wechsler D. Escala de Inteligencia de Wechsler para Niños – IV (WISC-IV) (TEA Edicio). Madrid, Spain; 2005.

- Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. Am J Clin Nutr. 1997; 65(4 Suppl):1220S–1228S; discussion 1229S–1231S

- Zadeh SS, & Begum K. Comparison of nutrient intake by sleep status in selected adults in Mysore, India. Nutr Res Pract 2011; 5:230–5

Table 1. General characteristics in subjects with attention-deficit hyperactivity disorder (ADHD) and in control subjects.[a]

	Cases (ADHD) n=60		Controls n=60		P-value
Gender, %male (n)	56.7	(34)	56.7	(34)	---
Age, years	9.3	(2.8)	9.3	(2.8)	---
Height, cm	136.5	(16.8)	138.6	(17.3)	0.496
Weight, kg	38.1	(16.2)	36.4	(14.5)	0.536
BMI[b], kg/m^2	19.6	(4.3)	18	(3.3)	**0.042**
BMI Z-Score	0.69	(1.1)	0.25	(1.1)	**0.027**
Physical activity, kcal/day[c]	1248.3	(824.6)	861.4	(483.3)	**0.013**
Nutrient blood and plasma levels					
Iron, μg/dl	80.1	(32.5)	85.4	(32.1)	0.366
Transferrin, mg/dl	266.2	(29.9)	265.7	(27.9)	0.922
Ferritin, μg/L	36.2	(21.0)	42.2	(36.9)	0.273
Zinc, μg/L	988.6	(182.0)	975.5	(162.0)	0.680
AA acid, nmol/g Hb	3200.4	(610.7)	2909.9	(772.9)	**0.031**
DHA acid, nmol/g Hb	889.2	(232.2)	822.9	(348.8)	0.243
AA/DHA	3.7	(1.0)	3.8	(1.1)	0.790

[a]Data are shown mean (SD) except for gender that is expressed as % of male participants (number) and body weight at birth; [b]Body mass index; [c] Measured with the actigraphy accelerometer ActiSleep; Bold face representing statistical differences with $p<0.05$.

Table 2. Energy (per day), nutrient (per day and per 1000 kcal) and caffeine (per day) intake in subjects with attention-deficit hyperactivity disorder and in control subjects.[a]

	Cases (ADHD) n=60	**Controls n=60**	**p-value**
Energy, kcal	1609.9 (375.3)	1626.9 (382.8)	0.806
Carbohydrates, g	122.7 (10.9)	119.6 (11.0)	0.114
Simple sugars, g	60.4 (16.7)	54.5 (10.5)	**0.022**
Protein, g	47.3 (7.3)	50.1 (7.0)	**0.031**
Animal protein, g	34.5 (7.1)	36.6 (7.8)	0.129
Vegetal protein, g	12.8 (2.2)	13.5 (2.6)	0.087
Fat, g	35.2 (3.9)	35.4 (4.1)	0.780
Saturated fat, g	14.0 (2.1)	14.2 (2.0)	0.448
Monounsaturated fat, g	11.5 (1.3)	11.5 (1.5)	0.793
Polyunsaturated fat, g	6.1 (1.7)	5.9 (1.6)	0.441
Dietary fiber, g	9.4 (2.1)	10.2 (2.5)	0.057
Iron, mg	6.1 (1.0)	6.4 (1.1)	0.129
Zinc, mg	5.1 (0.8)	5.4 (0.7)	0.066
Calcium, mg	527.8 (122.0)	542.3 (108.8)	0.474
Magnesium, mg	166.3 (22.9)	168.6 (24.9)	0.599
Vitamin C, mg	71.7 (36.8)	71.9 (31.3)	0.970
Riboflavin, mg	1.0 (0.2)	1.1 (0.2)	0.512
Thiamin, mg	0.8 (0.1)	0.8 (0.2)	0.387
Niacin, mg	10.4 (2.4)	11.1 (2.4)	0.133
Vitamin B6, mg	1.1 (0.2)	1.11 (0.2)	0.288
Folic acid, mg	144.5 (35.4)	153.3 (42.5)	0.218
Vitamin B12, µg	2.7 (0.7)	3.1 (0.7)	**0.013**
Vitamin A, µg	330.8 (105.9)	370.4 898.39	**0.036**
Vitamin D, µg	1.5 (0.8)	1.8 (0.9)	0.098
Vitamin E, mg	3.2 (0.8)	3.4 (0.9)	0.281
Caffeine, mg	3.8 (5.3)	1.6 (1.9)	**0.003**

[a]Data are given in mean and standard deviation; Bold face representing statistical differences with $p<0.05$.

Table 3. Sleep characteristics of subjects with attention-deficit hyperactivity disorder and in control subjects.[a]

	Cases (ADHD) n=60	Controls n=60	p-value
Actigraph sleep parameters			
Time in bed	22:36:36 (00:53)	22:21:00 (00:58)	0.137
Time out bed	07:40:12 (00:51)	07:28:48 (00:50)	0.226
Total sleep time, min	468.2 (50.9)	474.5 (60.9)	0.540
Latency, min	10.6 (9.8)	8.4 (6.9)	0.172
Efficiency, %	86.5 (3.7)	85.0 (11.9)	0.361
Wake after sleep onset, min	63.4 (20.0)	66.3 (22.7)	0.454
Awakenings	20.4 (5.4)	20.0 (5.5)	0.720
Average Awakenings, min	3.7 (0.9)	3.9 (1.1)	0.505
Sleep Disturbance Scale for Children (SDSC)			
STP	43.8 (12.2)	31.4 (4.3)	**<0.001**
BSP	11.9 (4.9)	8.2 (2.0)	**<0.001**
SBD	4.1 (2.2)	3.4 (1.3)	0.060
AD	3.4 (1.2)	3.2 (0.7)	0.315
SWTD	11.8 (4.5)	7.9 (2.3)	**<0.001**
EDS	9.2 (4.5)	6.1 (2.0)	**<0.001**
HYH	3.3 (2.5)	2.6 (1.7)	0.077
Percentages of individuals who meet cut-off criteria for sleep disturbances (SDSC)			
STP	55.0%	3.3%	**<0.001**
BSP	53.3%	11.7%	**<0.001**
SBD	21.7%	10.0%	0.080
AD	16.7%	10.0%	0.283
SWTD	76.7%	35.5%	**<0.001**
EDS	56.7%	20.0%	**<0.001**
HYH	26.7%	15.0%	0.116

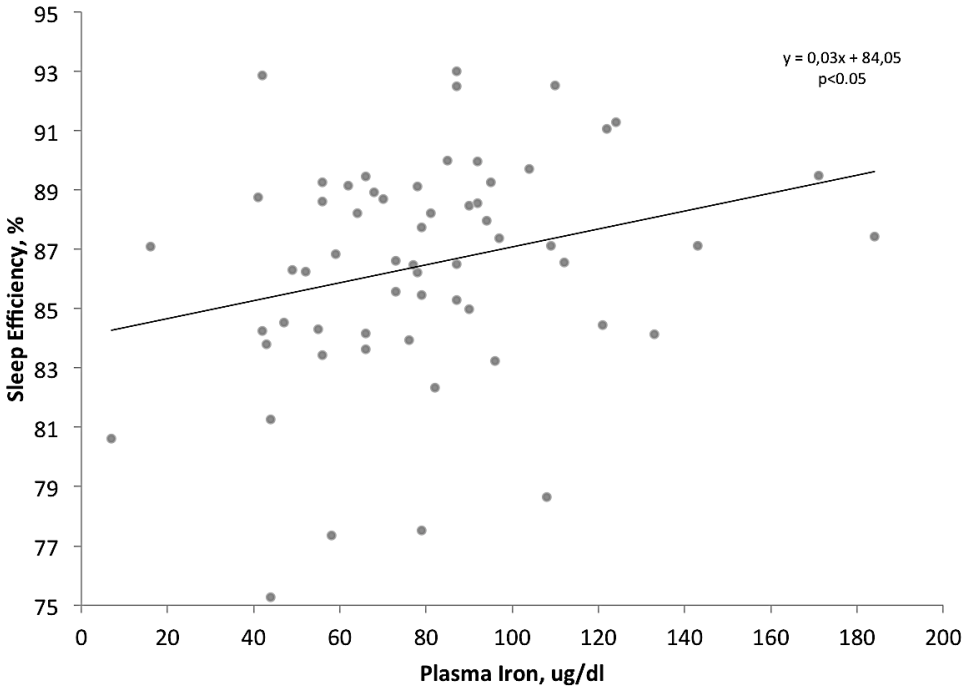
[a]Data are given in mean and standard deviation. **STP**: Sleep total problems; **BSP**: Behavioral Sleep Problems of Initiating and Maintaining Sleep; **SBD**: Sleep Breathing Disorders; **AD**: Arousal Disorders; **SWTD**: Sleep-Wake Transition Disorders; **EDS**: Excessive Daytime Somnolence; **HYH** Hyperhidrosis; Bold face representing statistical differences with $p<0.001$.

Table 4. Correlations between sleep disturbances measured with the Sleep Disturbance Scale for Children (SDSC) and daily energy and nutrient intake in subjects (n=60) with ADHD.

	STP	BSP	SDB	AD	SWTD	EDS	HYH
Energy, Kcal	0.06	0.06	-0.15	-0.10	0.11	0.05	0.05
Carbohydrates, g	-0.24	-0.20	-0.11	-0.04	-0.20	-0.15	-0.80
Simple sugars, g	-0.13	-0.09	-0.14	-0.02	-0.13	-0.04	-0.08
Protein, g	0.16	0.19	**0.28***	0.09	0.10	-0.05	0.07
Fat, g	0.18	0.09	-0.08	-0.04	0.18	0.14	0.06
Saturated, g	0.18	0.10	-0.10	-0.04	0.15	**0.28***	-0.02
Monounsaturated,g	0.22	0.13	0.10	-0.04	0.18	0.22	0.07
Polyunsaturated, g	-0.01	-0.03	-0.05	0.04	0.06	0.01	0.01
Dietary fiber, g	**-0.23***	-0.21	0.05	-0.04	-0.03	**-0.34****	-0.02
Vitamin C	-0.22	-0.08	-0.08	-0.12	-0.12	0.25	-0.04
Riboflavin	-0.03	-0.03	0.09	-0.07	0.03	-0.10	0.01
Thiamin	-0.25	-0.08	0.13	-0.07	-0.17	**-0.42****	-0.09
Niacin	0.01	0.07	0.09	-0.01	0.06	-0.19	0.08
Vitamin B6	-0.13	-0.06	0.06	-0.09	-0.02	**-0.29***	0.02
Folic acid	**-0.27***	-0.18	-0.05	-0.12	-0.08	**-0.37***	-0.04
Vitamin B12	-0.01	0.01	0.07	-0.15	-0.08	0.05	0.02
Vitamin A	0.01	-0.01	0.09	-0.09	0.01	-0.01	0.08
Vitamin D	-0.24	-0.16	-0.01	-0.07	-0.25	-0.27	-0.08
Vitamin E	-0.19	-0.13	0.02	0.04	-0.12	-0.27	-0.07
Calcium	0.06	0.08	0.13	0.02	0.21	-0.01	-0.01
Iron	**-0.21***	-0.16	0.05	-0.03	0.01	**-0.36****	-0.04
Zinc	0.01	0.05	0.19	-0.01	0.08	-0.19	0.02
Magnesium	-0.20	-0.16	0.28	-0.05	-0.01	**-0.34***	-0.02

*p<0.05; **p<0.01; STP: Sleep total problems; BSP: Behavioral Sleep Problems of Initiating and Maintaining Sleep; SBD: Sleep Breathing Disorders; AD: Arousal Disorders; SWTD: Sleep-Wake Transition Disorders; EDS: Excessive Daytime Somnolence; HYH Hyperhidrosis

Figure 1. Scatterplot depicting correlations between sleep efficiency (%) and serum iron levels (ug/dl) in subjects with ADHD.



4.3 Iron and ferritin associates with behavioral symptoms and cognitive abilities in children and adolescents with ADHD.

Iron and ferritin serum levels associates with behavioral symptoms and cognitive abilities in children and adolescents with ADHD

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Introduction

Attention deficit hyperactive disorder (ADHD) is characterized by continuous inattention and/or hyperactivity- impulsivity and also show features of oppositional defiance disorder (ODD), obsessive compulsive disorder (OCD) and aggressive behavior[144]. Cognitive impairments are also associated with this disorder[145]. The etiology of ADHD remains unknown, although several studies recognize the important influence of variety of factors such as genetic, biological and environmental, such as diet[4]. Several cross-sectional studies[39,40,45] conclude that ADHD patients show a tendency to have a poor quality diet, which could cause certain nutrient deficiencies. Micronutrients (vitamins and minerals) act as cofactors in many biochemical reactions; therefore nutritional deficiencies may interact with genetic pathways affecting the function of key enzymes involved in the brain function[146].

Iron deficiency has been reported to be a possible risk factor in the pathophysiology of ADHD, although thus far, the results regarding this issue have been contradictory[79,82,89,147]. Iron is an essential element that plays a number of roles in many biological processes, including the central nervous system. It has been suggested that iron deficiency might lead to ADHD symptoms via its impact on the metabolism of dopamine, since iron is a coenzyme of tyrosine hydroxylase, a critical step in dopamine synthesis[148] and of other cathecholamines, which have been involved into the pathophysiology of ADHD[81]. To date, a number of studies have shown that children with ADHD have lower iron levels, usually reported as ferritin levels, and also that iron deficiency affects cognitive, motor, development, and behavioral problems in children[79,90,149,150]. However, other studies have not confirmed such relationships[151,152].
Due to the controversial evidence on the relation of iron status in the pathophysiology of ADHD, the aim of the present study was to examine the association between different parameters indicators of iron deficiency and behavioral symptoms and cognitive indicators measured with a battery of ADHD specific diagnostic and assessment instruments in Spanish children and adolescents newly diagnosed with ADHD. Besides the traditional parameters of iron deficiency, we selected other variables such as mean corpuscular volume, mean corpuscular hemoglobin concentration or red cell distribution, which also inform about the iron status of individuals. We hypothesized that a low iron status levels would be associated with an increased in the severity of ADHD associated behaviors and with cognitive problems.

Methods

Subjects

Sixty children and adolescents (ages 6-16 years) newly diagnosed with ADHD (naïve) and 60 sex- and age- matched controls participated. Cases were recruited at the ADHD Unit of the Department of Child and Adolescent Psychiatry and Psychology of the Hospital of *Sant Joan de Deu* in Barcelona (Spain) and they were diagnosed for the first time and had never evaluated for psychiatric disorders or treated with psychopharmacological medicine. The ADHD diagnosis was performed according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR).[14] The Kiddie Schedule for Affective Disorders and Schizophrenia-Present and Lifetime version (K-SADS-PL) was also used to confirm the ADHD diagnosis and other comorbidities[16]. Exclusion criteria were: IQ<70, autism spectrum disorder, psychosis, developmental disorders, any ADHD drug treatment or nutrient (mineral/vitamin) complement.

Controls were recruited from the ADHD patients' classmates (40%) and from patients attending other hospital services (60%) (i.e. minor surgery ambulatory, etc.). Controls were screened for the absence of ADHD symptoms and the same exclusion criteria were applied also to controls. The study was approved by the Ethical Committee of the Hospital of *Sant Joan de Déu* (Barcelona, Spain). Written informed consent was obtained from participant's parents, and verbal assent from the participants. Demographic and clinical data were obtained from both subjects and parents. Participants underwent a physical examination, including height and weight. BMI was calculated as weight (kilograms) divided by height (meters) squared and BMI was standardized to BMI Z-score using age and gender.

Behavioral measures

Conners Parents Rating Scale-Revised (Short Version) (CPRS-R:S)[19]. It includes 27 items and evaluates behavior of children assessed by their parents. The scale includes oppositional behavior, inattentiveness, hyperactivity, psychosomatic and irritability domains.

Conners Teacher Rating Scale (CTRS)[20] includes 28 items and rates classroom behavior of children as assessed by teacher. There are three subscales of the form: eight items refer to inattentiveness, seven items refer to hyperactivity and eight items refer to conduct problems.

Child Behavior Checklist of Achenbach for parents (CBCL)[21] is created on 120 questions associated with behavioral and emotional problems. It includes eight different categories: anxious/depressed, withdrawn/depressed, somatic complaints, social problems, thought problems, attention problems, rule-breaking behavior, and aggressive behavior. The CBCL also show scores associated with anxiety, oppositional defiant disorder, conduct problems, somatic problems, affective problems, and attention deficit disorder.

Cognitive measures

Wechsler Intelligence Scale for Children-IV (WISC-IV)[22]. The scale consists of 10 subtests that assess verbal and performance abilities. Verbal subtests are: information, similarities, vocabulary, comprehension and digit span. Performance subtests are picture completion, picture arrangement, block design, object assembly, and digit symbol. Verbal and Performance IQ scores are obtained from the test.

Assessment iron status

Morning fasting blood samples were obtained by venipuncture in the forearm of each participant. Values of the different iron status parameters were determined in the Laboratory of Biochemistry of the Hospital *Sant Joan de Déu* (Barcelona).

Statistical analyses

Continuous variables were expressed as mean ± standard deviation (SD) whereas categorical variables were expressed as percentage. Differences in continuous variables were compared using the Student's T test. Categorical variables were compared using the χ^2 test. Multiple regression was used in order to evaluate the effects of age, gender, iron blood parameters and comorbid conditions, behavioral symptoms and cognitive measures. Model fit in the regression analysis was evaluated using Durbin-Watson test. Analyses were performed using the SPSS 21.0 statistical software package (SPSS Inc., Chicago, Ill., USA) and a p value$\leq$0.05 was considered statistically significant.

Results

General characteristics

The baseline characteristics of cases and controls are shown in **Table 1.** There were statistically significant differences associated with ADHD for BMI (and its Z-Score). On the other hand, the cases showed an average value of ADHD-RS (DMS-IV) of 34.2 (9.7) and the K-SADS confirmed the diagnosis of all the cases. In regard to comorbid diagnoses, 33.3% of ADHD patients met cut-off criteria for oppositional defiant disorder (ODD), 23.3% for anxiety, 3.3% for conduct disorder (CD) and 1.7% for depression**.**

Behavioral and cognitive assessment. Several significant group differences in ADHD symptoms were found, based on both clinical scores and questionnaires (**Table 2**) consistent with the diagnosis-based expectations. Moreover, children and adolescents with ADHD showed more difficulties that often co-occurring with ADHD, such as emotional or conduct problems or difficulties with peers (CBCL, **Table 2**). There were also significant group differences in terms of verbal comprehension, perceptual reasoning, working memory, speed processing and the IQ (WISC total score) (**Table 2**)

Iron serum status

Data on the different parameters to assess the iron status are shown in **Table 3**. When compared with controls, children and adolescents with ADHD had significantly lower levels of MHC, MCHC and RDW. No other differences were found, although cases showed slightly lower plasma concentrations of iron and ferritin but were not statistically significant.

Ferritin and iron serum levels are associated with behavioral symptoms in children and adolescents with ADHD

In children and adolescents with ADHD, hyperactivity measured with ADHD-RS and CPRS scales were significantly related with blood iron levels and remained significant after adjusting for potential confounding variables: age, sex and BMI (**Figure 1**). ADHD subjects with lower iron serum levels had higher scores, indicating more severe problems of hyperactivity. Moreover, inattention CTRS scores were correlated negatively with iron and ferritin serum levels (**Figure 1**). Those associations remained significant after adjusting for potential confounding variables. ADHD subjects with

lower levels of iron or ferritin showed higher scores of inattention, indicating more severe problems. All these associations were not found in the controls. Values of MVC were negatively associated with AHDH-RS hyperactivity (ß=-0.75 95%IC= -1.28;-0.21, p=0.007), but the association did not remain significant after adjusting for confounding variables.

Serum iron levels are associated with global intelligence quotient and working memory in children and adolescents with ADHD.

Lower intellectual quotient and working memory scores in children and adolescents with ADHD were associated with lower levels of serum iron (**Figure 2**). Moreover, values of MCHC were positively associated with processing speed (ß=3.7 95%IC=0.80; 6.60, p=0.013), remaining significant after adjusting for confounding variables. None of these associations were found in controls. On the other hand, none of the other parameters related to iron status (ferritin, hemoglobin, hematocrit, MCV, MHC, and RDW) was significantly related with WISC scores.

Discussion

This case-control study demonstrated, for the first time to our knowledge, that children and adolescents with ADHD with lower levels of iron stores (serum iron and MCHC) had significantly lower scores of intellectual quotient, working memory and processing speed. Those associations were not observed in the controls. Moreover, the study is further enhanced by the findings that ADHD children and adolescents with lower iron levels (iron and ferritin serum levels) have more severe symptoms of hyperactivity and inattention on three different scales: ADHD-RS, CPRS and CTRS. These results suggest that low iron stores, even if not associated with strictly iron deficiency or anemia, may contribute not only to the ADHD symptoms but also to the cognitive performance.

Former studies[23,24] have investigated the relationship between ferritin levels and parent CPRS and teaching CTRS ratings. All of them agreed that children with lower ferritin levels showed higher hyperactivity scores on CPRS and CTRS. Consistent with those previous studies, we have found also a significant correlation between low levels of serum iron no with serum ferritin levels and hyperactivity using two scales (ADHD-RS and CPRS). We have extended their findings because we have also found significant

associations between lower serum iron and ferritin levels and higher inattention scores on the CTRS. Konofal et al.[8] concluded that children with the most severe iron deficiency were the most inattentive, impulsive, and hyperactive. However, it should be note that some studies have not shown significant relationship between serum ferritin and the severity of ADHD symptoms[9,18].

It has been suggested that iron metabolism may have important role in ADHD pathophysiology, since iron is a coenzyme of tyrosine hydroxylase, which is critical in dopamine synthesis and is also related with monoamine oxidase, which is critical for the degradation of dopamine. Also, iron deficiency is associated with decreased dopamine transporter expression and this deficiency may lead to dysfunction in the basal ganglia[9,12]. Moreover, low plasma levels of ferritin have been reported in children with ADHD[8,25–27], although those lower serum ferritin levels not have been observed always, since several studies have reported no differences between ADHD patients and healthy control cases in terms of serum ferritin levels [9,17,23,28]. Our results are consistent with these latter studies since no significant differences were observed in the majority of iron store parameters between cases and controls. Donfrancesco et al.[9] stated that normal ferritin levels should not suggest that iron deficiency is not involved in the pathophysiology of ADHD. Indeed, serum ferritin is a marker of peripheral but not of the brain iron status, where iron is necessary as a cofactor. The extent to which serum ferritin correlates with iron levels in the brain remains unclear[29]. On the other hand, the iron supplementation received by ADHD children seems to improve significantly on ADHD symptomatology but the results are still not conclusive[30].

With regard of cognitive performance, we found that intellectual quotient, work memory and processing speed were positively associated with iron status in ADHD children after adjusting for confounding variables and not in controls. Konofal et al.[8] did also find a significant correlation between cognitive subscores on the CPRS and low ferritin levels in children with ADHD while Oner et al.[31] did not find any significant relationship among ferritin and other iron deficiency parameters with cognitive variables using different test battery, among them the WISC-Revised.

Many nutrients have been related to brain function, although, iron has been one of the most studied because of its critical role in the dopaminergic neurotransmission, brain

energy metabolism, and myelination[12,29]. Several studies have shown that iron deficiency, even without anemia, has been clearly associated to cognitive impairment, both in children and adults.[14,32,33] Lower score on mental and motor test has also been reported in development children in relation with iron status[14,32,34]. Surprisingly, no studies have investigated the relationship between iron levels and cognition in children with ADHD given the importance of this micronutrient in this pathology. It has been observed that ADHD patients have poorer cognition and school achievement and more difficulties in tasks which require planning, organization and problem solving[35–37].

Our results support the assumption that iron status may play a role in the pathophysiology of ADHD. Therefore, iron status should be included in the overall evaluation of children with ADHD. Some limitations of our design and methods should be acknowledged, the design of our study prevents our ability to assess cause-and-effect associations. Nonetheless, this study has several important strengths, including the fact that all the cases included were naïve, taking no medication. It have been suggested that the use of certain drugs, especially the psychostimulants used in the treatment of ADHD, may reduce dietary intake through the loss of appetite or affecting the children food choices[38], and therefore the blood iron status may be affected.

The present study implies that iron stores might be important in hyperactivity and inattention symptoms and in cognitive functions in children and adolescents with ADHD. The elucidation of these findings is considered to be important for both the etiology and treatment of ADHD. Our study adds to the findings in that serum iron and ferritin levels seems to be related with ADHD symptoms and cognitive measures even after adjusting for confounding variables. Iron supplementation could be considered as a part of the treatment for ADHD children even though further controlled studies with large sample sizes on this issue are necessary.

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References

1. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders, 5th Edition*. Arlington, VA; 2013.
2. Singh AP. Cognitive Functions among Children with Attention Deficit Hyperactive Disorder (ADHD) & Emotional Disorders.
3. R A B. *Attention Deficit Hyperactivity Disorder: A Handbook for Diagnosis and Treatment (3rd Ed.).* (Guilford Press, ed.). New York, NY; 2006.
4. Woo HD, Kim DW, Hong Y-S, et al. Dietary patterns in children with attention deficit/hyperactivity disorder (ADHD). *Nutrients*. 2014;6(4):1539-1553.
5. Park S, Cho S-C, Hong Y-C, et al. Association between dietary behaviors and attention-deficit/hyperactivity disorder and learning disabilities in school-aged children. *Psychiatry Res*. 2012;198(3):468-476.
6. Howard AL, Robinson M, Smith GJ, Ambrosini GL, Piek JP, Oddy WH. ADHD is associated with a "Western" dietary pattern in adolescents. *J Atten Disord*. 2011;15(5):403-411.
7. Benton D, a.i.s.b.l. IE. Micronutrient status, cognition and behavioral problems in childhood. *Eur J Nutr*. 2008;47(S3):38-50. doi:10.1007/s00394-008-3004-9.
8. Konofal E, Lecendreux M, Arnulf I, Mouren M-C. Iron deficiency in children with attention-deficit/hyperactivity disorder. *Arch Pediatr Adolesc Med*. 2004;158(12):1113-1115.
9. Donfrancesco R, Parisi P, Vanacore N, Martines F, Sargentini V, Cortese S. Iron and ADHD: time to move beyond serum ferritin levels. *J Atten Disord*. 2013;17(4):347-357.
10. Menegassi M, Mello ED De, Guimarães LR, et al. Food intake and serum levels of iron in children and adolescents with attention-deficit/hyperactivity disorder\rIngestão alimentar e níveis séricos de ferro em crianças e adolescentes com transtorno de déficit de atenção/hiperatividade. 2010;32(2):132-138.
11. Berner A, Kamal M, Bener H, Bhugra D. Higher prevalence of iron deficiency as strong predictor of attention deficit hyperactivity disorder in children. *Ann Med Health Sci Res*. 2015;4(3):291-297.
12. JM Wigglesworth HB. Iron dependent enzymes in the brain. In: Youdim MB (ed) Brain iron: neurochemical and behavioral aspects. *Taylor Fr New York*. 1998:25-66.
13. Cortese S, Lecendreux M, Bernardina BD, Mouren MC, Sbarbati A, Konofal E. Attention-deficit/hyperactivity disorder, Tourette's syndrome, and restless legs syndrome: The iron hypothesis. *Med Hypotheses*. 2008;70(6):1128-1132.
14. Halterman JS, Kaczorowski JM, Aligne CA, Auinger P, Szilagyi PG. Iron deficiency and cognitive achievement among school-aged children and adolescents in the United

States. *Pediatrics*. 2001;107(6):1381-1386.

15. S, Grantham-McGregor; C A. A review of studies on the effect of iron deficiency on cognitive development in children. *J Nutr*. 2001;131(6):49S-68S.
16. Oner O, Oner P, Bozkurt OH, et al. Effects of zinc and ferritin levels on parent and teacher reported symptom scores in attention deficit hyperactivity disorder. *Child Psychiatry Hum Dev*. 2010;41(4):441-447.
17. Menegassi M, De Mello ED, Guimarães LR, et al. Food intake and serum levels of iron in children and adolescents with attention-deficit/hyperactivity disorder. *Rev Bras Psiquiatr*. 2010;32(2):132-138.
18. Millichap JG, Yee MM, Davidson SI. Serum Ferritin in Children With Attention-Deficit Hyperactivity Disorder. *Pediatr Neurol*. 2006;34(3):200-203.
19. Conners CK, Sitarenios G, Parker JD, Epstein JN. The revised Conners' Parent Rating Scale (CPRS-R): factor structure, reliability, and criterion validity. *J Abnorm Child Psychol*. 1998;26(4):257-268.
20. Goyette CH, Conners CK, Ulrich RF. Normative data on revised Conners Parent and Teacher Rating Scales. *J Abnorm Child Psychol*. 1978;6(2):221-236.
21. Achenbach, M. T. *The Child Behavior Checklist and Related Instruments.* Lawrence Erlbaum Associates Publishers; 1999.
22. Wechsler D. *Escala de Inteligencia de Wechsler Para Niños – IV (WISC-IV)*. TEA Edicio. Madrid, Spain; 2005.
23. Percinel I, Yazici KU, Ustundag B. Iron Deficiency Parameters in Children and Adolescents with Attention-Deficit/Hyperactivity Disorder. *Child Psychiatry Hum Dev*. 2016;47(2):259-269.
24. Oner P, Oner O, Azik FM, Cop E, Munir KM. Ferritin and hyperactivity ratings in attention deficit hyperactivity disorder. *Pediatr Int*. 2012;54(5):688-692.
25. Bener A. Higher Prevalence of Iron Deficiency as Strong Predictor of Attention Deficit Hyperactivity Disorder in Children. *Eur Psychiatry*. 2015;30:691.
26. Juneja M, Jain R, Singh V, Mallika V. Iron deficiency in Indian children with attention deficit hyperactivity disorder. *Indian Pediatr*. 2010;47(11):955-958.
27. Cortese S, Angriman M, Lecendreux M, Konofal E. Iron and attention deficit/hyperactivity disorder: What is the empirical evidence so far? A systematic review of the literature. *Expert Rev Neurother*. 2012;12(10):1227-1240.
28. Kwon HJ, Lim MH, Ha M, et al. Transferrin In Korean Children With Attention Deficit Hyperactivity Disorder. *Psychiatry Investig*. 2011;8(4):366.
29. Beard J. Iron deficiency alters brain development and functioning. *J Nutr*. 2003;133(5 Suppl 1):1468S-72S.
30. Hariri M, Azadbakht L. Magnesium, Iron, and Zinc Supplementation for the Treatment

of Attention Deficit Hyperactivity Disorder: A Systematic Review on the Recent Literature. *Int J Prev Med*. 2015;6:83.

31. Oner O, Alkar OY, Oner P. Relation of ferritin levels with symptom ratings and cognitive performance in children with attention deficit–hyperactivity disorder. *Pediatr Int*. 2008;50(1):40-44.
32. Grantham-McGregor S, Ani C. A review of studies on the effect of iron deficiency on cognitive development in children. *J Nutr*. 2001;131(2S-2):649S-666S; discussion 666S-668S.
33. Scott SP, Murray-Kolb LE. Iron Status Is Associated with Performance on Executive Functioning Tasks in Nonanemic Young Women. *J Nutr*. 2016;146(1):30-37.
34. Lozoff B, Jimenez E, Hagen J, Mollen E, Wolf AW. Poorer Behavioral and Developmental Outcome More Than 10 Years After Treatment for Iron Deficiency in Infancy. *Pediatrics*. 2000;105(4).
35. Jakobson A, Kikas E. Cognitive functioning in children with and without Attention-deficit/Hyperactivity Disorder with and without comorbid learning disabilities. *J Learn Disabil*. 40(3):194-202.
36. Klenberg L, Hokkanen L, Lahti-Nuuttila P, Närhi V. Teacher Ratings of Executive Function Difficulties in Finnish Children with Combined and Predominantly Inattentive Symptoms of ADHD. *Appl Neuropsychol Child*. May 2016:1-10.
37. Barkley RA, A. R. Behavioral inhibition, sustained attention, and executive functions: Constructing a unifying theory of ADHD. *Psychol Bull*. 1997;121(1):65-94.
38. Pozzi M, Bertella S, Cattaneo D, et al. Are Non-Serious Adverse Reactions to Psychiatric Drugs Really Non-Serious? *J Child Adolesc Psychopharmacol*. 2013;23(6):394-400.

Table 1. General characteristics in subjects with attention-deficit hyperactivity disorder (ADHD) and in control subjects.[a]

	Cases (ADHD) n=60	Controls n=60	p-value
Gender, %male (n)	56.7 (34)	56.7 (34)	---
Age, years	9.3 (2.8)	9.3 (2.8)	---
Height, cm	136.5 (16.8)	138.6 (17.3)	0.496
Weight, kg	38.1 (16.2)	36.4 (14.5)	0.536
BMI[b], kg/m^2	19.6 (4.3)	18 (3.3)	**0.042**
BMI Z-Score	0.69 (1.1)	0.25 (1.1)	**0.027**
Body weight at birth			
Lower than 2.5kg, %	13.3	5.0	0.114
Higher than 2.5kg, %	86.7	95.0	

[a]Data are shown mean (SD) except for gender that is expressed as % of male participants (number) and body weight at birth;

Table 2. Values (mean and standard deviations) of behavioral and cognitive measures in subjects with attention-deficit hyperactivity disorder (ADHD) and in control subjects.

	Cases (ADHD) n=60	Controls n=60	p-value
Behavioral measures			
ADHD Parent Rating Scales			
Inattention	18.1 (5.9)	2.5 (3.1)	**0.001**
Hyperactivity	14.2 (7.6)	2.5 (3.0)	**0.001**
Total	32.4 (11.3)	5.0 (5.2)	**0.001**
Conners Parent Rating Scale			
Inattention	71.7 (11.7)	46.3 (6.5)	**0.001**
Hyperactivity	69.5 (15.0)	47.4 (4.9)	**0.001**
Oppositional	60.5 (13.9)	44.5 (6.1)	**0.001**
Total	71.5 (9.9)	45.4 (4.9)	**0.001**
Conners Teacher Rating Scale			
Inattention	58.6 (10.7)	46.3 (5.7)	**0.001**
Hyperactivity	60.7 (14.0)	48.0 (7.5)	**0.001**
Oppositional	56.6 (13.9)	48.7 (6.0)	**0.001**
Total	64.2 (14.3)	47.6 (7.8)	**0.001**
Child Behavior Checklist (CBCL)			
Attention Problems	72.0 (9.4)	52.3 (3.7)	**0.001**
ADHD	69.1 (7.1)	51.6 (3.5)	**0.001**
Defiant	63.7 (9.1)	52.8 (4.5)	**0.001**
Anxiety	62.2 (7.3)	54.7 (6.0)	**0.001**
Depression	59.4 (7.8)	54.1 (7.3)	**0.001**
Attention problems	72.0 (9.4)	52.3 (3.7)	**0.001**
Breaking rules	60.5 (7.0)	52.1 (3.6)	**0.001**
Aggressive behavior	65.4 (8.8)	52.6 (4.1)	**0.001**
Oppositional Defiant	63.7 (9.1)	52.8 (4.5)	**0.001**
Conduct problems	60.9 (7.4)	51.5 (3.3)	**0.001**
Cognitive measures			
Wechsler Intelligence Scale for Children (WISC)			
Verbal comprehension	103.0 (16.9)	117.6 (15.5)	**0.001**
Perceptual reasoning	98.4 (12.9)	113.1 (14.5)	**0.001**
Work memory	92.7 (14.9)	108.7 (14.5)	**0.001**
Processing speed	98.1 (12.4)	110.1 (13.8)	**0.001**
WISC total score	97.3 (13.4)	114.6 (17.6)	**0.001**

Bold face representing statistical differences with $p<0.001$.

Figure 1. Scatterplot depicting correlations between iron and ferritin serum levels and behavioral measures in subjects with ADHD.

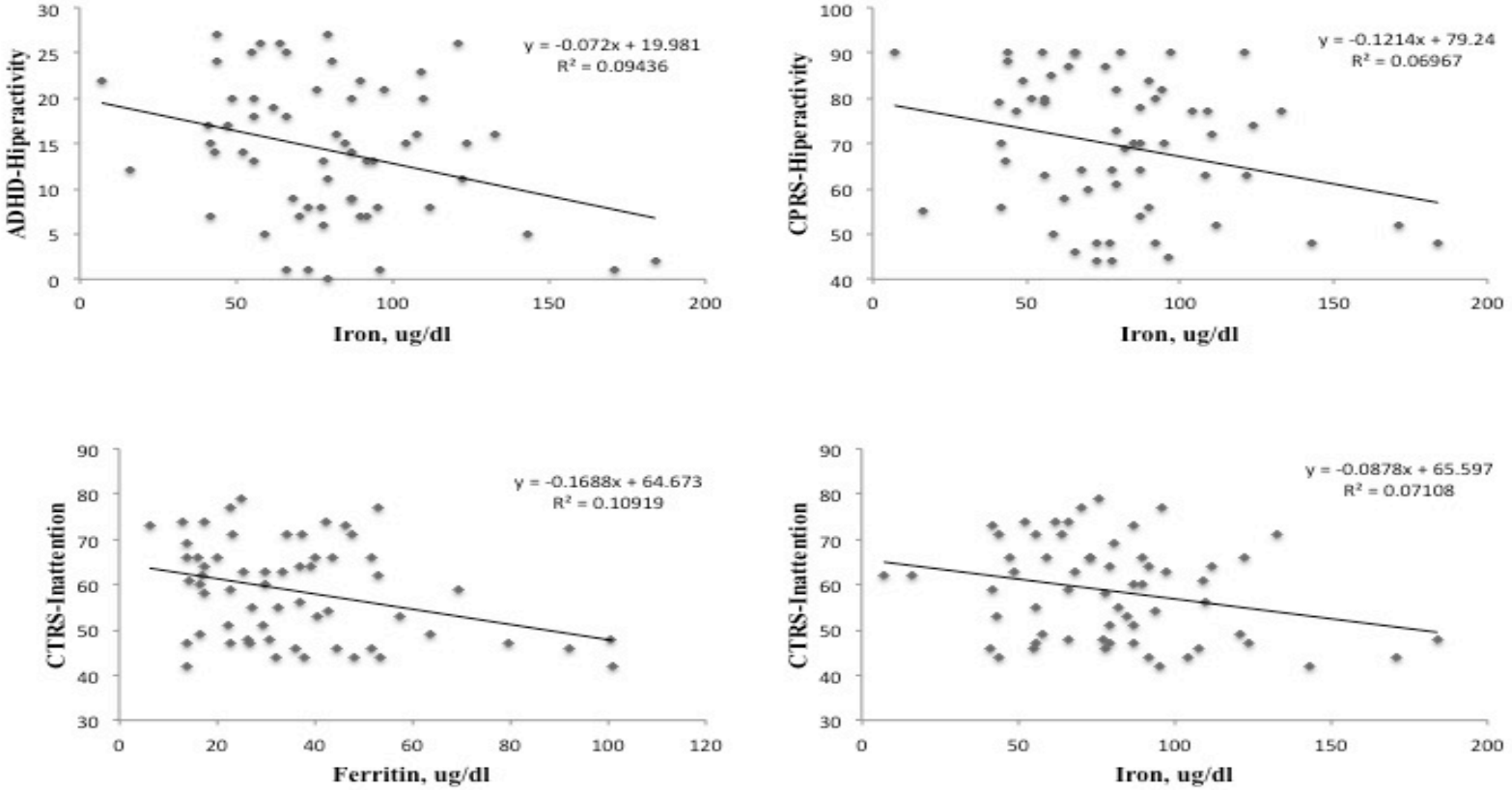
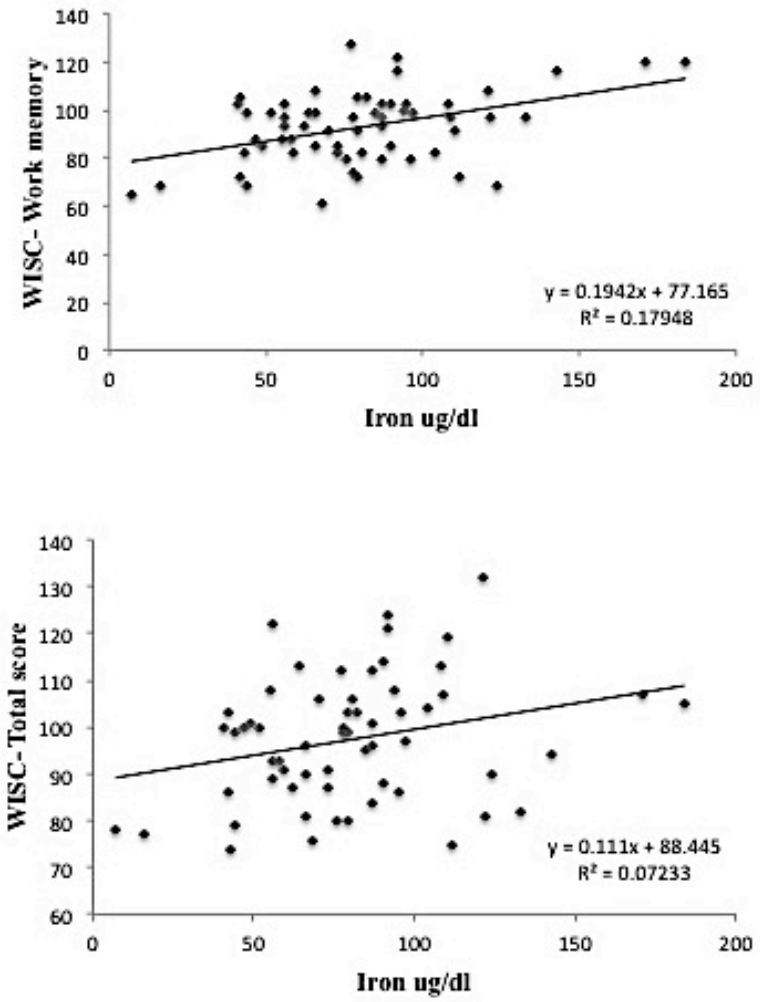


Figure 2. Scatterplot depicting correlations between iron serum levels and cognitive measures (Wechsler Intelligence Scale for Children) in subjects with ADHD.



5. Discussion

DISCUSSION

This thesis research project presents the results obtained in the study of the relationships among diet, sleep and attention-deficit hyperactivity disorder (ADHD) in children and adolescents. The design is a control-case study were 60 newly diagnosed with ADHD (naïve) from the Hospital of Sant Joan de Déu (Barcelona, Spain) were sex- and age- matched with 60 controls. In this discussion section, we will consider the results of the manuscripts included in the project from a general point of view, provide and update on current research on the topic, and present a broader interpretation of the main results.

In this research, we first examined the association between the Mediterranean diet and ADHD diagnosis in children and adolescents. Second, we investigated the potential associations between diet, nutrients and ADHD symptomatology and comorbidities as well as cognitive abilities. Finally, we assessed the influence of diet and nutrient deficiencies on the sleep quality on the ADHD subjects.

5.1. **The Mediterranean diet and ADHD in children and adolescents**

Several advantages supporting the study of dietary pattern versus single nutrients in health promotion, including mental health, have previously been discussed (Howard et al., 2011, Woo et al., 2014). It seems that besides analyzing the impact that a single food component may have on ADHD, the role of dietary patterns as a whole can be more interesting. Studies have uniformly demonstrated that low quality diets are persistently associated to a higher risk of ADHD (Howard et al., 2011; Azadbakht & Esmaillzadeh, 2012; Woo et al., 2014; Ghanizadeh & Haddad, 2015). Consistent with these results, a **low adherence to the Mediterranean diet** is also associated with the odds of ADHD diagnosis in children and adolescents (**Manuscript I**).

Although the mechanisms linking low quality diet and ADHD are still unknown, an unbalanced diet, can lead to deficiencies in essential nutrients or higher intakes of certain food components (Izquierdo-Pulido et al., 2015). There are numerous potential biological pathways by which diet quality may have an impact on mental health (O'Neil

et al., 2014; Sarris et al., 2015). For instance, low levels of serum iron, ferritin, zinc or long-chain omega-3 seem to have a relationship with ADHD, since they contribute to a healthy neurocognitive and physical growth. However, in our study, no significant differences regarding the intakes of those nutrients or their plasma levels were observed between cases and controls.

We observed that children and adolescents with ADHD consumed lesser amounts of vegetables, citrus fruit's and blue fish, but larger amounts of soft-drinks (including cola drinks), sugar and candy than healthy individuals. It might occur that a high intake of these low nutrient products could be indicating that what really exists is a poor micronutrient intake. Two recent studies have found that a vitamin and mineral supplementation resulted in significant reductions in ADHD symptoms in both children and adults with ADHD (Rucklidge et al., 2014; Gordon et al., 2015). This kind of approach makes physiological sense considering that nutrients are required for many critical biochemical reactions to occur and also because it is unlikely that one nutrient by itself would resolve all vulnerabilities present in a complex disorder such as ADHD (Rucklidge & Kaplan, 2014).

It should be note that the relationship found between diet and ADHD could be both ways (Howard et al., 2011). Individuals with ADHD are often characterized by impulsivity traits and emotional distress that may lead to poor dietary choices (i.e. fat-rich or sugar-rich snack foods) to compensate their emotions, as a form of self-medication (Barkley, 2014; O'Neil et al., 2014). In our population, we found that the intake of sugary beverages and foods was significantly higher in cases than in controls, and those higher intakes were reflected in a larger amount of sugar intake. On the other hand, the role of the family cannot be dismissed considering that a healthy diet is related to a better functioning family (Ambrosini et al., 2009; Russell et al., 2014). Parents of individuals with ADHD often report a more dysfunctional family environment (Wehmeier et al., 2010; Foley, 2011; Singer-Leshinsky, 2011;), so it is plausible that the relationship between low adherence to a healthy diet and ADHD diagnosis may be orchestrated by a dysfunctional family environment (Howard et al., 2011). All of these factors could support a vicious cycle: impulsiveness and family dysfunction could lead to a worse choice of foods, lowering the diet quality, which eventually could provoke a

low intake of certain nutrients. This may induce certain nutritional subclinical deficiencies and hence, worsening ADHD symptomatology.

Our findings suggest that certain dietary habits may play a role in ADHD development, even though further work is required to investigate causality and also to figure out if diet manipulation could reverse the symptoms of ADHD taking in consideration all potential factors. All this will help to establish specific dietary strategies for families and children with ADHD that might help improve their quality of life.

5.2. Iron deficiency associates with behavioral symptoms and cognitive abilities in children and adolescents with ADHD.

Iron deficiency has been reported to be a possible risk factor in the pathophysiology of ADHD. However, to date, the results regarding the potential relationship between iron deficiency and ADHD have shown some inconsistency. Thus, it has been pointed out that low iron levels are related with cognitive, motor, development, and behavioral problems in children with ADHD (Grantham-McGregor et al., 2001; Halterman et al., 2001; Konofal et al., 2004; Oner et al., 2010) while other authors have not confirmed such relationships (Millichap et al., 2006; Menegassi et al., 2010). In line with the authors that found relationship between iron and ADHD symptomatology, we have found that ADHD children and adolescents with **lower iron serum levels showed not only more severe symptoms of hyperactivity but also of inattention** (**Manuscript II**). Interesting was that only a 1.5% of the cases had a strictly iron deficiency. These results suggest that low iron stores, even if not associated with iron deficiency or anemia, may contribute only to the ADHD symptomatology.

The mechanisms by which iron could play a role in ADHD pathophysiology are unknown. It has been postulated that iron deficiency might lead to ADHD symptoms via its impact on the metabolism of dopamine and of other cathecholamines (Cortese et al., 2008). Moreover, iron deficiency is also associated with decreased dopamine transporter expression and this deficiency may lead to dysfunction in the basal ganglia (Wigglesworth et al., 1998; Donfrancesco et al., 2013). Nevertheless, there is a certain controversy about which may be the best biomarker of iron levels for ADHD subjects.

It has been suggested that normal serum ferritin levels should not suggest that iron deficiency is not involved in the pathophysiology of ADHD (Donfrancesco et al., 2013). Indeed, serum ferritin is a marker of peripheral but not of the brain iron status, where iron is necessary as a cofactor. The extent to which serum ferritin correlates with iron levels in the brain remains unclear (Beard, 2003). In fact, we did not find significant differences in the iron store parameters studied between ADHD children and their controls.

Another issue that deserves discussion is the association between iron levels and cognitive performance. Konofal et al. (2004) found a significant correlation between cognitive sub-scores on the CPRS scale and low ferritin levels in children with ADHD, while Oner et al. (2008) did not find any significant relationship among ferritin and other iron deficiency parameters with cognitive variables using several test batteries. In our case, we found that **intellectual quotient, work memory and processing speed were positively associated with iron status in ADHD children** after adjusting for confounding variables. Those associations were not significant in controls (**Manuscript II**). Many nutrients have been related to brain function, although, iron has been one of the most studied due to its participation in the dopaminergic neurotransmission, brain energy metabolism, and myelination (Wigglesworth et al, 1998; Beard, 2003).

Several studies have confirmed the association between cognitive impairment and iron deficiency, even without anemia, in children and adults (Grantham-McGregor & Ani, 2001; Halterman et al., 2001; Scott & Murray-Kolb, 2016). Lower score on mental and motor test has also been reported in children in relation with iron status (Lozoff et al., 2000; Grantham-McGregor & Ani, 2001; Halterman et al., 2001;). Surprisingly, no studies have investigated the relationship between iron levels and cognition in children with ADHD given the importance of this micronutrient in this pathology, knowing that ADHD patients have poorer cognition and school achievement and more difficulties in tasks which require planning, organization and problem solving (Barkley, 1997; Jakobson & Kikas, 2007; Klenberg et al., 2016). Our results support the assumption that iron status plays a role in the pathophysiology of ADHD.

Iron stores might be important in hyperactivity and inattention symptoms and in cognitive functions in children and adolescents with ADHD. Low iron stores, even if not associated with iron deficiency or anemia, may contribute not only to the ADHD symptoms but also to the cognitive performance. Therefore, iron status should be included in the overall evaluation of children with ADHD.

5.3. Sleep quality and sleep disturbances associate with diet in children and adolescents with ADHD: a control-case study

Sleep disturbances are particularly widespread in ADHD (Miano et al., 2016; Vélez-Galarraga et al., 2016) and they contribute to ADHD symptomatology. On the other hand, there is evidence that suggest an additional and independent relationship between diet and sleep (Peuhkuri et al., 2012a; Peuhkuri et al., 2012b; Grandner et al., 2013; St-Onge et al., 2016). Given that both sleep disturbances and diet are contributing factors to ADHD symptomology, and diet has been shown to affect sleep, in our **Manuscript III**, we have investigated the relationships between sleep and dietary intake in children and adolescents with ADHD in comparison with controls.

Blunden et al. (2011) found associations between more sleep disturbance and higher intakes of carbohydrates, fat, and, most particularly, sugar which was also a significant predictor of nighttime sweating. In contrast to Bluden's findings, only a relationship between saturated fat intake and excessive daytime somnolence was found in our study. Most interesting was the fact that children and adolescents with ADHD who had higher **intake** of dietary fiber and **higher intake** of **certain vitamins and minerals** showed less total sleep problems and less excessive daytime somnolence (**Manuscript III**). Specifically, lower intakes of thiamine, vitamin B6, folate, iron and magnesium were related with sleep disturbance. Those associations were not found in the controls.

Evidence supporting a beneficial influence of macronutrient composition of the diet on sleep parameters is weak. Grandner et al. (2014) found that high intakes of palmitic acid, a saturated fatty acid, were related to sleep problems, such as difficulty in falling sleep. Interestingly, the same authors showed that reduced intake of lauric acid, another saturated fatty acid, was associated with both difficulties falling asleep and maintaining

sleep. Future studies examining the roles of diets containing saturated fatty acids are need and the possible mechanisms, which could explain these relationships. Regarding micronutrients, deficiencies of group B vitamins and certain minerals (e.g. magnesium) may disrupt sleep, and their effect seems to be based on their influence on the secretion of melatonin (Peuhkuri et al., 2012a). In the brain, vitamin B6 is needed in the synthesis of serotonin from tryptophan. In a study comparing poor sleepers with good sleepers, lower vegetable consumption and vitamin B6 intakes were found in the poor sleeper group (Huang et al., 2013). In the same line, folate, vitamin B6, magnesium and zinc deficiencies have been linked with lower melatonin levels in rodents (Peuhkuri et al., 2012b), linking those micronutrients with sleep. However, in humans, the role of these vitamins and minerals is less well studied. Rondanelli et al. (2011) found that the administration of melatonin, magnesium, and zinc appeared to improve the quality of sleep and the quality of life in long-term care facility residents with primary insomnia compare with a placebo capsule. Magnesium and zinc would enhance the secretion of melatonin from the pineal gland by stimulating melatonin synthesis key enzymes (Peuhkuri et al., 2012a).

Evidence suggests that the balance of DHA and AA in the pineal gland regulates melatonin production, with higher levels of DHA relating to increased levels of melatonin (Montgomery et al. 2014). DHA seems needed for one of the enzymes which transforms serotonin into melatonin (Peuhkuri et al., 2012b). Epidemiological studies find higher levels of omega-3 fatty acids associated with fewer sleep problems in infants (Cheruku et al., 2002) and also in children with ADHD (Burgess et al. 2000). Consistent with the fact that DHA seems to be needed for the synthesis of melatonin, we that children and adolescents **with a higher ratio of AA/DHA showed more difficulties in initiating and maintaining sleep (Manuscript III)**. Thus, an increase in DHA levels would decrease AA/DHA ratio and, eventually, the difficulties would decrease.

Although iron has been one of the most studied micronutrient involved in the pathophysiology of ADHD, only few works have explored the association between iron and sleep disturbances in patients with ADHD. In our study, **we found that lower iron serum levels were associated with lower sleep quality** (measured as sleep efficiency) (**Manuscript III**). Consistent with our results, other authors have found associations between low serum of ferritin and restless legs syndrome in ADHD children (Oner et

al., 2007) and sleep wake transition disorders, which includes items of abnormal movements and small awakenings during sleep (Cortese et al., 2009). The same authors carried out a study in which they evaluated motor activity during sleep in children with ADHD (Cortese et al., 2009). They concluded that ferritin levels below 45 µg/l might indicate a risk for disruption of normal sleep patterns (abnormal movements and small awakenings) in children with ADHD. However, significant differences regarding ferritin levels and disruption of normal sleep patters were found in our study.

Our findings suggest that diet may influence in the sleep patterns of children and adolescents with ADHD. Moreover, serum iron stores and levels of DHA also can have a role in the sleep quality of ADHD subjects. Given the importance of treating ADHD from a holistic and multidisciplinary perspective and the fact that both sleep and diet are potential modifiable behaviors, our findings suggest the need for future studies to further understand and enable a more holistic approach to the treatment of children and adolescents with ADHD.

In summary, this thesis provides not only a global but also a detailed vision of **different factors** that may play a role in the **ADHD development**. In particular, we demonstrated a positive relationship between a lower adherence to the Mediterranean diet and ADHD diagnoses. Additionally, a deficiency of iron seem to be related with ADHD symptoms and cognitive measures and finally, our results suggest that the interrelationship between diet and sleep in children wit ADHD may be of consequence and warrants further investigation. Given the importance of treating ADHD from a holistic and multidisciplinary perspective and the fact that both sleep and dietary intake are potentially modifiable behaviors, our findings suggest the need for future studies to further understand and enable a more holistic approach to the treatment of children and adolescent with ADHD symptoms.

5.4 Limitations and strengths

The conclusions derived from this thesis are conditioned by some limitations. Our case-control study design prevents our ability to assess cause-and-effect associations. Furthermore, all dietary instruments such as food frequency questionnaires measuring

past food intake are vulnerable both to random and systematic measurement errors. Neither the timing of food consumption nor the food additive content were measured and they might affect sleep (Harada et al., 2007) and behavior (Bateman et al., 2004). Nonetheless, this study has several important strengths: **1)** All the cases included were naïve, taking no medication. The use of certain drugs might affect the food choices and provoke changes to the child and adolescent's diet as well as the sleep patterns (Pozzi et al., 2013); **2)** Well-trained, experienced psychiatrists and psychologists performed the evaluation of all the participants of this study.

6. Conclusions

The results obtained from the present study have enabled us to draw the following conclusions:

1. **There is a positive relationship between a lower adherence to the Mediterranean diet and ADHD diagnosis in children and adolescents**. Our data supports the notion that not only "specific nutrients" but also the "whole diet" should be considered in ADHD.

    a. Children and adolescents with ADHD showed a greater number of habits that move away from the Mediterranean dietary pattern. Individuals with ADHD more often missed having a second serving of fruit daily and showed reduced intake of vegetables, pasta and rice almost every day when compared to controls. Moreover, subjects with ADHD went to fast-food restaurants and skipped breakfast more often than controls.

    b. A high consumption of sugar and candy, cola beverages, and soft drinks and a low consumption of blue fish were also associated with a higher prevalence of ADHD diagnosis.

    c. Clinicians should focus also on diet not with the expectation of dietary changes improving the behavior, but with the concern that children with ADHD are more likely to be eating unhealthy diets and that it should be a part of the evaluation to improve their health.

    d. Further studies are necessary to establish specific dietary strategies for families and children with ADHD that might help improve their quality of life.

2. **Low serum iron stores** may play an important role not only in the **severity of ADHD symptomatology** but also in **certain cognitive functions** of children and adolescents with AHDH

    a. Subjects with ADHD who had lower levels of serum iron stores showed more severe symptoms of hyperactivity and inattention scored in three

different scales: ADHD-RS, CPRS, and CTRS.

b. Lower scores of intellectual quotient, working memory and processing speed were found in ADHD children and adolescents with lower serum iron levels. Those associations were not observed in the controls.

c. Our results support the assumption that serum iron status may play a role in the pathophysiology of ADHD, due to its critical functions in the dopaminergic neurotransmission, brain energy metabolism, and myelination.

d. Iron supplementation and dietary strategies to increase the intake of iron could be considered when necessary as a part of the treatment of ADHD, even though further clinical controlled studies are necessary.

3. **Dietary intake** and serum levels of **iron** and of **long-chain polyunsaturated fatty acids** can influence **the sleep quality** of the children and adolescents with ADHD.

   a. Lower serum iron levels were significantly associated with poorer sleep efficiency in ADHD subjects. This association was no find in healthy subjects. Deficiencies of iron have been related with disruption of normal sleep patterns.

   b. A higher ratio of AA/DHA showed a significant relationship with higher sleep problems of initiating and maintaining sleep in children and adolescents with ADHD. DHA seems to have an important role in the synthesis of melatonin.

   c. Low intakes of certain micronutrients (thiamin, vitamin B6, acid folic, iron and magnesium) were associated with excessive daytime somnolence, which is a sleep disturbance. Deficiencies of these micronutrients have been associated with sleep disorders.

d. Sleep disturbances may represent a source of distress for the ADHD children and adolescents and their families and they may aggravate daytime ADHD symptoms, it would be of interest to give specifically dietary counseling to the patients to ensure a correct, sufficient intake of nutrients related to healthy sleep patterns.

7. Bibliography

Afaghi, A., O'Connor, H., & Chow, C. M. (2007). High-glycemic-index carbohydrate meals shorten sleep onset. *The American Journal of Clinical Nutrition*, *85*(2), 426–430.

Ahn, S. I., Kim, Y. S., Jon, D.-I., Jung, M. H., Hong, N., & Hong, H. J. (2016). The relation of blood iron level with frontal function in children with attention-deficit/hyperactivity disorder. *Journal of Korean Neuropsychiatric Association*, *55*(1), 51.

Akhondzadeh, S., Mohammadi, M., & Khademi, M. (2004). Zinc sulfate as an adjunct to methylphenidate for the treatment of attention deficit hyperactivity disorder in children : A double blind and randomized trial. *BCM Psychiatry*, 4(9), 1–6.

Ambrosini, G. L., Oddy, W. H., Robinson, M., O'Sullivan, T. A., Hands, B. P., de Klerk, N. H., Silburn, S. R., Zubrick, S. R., Kendall, G. E., Stanley, F. J., Beilin, L. J. (2009). Adolescent dietary patterns are associated with lifestyle and family psycho-social factors. *Public Health Nutrition*, *12*(10), 1807–1815.

Arnold, L. E., Amato, A., Bozzolo, H., Hollway, J., Cook, A., Ramadan, Y., Crowl, L., Zhang, D., Thompson, S., Testa, G., Kliewer, V., Wigal, T., McBurnett, K., Manos, M. (2007). Acetyl-L-carnitine in attention-deficit/hyperactivity disorder: a multi-site, placebo-controlled pilot trial. *Journal of Child and Adolescent Psychopharmacology*, *17*(6), 791–802.

Arnold, L. E., Bozzolo, H., Hollway, J., Cook, A., DiSilvestro, R. A., Bozzolo, D. R., Crowl, L., Ramadan, Y., Williams, C. (2005). Serum zinc correlates with parent- and teacher- rated inattention in children with attention-deficit/hyperactivity disorder. *Journal of Child and Adolescent Psychopharmacology*, *15*(4), 628–636.

Arnold, L. E., Disilvestro, R. a, Bozzolo, D., Bozzolo, H., Crowl, L., Fernandez, S., Ramadan, Y., Thompson, S., Mo, X., Abdel-Rasoul, M., Joseph, E. (2011). Zinc for attention-deficit/hyperactivity disorder: placebo-controlled double-blind pilot trial alone and combined with amphetamine. *Journal of Child and Adolescent Psychopharmacology*, *21*(1), 1–19.

Arnold, L. E., Hurt, E., & Lofthouse, N. (2013). Attention-deficit/hyperactivity disorder. *Child and Adolescent Psychiatric Clinics of North America*, *22*(3), 381–402.

Arnsten, A. F.T, & Rubia, K. (2012). Neurobiological circuits regulating attention, cognitive control, motivation, and emotion: disruptions in neurodevelopmental psychiatric. *Journal of the American Academy of Child and Adolescent Psychiatry*, *51*(4), 356–367.

Arnsten, A. F. T. (2007). Catecholamine and second messenger influences on prefrontal cortical networks of "representational knowledge'': a rational bridge between genetics and the symptoms of mental illness. *Cerebral Cortex*, 17 Suppl, i6-15.

Arnsten, A. F. T. (2009a). The emerging neurobiology of attention deficit hyperactivity disorder: the key role of the prefrontal association cortex. *The Journal of Pediatrics*, *154*(5), I-S43.

Arnsten, A. F. T. (2009b). Toward a new understanding of attention-deficit hyperactivity disorder pathophysiology: an important role for prefrontal cortex dysfunction. *CNS Drugs*, *23 Suppl* , 33–41.

American Psychiatric Association. (2000). Diagnostic and statistical manual of mental disorders, 4th edn, text revision: DSM-IV-TR. *American Psychiatric Association*, Washington, DC

Azadbakht, L., & Esmaillzadeh, A. (2012). Dietary patterns and attention deficit hyperactivity disorder among Iranian children. *Nutrition*, *28*(3), 242–249.

Bader, A., & Adesman, A. (2012). Complementary and alternative therapies for children and adolescents with ADHD. *Current Opinion in Pediatrics*, *24*(6), 760–769.

Barkley, R. A. (2006). Attention deficit hyperactivity disorder: a handbook for diagnosis and treatment, 3rd edn. *Guilford Press*, New York, NY.

Barkley, R. A. (2014). ADHD, obesity, and eating pathology. *The ADHD report*, *22*(5), 1–6.

Barkley, R. A. (1997). Behavioral inhibition, sustained attention, and executive functions: Constructing a unifying theory of ADHD. *Psychological Bulletin*, *121*(1), 65–94.

Bateman, B., Warner, J. O., Hutchinson, E., Dean, T., Rowlandson, P., Gant, C., … Stevenson, J. (2004). The effects of a double blind, placebo controlled, artificial food colourings and benzoate preservative challenge on hyperactivity in a general population sample of preschool children. *Archives of Disease in Childhood*, *89*(6), 506–511.

Beard, J. (2003). Iron deficiency alters brain development and functioning. *The Journal of Nutrition*, *133*(5 Suppl 1), 1468S–1472S.

Beebe, D. W., Simon, S., Summer, S., Hemmer, S., Strotman, D., Dolan, L. M. (2013). Dietary intake following experimentally restricted sleep in adolescents. *Sleep*, *36*(6), 827–834

Bendz, L. M., & Scates, A. C. (2010). Melatonin treatment for insomnia in pediatric patients with attention-deficit/hyperactivity disorder. *Annals of Pharmacotherapy*, *44*(1), 185–191.

Bener, A. (2015). Higher prevalence of iron deficiency as strong predictor of attention deficit hyperactivity disorder in children. *European Psychiatry*, *30(Suppl 1)*, 28-31.

Berner, A., Kamal, M., Bener, H., & Bhugra, D. (2014). Higher prevalence of iron deficiency as strong predictor of attention deficit hyperactivity disorder in children. *Annals of Medical and Health Sciences Research*, *4(Suppl 3)*, S291-S297

Beydoun, M. A., Gamaldo, A. A., Canas, J. A., Beydoun, H. A., Shah, M. T., McNeely, J. M., Zonderman, A. B. (2014). Serum nutritional biomarkers and their associations with sleep among US adults in recent national surveys. *PLoS ONE*, *9*(8), e103490.

Biederman, J., & Faraone, S. V. (2005). Attention-deficit hyperactivity disorder. *Lancet*, *366*(9481), 237-248.

Bilici, M., Yildirim, F., Kandil, S., Bekaroğlu, M., Yildirmiş, S., Değer, O., Ulgen, M., Yildiran, A., Aksu, H. (2004). Double-blind, placebo-controlled study of zinc sulfate in the treatment of attention deficit hyperactivity disorder. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *28*(1), 181–190.

Bloch, M. H., & Qawasmi, A. (2011). Omega-3 fatty acid supplementation for the treatment of children with attention-deficit/hyperactivity disorder symptomatology: systematic review and meta-analysis. *Journal of the American Academy of Child and Adolescent Psychiatry*, *50*(10), 991–1000.

Blunden, S. L., Milte, C. M., & Sinn, N. (2011). Diet and sleep in children with attention deficit hyperactivity disorder: preliminary data in Australian children. *Journal of Child Health Care*, *15*(1), 14–24.

Boomsma, D. I., Saviouk, V., Hottenga, J. J., Distel, M. A., de Moor, M. H. M., Vink, J. M., Geels, L. M., van Beek, J. H., Bartels, M., de Geus, E. J., Willemsen, G. (2010). Genetic epidemiology of attention deficit hyperactivity disorder (ADHD index) in adults. *PloS One*, *5*(5), e10621.

Bruni, O., Ottaviano, S., Guidetti, V., Romoli, M., Innocenzi, M., Cortesi, F., & Giannotti, F. (1996). The sleep disturbance scale for children (SDSC). construction and validation of an instrument to evaluate sleep disturbances in childhood and adolescence. *Journal of Sleep Research*, *5*(4), 251–261.

Burgess, J. R., Stevens, L., Zhang, W., & Peck, L. (2000). Long-chain polyunsaturated fatty acids in children with attention-deficit hyperactivity disorder. *The American Journal of Clinical Nutrition*, *71*(1 Suppl), 327S–30S.

Cantós, D., Farran, A., & Palma, I. (2004). *PCN Pro*. Barcelona: Universitat de Barcelona.

Castle, L., Aubert, R. E., Verbrugge, R. R., Khalid, M., & Epstein, R. S. (2007). Trends in medication treatment for ADHD. *Journal of Attention Disorders*, *10*(4), 335–342.

Catalá, A. (2010). The function of very long chain polyunsaturated fatty acids in the pineal gland. *Biochimica et Biophysica Acta*, *1801*(2), 95–99.

Centers for Disease Control and Prevention. (2009). Clinical growth charts. Retrieved from http://www.cdc.gov/growthcharts/clinical_charts.htm

Chalon, S. (2006). Omega-3 fatty acids and monoamine neurotransmission. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, *75*(4–5), 259–269.

Cheruku, S. R., Montgomery-Downs, H. E., Farkas, S. L., Thoman, E. B., & Lammi-Keefe, C. J. (2002). Higher maternal plasma docosahexaenoic acid during pregnancy is associated with more mature neonatal sleep-state patterning. *The American Journal of Clinical Nutrition*, *76*(3), 608–613.

Chollet, D., Franken, P., Raffin, Y., Henrotte, J. G., Widmer, J., Malafosse, A., & Tafti, M. (2001). Magnesium involvement in sleep: genetic and nutritional models. *Behavior Genetics*, *31*(5), 413–425.

Cohen-Zion, M., & Ancoli-Israel, S. (2004). Sleep in children with attention-deficit hyperactivity disorder (ADHD): a review of naturalistic and stimulant intervention studies. *Sleep Medicine Reviews*, *8*(5), 379–402.

Cole, T. J., & Lobstein, T. (2012). Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. *Pediatric Obesity*, *7*(4), 284–294.

Colter, A. L., Cutler, C., & Meckling, K. A. (2008). Fatty acid status and behavioural symptoms of attention deficit hyperactivity disorder in adolescents: a case-control study. *Nutrition Journal*, *7*, 8.

Conners, C. K., Sitarenios, G., Parker, J. D., & Epstein, J. N. (1998). The revised Conners' Parent Rating Scale (CPRS-R): factor structure, reliability, and criterion validity. *Journal of Abnormal Child Psychology*, *26*(4), 257–268.

Cooper, R. E., Tye, C., Kuntsi, J., Vassos, E., & Asherson, P. (2016). The effect of omega-3 polyunsaturated fatty acid supplementation on emotional dysregulation, oppositional behaviour and conduct problems in ADHD: a systematic review and meta-analysis. *Journal of Affective Disorders*, *190*, 474–482.

Cornu, C., Remontet, L., Noel-Baron, F., Nicolas, A., Feugier-Favier, N., Roy, P., … Kassaï, B. (2010). A dietary supplement to improve the quality of sleep: a randomized placebo controlled trial. *BMC Complementary and Alternative Medicine*, *10*(1), 29.

Cortese, S., Angriman, M., Lecendreux, M., & Konofal, E. (2012). Iron and attention deficit/hyperactivity disorder: what is the empirical evidence so far? a systematic review of the literature. *Expert Review of Neurotherapeutics*, *12*(10), 1227–1240.

Cortese, S., Konofal, E., Bernardina, B. D., Mouren, M.-C., & Lecendreux, M. (2009). Sleep disturbances and serum ferritin levels in children with attention-deficit/hyperactivity disorder. *European Child & Adolescent Psychiatry*, *18*(7), 393–399.

Cortese, S., Lecendreux, M., Bernardina, B. D., Mouren, M. C., Sbarbati, A., & Konofal, E. (2008). Attention-deficit/hyperactivity disorder, Tourette's syndrome, and restless legs syndrome: the iron hypothesis. *Medical Hypotheses*, *70*(6), 1128–1132.

De Sousa, A., & Kalra, G. (2012). Drug therapy of attention deficit hyperactivity disorder: current trends. *Mens Sana Monographs*, *10*(1), 45–69.

American Psychiatric Association. (2013). Diagnostic and statistical manual of mental disorders, 5th edn (DSM-5). *American Psychiatric Publishing*, Arlington

Dodig-Curković K, Dovhanj J, Curković M, Dodig-Radić J, D. D. (2009). The role of zinc in the treatment of hyperactivity disorder in children. *Acta Medica Croatica*, *63*(4), 307–313.

Donfrancesco, R., Parisi, P., Vanacore, N., Martines, F., Sargentini, V., & Cortese, S. (2013). Iron and ADHD: time to move beyond serum ferritin levels. *Journal of Attention Disorders*, *17*(4), 347–357.

Bloch, M. H., & Mulqueen, J. (2014). Nutritional supplements for the treatment of attention-deficit hyperactivity disorder. Child Adolescent Psychiatry Clinics of North America. *23*(4), 883–897.

DuPaul, G. J., Power, T. J., Anastopoulos, A. D., & Reid, R. (1998). *ADHD rating scale-IV: checklists, norms and clinical interpretation*. New york: The Guilford Press.

Ebben, M., Lequerica, A., & Spielman, A. (2002). Effects of pyridoxine on dreaming: a preliminary study. *Perceptual and Motor Skills*, *94*(1), 135–140.

Elbaz, F., Zahra, S., & Hanafy, H. (2016). Magnesium, zinc and copper estimation in children with attention deficit hyperactivity disorder (ADHD). *Egyptian Journal of Medical Human Genetics*, 17(1), 63-70.

Faraone, S. V., & Biederman, J. (2000). Nature, nurture, and attention deficit hyperactivity disorder. *Developmental Review*, 20(4), 568-581.

Faraone, S. V., Perlis, R. H., Doyle, A. E., Smoller, J. W., Goralnick, J. J., Holmgren, M. A., & Sklar, P. (2005). Molecular genetics of attention-deficit/hyperactivity disorder. *Biological Psychiatry*, *57*(11), 1313–1323.

Farran, A., Zamora, R., & Cervera, P. (2004). Tablas de composición de alimentos del CESNID. *McGraw-Hill/Interamericana de España - Edicions UB*. Barcelona.

Foley, M. (2011). A comparison of family adversity and family dysfunction in families of children with attention deficit hyperactivity disorder (ADHD) and families of children without ADHD. *Journal for Specialists in Pediatric Nursing : JSPN*, *16*(1), 39–49.

Gapin, J., & Etnier, J. L. (2010). The relationship between physical activity and executive function performance in children with attention-deficit hyperactivity disorder. *Journal of Sport & Exercise Psychology*, *32*(6), 753–763.

Ghanizadeh, A., & Haddad, B. (2015). The effect of dietary education on ADHD, a randomized controlled clinical trial. *Annals of General Psychiatry*, *14*(1), 1–7.

Gillies, D., Sinn, J. Kh., Lad, S. S., Leach, M. J., Ross, M. J. (2012). Polyunsaturated fatty acids (PUFA) for attention deficit hyperactivity disorder (ADHD) in children and adolescents. *Cochrane Database of Systematic Reviews,* 11(7):CD007986.

Golan, N., Shahar, E., Ravid, S., & Pillar, G. (2004). Sleep disorders and daytime sleepiness in children with attention-deficit/hyperactive disorder. *Sleep*, *27*(2), 261–266.

Gominak, S. C., & Stumpf, W. E. (2012). The world epidemic of sleep disorders is linked to vitamin D deficiency. *Medical Hypotheses*, *79*(2), 132–135.

González C., R., Rodríguez S., A., & Sánchez G., J. (2015). Epidemiología del TDAH. *Revista Española de Pediatría*, *71*(2), 58–61.

Gordon, H. A., Rucklidge, J. J., Blampied, N. M., & Johnstone, J. M. (2015). Clinically significant symptom reduction in children with attention-deficit/hyperactivity disorder treated with micronutrients: an open-label reversal design study. *Journal of Child and Adolescent Psychopharmacology*, *25*(10), 783–798.

Grandner, M. A., Jackson, N., Gerstner, J. R., & Knutson, K. L. (2014). Sleep symptoms associated with intake of specific dietary nutrients. *Journal of Sleep Research*, *23*(1), 22–34.

Grandner, M. A., Kripke, D. F., Naidoo, N., & Langer, R. D. (2010). Relationships among dietary nutrients and subjective sleep, objective sleep, and napping in women. *Sleep Medicine*, *11*(2), 180–184.

Grandner, M. A, Jackson, N., Gerstner, J. R., & Knutson, K. L. (2013). Dietary nutrients associated with short and long sleep duration. Data from a nationally representative sample. *Appetite, 64*, 71–80.

Grantham-McGregor, S., & Ani, C. (2001). A review of studies on the effect of iron deficiency on cognitive development in children. *The Journal of Nutrition*, *131*(6), 49S–68S.

Halterman, J. S., Kaczorowski, J. M., Aligne, C. A., Auinger, P., & Szilagyi, P. G. (2001). Iron deficiency and cognitive achievement among school-aged children and adolescents in the United States. *Pediatrics*, *107*(6), 1381–1386.

Harada, T., Hirotani, M., Maeda, M., Nomura, H., & Takeuchi, H. (2007). Correlation between breakfast tryptophan content and morning-evening in Japanese infants and students aged 0-15 yrs. *Journal of Physiological Anthropology*, *26*(2), 201–207.

Hariri, M., & Azadbakht, L. (2015). Magnesium, iron, and zinc supplementation for the treatment of attention deficit hyperactivity disorder: a systematic review on the recent literature. *International Journal of Preventive Medicine*, *6*, 83.

Hawkey, E., & Nigg, J. T. (2014). Omega−3 fatty acid and ADHD: blood level analysis and meta-analytic extension of supplementation trials. *Clinical Psychology Review*, *34*(6), 496–505.

Hillman, C. H., Pontifex, M. B., Castelli, D. M., Khan, N. a., Raine, L. B., Scudder, M. R., Drollette, E. S., Moore, R. D., Wu, C-T., Kamijo, K. (2014). Effects of the FITKids randomized controlled trial on executive control and brain function. *Pediatrics*, *134*(4), e1063–e1071.

Hogenkamp, P. S., Nilsson, E., Nilsson, V. C., Chapman, C. D., Vogel, H., Lundberg, L. S., Zarei, S., Cedernaes, J., Rångtell, F.H., Broman, J. E., Dickson, S. L., Brunstrom, J. M., Benedict, C., Schiöth, H. B. (2013). Acute sleep deprivation increases portion size and affects food choice in young men. *Psychoneuroendocrinology*, *38*(9), 1668–1674.

Howard, A. L., Robinson, M., Smith, G. J., Ambrosini, G. L., Piek, J. P., & Oddy, W. H. (2011). ADHD is associated with a "Western" dietary pattern in adolescents. *Journal of Attention Disorders*, *15*(5), 403–411.

Huang, Y. C., Wahlqvist, M. L., & Lee, M. S. (2013). Sleep quality in the survival of elderly taiwanese: roles for dietary diversity and pyridoxine in men and women. *Journal of the American College of Nutrition*, *32*(6), 417–427.

Huss, M., Völp, A., & Stauss-Grabo, M. (2010). Supplementation of polyunsaturated fatty acids, magnesium and zinc in children seeking medical advice for attention-deficit/hyperactivity problems - an observational cohort study. *Lipids in Health and Disease*, *9*, 105.

Hvolby, A. (2014). Associations of sleep disturbance with ADHD: implications for treatment. *ADHD Attention Deficit and Hyperactivity Disorders*, 7(1), 1–18.

Izquierdo-Pulido, M. L., Ríos-Hernández, A., Farran, A., & Alda, J. Á. (2015). The role of diet and physical activity in children and adolescents with ADHD. *Recent Advances in Pharmaceutical Sciences V*. *Research Signpost*. Chapter 4, 51-64

Jakobson, A., & Kikas, E. (2007). Cognitive functioning in children with and without attention-deficit/hyperactivity disorder with and without comorbid learning disabilities. *Journal of Learning Disabilities*, *40*(3), 194–202.

Juneja, M., Jain, R., Singh, V., & Mallika, V. (2010). Iron deficiency in Indian children with attention deficit hyperactivity disorder. *Indian Pediatrics*, *47*(11), 955–958.

Kanarek, R. B. (2011). Artificial food dyes and attention deficit hyperactivity disorder. *Nutrition Reviews*, *69*(7), 385–391.

Kaufman, J., Birmaher, B., Brent, D., Rao, U., Flynn, C., Moreci, P., Williamson, D., Ryan, N. (1997). Schedule for affective disorders and schizophrenia for school-age children-present and lifetime version (K-SADS-PL): initial reliability and validity data. *Journal of the American Academy of Child and Adolescent Psychiatry*, *36*(7), 980–988.

Kiddie, J. Y., Weiss, M. D., Kitts, D. D., Levy-Milne, R., & Wasdell, M. B. (2010). Nutritional status of children with attention deficit hyperactivity disorder: a pilot study. *International Journal of Pediatrics*, 767318 doi.org/10.1155/2010/767318

Kiluk, B. D., Weden, S., & Culotta, V. P. (2009). Sport participation and anxiety in children with ADHD. *Journal of Attention Disorders*, *12*(6), 499–506.

Kim, Y., & Chang, H. (2011). Correlation between attention deficit hyperactivity disorder and sugar consumption, quality of diet, and dietary behavior in school children. *Nutrition Research and Practice*, *5*(3), 236–245.

Klenberg, L., Hokkanen, L., Lahti-Nuuttila, P., & Närhi, V. (2016). Teacher ratings of executive function difficulties in finnish children with combined and predominantly inattentive symptoms of ADHD. *Applied Neuropsychology: Child*, 1–10. doi:10.1080/21622965.2016.1177531

Konofal, E., Lecendreux, M., Arnulf, I., & Mouren, M. C. (2004). Iron deficiency in children with attention-deficit/hyperactivity disorder. *Archives of Pediatrics & Adolescent Medicine*, *158*(12), 1113–1115.

Konofal, E., Lecendreux, M., Deron, J., Marchand, M., Cortese, S., Zaïm, M., … Arnulf, I. (2008). Effects of iron supplementation on attention deficit hyperactivity disorder in children. *Pediatric Neurology*, *38*(1), 20–26.

Kozielec, T., & Starobrat-Hermelin, B. (1997). Assessment of magnesium levels in children with attention deficit hyperactivity disorder (ADHD). *Magnesium Research*, *10*(2), 143–148.

Kwon, H. J., Lim, M. H., Ha, M., Kim, E. J., Yoo, S. J., Kim, J. W & Paik, K. C. (2011). Transferrin in Korean children with attention deficit hyperactivity disorder. *Psychiatry Investigation*, *8*(4), 366-371

LaChance, L., McKenzie, K., Taylor, V. H., & Vigod, S. N. (2016). Omega-6 to omega-3 fatty acid ratio in patients with ADHD: a meta-analysis. *Journal of the Canadian Academy of Child and Adolescent Psychiatry, 25*(2), 87–96.

LeBourgeois, M. K., Avis, K., Mixon, M., Olmi, J., & Harsh, J. (2004). Snoring, sleep quality, and sleepiness across attention-deficit/hyperactivity disorder subtypes. *Sleep*, *27*(3), 520–525.

Lim, C. G., Ooi, Y. P., Fung, D. S. S., Mahendran, R., & Kaur, A. (2008). Sleep disturbances in Singaporean children with attention deficit hyperactivity disorder. Annals Academy of Medicine Singapore, *37*(8), 655–661.

Liu, J., He, P., Li, L., Shen, T., Wu, M., Hu, J., Zhuang, Y., He, G. (2014). Study on the association between diet, nutrient and attention deficit hyperactivity disorder among children in Shanghai, Kunshan, Wuxi three kindergarten. *Journal of Hygiene Research*, *43*(2), 235–239.

Lozoff, B., Jimenez, E., Hagen, J., Mollen, E., & Wolf, A. W. (2000). Poorer behavioral and developmental outcome more than 10 years after treatment for iron deficiency in infancy. *Pediatrics*, *105*(4).

Mariscal-Arcas, M., Rivas, A., Velasco, J., Ortega, M., Caballero, A. M., & Olea-Serrano, F. (2009). Evaluation of the Mediterranean diet quality index (KIDMED) in children and adolescents in Southern Spain. *Public Health Nutrition*, *12*(9), 1408–1412.

Massa, J., Stone, K. L., Wei, E. K., Harrison, S. L., Barrett-Connor, E., Lane, N. E., … Schernhammer, E. (2015). Vitamin D and actigraphic sleep outcomes in older community-Dwelling men: the MrOS sleep study. *Sleep*, 38(2), 251-257

Mazefsky, C. A., Anderson, R., Conner, C. M., & Minshew, N. (2011). Child behavior checklist scores for school-aged children with autism: preliminary evidence of patterns suggesting the need for referral. *Journal of Psychopathology and Behavioral Assessment*, *33*(1), 31–37.

McCann, D., Barrett, A., Cooper, A., Crumpler, D., Dalen, L., Grimshaw, K., Kitchin, E., Lok, K., Porteous, L., Prince, E., Sonuga-Barke., Warner, J. O., Stevenson, J. (2007). Food additives and hyperactive behaviour in 3-year-old and 8/9-year-old children in the community: a randomised, double-blinded, placebo-controlled trial. *Lancet*, *370*(9598), 1560–1567.

Menegassi, M., De Mello, E. D., Guimarães, L. R., Matte, B. C., Driemeier, F., Pedroso, G. L., Rohde, L. A., Schmitz, M. (2010). Food intake and serum levels of iron in children and adolescents with attention-deficit/hyperactivity disorder. *Revista Brasileira de Psiquiatria*, *32*(2), 132–138.

Miano, S., Esposito, M., Foderaro, G., Ramelli, G. P., Pezzoli, V., & Manconi, M. (2016). Sleep-related disorders in children with attention-deficit hyperactivity disorder: preliminary results of a full sleep assessment study. *CNS Neuroscience & Therapeutics*, *22*(11), 906–914.

Mick, E., & Faraone, S. V. (2008). Genetics of attention deficit hyperactivity disorder. *Child and Adolescent Psychiatric Clinics of North America*, *17*(2), 261–284.

Millichap, J. G., & Yee, M. M. (2012). The diet factor in attention-deficit/hyperactivity disorder. *Pediatrics*, *129*(2), 330–337.

Millichap, J. G., Yee, M. M., & Davidson, S. I. (2006). Serum Ferritin in Children With Attention-Deficit Hyperactivity Disorder. *Pediatric Neurology*, *34*(3), 200–203.

Montgomery, P., Burton, J. R., Sewell, R. P., Spreckelsen, T. F., & Richardson, A. J. (2014). Fatty acids and sleep in UK children: subjective and pilot objective sleep results from the DOLAB study - a randomized controlled trial. *Journal of Sleep Research*, *23*(4), 364–388.

Moran, H., V., & Lowe, N. M. (2016). Nutrition and the developing Brain. Boca Raton, FL. *CRC Press Taylor & Francis Group.*

Mousain-Bosc, M., Roche, M., Rapin, J., & Bali, J. P. (2004). Magnesium VitB6 intake reduces central nervous system hyperexcitability in children. *Journal of the American College of Nutrition*, *23*(5), 545S–548S.

National Institute of Mental Health. Attention deficit hyperactivity disorder (ADHD) publication. Retrieved from http://www.nimh.nih.gov/health/publications/attention-deficit-hyperactivity-disorder/adhd_booklet_cl508.pdf

Newmark, S. C. (2009). Nutritional intervention in ADHD. *Explore*, *5*(3), 171–174.

Nigg, J. T., Lewis, K., Edinger, T., & Falk, M. (2012). Meta-analysis of attention-deficit/hyperactivity disorder or attention-deficit/hyperactivity disorder symptoms, restriction diet, and synthetic food color additives. *Journal of the American Academy of Child and Adolescent Psychiatry*, *51*(1), 86–97.

O'Neil, A., Quirk, S. E., Housden, S., Brennan, S. L., Williams, L. J., Pasco, J. A., Berk, M., Jacka, F. N. (2014). Relationship between diet and mental health in children and adolescents: a systematic review. *American Journal of Public Health*, *104*(10), e31–e42.

Oner, O., Alkar, O. Y., & Oner, P. (2008). Relation of ferritin levels with symptom ratings and cognitive performance in children with attention deficit–hyperactivity disorder. *Pediatrics International*, *50*(1), 40–44.

Oner, O., Oner, P., Bozkurt, O. H., Odabas, E., Keser, N., Karadag, H., & Kizilgün, M. (2010). Effects of zinc and ferritin levels on parent and teacher reported symptom scores in attention deficit hyperactivity disorder. *Child Psychiatry and Human Development*, *41*(4), 441–447.

Oner, P., Dirik, E. B., Taner, Y., Caykoylu, A., & Anlar, O. (2007). Association between low serum ferritin and restless legs syndrome in patients with attention deficit hyperactivity disorder. *The Tohoku Journal of Experimental Medicine*, *213*(3), 269–276.

Oner, P., Oner, O., Azik, F. M., Cop, E., & Munir, K. M. (2012). Ferritin and hyperactivity ratings in attention deficit hyperactivity disorder. *Pediatrics International : Official Journal of the Japan Pediatric Society*, *54*(5), 688–692.

Owens, J. A., Maxim, R., Nobile, C., McGuinn, M., & Msall, M. (2000). Parental and self-report of sleep in children with attention-deficit/hyperactivity disorder. *Archives of Pediatrics & Adolescent Medicine*, *154*(6), 549–555.

Park, S., Cho, S. C., Hong, Y. C., Oh, S. Y., Kim, J. W., Shin, M. S., Kim, B. N., Yoo, H. J., Cho, I. H., Bhang, S.Y. (2012). Association between dietary behaviors and attention-deficit/hyperactivity disorder and learning disabilities in school-aged children. *Psychiatry Research*, *198*(3), 468–476.

Parletta, N., Niyonsenga, T., & Duff, J. (2016). Omega-3 and omega-6 polyunsaturated fatty acid levels and correlations with symptoms in children with Attention deficit hyperactivity disorder, autistic spectrum disorder and typically developing controls. *PLoS ONE*, *11*(5), e0156432.

Partinen, M., & Westermarck, T. (2014). Nutrition, sleep and sleep disorders – relations of some food constituents and sleep, pharmacology and nutritional intervention in the treatment of disease. *Faik Atroshi (Ed), InTech*. doi:10,5772/58345

Patte, K. A., Davis, C. A., Levitan, R. D., Kaplan, A. S., Carter-Major, J., & Kennedy, J. L. (2016). A behavioral genetic model of the mechanisms underlying the link between obesity and symptoms of ADHD. *Journal of Attention Disorders*, 1087054715618793. doi.org/10.1177/1087054715618793

Pelham, W. E., Fabiano, G. A., Waxmonsky, J. G., Greiner, A. R., Gnagy, E. M., Pelham, W. E., Coxe, S., Verley, J., Bhatia, I., Hart, K., Karch, K., Konijnendijk, E., Tresco, K., Nahum-Shani, I., Murphy, S. A. (2016). Treatment sequencing for childhood ADHD: a multiple-randomization study of adaptive medication and behavioral interventions. *Journal of Clinical Child & Adolescent Psychology*, *45(4), 396-415*.

Pelsser, L. M., Frankena, K., Buitelaar, J. K., & Rommelse, N. N. (2010). Effects of food on physical and sleep complaints in children with ADHD: a randomised controlled pilot study. *European Journal of Pediatrics*, *169*(9), 1129–38.

Percinel, I., Yazici, K. U., & Ustundag, B. (2016). Iron deficiency parameters in children and adolescents with attention-deficit/hyperactivity disorder. *Child Psychiatry & Human Development*, *47*(2), 259–269.

Peuhkuri, K., Sihvola, N., & Korpela, R. (2012a). Diet promotes sleep duration and quality. *Nutrition Research*, *32*(5), 309–19.

Peuhkuri, K., Sihvola, N., & Korpela, R. (2012b). Dietary factors and fluctuating levels of melatonin. *Food & Nutrition Research*, *56*. doi.org/10.3402/fnr.v56i0.17252

Polanczyk, G. V, Salum, G. A., Sugaya, L. S., Caye, A., & Rohde, L. A. (2015). Annual research review: a meta-analysis of the worldwide prevalence of mental disorders in children and adolescents. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, *56*(3), 345–365.

Pontifex, M. B., Saliba, B. J., Raine, L. B., Picchietti, D. L., & Hillman, C. H. (2013). Exercise improves behavioral, nuerocognitive, and scholastic performance in children with ADHD. *The Journal of Pediatrics*, *162*(3), 543–551.

Ponto, L. L. B., & Schultz, S. K. (2003). Ginkgo biloba extract: review of CNS effects. *Annals of Clinical Psychiatry*, *15*(2), 109–119.

Pozzi, M., Bertella, S., Cattaneo, D., Molteni, M., Perrone, V., Carnovale, C., Antoniazzi, S., Clementi, E., Radice, S. (2013). Are non-serious adverse reactions to psychiatric drugs really non-serious?. *Journal of Child and Adolescent Psychopharmacology*, *23*(6), 394–400.

Rietveld, M. J. H., Hudziak, J. J., Bartels, M., Beijsterveldt, C. E. M., & Boomsma, D. I. (2004). Heritability of attention problems in children: longitudinal results from a study of twins, age 3 to 12. *Journal of Child Psychology and Psychiatry*, *45*(3), 577–588.

Rodríguez, I. T., Ballart, J. F., Pastor, G. C., Jordà, E. B., & Arija, V. (2008). Validación de un cuestionario de frecuencia de consumo alimentario corto : reproducibilidad y validez, *23*(3), 242–252.

Rondanelli, M., Opizzi, A., Monteferrario, F., Antoniello, N., Manni, R., & Klersy, C. (2011). The effect of melatonin, magnesium, and zinc on primary insomnia in long-term care facility residents in Italy: a double-blind, placebo-controlled clinical trial. *Journal of the American Geriatrics Society*, *59*(1), 82–90.

Rowe, K., & Rowe, K. (1994). Synthetic food coloring and behavior: a dose response effect in a double-blind, placebo-controlled, repeated-measures study. *Pediatrics*, 691–698.

Rucklidge, J. J., Frampton, C. M., Gorman, B., & Boggis, A. (2014). Vitamin-mineral treatment of attention-deficit hyperactivity disorder in adults: double-blind randomised placebo-controlled trial. *The British Journal of Psychiatry*, *204*(4), 306–315.

Rucklidge, J. J., & Kaplan, B. J. (2014). Broad-spectrum micronutrient treatment for attention-deficit/hyperactivity disorder: rationale and evidence to date. *CNS Drugs*, *28*(9), 775–785.

Russell, G., Ford, T., Rosenberg, R., & Kelly, S. (2014). The association of attention deficit hyperactivity disorder with socioeconomic disadvantage: alternative explanations and evidence. *Journal of Child Psychology and Psychiatry*, *55*(5), 436–445.

Salehi, B., Imani, R., Mohammadi, M. R., Fallah, J., Mohammadi, M., Ghanizadeh, A., Tasviechi, A. A., Vossoughi, A., Rezazadeh, S. A., Akhondzadeh, S. (2010). Ginkgo biloba for attention-deficit/hyperactivity disorder in children and adolescents: a double blind, randomized controlled trial. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *34*(1), 76–80.

Salehi, B., Mohammadbeigi, A., Sheykholeslam, H., Moshiri, E., & Dorreh, F. (2016). Omega-3 and zinc supplementation as complementary therapies in children with attention-deficit/hyperactivity disorder. *Journal of Research in Pharmacy Practice*, *5*(1), 22–26.

Sarris, J., Logan, A. C., Akbaraly, T. N., Amminger, G. P., Balanzá-Martínez, V., Freeman, M. P., Hibbeln, J., Matsuoka, Y., Mischoulon, D., Mizoue, T., Nanri, A., Nishi, D., Ramsey, D., Rucklidge, J., Sanchez-Villegas, A., Scholey, A., Su, K-P., Jacka, F. N. (2015). Nutritional medicine as mainstream in psychiatry. *The Lancet Psychiatry*, 2(3), 271-274.

Scott, S. P., & Murray-Kolb, L. E. (2016). Iron status is associated with performance on executive functioning tasks in nonanemic young women. *Journal of Nutrition*, *146*(1), 30–37.

Sever, Y., Ashkenazi, A., Tyano, S., & Weizman, A. (1997). Iron treatment in children with attention deficit hyperactivity disorder. A preliminary report. *Neuropsychobiology*, *35*(4), 178–180.

Siddiqui, S. V., Chatterjee, U., Kumar, D., Siddiqui, A., & Goyal, N. (2008). Neuropsychology of prefrontal cortex. *Indian Journal of Psychiatry*, *50*(3), 202–208.

Singer-Leshinsky, S. (2011). Attention-deficit/hyperactivity disorder: helping families to achieve success. *JAAPA*, *24*(3), 52–57.

Smith, A. L., Hoza, B., Linnea, K., McQuade, J. D., Tomb, M., Vaughn, A. J., Shoulberg, E.K., Hook, H. (2013). Pilot physical activity intervention reduces severity of ADHD symptoms in young children. *Journal of Attention Disorders*, *17*(1), 70–82.

Song, C. H., Kim, Y. H., & Jung, K. I. (2012). Associations of zinc and copper levels in serum and hair with sleep duration in adult women. *Biological Trace Element Research*, *149*(1), 16–21.

Sonuga-Barke, E. J. S. (2015). Editorial: Diet and children's behaviour problems - disentangling urban myth from clinical reality. *Journal of Child Psychology and Psychiatry*, *56*(5), 497–499.

Sonuga-Barke, E. J. S., Brandeis, D., Cortese, S., Daley, D., Ferrin, M., Holtmann, M., Stevenson, J., Danckaerts, M., van der Oord, S., Döpfner, M., Dittman, R. W., Simonoff, E., Zuddas, A., Banaschewski, T., Buitelaar, J., Coghill, D., Konofal, E., Lecendreux, M., Wong, I. C., Sergeant, J; European ADHD Guidelines Group. (2013). Nonpharmacological interventions for ADHD: systematic review and meta-analyses of randomized controlled trials of dietary and psychological treatments. *The American Journal of Psychiatry*, *170*(3), 275–289.

St-Onge, M.-P., Roberts, A., Shechter, A., & Choudhury, A. R. (2016). Fiber and saturated fat are associated with sleep arousals and slow wave sleep. *Journal of Clinical Sleep Medicine*, *12*(1), 19–24.

Stevens, L. J., Kuczek, T., Burgess, J. R., Stochelski, M. a., Arnold, L. E., & Galland, L. (2013). Mechanisms of behavioral, atopic, and other reactions to artificial food colors in children. *Nutrition Reviews*, *71*(5), 268–281.

Stevenson, J., Buitelaar, J., Cortese, S., Ferrin, M., Konofal, E., Lecendreux, M., Simmonoff, E., Wong, I. C., Sonuga-Barke, E. (2014). Research review: the role of diet in the treatment of attention-deficit/hyperactivity disorder-an appraisal of the evidence on efficacy and recommendations on the design of future studies. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, *55*(5), 416–427.

Sung, V., Hiscock, H., Sciberras, E., & Efron, D. (2008). Sleep problems in children with attention-deficit/hyperactivity disorder: prevalence and the effect on the child and family. *Archives of Pediatrics & Adolescent Medicine*, *162*(4), 336–342.

Tenenbaum, S., Paull, J. C., Sparrow, E. P., Dodd, D. K., & Green, L. (2002). An experimental comparison of Pycnogenol and methylphenidate in adults with attention-deficit/hyperactivity disorder (ADHD). *Journal of Attention Disorders*, *6*(2), 49–60.

Transler, C., Eilander, A., Mitchell, S., & van de Meer, N. (2010). The impact of polyunsaturated fatty acids in reducing child attention deficit and hyperactivity disorders. *Journal of Attention Disorders*, *14*(3), 232–246.

Trebatická, J., Kopasová, S., Hradecná, Z., Cinovský, K., Skodácek, I., Suba, J., Muchová, J., Zitnanová, I., Waczuliková, I., Rohdewald, P., Duracková, Z. (2006). Treatment of ADHD with French maritime pine bark extract, Pycnogenol. *European Child & Adolescent Psychiatry*, *15*(6), 329–335.

Van der Heijden K. B., Smits, M. G., Van Someren, E. J. W., Ridderinkhof, K. R., & Gunning, W. B. (2007). Effect of melatonin on sleep, behavior, and cognition in ADHD and chronic sleep-onset insomnia. *Journal of the American Academy of Child & Adolescent Psychiatry*, *46*(2), 233–241.

Van Egmond-Fröhlich, A. W. A, Weghuber, D., & de Zwaan, M. (2012). Association of symptoms of attention-deficit/hyperactivity disorder with physical activity, media time, and food Intake in children and adolescents. *PLoS One*, *7*(11), e49781.

Van Oudheusden, L. J., & Scholte, H. R. (2002). Efficacy of carnitine in the treatment of children with attention-deficit hyperactivity disorder. *Prostaglandins, Leukotrienes, and Essential Fatty Acids*, *67*(1), 33–38.

Vélez-Galarraga, R., Guillén-Grima, F., Crespo-Eguílaz, N., & Sánchez-Carpintero, R. (2016). Prevalence of sleep disorders and their relationship with core symptoms of inattention and hyperactivity in children with attention-deficit/hyperactivity disorder. *European Journal of Paediatric Neurology*, *20*(6), 925–937.

Verret, C., Gardiner, P., & Béliveau, L. (2010). Fitness level and gross motor performance of children with attention-deficit hyperactivity disorder. *Adapted Physical Activity Quarterly : APAQ*, *27*(4), 337–351.

Verret, C., Guay, M.-C., Berthiaume, C., Gardiner, P., & Béliveau, L. (2012). A physical activity program improves behavior and cognitive functions in children with ADHD: an exploratory study. *Journal of Attention Disorders*, *16*(1), 71–80.

Visser, S. N., Danielson, M. L., Bitsko, R. H., Holbrook, J. R., Kogan, M. D., Ghandour, R. M., Perou, R., Blumberg, S. J. (2014). Trends in the parent-report of health care provider-diagnosed and medicated attention-deficit/hyperactivity disorder: United States, 2003-2011. *Journal of the American Academy of Child and Adolescent Psychiatry*, *53*(1), 34–46.

Ward, N. I., Soulsbury, K. A., Zettel, V. H., Colquhoun, I. D., Bunday, S., & Barnes, B. (2009). The influence of the chemical additive tartrazine on the zinc status of hyperactive children a double-blind placebo-controlled study. *Journal of Nutritional Medicine*, 1(1), 51-57.

Wechsler, D. (2005). *Escala de Inteligencia de Wechsler para Niños – IV (WISC-IV). TEA Edicio*. Madrid, Spain.

Wehmeier, P. M., Schacht, A., & Barkley, R. A. (2010). Social and emotional impairment in children and adolescents with ADHD and the impact on quality of life. *The Journal of Adolescent Health*, *46*(3), 209–217.

Wender, E. H., & Solanto, M. V. (1991). Effects of sugar on aggressive and inattentive behavior in children with attention deficit disorder with hyperactivity and normal children. *Pediatrics*, *88*(5), 960–966.

Wesnes, K. A., Pincock, C., Richardson, D., Helm, G., & Hails, S. (2003). Breakfast reduces declines in attention and memory over the morning in schoolchildren. *Appetite*, 41(3), 329-331.

Widenhorn-Müller, K., Schwanda, S., Scholz, E., Spitzer, M., & Bode, H. (2014). Effect of supplementation with long-chain ω-3 polyunsaturated fatty acids on behavior and cognition in children with attention deficit/hyperactivity disorder (ADHD): a randomized placebo-controlled intervention trial. *Prostaglandins Leukotrienes and Essential Fatty Acids*, *91*(1–2), 49–60.

Wigglesworth, J. M. & Baum, H. (1998). Iron dependent enzymes in the brain. In: brain iron: neurochemical and behavioral aspects. Yodium, M. B. H., ed, *Taylor and Francis; New York*, 25–66.

Willett, W. C., Howe, G. R., & Kushi, L. H. (1997). Adjustment for total energy intake in epidemiologic studies. *The American Journal of Clinical Nutrition*, *65*(4 Suppl), 1220S–1228S.

Wolraich, M. L. (1995). The effect of sugar on behavior or cognition in children. *JAMA*, *274*(20), 1617.

Woo, H. D., Kim, D. W., Hong, Y.-S., Kim, Y.-M., Seo, J.-H., Choe, B. M., Park, J. H., Kang, J-E., Yoo, J-H., Chueh, H. W., Lee, J. H., Kwak, J. M., Kim, J. (2014). Dietary patterns in children with attention deficit/hyperactivity disorder (ADHD). *Nutrients*, *6*(4), 1539–1553.

Wu, J., Xiao, H., Sun, H., Zou, L., & Zhu, L. Q. (2012). Role of dopamine receptors in ADHD: a systematic meta-analysis. *Molecular Neurobiology*, *45*(3), 605–620.

Yoon, S. Y. R., Jain, U., & Shapiro, C. (2012). Sleep in attention-deficit/hyperactivity disorder in children and adults: past, present, and future. *Sleep Medicine Reviews*, *16*(4), 371–388.

Young, G & Conquer, J. (2005). Omega-3 fatty acids and neuropsychiatric disorders. *Reproduction Nutrition Development.*, *45*(1), 1–28.

Yürümez, E., & Kılıç, B. G. (2016). Relationship between sleep problems and quality of life in children with ADHD. *Journal of Attention Disorders*, *20*(1), 34–40.

Zadeh, S. S., & Begum, K. (2011). Comparison of nutrient intake by sleep status in selected adults in Mysore, India. *Nutrition Research and Practice*, *5*(3), 230–235.

8. Appendix

A. Clinical test

A1. KIDMED Test

Adherencia a la DIETA MEDITERRÁNEA en la infancia Puntos
Toma una fruta o un zumo natural todos los días. +1
Toma una 2a pieza de fruta todos los días. +1
Toma verduras frescas (ensaladas) o cocinadas regularmente una +1 vez al día.
Toma verduras frescas o cocinadas de forma regular más de una +1 vez al día.
Consume pescado con regularidad (por lo menos 2-3 veces al a +1 semana).
Acude una vez o mas a la semana a un centro de comida rápida -1 (*fast food*) tipo hamburguesería.
Le gustan las legumbres y las toma más de 1 vez a la semana. +1
Toma pasta o arroz casi a diario (5 dias o más a la semana) +1
Desayuna un cereal o derivado (pan, etc) +1
Toma frutos secos con regularidad (al menos 2-3 veces a la +1 semana).
Se utiliza aceite de oliva en casa. +1
No desayuna -1
Desayuna un lácteo (yogurt, leche, etc). +1
Desayuna bollería industrial, galletas o pastelitos. -1
Toma 2 yogures y/o 40 g queso cada día. +1
Toma golosinas y/o caramelos varias veces al día -1

Valor del índice KIDMED

≤ 3: Dieta de muy baja calidad
4 a 7: Necesidad de mejorar el patrón alimentario para ajustarlo al modelo mediterráneo.
≥ 8: Dieta mediterránea óptima

Fuente:

Serra Majem L, Ribas Barba L, Ngo de la Cruz J, Ortega Anta RM, Pérez Rodrigo C, Aranceta Bartrina J. Alimentación, jóvenes y dieta mediterránea enEspaña. Desarrollo del KIDMED, índice de calidad de la dieta mediterráena en la infancia y la adolescencia. In: Serra Majem L, Aranceta Bartrina J, editores. Alimentación infantil y juvenil. Masson; 2004(reimpresión). p. 51-59

A.2 Food Frequency Questionnaire

Frecuencia de consumo de alimentos - infantil

ID:	(a rellenar por el personal investigador)	**Fecha:**	
Apellidos:	(escriba apellidos en MAYÚSCULAS)		
Nombre:	(escriba nombre en MAYÚSCULAS)		

Instrucciones

1. Este cuestionario NO debe ser autoadministrado. Debe ser rellenado por el personal investigador.

2. Cuando estime las frecuencias de consumo, piense en todas las formas en que puede consumir un alimento. Por ejemplo, las verduras crudas pueden consumirse como plato o como acompañamiento.

3. Tenga en cuenta las cantidades cuando estime las frecuencias. Por ejemplo, si un individuo suele consumir un plato de verduras crudas cada día y 3 veces a la semana como acompañamiento entonces serán 7 raciones más 3 medias raciones (total: 8,5 raciones/semana).

4. Introduzca un sólo dato de frecuencia para cada alimento, usando la columna que más le convenga.

1a. Sigue el niño/a alguna dieta especial? No Sí, por iniciativa de los padres Sí, por indicación de un profesional **1b. Cual?** Sin gluten Sin lactosa Sin proteína láctea Sin huevo Baja en energía Vegetariana Ovolactovegetariana Otra….....
.....

2. Qué comidas hace habitualmente el niño/a? Desayuno Media-mañana Comida Merienda Cena Resopón Entre horas

3. Con qué frecuencia come el niño fuera de casa?: Nunca A veces Habitualmente Fin de semana

4. Si come habitualmente fuera de casa, indique qué comidas
Desayuno Media-mañana Comida Merienda Cena Resopón Entre horas

5. Qué aceite/grasa acostumbra a utilizar para: Freír..... Aliñar..... Guisar.....

6. Acostumbra a eliminar la grasa o la piel de los diferentes alimentos (jamón, pollo, etc.)? Sí No A veces

7a. Toma algún complejo vitamínico-mineral o suplemento nutricional?
Sí No A veces

7b. En caso afirmativo, indique el nombre comercial del producto y la frecuencia de consumo (pastilla/cápsula):

NOMBRE	FRECUENCIA	Cuanto tiempo hace que lo toma?
	1 2 3 4 5 D S M A	**…… meses**
	1 2 3 4 5 D S M A	**…… meses**
	1 2 3 4 5 D S M A	**…… meses**

D, diario; S, semanal; M, mensual; A, anual

A continuación le preguntaré con que frecuencia **(poner un número [N] o una cruz [X])** acostumbra a
tomar los siguientes alimentos:

	A LA SEMANA	AL MES	NUNCA
Leche			
Yogurt (también DANONINO considerar ½ ración)			
Chocolate: tableta, bombones, “Kit Kat”, “Mars”…			
Cereales de desayuno (“Corn-Flakes”, “Kellog’s”)			
Galletas tipo “maría”			
Galletas con chocolate, crema…			
Magdalenas, bizcocho, melindros…			
Ensaimada, donut, croissant…			
“Phoskitos” “Pantera Rosa” “Bony” Tigretón			
	A LA SEMANA	**AL MES**	**NUNCA**
Ensalada: lechuga, tomate, escarola…			
Judías verdes, guisantes, brócoli, zanahoria			
Acelgas, espinacas			
Verduras de guarnición: berenjena, champiñones			
Patatas al horno, fritas o hervidas			
Legumbres: lentejas, garbanzos, judías…			
Arroz blanco, paella			
Pasta: fideos, macarrones, espaguetis…			
Sopas y cremas de verduras			
	A LA SEMANA	**AL MES**	**NUNCA**
Huevos			
Pollo o pavo (incluir el fiambre de pollo y pavo también)			
Ternera, cerdo, cordero (bistec, empanada,…)			
Carne picada, salchichas, hamburguesa, albóndigas			
Pescado blanco: merluza, panga, rape, bacalao…			
Pescado azul: sardinas, atún, salmón (conservas tb)			
Marisco: mejillones, gambas, langostinos, calamares,…			
Croquetas, empanadillas, pizza			
Pan (en bocadillo, con las comidas,…)			

	A LA SEMANA	AL MES	NUNCA
Jamón salado, dulce, embutidos			
Queso blanco o fresco (Burgos,…) o bajo en calorías			
Otros quesos: curados o semicurado, cremosos			
	A LA SEMANA	**AL MES**	**NUNCA**
Frutas cítricas: naranja, mandarina, (kiwi también)			
Otras frutas: manzana, pera, melocotón, plátano…			
Frutas en conserva (en almíbar…)			
Zumos de fruta natural			
Zumos de fruta comercial			
Frutos secos: cacahuetes, avellanas, almendras,…			
Postres lácteos: natillas, flan, mató, arroz con leche,…			
Pasteles de crema o chocolate (tartas, brazo gitano, etc			
Golosinas: gominolas, caramelos…			
Bolsas de aperitivos (“chips”, “Cheetos”, “Fritos”…)			
Helados			
Azúcar			
	A LA SEMANA	**AL MES**	**NUNCA**
Bebidas azucaradas (“Coca-cola”, “Fanta”, Trina,…)			
Bebidas bajas en calorías ("Coca-cola light")			
OTROS	**A LA SEMANA**	**AL MES**	**NUNCA**

B. Article/ Congress communications

B1. Communication I. Poster

Title: Diet and sleep in children and adolescents with Attention and deficit disorder

Authors: Ríos-Hernández A, Carpio-Arias T, Alda JA, Farran-Codina, A, Izquierdo-Pulido M.

Congress: II Workshop Anual sobre "Cacao y chocolate: Ciencia y Gastronomía"
November, 2016

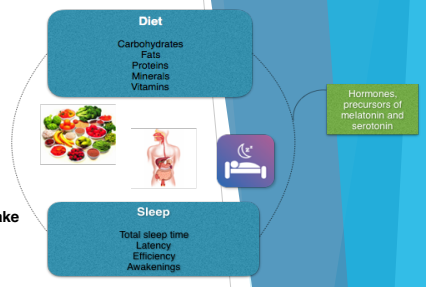
DIET AND SLEEP IN SPANISH CHILDREN AND ADOLESCENTS WITH ATTENTION DEFICIT DISORDER.

Ríos-Hernández A.[1], Carpio-Arias T.[1*] Alda JA.[2], Farran-Codina, A[1], Izquierdo-Pulido M.[1,3]

[1]Department of Nutrition, Food Science and Gastronomy and Institut de Recerca en Nutrició i Seguretat Alimentària. University of Barcelona, Spain. [2]ADHD Unit. Child and Adolescent Psychiatry Department. Hospital Sant Joan de Deu Barcelona, Spain; [3]CIBER Physiopathology of Obesity and Nutrition (CIBEROBN), Instituto de Salud Carlos III, Spain

Background and objectives:

Nutrient deficiencies and unhealthy diets have been related with attention–deficit/ hyperactivity disorder (ADHD). Sleep disorders also might influence in ADHD symptoms. To date little is known about the impact of diet and sleep on ADHD symptoms. The aim of this study was to assess the relationship between diet and sleep disturbances in children and adolescents with ADHD.



Subjects & Methods

55 Cases

55 Controls

→ **Matched by sex and age 6-16 years** →

Energy and dietary intake
Anthropometric measures
Sleep characteristics

Results

Sleep disorders were more prevalent in ADHD children compared to control **(Figure 1)**. It was found that a lower consumption of: fiber, vegetable protein, thiamin, vitamin D, iron, folate, potassium, magnesium is associated with a higher prevalence of total sleep problems (p<0, 05) **(Figure 2)**. In the same way, a lower consumption of fiber, vegetable protein, thiamin, vitamin B6, vitamin E, iron, folate, magnesium and zinc intake was associated with a higher prevalence of "Excessive Daytime Sleepiness" only in children and adolescents with ADHD (p<0,05) (**Table 1**).

Figure 1. Prevalence of Sleep Disorders

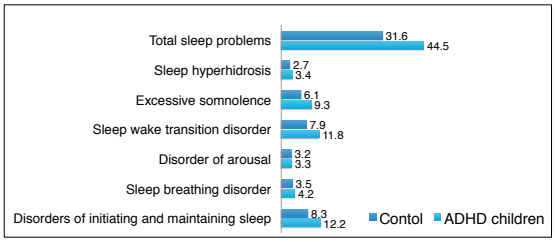
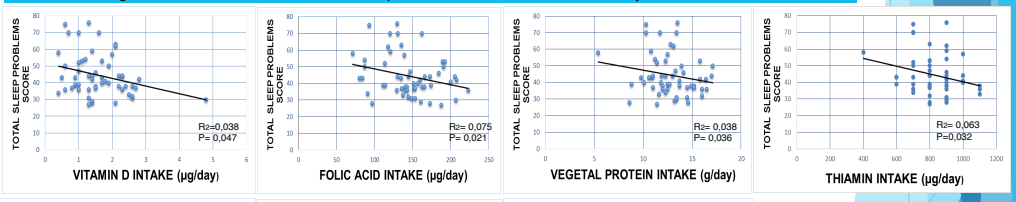
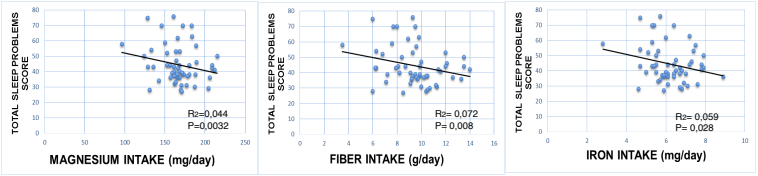


Table 1. Association of Excessive Daytime Sleepiness and diet*

n=55	B	95% CL	P-value
Fiber	-0.80	-1.35, -0.24	0.006
Vegetal protein	-0.75	-1.30, -0.23	0.005
Saturated fat	0.70	0.11, 1.30	0.022
Thiamin	-15.26	-24.10, -6.45	0.001
Vitamin B6	-8.30	-15.30, -1.30	0.022
Iron	-1.63	-2.75, -0.51	0.005
Folate	-0.04	-0.08- 0.01	0.008
Magnesium	-0.07	-0.12, -0.01	0.016

Figure 2. Associations between Total sleep disorders and nutrient intake in subjects with ADHD *





*Adjusted for sex, age and ADHD subtype

Conclusions

1. Children and adolescents with ADHD have higher prevalence of sleep disorders than healthy subjects.
2. Our results are in accordance with published data that showed that nutritional deficiencies such as that of vitamin B group, magnesium and zinc may impair sleep by altering neural responses via circulating intestinal hormones (e.g. insulin, CCK, ghrelin) or by affecting the synthesis of serotonin and melatonin.
3. Taking in consideration that diet is a modifiable factor, specific dietary strategies for families and children with AHDH might help their sleep patterns and, in consequence, their quality of life.

References:

Izquierdo-Pulido et al. The role of diet and physical activity in children and adolescents with ADHD. *Recent Advances in Pharmaceutical Sciences V*. Research Signpost. 2015; 4:51-64; Blunden et al. Diet and sleep in children with attention deficit hyperactivity disorder: preliminary data in Australian children. *Journal of child health care*. 2011;*15*:14–24; Golley et al. Sleep duration or bedtime? Exploring the association between sleep timing behaviour, diet and BMI in children and adolescents. *International Journal of Obesity*. 2013; 37:546-551

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Instituto de Salud Carlos III, Ministerio de Ciencia e Innovación (PI11/2009)

B2. Communication II. Poster

Title: Association between 5-HTTLPR L/S polymorphism and ADHD in children and adolescents: Implications in diet and obesity

Authors: Carpio-Arias T, Torres-Anguiano J, Ríos-Hernández A, Farran-Codina A, Alda JA, Izquierdo-Pulido M.

Congress: NuGOweek, September, 2016; Copenhagen, Denmark

Association between 5-HTTLPR L/S polymorphism and ADHD in children and adolescents: Implications in diet and obesity

Carpio-Arias TV[1], Torres-Anguiano J[1], Ríos-Hernández A[1], Farran-Codina A[1], Alda JA[2], Izquierdo-Pulido M [1,3]

[1]Department of Nutrition, Food Science and Gastronomy. University of Barcelona, Spain; [2]ADHD Unit. Child and Adolescent Psychiatry Department. Hospital Sant Joan de Deu Barcelona, Spain; [3]CIBER Physiopathology of Obesity and Nutrition (CIBEROBN), Instituto de Salud Carlos III, Spain.

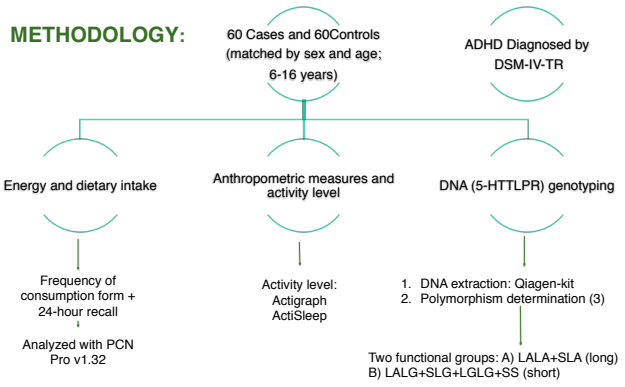
INTRODUCTION:

It has been postulated that the presence of functional polymorphism in the promoter region HTTLPR gene serotonin transporter (*SLC6A4*) may be related to certain compulsive eating behaviors and increased obesity (1,2).

AIM:

To study the presence of this polymorphism in children and adolescents with attention deficit disorder and hyperactivity disorder (ADHD) and its potential association with the presence of overweight / obesity and eating habits in these patients.

METHODOLOGY:



RESULTS:

Figure 1. Percentage of genetic variations polymorphism 5-HTTLPR gene between cases and controls.

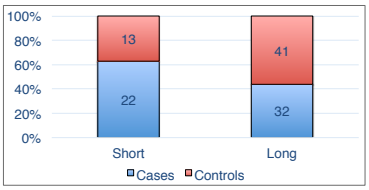


Table 1. BMI and physical activity of subjects with ADHD and control subjects

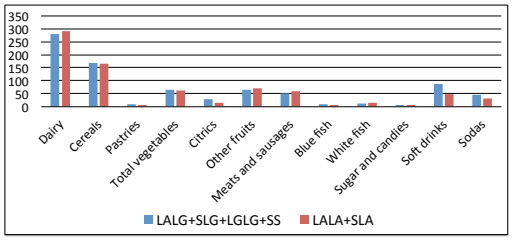
	Cases ADHD	Controls	P value
BMI Z – score	0.73 (1.04)	0.24 (1.09)	0.018
Physical activity, kcal/ day	1213.2 (810.4)	866.2 (486.3)	0.008

Table 2. ADHD symptomatology and 5-HTTLPR L/S polymorphism

	A* n=32	B* n=22	P value
ADHD Parents			
ADHD (Total)	30.9 (11.3)	34.2 (11.5)	NS
Hiperactivity	14.3 (6.5)	13.8 (9.3)	NS
Inattention	16.6 (6.6)	20.4 (4.1)	0.01
Conners parents			
ADHD (Total)	69.1 (10.7)	74.9 (8.0)	0.03
Hiperactivity	70.5 (13.5)	67.3 (17.4)	NS
Inattentión	68.7 (12.9)	76.4 (8.6)	0.01

A=LALA+SLA; B=LALG+SLG+LGLG+SS

Figure 2. Food consumption in children with ADHD classified by 5-HTTLPR L/S, (data are presented in mean)



CONCLUSIONS:

1. The "short" variant predominates in children and adolescents with ADHD
2. The presence of the short variant could not relate to aspects of diet or obesity
3. The presence of the short variant allele, predominant in cases of children with ADHD could bring as consequences symptoms characteristic of the disease such as inattention that could have long-term consequences of obesity.

REFERENCES:

1. Cortese S et al. Association between ADHD and obesity: A systematic review and meta-analysis. Am J Psychiatry, 2016; 173:1.
2. Lee YG et al. Meta-Analysis of case-control and family based associations between the 5-HTTLPR L/S polymorphism and susceptibility to ADHD. J Attent Dis. 2015, DOI: 10.1177/1087054715587940
3. Wendland JR et al. Simultaneous genotyping of four functional loci of human*SLC6A4*, with a reappraisal of *5-HTTLPR* and rs25531. Mol. Psychiatry, 2016; 11:224–226.

Abstract number: 24948

B3. International research. "Growing Up in New Zealand"

Results from the internship performed at the University of Auckland and in the Starship Children´s Hospital in New Zealand during the period from June to September of 2015 and from August to September 2016 under the supervision of Dr C Grant and Dr C Wall.

The relationship of dietary practices with sleep in 2-year-old children

Abstract

Aims: To determine if dietary practices are associated with sleep duration and night-time wakenings in young children.

Methods: An ethnically and socioeconomically diverse cohort of pregnant women in New Zealand (n=6822) were enrolled in 2009 and 2010. Breastfeeding and dietary practices were described with data collected using a dietary history when the children were aged 9 months and a food frequency questionnaire when they were aged 24 months.

Sleep duration and number of night-time wakenings were determined by parental report at age two years. Short sleep duration was defined as <11 hours in each day and night and increased night-time wakenings as ≥ 2 per night.

Multivariate logistic analysis was used to examine the association of dietary practices with sleep duration and night-time wakenings, with associations described using adjusted odds ratios (OR) and 95% confidence intervals (CI).

Results: Among the 6288 children for whom complete data were available median (interquartile range (IQR) sleep duration was 12.5 (11.5-13.5). Sleep duration of < 11 years was reported for 734 (12%) children and ≥ night-time wakenings for 1063 (17%) children. The odds of the child having sleep duration <11 hours decreased with each increase of serving of spreads per day (OR=0.90), and increased for each increase of serving of soft drinks and snacks per day (OR=1.14). Children having woken two or more times the odds decreased as the number of daily servings of vegetables increased per day (OR=0.92) and increased as the number of servings of milk, cheese and yogurt increased per day (OR= 1.15).

Conclusions: Our findings have important public health implications as they show that NZ children at two years have a high frequency of consumption of soft drinks and snacks and short sleep duration at night.

Introduction

Consolidation of night-time sleep patterns occurs during the first year of life, alongside reductions in daytime sleep duration. Inadequate sleep during early childhood has a negative impact in several areas of health and functioning, including physical growth, cognitive functioning, behaviour and school performance.[1] Moreover, short sleep duration in childhood has been associated with an increased risk of obesity, poor academic performance and behavioural problems.[2]

A gradual decline in average sleep duration for children and adolescents has been reported from several countries in recent decades. For example, in a Swiss longitudinal study, Iglowstein et al.[3] showed a decreasing trend in total sleep duration and a delay in bedtime without changing wakeup-time, across three consecutive cohorts (1974, 1979 and 1986). Similar trends have also been reported in Australian children, with a decline in week-day sleep duration of about 30 min between 1985 and 2004 among 10-to 15 year-old; and from Spain, where a decrease of 20 min sleep in in 24 hours was reported from 1987 to 2011.[4] A meta-analysis by Matricciani et al.[5] using data from 20 Pacific rim and European countries identified that children´s sleep has decreased 0.75 min per year over the past century, with the rate of change being greatest on school days, for older children and for boys.

A recent systematic literature review conducted by the National Sleep Foundation recommends that newborns sleep for 14 -17 h per night, infants for 12-15 h, toddlers for 11-14 h and preschoolers for 10- 13 h per night.[7] Children´s sleep/wake up patterns are influenced by several socio-cultural variables including child age and sex, parent characteristics and behavior, and socioeconomic status.[6] In addition to these factors there is also a relationship between the child's dietary practices and their sleep.

This relationship between sleep and diet is complex. Sleep has an influence on dietary choices[9–11] but also diet and the timing of meals may influence sleep.[12–15] Recently, it was reported that in children aged six years higher intakes of carbohydrates, sugar, fat, cholesterol and lower intake of dietary fibre were associated with either sleep disturbances (problems of initiating sleep, excessive sleepiness and sleep disordered breathing) or shorter sleep duration have.[9,13] Unhealthy dietary patterns (lower intake of

vegetables and fruit and higher intake of meat/alternatives and soft-drinks) have also been shown to be associated with insufficient sleep in 10 to 14 year old children.[16] Some studies have suggested that specific foods such as milk, fruits and herbal products promote longer sleep.[15,17] However, it remains unclear if eating behaviors, for example, snaking between meals and after supper, rather than the specific foods eaten are responsible for relationships between dietary practices and sleep that have been reported in school aged children.[18]

The relationship between dietary practices and sleep in preschool aged children is even less completely understood. Breastfeeding has been shown to be associated with more frequent night time wakenings in some,[19–21 23], but not all studies where this relationship has been investigated.[24,25] The relationship between other components of the infant and toddler diet and night time sleep has not been described. The aim of our study was to determine the relationship between dietary practices during the first two years of life with sleep duration and night-time wakenings at age two years.

Methods

We completed our study within a cohort into which were enrolled a nationally representative sample of children born in New Zealand 2009 and 2010.[26,27] Ethics approval was obtained from the Ministry of Health Northern Y Regional Ethics Committee (NTY/08/06/055) and written informed consent was obtained from all enrolled women.

Recruitment into the cohort occurred antenatally. Pregnant women were eligible if residing within a geographically defined region of New Zealand, where one-third of the national population lives. This region was chosen for its ethnic and socio-economic diversity. There were no other inclusion or exclusion criteria. The 6853 children born to the 6822 pregnant women who were enrolled included approximately 11% of the national birth cohort over the recruitment period. For this analysis of dietary intake and sleep we used the data from the (n=6327, 92% of the child cohort) for whom the two year data collection waves up to and including age two years were completed.

Data collection and measurement

Data were collected using: computer-assisted face-to-face interviews, completed with each woman at enrolment and then when their children were nine and 24 months old; computer-assisted telephone interviews completed with each enrolled woman when their child was 6 and 35 weeks, and then 16 and 23 months old; by linkage with maternity hospital and infant healthcare records; and by child anthropometric measurements obtained at age 2 years.

Measurement of sleep duration and night time wakenings

At the interview completed when the children were two years old, each mothers was asked to describe, on average, how much time their child spent asleep at night in total and during the day, and, on average, how many time their child woke at night. These items, were adapted from The Southampton Women's Study & Brief Infant Sleep Questionnaire, a validated screening tool for clinical and research purposes in paediatric settings.[28] Average sleep duration per day and night of less than 11 hours was defined as shorter than normal based upon the Sleep Health Foundation recommendations for children aged one to two years.[29]

Measurement of dietary practices

The duration, in months, of any and of exclusive breastfeeding were described. Exclusive breastfeeding was defined as only breast milk and no other milk, solids, fluids or water.[30] Information on breastfeeding duration were obtained through maternal report at the interviews completed when the children were 6 weeks, 9, 31 and 45 months old. The duration of exclusive breastfeeding (in months) was corrected for the age of introduction of foods or drinks reported when the children were 9 months old.

At the 9 month interview each mother was asked how old their child was when they first tried one of 25 food items (infant milk formula or milk; baby rice; baby breakfast cereal; other cereal; bread or toast; rusks; biscuits; vegetables; fruit; meat; fish; eggs; puddings; nuts or peanut butter; shellfish; soy foods; sweets; chocolate; hot chips; potato chips-crisps; fruit juices; herbal drinks; tea; coffee; soft drinks). This food list

was designed by a dietitian (C.R.W) who selected the food items based on foods and beverages commonly fed to infants in New Zealand at the time of the interview.

At the two-year interview the child's dietary intake over the last four weeks was described using a semi-quantitative, sixty-two-item food frequency questionnaire (FFQ). The FFQ collected data that allowed description of the number of servings per day from each of the four main food groups: vegetables and fruits; milk and milk products; breads and cereals; lean meat, meat alternatives and eggs and also the number of servings per day of spreads, soft drinks and snacks. Cards with pictures of standard serving sizes were used to assist description of portion size consumed.

Anthropometry

Anthropometric measurements of children were undertaken at birth, and age nine months and two years. Birth weight and length and gestational age were determined from data recorded at the maternity hospitals where the cohort children were born and from parental report of birth weight obtained at the interview completed when the cohort children were six weeks old. At the nine-month interview the child's most recent weight and age as recorded in their health and development record book were documented. At the two-year interview each child's height and weight were measured in light clothes (no shoes, hats, jumper or jacket). Weight was measured twice to the nearest 0.1 kg, using a calibrated Tanita digital scale. If the two weight measurements differed by more than 200 grams, a third weight measurement was obtained. When it was not possible to measure the child's weight, their weight was estimated by calculating the difference between the parent`s weight with the parent holding the child and then the parent's weight without the child. Height was measured twice to the nearest 0.1 centimetres using a laser (PRECASTER CA601). If the two height measurements differed by 5 mm a third measurement was performed.

Weight-for-age, length-for-age, weight-for-length and BMI-for-age z-scores were calculated using the WHO Child Growth Standards macro programme for SAS.[31] With the observed mean z-score for each of these variables being > -1.5 we applied the WHO recommended cut-offs for extreme values (Height-for-age: <-5.0 and >+3.0; weight-for-age: <-5.0 and >+5.0; weight-for-height: <-4.0 and >+5.0; and BMI-for-age <-4.0 and >+5.0.[32]

BMI-for-age z-score was classified into five categories following the WHO Child Growth Standards: Underweight (BMI < 18.5)**,** Normal (18.5 ≤ BMI < 25)**,** Overweight (25 ≤ BMI < 30)**,** Obese (BMI ≥30) [33]

Covariate measurement

Multiple potential confounding factors from across the life course were collected.
Maternal and household demographics measured included maternal ethnicity, age, highest educational qualification, and socio-economic deprivation.
At the antenatal interview mothers were asked to describe to the most detailed level possible, with this then coded into six level 1 categories following the statistics NZ coding criteria: (i) European; (ii) Māori; (iii) Pacific Peoples; (iv) Asian; (v) Middle Eastern; Latin American and African and (vi) Other.
Parental socioeconomic status, educational qualifications, and household income items were based on measures taken from Statistics New Zealand's 2006 national census[34] and 2008 General Social Survey.[35] Area-level socio-economic deprivation was measured using the 2006 NZ Index of Deprivation (NZDep06), grouped as quintiles.[36] NZDep06 is derived from 2006 census data on nine socioeconomic characteristics and is a well-validated measure of small area socioeconomic deprivation in NZ.

Maternal health and health behaviour measures included cigarette smoking and alcohol consumption during pregnancy and infancy and physical activity during pregnancy. At the antenatal interview each mothers was asked their self-reported weight and height[37].

Child characteristics measured included gender, total of hours the child spend at home watching television and video screens.

Study size

The sample size for the cohort was sufficient to provide adequate power to undertake complex analyses over time across the whole cohort as well as within ethnic and socioeconomic subgroups.[26]

Statistical Analysis

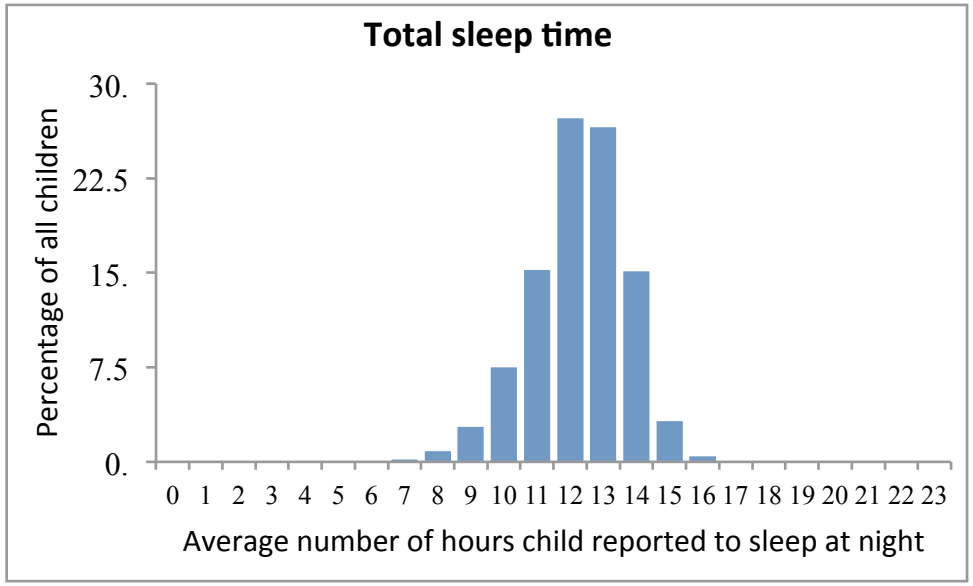
For the purposes of analysis comparisons were made of children with normal (≥ 11 hours) sleep duration per 24 hours versus those with short (< 11 hours) sleep duration, and those children with two or more night time wakenings were compared with those with one or no night time wakenings.

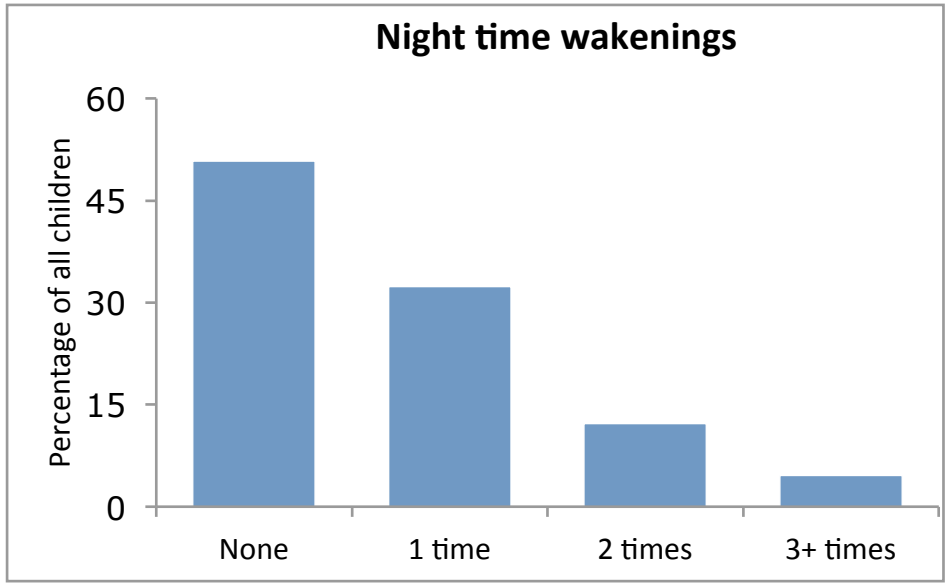
We used a hierarchical approach to model our conceptual framework.[38] Potential variables of relevance to the relationship of food consumption patterns with sleep duration and night time wakenings at age two years were grouped by their proximity to the child's sleeping patterns at age two years. We grouped variables into those describing: (i) maternal and household demographics; (ii) maternal health characteristics and behaviours; (iii) child characteristics, the presence of siblings when the child was an infant and the mother having a current partner when the child was two years old. Within each of these three groupings we performed multivariable variable analyses to identify those variables independently associated with sleep duration and night time wakenings. These analyses began with the most distal groups, with variables identified as significant in the more distal models were then included in multivariate models created from each of the subsequent more proximal groupings.

All data manipulation and statistical analysis were conducting using the SAS statistical software package version 9.2. Statistical significance was considered at a 2-tailed p-value of <0.05. Independent associations were described using adjusted odds ratios and 95% confidence intervals.

Results

Figure 1. Parental report of average number of hours per night that the child sleeps and average number of wakenings per night at age two





Sleep duration at night was reported by the parents of 6288 (100%) and number of night-time wakening's by 6318 (100%) of the 6327 children (Figure 1). The median (interquartile range (IQR)) number of hours of sleep per night was 12.5 (11.5-13.5) hours. The sleep duration for 5306 (84%) of the children, met the recommendations for this age group of 11 to 14 hours of sleep per night.[34] Parents reported, on average, no night-time wakening's were reported by 5255 (83%).

Table 1. Frequency of consumption of food groups at age two years in the Growing Up in New Zealand cohort children

	Number of servings per day*								
Food category	**None n (row %)**	**<1 n (row %)**	**1 n (row %)**	**2 n (row %)**	**3 n (row %)**	**4 n (row %)**	**5 n (row %)**	**6 or more n (row %)**	**Total**
Vegetables	35 (1)	74 (1)	501 (8)	1609 (26)	2665 (43)	850 (14)	289 (5)	217 (3)	6240
Fruit	25 (<1)	31 (<1)	291 (5)	1144 (18)	2126 (34)	1284 (21)	607 (10)	714 (11)	6222
Milk, cheese and yogurt	11 (<1)	19 (<1)	219 (4)	1218 (20)	1497 (24)	1402 (22)	948 (15)	919 (15)	6233
Bread, rice, pasta	4 (<1)	3 (<1)	31 (<1)	172 (3)	663 (11)	1249 (20)	1343 (22)	2753 (44)	6218
Meat, meat alternatives, eggs	0 (0)	1 (<1)	100 (2)	1187 (21)	2728 (47)	1445 (25)	214 (4)	93 (2)	5768
Spreads	169 (3)	257 (4)	1491 (24)	2149 (34)	1055 (17)	576 (9)	305 (5)	232 (4)	6234
Soft drinks and snacks	61 (1)	257 (4)	3094 (50)	2238 (36)	372 (6)	136 (2)	58 (1)	25 (<1)	6241

The recommended number of daily servings of vegetables was met by 90%, of fruit by 95%, of bread and cereals by 86%, of milk and milk products by 98% and of meat, meat alternatives or eggs by 100%. Spreads were consumed at least once daily by 97% and soft drinks and snacks were consumed at least daily by 95% (Table 1). The median (IQR) number of daily servings of each food group were vegetables 3.0 (2.5-3.5), fruit 3.0 (2.0-3.5), bread and cereals 5.5 (4.5-7.0), milk and milk products 4.0 (3.0-5.0), meat, meat alternatives or eggs 3.5 (3.0-4.0). The median (IQR) number of daily servings of spreads was 2.0 (1.5-3.0) and of soft drinks and snacks was 1.5 (1.5-2.0).

Table 2. Associations of maternal and household demographics with child's sleep duration and night time wakening

Antenatal maternal characteristics		Child's night time sleep duration meets recommendations*				Child wakens two or more times at night			
		Less than 11 hours n = 734	11 to 14 hours n = 5554	Univariate odds ratio (95% CI)	Multiivariate odds ratio (95% CI)	Yes n = 1063	No n = 5255	Univariate odds ratio (95% CI)	Multiivariate odds ratio (95% CI)
Relationship with partner (n = 5564), n (%)				< 0.001	0.76			0.21	0.62
Single parent	283 (5)	48 (17)	232 (83)	1.80 (1.29-2.49)	0.99 (0.63-1.51)	60 (21)	222 (79)	1.35 (0.99-1.80)	1.30 (0.87-1.91)
Dating but not cohabiting	213 (4)	44 (21)	169 (79)	2.28 (1.59-3.20)	1.11 (0.72-1.69)	36 (17)	177 (83)	1.01 (0.69-1.45)	1.04 (0.67-1.59)
Cohabiting with partner	1528 (27)	183 (12)	1334 (88)	1.20 (1.00-1.44)	0.91 (0.73-1.15)	247 (16)	1280 (84)	0.96 (0.82-1.13)	1.03 (0.86-1.24)
Married	3540 (64)	362 (10)	3164 (90)	1.00	1.00	591 (17)	2946 (83)	1.00	1.00
Household structure (n=6154), n (%)				< 0.001	0.75			0.01	0.38
Solo parent	206 (3)	40 (19)	165 (80)	2.42 (1.67-3.44)	1.18 (0.70-1.96)	44 (21)	161 (78)	1.46 (1.02-2.04)	1.12 (0.69-1.80)
Two parents alone	4156 (67)	376 (9)	3760 (91)	1.00	1.00	654 (16)	3497 (84)	1.00	1.00
Parent(s) with extended family	1464(24)	259 (18)	1193 (82)	2.17 (1.83-2.57)	1.10 (0.88-1.38)	274 (19)	1188 (81)	1.23 (1.05-1.44)	1.18 (0.97-1.43)
Parent non kin	328 (5)	36 (11)	289 (89)	1.25(0.85-1.77)	0.94 (0.62-1.39)	62 (19)	265 (81)	1.25 (0.93-1.66)	1.17 (0.85-1.59)
Parity (n=6154), n (%)				< 0.001	0.03			0.00	0.05
First child	2590 (42)	250 (10)	2328 (90)	0.72 (0.61-0.84)	0.80 (0.66-0.98)	389 (15)	2196 (85)	0.80 (0.70-0.92)	0.79 (0.68-0.93)
Subsequent child	3564 (58)	461 (13)	3079 (87)	1.00	1.00	645 (18)	2915 (82)	1.00	1.00

Age group (years) (n=6161), n (%)				< 0.001	0.02			0.00	0.00
<20	260 (4)	38 (15)	221 (85)	0.94 (0.64-1.33)	0.69 (0.45-1.05)	33 (13)	227 (87)	0.68 (0.45-0.98)	0.64 (0.41-0.97)
20 – 29	2321 (38)	357 (15)	943 (84)	1.00	1.00	407 (17)	1912 (82)	1.00	1.00
30 – 39	3309 (54)	292 (9)	3004 (91)	0.53 (0.45-0.63)	0.77 (0.63-0.94)	528 (16)	2775 (84)	0.89 (1.12-2.04)	0.97 (0.83-1.15)
≥40	271 (4)	25 (9)	245 (91)	0.55 (0.35-0.83)	0.79 (0.48-1.24)	66 (24)	204 (75)	1.52 (1.12-2.04)	1.77 (1.27-2.45)
Self-prioritized ethnicity (n=6145), n (%)				< 0.001	< 0.001			< 0.001	< 0.001
European	3495 (57)	209 (6)	3270 (94)	1.00	1.00	486 (14)	3005 (86)	1.00	1.00
Maori	815 (13)	161 (20)	645 (80)	3.9 (3.12-4.87)	2.70 (2.06-3.54)	160 (20)	652 (80)	1.52 (1.24-1.84)	1.33 (1.05-1.68)
Pacific	787 (13)	203 (26)	576 (74)	5.51 (4.46-6.82)	3.12 (2.34-4.17)	149 (19)	638 (81)	1.44 (1.18-1.76)	1.08 (0.82-1.41)
Asian	836 (14)	113 (13)	721 (86)	2.45 (1.92-3.12)	2.15 (1.55-2.98)	198 (24)	637 (76)	1.92 (1.59-2.31)	1.75 (1.35-2.60)
Other[†]	212 (3)	25 (12)	186 (88)	2.10 (1.32-3.21)	1.83 (1.09-2.93)	41 (19)	170 (80)	1.49 (103-2.10)	1.42 (0.96-2.05)
Country of mother's birth (n= 6154), n (%)				0.002	0.73			< 0.001	0.31
New Zealand	4065 (66)	433 (11)	3606 (89)	1.00	1.00	624 (15)	3434 (85)	1.00	1.00
Other	2089 (34)	278 (13)	1801 (87)	1.29 (1.09-1.51)	1.04 (0.90-1.36)	410 (20)	1677 (80)	1.34 (1.17-1.54)	1.11 (0.91-1.34)
Pregnancy planning (n=6133), n (%)				< 0.001	0.01			0.00	0.74
Planned	3806 (62)	330 (9)	3463 (91)	1.00	1.00	597 (16)	3202 (84)	1.00	1.00
Unplanned	2327 (38)	378 (16)	1927 (84)	2.06 (1.76-2.41)	0.77 (0.64-0.94)	433 (19)	1892 (81)	1.23 (1.07-1.41)	0.97 (0.82-1.15)

Education (n=6143), n (%)				< 0.001	0.02			0.03	0.33
No secondary education	394 (6)	84 (22)	301 (78)	2.63 (2.02-3.41)	1.51 (1.10-2.08)	70 (18)	322 (82)	1.14 (0.86-1.48)	1.01 (0.73-1.39)
Secondary education	1400 (23)	209 (15)	1180 (85)	1.67 (1.40-2.00)	1.11 (0.90-1.36)	267 (19)	1132 (81)	1.23 (1.05-1.44)	1.14 (0.95-1.36)
Tertiary education	4349 (71)	415 (10)	3918 (90)	1.00	1.00	697 (16)	3646 (84)	1.00	1.00
Employment Status (n=5881), n (%)				< 0.001	0.06			0.24	0.80
Employed	3437 (58)	342 (10)	3078 (90)	1.00	1.00	553 (16)	2879 (84)	1.00	1.00
Unemployed	438 (7)	79 (18)	355 (82)	2.00 (1.52-2.61)	1.02 (0.75-1.37)	84 (19)	353 (81)	1.23 (0.95-1.59)	1.02 (0.77-1.34)
Student	418 (7)	32 (8)	384 (92)	0.75 (0.50-1.08)	0.58 (0.39-0.85)	77 (18)	341 (82)	1.18 (0.89-1.52)	1.13 (0.86-1.48)
Not in workforce	1588 (27)	226 (14)	1353 (86)	1.50 (1.25-1.80)	0.98(0.80-1.80)	276 (17)	1311 (83)	1.10 (0.93-1.28)	0.98 (0.82-1.17)
Household deprivation[‡] (n=6159), n (%)				< 0.001	0.00			0.01	0.52
Dep 1 & 2	1043 (17)	60 (6)	982 (94)	1.00	1.00	151 (14)	891 (85)	1.00	1.00
Dep 3 & 4	1179 (19)	96 (8)	1082 (92)	1.45 (1.04-2.04)	1.32 (0.92-1.90)	185 (16)	992 (84)	1.10 (0.87-1.39)	1.08 (0.84-1.39)
Dep 5 & 6	1085 (18)	91 (8)	986 (91)	1.51 (1.08-2.13)	1.19 (0.82-1.73)	175 (16)	909 (84)	1.14 (0.90-1.44)	1.13 (0.88-1.46)
Dep 7 & 8	1292 (21)	158 (12)	1126 (88)	2.30 (1.69-3.15)	1.36 (0.96-1.94)	221 (17)	1068 (83)	1.22 (0.98-1.53)	1.08 (0.84-1.39)
Dep 9 & 10	1560 (25)	307 (20)	1235 (80)	4.07 (3.07-5.48)	1.90 (1.35-2.69)	302 (19)	1256 (81)	1.42 (1.15-1.76)	1.24 (0.96-1.60)

***** 11 to 14 hours per night

† Adjusted for **parity, age, ethnicity, pregnancy planning, education, and household deprivation**

‡ Adjusted for **parity, age, and ethnicity**

§ ≥5 moderate activity or ≥2 vigorous sessions of activity per week) Women who engaged in moderate activity for at least 30 minutes on at least five out of seven days, or who engaged in vigorous activity for at least 30 minutes at least two out of seven days were classified as engaging in 'regular' activity [59–6]

CI – confidence interval

Table 3. Associations of maternal health characteristics and behaviours with child's sleep duration and night time wakening

Maternal health characteristics		Child's night time sleep duration meets recommendations*				Child wakens two or more times at night			
		Less than 11 hours n = 734	11 to 14 hours n = 5554	Univariate odds ratio (95% CI)	Multiivariate odds ratio (95% CI)	Yes n = 1063	No n = 5255	Univariate odds ratio (95% CI)	Multiivariate odds ratio (95% CI)
Maternal pre-pregnancy BMI in kg/m^2 (n=5589), n (%)				< 0.001	< 0.001			0.03	0.08
Underweight (BMI < 18.5)	219 (4)	27 (12)	191 (88)	1.50 (0.97-2.24)	1.44 (0.90-2.23)	48 (22)	171 (78)	1.46 (1.03-2.02)	1.41 (0.97-2.00)
Normal (18.5 ≤ BMI < 25)	3079 (55)	265 (9)	2806 (91)	1.00	1.00	496 (16)	2580 (84)	1.00	1.00
Overweight (25 ≤ BMI < 30)	1277 (23)	139 (11)	1131 (89)	1.30 (1.05-1.61)	1.24 (0.98-1.56)	207 (16)	1068 (84)	1.01 (0.84-1.20)	0.95 (0.78-1.14)
Obese (BMI ≥30)	1014 (18)	162 (16)	840 (84)	2.04 (1.65-2.52)	1.91 (1.52-2.40)	192 (19)	819 (81)	1.22 (1.01-1.46)	1.17 (0.96-1.42)
Met regular activity guidelines[§] (n=5723), n (%)									
Before pregnancy				0.81	0.37			0.14	0.19
Yes before pregnancy	3304 (58)	377 (12)	2913 (88)	1.00	1.00	534 (16)	2766 (83)	1.00	1.00
No before pregnancy	2419 (42)	280 (12)	2121 (88)	1.02 (0.86-1.20)	1.11 (0.89-1.38)	427 (18)	1991 (82)	1.11 (0.96-1.27)	1.12 (0.94-1.34)
During first trimester				0.24	0.16			0.76	0.57
Yes during first trimester	1792 (31)	219 (12)	1566 (88)	1.00	1.00	297 (17)	1494 (83)	1.00	1.00
No during first trimester	3931 (69)	438 (11)	3468 (89)	0.90 (0.76-1.07)	0.83 (0.65-1.08)	664 (17)	3263 (83)	1.02 (0.88-1.19)	0.94 (0.76-1.16)
During rest of pregnancy				0.88	0.90			0.30	0.50

Yes during rest of pregnancy	1337 (23)	155 (12)	1175 (88)	1.00	1.00	212 (16)	1123 (84)	1.00	1.00
No during rest of pregnancy	4386 (77)	502 (11)	3859 (88)	0.99 (0.82-1.20)	1.07 (0.79-1.32)	749 (17)	3634 (83)	1.09 (0.93-1.29)	1.07 (0.87-1.34)
General Health (n=6149), n (%)				0.00	0.25			0.00	0.12
Poor or Fair	581 (9)	90 (16)	484 (84)	1.47 (1.15-1.87)	1.20 (0.88-1.61)	122 (21)	457 (78)	1.36 (1.10-1.68)	1.22 (0.96-1.53)
Good or Excellent	5568 (91)	620 (11)	4919 (89)	1.00	1.00	911 (16	4650 (84)	1.00	1.00
Long term illness (n=6147), n (%)				0.15	0.06			0.00	0.10
Yes	640 (10)	63 (10)	574 (90)	0.82 (0.62-1.07)	0.74 (0.53-1.00)	134 (21)	505 (79)	1.36 (1.10-1.66)	1.22 (0.96-1.53)
No	5507 (90)	647 (12)	4827 (88)	1.00	1.00	899 (16)	4600 (84)	1.00	1.00
Long term disability (n=6150), n (%)				0.92	0.59			0.01	0.44
Yes	257 (4)	29 (11)	225 (89)	0.98 (0.65-1.43)	0.88 (0.55-1.36)	58 (17)	199 (77)	1.47 (1.08-1.97)	1.14 (0.80-1.60)
No	5889 (96)	681 (12)	5175 (88)	1.00	1.00	974 (17)	4906 (83)	1.00	1.00
Ante-or postnatal depression (n=5723), n (%)				0.01	< 0.001			0.00	0.00
EPDS normal both time points	4625 (83)	484 (10)	4141 (89)	1.00	1.00	739 (16)	3901 (84)	1.00	1.00
EPDS high antenatally	482 (9)	77 (16)	405 (84)	1.63 (1.24-2.10)	1.63 (1.21-2.18)	109 (22)	377 (78)	1.53 (1.21-1.91)	1.58 (1.23-2.01)
EPDS high postnatally	263 (5)	47 (18)	216 (82)	1.86 (1.33-2.56)	1.89 (1.29-2.71)	56 (21)	209 (79)	1.41 (1.03-1.90)	1.42 (1.01-1.96)
EPDS high both ante- and postnatally	169 (3)	30 (18)	139 (82)	1.85 (1.21-2.73)	1.70 (1.01-2.73)	30 (17)	142 (83)	1.11 (0.73-1.64)	0.93 (0.56-1.48)

Maternal smoking (n=5706), n (%)				< 0.001	0.42			0.40	0.95
No smoking (before or during)	4598 (81)	490 (11)	4085 (89)	1.00	1.00	760 (16)	3833 (83)	1.00	1.00
Stopped smoking for pregnancy	550 (10)	68 (12)	479 (88)	1.18 (0.90-1.54)	1.14 (0.82-1.56)	92 (17)	458 (83)	0.95 (0.71-1.26)	0.93 (0.75-1.15)
Continued smoking during pregnancy	558 (10)	94 (17)	458 (83)	1.71 (1.34-2.17)	1.37 (0.85-2.21)	105 (19)	453 (81)	0.97 (0.62-1.50)	1.08 (0.88-1.32)
Alcohol use before pregnancy (n= 6297), n (%)				< 0.001	0.00			< 0.001	< 0.001
No alcohol	1762 (28)	289 (16)	1462 (83)	1.00	1.00	407 (23)	1351 (77)	1.00	1.00
Less than 1 drink per week	1150 (18)	126 (11)	1018 (89)	0.53 (0.45-0.62)	0.79 (0.61-1.00)	182 (16)	967 (84)	0.62 (0.51-0.76)	0.68 (0.55-0.85)
1-3 drinks per week	1527 (24)	124 (8)	1395 (92)	0.37 (0.32-0.42)	0.60 (0.45-0.78)	197 (13)	1328 (87)	0.49 (0.41-0.59)	0.57 (0.46-0.72)
4+ drinks per week	1858 (30)	188 (10)	1656 (90)	0.45 (0.39-0.52)	0.74 (0.56-0.97)	273 (15)	1583 (53)	0.57 (0.48-0.68)	0.64 (0.51-0.81)
Alcohol use during first trimester (n= 6295), n (%)				0.03	0.07			0.01	0.61
No alcohol	4868 (77)	577 (12)	4260 (88)	1.00	1.00	854 (14)	4006 (82)	1.00	1.00
Less than 1 drink per week	579(9)	47 (8)	531 (92)	0.60 (0.43-0.80)	0.90 (0.64-1.26)	76 (13)	502 (87)	0.68 (0.53-0.87)	0.92 (0.70-1.20)
1-3 drinks per week	439 (7)	49 (11)	389 (89)	0.64 (0.38-1.03)	1.36 (0.96-1.89)	58 (13)	381 (87)	0.83 (0.55-1.20)	0.93 (0.68-1.26)
4+ drinks per week	409 (6)	55 (14)	348 (86)	2.10 (1.02-4.00)	1.45 (1.01-2.07)	70 (17)	339 (83)	1.50 (0.75-2.81)	1.18 (0.85-1.60)

Alcohol use during rest of pregnancy (n= 6301), n (%)				0.00	0.32			0.01	0.51
No alcohol	5435 (86)	655 (12)	4745 (88)	1.00	1.00	941 (17)	4486 (83)	1.00	1.00
Less than 1 drink per week	607 (10)	46 (8)	559 (92)	0.57 (0.47-0.68)	0.86 (0.61-1.19)	76 (12)	530 (87)	0.82 (0.69-0.97)	0.88 (0.69-1.15)
1-3 drinks per week	209 (3)	17 (8)	191 (92)	0.60 (0.44-0.81)	0.78 (0.44-1.30)	31 (15)	178 (85)	0.99 (0.75-1.32)	1.11 (0.73-1.66)
4+ drinks per week	50 (1)	11 (22)	38 (77)	2.10 (1.18-3.84)	1.67 (0.75-3.45)	12 (24)	38 (76)	1.35 (0.77-2.41)	1.45 (0.68-2.90)
9 month maternal characteristics									
Cigarettes smoked per day (n=6145), n (%)				< 0.001	0.60			0.10	0.56
0 cigarettes per day	5308 (86)	573 (11)	4709 (89)	1.00	1.00	872 (16)	4429 (83)	1.00	1.00
1 to 4 cigarettes per day	198 (3)	25 (13)	173 (87)	1.18 (0.76-1.79)	0.87 (0.49-1.48)	35 (18)	163 (82)	1.09 (0.74-1.56)	1.29 (0.80-2.04)
5 to 9 cigarettes per day	312 (5)	49 (16)	259 (84)	1.55 (1.12-2.12)	0.89 (0.52-1.52)	53 (17)	257 (82)	1.05 (0.76-1.41)	1.08 (0.65-1.77)
10 to 14 cigarettes per day	189 (3)	41 (22)	145 (78)	2.32 (1.61-3.29)	1.28 (0.70-2.31)	43 (23)	146 (77)	1.50 (1.04-2.10)	1.51 (0.86-2.63)
15 or more cigarettes per day	138 (2)	21 (16)	114 (84)	1.51 (0.92-2.38)	0.83 (0.39-1.71)	30 (22)	108 (78)	1.41 (0.92-2.10)	1.25 (0.64-2.39)
Currently Alcoholic status (n=6149), n(%)				< 0.001	< 0.001			< 0.001	0.09
No alcohol	2327 (38)	375 (16)	1937 (84)	1.00	1.00	480 (21)	1843 (79)	1.00	1.00
Alcohol	3822 (62)	335 (9)	3466 (91)	0.50 (0.42-0.58)	0.60 (0.49-0.74)	1764 (46)	3264 (85)	0.65 (0.57-0.74)	0.86 (0.72-1.02)

Maternal characteristic at 2 years									
Cigarettes smoked per day (n=6295), n (%)				< 0.001	0.74			0.00	0.03
0 cigarettes per day	5456 (87)	589 (11)	4840 (89)	1.00	1.00	905 (17)	4544 (83)	1.00	1.00
1 to 4 cigarettes per day	208 (3)	31 (15)	176 (85)	1.45 (0.96-2.11)	1.27 (0.74-2.10)	36 (17)	172 (83)	1.05 (0.72-1.50)	0.95 (0.59-1.52)
5 to 9 cigarettes per day	307 (5)	46 (15)	255 (85)	1.48 (1.05-2.03)	1.34 (0.80-2.22)	52 (17)	253 (83)	1.03 (0.75-1.39)	0.94 (0.58-1.50)
10 to 14 cigarettes per day	197 (3)	37 (19)	158 (81)	1.92 (1.31-2.75)	1.29 (0.71-2.31)	27 (14)	170 (86)	0.80 (0.52-1.18)	0.66 (0.37-1.18)
15 or more cigarettes per day	127 (2)	25 (20)	99 (80)	2.07 (1.30-3.19)	1.48 (0.74-2.91)	38 (30)	89 (70)	2.14 (1.44-3.13)	1.84 (1.01-3.32)

Table 4. Associations of child characteristics, presence of siblings and current maternal partner with night-time sleeping duration and night time wakening

Child characteristics		Child's night time sleep duration meets recommendations*				Child wakens two or more times at night			
		Less than 11 hours n = 734	11 to 14 hours n = 5554	Univariate odds ratio (95% CI)	Multiivariate odds ratio (95% CI)	Yes n = 1063	No n = 5255	Univariate odds ratio (95% CI)	Multiivariate odds ratio (95% CI)
Current partner (n=6307), n(%)				0.01	0.25			0.00	0.12
Yes	5689 (90)	639 (11)	5019 (89)	1.00	1.00	931 (16)	4750 (84)	1.00	1.00
No	618 (10)	91 (15)	519 (85)	1.38 (1.08-1.74)	1.20 (0.87-1.61)	129 (21)	488 (79)	1.35 (1.09-1.65)	1.23 (0.94-1.59)
Video (n=6147), n(%)				< 0.001	0.01			0.00	0.73
Seldom or never	3896 (63)	347 (9)	3527 (91)	1.00	1.00	606 (16)	3283 (84)	1.00	1.00
Once a week	557 (9)	86 (16)	465 (84)	1.88 (1.45-2.42)	1.45 (1.04-2.00)	94 (17)	462 (83)	1.10 (0.86-1.39)	1.02 (0.75-1.37)
Several times a week	622 (10)	104 (17)	515 (83)	2.05 (1.61-2.59)	1.61(1.19-2.17)	108 (17)	514 (83)	1.14 (0.90-1.42)	1.04 (0.77-1.38)
Once a day	671 (11)	106 (16)	565 (84)	1.91 (1.50-2.40)	1.22 (0.88-1.68)	129 (19)	541 (81)	1.29 (1.04-1.59)	1.15 (0.86-1.53)
Several times a day	401 (6)	65 (16)	331 (84)	2.00 (1.49-2.65)	1.46 (0.98-2.16)	96 (24)	305 (76)	1.70 (1.33-2.17)	1.65 (1.17-2.32)

TV (n=6155), n(%)				< 0.001	< 0.001			0.00	0.39
Seldom or never	3007 (49)	226 (8)	2765 (92)	1.00	1.00	461 (15)	2542 (85)	1.00	1.00
Once a week	540 (9)	56 (10)	481 (90)	1.42 (1.03-1.92)	1.15 (0.77-1.67)	84 (16)	455 (84)	1.02 (0.79-1.30)	1.02 (0.74-1.38)
Several times a week	708 (12)	113 (16)	590 (84)	2.34 (1.83-2.98)	1.82 (1.33-2.48)	121 (17)	585 (83)	1.14 (0.91-1.42)	1.04 (0.78-1.38)
Once a day	1186 (19)	189 (16)	991 (84)	2.33 (1.90-2.87)	1.91 (1.45-2.52)	215 (18)	969 (82)	1.22 (1.02-1.46)	1.22 (0.97-1.57)
Several times a day	703 (11)	124 (18)	573 (82)	2.65 (2.08-3.35)	1.78 (1.25-2.50)	152 (22)	551 (78)	1.52 (1.24-1.86)	1.25 (0.92-1.68)
Breast feeding duration (months) (n= 5902)				0.04	0.98			0.03	0.02
Duration of exclusive breastfeeding in months		3.70 (3.53-3.86)	3.87 (3.81-3.93)	0.96 (0.93-1.00)	1.00 (0.96-1.05)	3.98 (3.85-4.12)	3.83 (3.77-3.88)	1.04 (1.00-1.07)	1.05 (1.01-1.09)
Gestation age in weeks (n=6315), n(%)				0.96	0.59			0.72	0.62
<37	404 (6)	45 (11)	355 (89)	0.96 (0.69-1.30)	0.90 (0.54-1.44)	72 (18)	330 (82)	1.08 (0.82-1.40)	1.00 (0.66-1.48)
37-41	5759 (91)	670 (12)	5056 (88)	1.00	1.00	968 (17)	4784 (83)	1.00	1.00
>41	152 (2)	17 (11)	133 (89)	0.96 (0.56-1.56)	0.72 (0.33-1.36)	23 (15)	129 (85)	0.88 (0.55-1.35)	0.76 (0.42-1.28)

Birth weight (n=6321), n(%)				0.99	0.57			0.22	0.94
1	316 (5)	36 (11)	276 (88)	0.99 (0.68-1.39)	0.93 (0.52-1.60)	64 (20)	250 (80)	1.28 (0.96-1.69)	1.07 (0.67-1.68)
2	4959 (78)	576 (12)	4356 (88)	1.00	1.00	826 (17)	4130 (83)	1.00	1.00
3	1046 (16)	122 (12)	916 (88)	1.01 (0.81-1.24)	0.87 (0.67-1.12)	172 (16)	870 (83)	0.99 (0.82-1.81)	1.02 (0.82-1.26)
Birth weight Z-score (n=6294)				0.04	0.99			0.28	0.99
		1.20 (1.11-1.29)	1.22 (1.19-1.25)	0.94 (0.88-1.00)	1.00 (0.92-1.09)	1.23 (1.15-1.30)	1.21 (1.18-1.25)	0.97 (0.92-1.02)	1.00 (0.93-1.07)
Siblings (n=6252), n(%)				< 0.001	0.00			0.04	0.35
None	2331 (37)	205 (9)	2112 (91)	1.00	1.00	361 (15)	1965 (84)	1.00	1.00
One or more	3921 (63)	514 (13)	3383 (87)	1.56 (1.32-1.86)	1.48 (1.21-1.83)	686 (17)	3231 (82)	1.16 (1.01-1.33)	1.08 (0.92-1.28)

Table 6. Multivariate adjusted model of the association of night time sleeping duration and night time wakening with maternal and child characteristics.

		Multivariable odds ratio (95% CI) for association with meeting recommended duration of night-time sleep	Multivariable odds ratio (95% CI) for association with no night time wakenings
Parity (n=6154), n (%)		---	0.14
First child	2590 (42)		0.88 (0.73-1.05)
Subsequent child	3564 (58)		1.00
Age group (years) (n=6161), n (%)		0.00	0.00
<20	260 (4)	0.59 (0.36-0.94)	1.91 (1.17-3.28)
20 - 29	2321 (38)	1.00	1.00
30 - 39	3309 (54)	0.75 (0.60-0.93)	1.88 (1.13-3.26)
≥40	271 (4)	0.57 (0.32-0.96)	3.43 (1.89-6.45)

Self-prioritized ethnicity (n=6145), n (%)		< 0.001	0.00
European	3495 (57)	1.00	1.00
Maori	815 (13)	2.61 (1.96-3.47)	1.32 (1.02-1.71)
Pacific	787 (13)	2.66 (1.95-3.62)	1.05 (0.78-1.39)
Asian	836 (14)	1.36 (0.96-1.91)	1.71 (1.31-2.22)
Other[†]	212 (3)	1.65 (0.96-2.68)	1.33 (0.87-1.98)
Pregnancy planning (n=6133), n (%)		0.01	---
Planned	3806 (62)	1.00	
Unplanned	2327 (38)	0.76 (0.62-0.94)	
Education (n=6143), n (%)		0.16	---
No secondary education	394 (6)	1.37 (0.95-1.95)	
Secondary education	1400 (23)	1.15 (0.92-1.45)	
Tertiary education	4349 (71)	1.00	

Employment Status (n=5881), n (%)		0.02	---
Employed	3437 (58)	1.00	
Unemployed	438 (7)	1.12 (0.79-1.54)	
Student	418 (7)	0.50 (0.30-0.77)	
Not in workforce	1588 (27)	0.98 (0.78-1.23)	
Household deprivation[ǂ] (n=6159), n (%)		0.00	---
Dep 1 & 2	1043 (17)	1.00	
Dep 3 & 4	1179 (19)	1.25 (0.84-1.86)	
Dep 5 & 6	1085 (18)	1.30 (0.88-1.95)	
Dep 7 & 8	1292 (21)	1.43 (0.98-2.10)	
Dep 9 & 10	1560 (25)	1.90 (1.32-2.79)	

Long term illness (n=6147), n (%)		---	0.09
Yes	640 (10)		1.24 (0.96-1.59)
No	5507 (90)		1.00
Ante-or postnatal depression (n=5723), n (%)		---	0.13
EPDS normal both time points	4625 (83)		1.00
EPDS high antenatally	482 (9)		1.28 (0.97-1.67)
EPDS high postnatally	263 (5)		1.37 (0.95-1.93)
EPDS high both ante- and postnatally	169 (3)		1.02 (0.60-1.64)
Alcohol use before pregnancy (n= 6297), n (%)		---	< 0.001
No alcohol	1762 (28)		1.00
Less than 1 drink per week	1150 (18)		0.64 (0.50-0.82)
1-3 drinks per week	1527 (24)		0.58 (0.46-0.74)
4+ drinks per week	1858 (30)		0.74 (0.59-0.93)

9 months alcoholic status (n=6160), n(%)		0.01	---
No alcohol	2332 (38)	1.00	
Alcohol	3828 (62)	0.75 (0.61-0.93)	
Cigarettes smoked per day at 2 years (n=6295), n (%)		---	0.12
0 cigarettes per day	5456 (87)		1.00
1 to 4 cigarettes per day	208 (3)		1.19 (0.75-1.84)
5 to 9 cigarettes per day	307 (5)		1.28 (0.85-1.90)
10 to 14 cigarettes per day	197 (3)		0.96 (0.57-1.57)
15 or more cigarettes per day	127 (2)		1.94 (1.12-3.27)
Breast feeding duration (months) (n= 5902)		---	0.01
Duration of exclusive breastfeeding in months			1.05 (1.01-1.09)
Current partner (n=6307), n(%)		---	0.03
Yes	5689 (90)		1.00
No	618 (10)		1.36 (1.02-1.80)
TV (n=6155), n(%)		0.01	---
Seldom or never	3007 (49)	1.00	
Once a week	540 (9)	0.80 (0.53-1.17)	
Several times a week	708 (12)	1.49 (1.11-1.99)	

Once a day	1186 (19)	1.27 (0.97-1.65)	
Several times a day	703 (11)	1.24 (0.90-1.69)	
Siblings (n=6252), n(%)		0.04	---
None	2331 (37)	1.00	
One or more	3921 (63)	1.27 (1.01-1.60)	
Servings per day of food groups			
Servings of vegetables/day, (n=6240)		0.73	0.03
		1.02 (0.93-1.11)	0.92 (0.85-0.99)
Servings of fruit/day, (n=6222)		0.40	0.92
		1.02 (0.97-1.08)	1.00 (0.95-1.06)
Servings of milk cheese and yoghurt/day, (n=6233)		0.23	< 0.001
		1.04 (0.98-1.10)	1.15 (1.09-1.21)
Servings of bread, rice, pasta and cereals/day, (n=6218)		0.22	0.14
		0.97 (0.92-1.02)	0.96 (0.92-1.01)

Servings of spreads/day, (n=6234)	0.01	0.19
	0.90 (0.83-0.97)	1.04 (0.98-1.11)
Servings of meats, meat alternatives and eggs/day, (n=5768)	0.72	0.72
	0.98 (0.87-1.09)	0.98 (0.89-1.08)
Servings of soft drinks and snacks/day, (n=6241)	0.03	0.22
	1.14 (1.01-1.28)	1.07 (0.96-1.19)

References

1. Shochat T, Cohen-Zion M, Tzischinsky O. Functional consequences of inadequate sleep in adolescents: a systematic review. *Sleep Med Rev*. 2014;18(1):75-87. doi:10.1016/j.smrv.2013.03.005.
2. Chaput J-P, Gray CE, Poitras VJ, et al. Systematic review of the relationships between sleep duration and health indicators in school-aged children and youth. *Appl Physiol Nutr Metab*. 2016;41(6 (Suppl. 3)):S266-S282. doi:10.1139/apnm-2015-0627.
3. Iglowstein I, Jenni OG, Molinari L, Largo RH. Sleep duration from infancy to adolescence: reference values and generational trends. *Pediatrics*. 2003;111(2):302-307. doi:10.1542/peds.111.2.302.
4. de Ruiter I, Olmedo-Requena R, Sánchez-Cruz JJ, Jiménez-Moleón JJ. Changes in sleep duration in Spanish children aged 2–14 years from 1987 to 2011. *Sleep Med*. 2016;21:145-150. doi:10.1016/j.sleep.2015.12.021.
5. Matricciani L, Olds T, Petkov J. In search of lost sleep: Secular trends in the sleep time of school-aged children and adolescents. *Sleep Med Rev*. 2012;16(3):203-211. doi:10.1016/j.smrv.2011.03.005.
6. Jenni OG, O'Connor BB. Children's Sleep: An Interplay Between Culture and Biology. *Pediatrics*. 2011;115(Supplement 1).
7. Hirshkowitz M, Whiton K, Albert SM, et al. National sleep foundation's sleep time duration recommendations: Methodology and results summary. *Sleep Heal*. 2015;1(1):40-43. doi:10.1016/j.sleh.2014.12.010.
8. Gruber R, Carrey N, Weiss SK, et al. Position statement on pediatric sleep for psychiatrists. *J Can Acad Child Adolesc Psychiatry*. 2014;23(3):174-195.
9. Blunden SL, Milte CM, Sinn N. Diet and sleep in children with attention deficit hyperactivity disorder: preliminary data in Australian children. *J Child Health Care*. 2011;15(1):14-24. doi:10.1177/1367493510385020.
10. Hogenkamp PS, Nilsson E, Nilsson VC, et al. Acute sleep deprivation increases portion size and affects food choice in young men. *Psychoneuroendocrinology*. 2013;38(9):1668-1674. doi:10.1016/j.psyneuen.2013.01.012.
11. Beebe DW; Simon S; Summer S; Hemmer S; Strotman D; Dolan LM. Dietary Intake Following Experimentally Restricted Sleep in Adolescents. *Sleep*. 2013;36(6):827-834.
12. Afaghi A, O'Connor H, Chow CM. High-glycemic-index carbohydrate meals shorten sleep onset. *Am J Clin Nutr*. 2007;85(2):426-430.
13. Grandner M a, Jackson N, Gerstner JR, Knutson KL. Dietary nutrients associated with short and long sleep duration. Data from a nationally representative sample. *Appetiteiosher9.alumnes*. 2013;64:71-80. doi:10.1016/j.appet.2013.01.004.
14. Kim S, DeRoo LA, Sandler DP. Eating patterns and nutritional characteristics associated with sleep duration. *Public Health Nutr*. 2011;14(5):889-895. doi:10.1017/S136898001000296X.
15. Peuhkuri K, Sihvola N, Korpela R. Diet promotes sleep duration and quality. *Nutr Res*. 2012;32(5):309-319. doi:10.1016/j.nutres.2012.03.009.
16. Franckle RL, Falbe J, Gortmaker S, et al. Insufficient sleep among elementary and middle school students is linked with elevated soda consumption and other unhealthy dietary behaviors. *Prev Med (Baltim)*. 2015;74:36-41. doi:10.1016/j.ypmed.2015.02.007.
17. Sánchez-Ortuño MM, Bélanger L, Ivers H, LeBlanc M, Morin CM. The use of natural products for sleep: A common practice? *Sleep Med*. 2009;10(9):982-987.

doi:10.1016/j.sleep.2008.10.009.
18. Khan MKA, Faught EL, Chu YL, Ekwaru JP, Storey KE, Veugelers PJ. Is it nutrients, food items, diet quality or eating behaviours that are responsible for the association of children's diet with sleep? *J Sleep Res*. October 2016. doi:10.1111/jsr.12466.
19. Ramamurthy MB, Sekartini R, Ruangdaraganon N, et al. Effect of current breastfeeding on sleep patterns in infants from Asia-Pacific regionj pc_2453 669..674. doi:10.1111/j.1440-1754.2012.02453.x.
20. Mindell JA, Du Mond C, Tanenbaum JB, Gunn E. Long-Term Relationship Between Breastfeeding and Sleep. *Child Heal Care*. 2012;41(3):190-203. doi:10.1080/02739615.2012.685038.
21. Kohyama J, Mindell J a., Sadeh A. Sleep characteristics of young children in Japan: Internet study and comparison with other Asian countries. *Pediatr Int*. 2011;53(5):649-655. doi:10.1111/j.1442-200X.2010.03318.x.
22. World Health Organization. *The Optimal Duration of Exclusive Breastfeeding*. Geneva, Switzerland: World Health Organization; 2001.
23. Galbally M, Lewis AJ, Mcegan K, Scalzo K, Islam FA. Breastfeeding and infant sleep patterns: an Australian population study. *J Pediatr Child Heal*. 2013:147-152. doi:10.1111/jpc.12089.
24. Montgomery-Downs HE, Clawges HM, Santy EE. Infant feeding methods and maternal sleep and daytime functioning. *Pediatrics*. 2010;126(6):e1562-8. doi:10.1542/peds.2010-1269.
25. Brown A, Harries V. Infant Sleep and Night Feeding Patterns During Later Infancy: Association with Breastfeeding Frequency, Daytime Complementary Food Intake, and Infant Weight. doi:10.1089/bfm.2014.0153.
26. Morton SMB, Atatoa Carr PE, Grant CC, et al. Cohort profile: growing up in New Zealand. *Int J Epidemiol*. 2013;42(1):65-75. doi:10.1093/ije/dyr206.
27. Morton SMB, Ramke J, Kinloch J, et al. Growing Up in New Zealand cohort alignment with all New Zealand births. *Aust N Z J Public Health*. 2015;39(1):82-87. doi:10.1111/1753-6405.12220.
28. Sadeh A. A Brief Screening Questionnaire for Infant Sleep Problems: Validation and Findings for an Internet Sample. *Pediatrics*. 2004;113(6):e570-e577. doi:10.1542/peds.113.6.e570.
29. Sleep Health Foundation. Sleep Needs Across the Lifespan. 2015.
30. World Health Organization. *Indicators for Assessing Breast Feeding Practices WHO/CDD/SER/91.14*. Geneva: World Health Organization; 1991.
31. WHO. *WHO Anthro for Personal Computers, Version 3.2.2, 2007: Software for Assessing Growth and Development of the World's Children*. Geneva; 2007.
32. WHO. *Physical Status: The Use and Interpretation of Anthropometry. WHO Technical Report Series: 854*. Geneva, Switzerland; 2007.
33. World Health Organization. *Physical Status:the Use and Interpretation of Anthropometry*. Geneva; 1995.
34. Statistics New Zealand. *2006 Census Questionnaires*. Christchurch: Information Centre; 2008.
35. Statistics New Zealand. *General Social Survey*.; 2008.
36. Salmond C, Crampton P, Atkinson J. *NZDep2006 Index of Deprivation*. Wellington; 2007.
37. Brunner Huber LR. Validity of Self-reported Height and Weight in Women of Reproductive Age. *Matern Child Health J*. 2007;11(2):137-144. doi:10.1007/s10995-006-0157-0.

38. Victora CG, Huttly SR, Fuchs SC, Olinto MT. The role of conceptual frameworks in epidemiological analysis: a hierarchical approach. *Int J Epidemiol*. 1997;26(1):224-227.
39. Ministry of Health. Food and Nutrition Guidelines for Healthy Children and Young People (Aged 2–18 years): A background paper. 2012.

B4. Article

The role of diet and physical activity in children and adolescents with ADHD

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Abstract. ADHD (Attention Deficit and Hyperactive Disorder) is the most common neurobehavioral disorder of childhood, presenting with pervasive and impairing symptoms of inattention, hyperactivity, impulsivity, or a combination. There is scientific evidence that some dietary and physical activity strategies may be useful to improve the symptoms of ADHD and benefit the social, cognitive and academic performance of children and adolescents with ADHD. The purpose of our study was to review the scientific literature on the role of diet and physical activity in ADHD symptomatology up to date.

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4. The role of diet and physical activity in children and adolescents with ADHD

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Abstract. ADHD (Attention Deficit and Hyperactive Disorder) is the most common neurobehavioral disorder of childhood, presenting with pervasive and impairing symptoms of inattention, hyperactivity, impulsivity, or a combination. There is scientific evidence that some dietary and physical activity strategies may be useful to improve the symptoms of ADHD and benefit the social, cognitive and academic performance of children and adolescents with ADHD. The purpose of our study was to review the scientific literature on the role of diet and physical activity in ADHD symptomatology up to date.

Introduction

Attention deficit and hyperactivity disorder (ADHD) is one of the most common psychiatric disorders in early childhood and adolescence with a prevalence rate exceeding 5% [1]. Some of the most common symptoms

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associated with ADHD are hyperactivity, attention deficit, cognitive deficit and poor impulse control [2]. The etiology of ADHD is still unknown, although there are several factors which may have a certain influence in the symptomatology, including diet and physical activity [2].

The research aimed to study the association between diet and ADHD has been growing in the last decades. Thus, children and adolescents with ADHD seem to have lower levels of certain nutrients such as: iron [3], zinc [4,5], and omega 3 [6,7], among others. In some cases, the supplementation with these nutrients, especially with omega 3 [6], has showed to improve the ADHD symptomatology. The most significant research done in this field is summarized in Table 1.

Table 1. Relevant scientific evidence of deficiency or supplementation in essential nutrients observed in ADHD children and adolescents.

Study	Nutrients	Study design	Main Findings
Colter *et al.*[8]	Omega 3-Omega 6	Case-control	Children with ADHD have lower omega 3 and 6 plasma levels than healthy children.
Transler *et al.* [9]		Placebo-controlled studies	A daily supplementation (for 4 months) with a mixture of omega-3 and 6 decreased frequency and severity of symptoms.
Bloch and Qawasmi [6]		Systematic review and meta-analysis	Supplementation was statistically significant beneficial for children with ADHD. EPA's effect was bigger than DHA' effect.
Gillies *et al.* [7]		Systematic review	A combination of omega-3 and -6 is not statistically significant in the treatment of ADHD.
Widenhorn-Müller *et al.* [10]		Randomized placebo-controlled intervention trial	Supplementation improves working memory function in ADHD children.
Konofal *et al.* [11]	Iron	Case- control	Serum ferritin levels were lower in children with ADHD than controls. Lower ferritin levels were correlated with more severe ADHD symptoms.
Konofal *et al.* [3]		Double-blind, placebo-controlled, randomized trial	Supplementation (80 mg/day) appeared to improve ADHD symptoms in children with low serum ferritin levels.
Oner *et al.* [12]		Cross-sectional	Hyperactivity was significantly associated with ferritin levels but not with cognitive measures.
Donfrancesco *et al.* [13]		Case-control	No significant relationship between serum ferritin levels and ADHD.
Bilici *et al.* [4]	Zinc	Placebo-controlled double-blind study	Supplementation was significantly better to placebo in reducing symptoms of hyperactivity, impulsivity and impaired socialization in patients with ADHD.
Akhondzadeh *et al.* [5]		Double blind and randomized trial	Supplementation might be beneficial in the treatment of children with ADHD
Arnold et al.[14]		Placebo-controlled double-blind	Zinc supplementation alone (8 weeks) did not improve inattention, but when combined with pharmacological treatment, the optimal dose of the drug was reduced by 37%.
Kozielec and Starobrat-Hermelin [15]	Magnesium	Case-control	95% of ADHD children showed deficiency in magnesium comparing to controls. After 6 months of Mg supplementation (200 mg/day) hyperactivity was reduced.
Mousain-Bosc et al.[16]		Placebo-controlled double-blind	Mg supplementation (100 mg/day) combined with vitamin B6 for 6 months improved symptomatology
Huss et al.[17]		Observational study	Hyperactivity and inattention of most of patients was reduced after supplementation for 12 weeks with a combination of omega-3 and 6, magnesium and zinc.

Other studies suggest that diets rich in sugars and artificial colorings increase the hyperactivity of children [18–22]; however, those findings are still inconsistent and more data are needed. Research about the associations of different dietary patterns and ADHD has also been conducted [22–25]. This approach is really interesting since it assesses the influence of the whole diet.

Besides the potential benefits that a healthy dietary pattern may have on neurocognitive, behavioral and physical growth, it has also been suggested that physical activity might have a positive impact in behavior, neurocognitive function, motor skills, and school performance of children and adolescents with ADHD [26–29], as it will be discussed further.

Although the pathophysiology of ADHD has been not fully demonstrated, there is an important hypothesized mechanism of deregulated dopamine in the prefrontal cortex (PFC). The PFC has the function to regulate behavior, inhibit inappropriate emotions, impulses and habits [30]. Several studies indicate that patients with ADHD present anatomic abnormalities or neurochemical brain dysfunction [31]. Stimulant medications (such as methylphenidate and amphetamines) are used to treat the majority of the symptoms of children with ADHD, but not all the patients have a good response to them and some parents have the concern about the side effects of these drugs in the growth, nervous and cardiovascular system of their children [32]. There is an important need to develop other interventions that do not have repercussion in the health and wellness of the children. Thus, it has been proposed that the combination of diet and physical activity could help children and adolescents with ADHD to improve the symptomatology and their whole quality of life. The aim of this chapter is to review the scientific literature regarding the possible benefits of different dietary approaches and physical activity for symptom management for children and adolescents with ADHD.

1. Role of the whole diet on ADHD: healthy patterns *versus* restrictive elimination diets?

1.1. Dietary patterns and ADHD

As mentioned above, several studies have analyzed the beneficial or detrimental effects of specific single nutrients on ADHD symptomology [33]. Moreover, associations between dietary patterns and ADHD have been recently examined in several cross-sectional studies (Table 2). This new approach is of great interest since nutrients are nearly always consumed together, and they are highly interrelated in the food matrix. Therefore, the

study of dietary patterns is really useful for understanding much better the role of diet in ADHD. Assessing the whole diet instead of the effects of a single nutrient on the relation between diet and ADHD may contribute even more to understand this complex relationship.

The majority of studies on diet and ADHD conclude that ADHD patients have a tendency to have a poor quality diet, which could cause certain nutrient deficiencies. Those deficiencies might affect the neurocognitive, behavioral and physical development at this important stage of life. Indeed, Park *et al.* [35] found that higher intakes of sweetened desserts, fried food, and salt were associated with more learning, attention, and behavioral problems. On the other side, a balanced diet, regular meals, and a high intake of dairy products and vegetables were associated with less learning, attention, and behavioral problems.

Table 2. Summary of the main studies on the influence of the diet on children and adolescents with attention-deficit and hyperactive disorder (ADHD).

Reference	Design	N; age	Country	Main Findings
Howard *et al.* [24]	Cross-sectional study	115; 14y follow-up	Australia	A Western-style diet[a] may be associated with ADHD.
Azadbakht & Esmaillzadeh [23]	Cross-sectional study	375; 6-11y	Iran	Significant independent associations between the sweet[b] and fast food[c] dietary patterns and the prevalence of ADHD.
van Egmond-Fröhlich *et al.* [34]	Cross-sectional study	9,428; 6-17y	Germany	Poor nutrition quality and high-energy intake appear to be independently associated with ADHD symptoms.
Park *et al.* [35]	Cross-sectional study	986; 8-11y	Korea	High intake of sweetened desserts, fried food, and salt is associated with more learning, attention, and behavioral problems, whereas a balanced diet, regular meals, high intake of dairy products and vegetables is associated with fewer problems.
Woo *et al.* [25]	Case-Control study	192; 7-12y	Korea	The traditional-healthy Korean[d] dietary pattern was associated with lower odds having ADHD
Liu *et al.* [36]	Cross-sectional study	417; 6-11y	China	Positive correlation between diet intake (processed meat, salty snacks) and hyperactivity index. Children's diet pattern is an important environmental impact factor for ADHD.
Ghanizadeh and Haddad [37]	Randomized controlled clinical trial	106; 5-14y	Iran	Encouraging the children with ADHD to increase their intake of recommended diet markedly improves their attention.

[a]High in total fat, saturated fat, refined sugar, and sodium; [b]High in ice cream, refined grains sweet desserts, sugar, and soft drinks; [c]High in processed meat, commercially produced fruit juices, pizza, snacks, sauces and soft drinks; [d]High intake of kimchi, grains, and bonefish, and low intake of fast-food and beverages.

The “unhealthy” dietary patterns identified in the different studies (such as “Western”, “fast food” or “sweet” patterns) were generally high in total fat, saturated fat, refined sugars, and sodium. The relationship observed between higher scores for the “unhealthy” dietary pattern and an increased odds for ADHD supports the hypothesis that highly processed and energy-dense foods are linked with ADHD symptomatology [24,34,35].

Howard *et al.* [24] suggested that children eating a “Western” diet, high in fried food, sweetened desserts and unbalanced, are also likely to have micronutrient and/or PUFA deficiencies. Iron, zinc or magnesium deficiencies and lower circulating levels of omega-3, higher levels of omega-6, and a lower omega-3 *versus* omega-6 ratio has been reported in children and adolescents with ADHD [8]. An inadequate micronutrient intake, coming from an unbalanced dietary pattern, could result in suboptimal brain function in children and adolescents [23]. Futhermore, Van Egmond-Fröhlich *et al.* [34] pointed out that ADHD symptoms might be associated with poor food selection rather than overeating in terms of volume.

“Unhealthy” or “junk foods” besides being usually high in fat and sugars may be rich also in artificial food colorings and preservatives, which could negatively affect ADHD symptoms [38]. It has been suggested, as it will be discussed below, that certain food additives may lead to hyperactivity or changes in neurotransmitter function [21]. An interesting point is that the relationship observed between poor dietary choices and ADHD may be bidirectional [24]. The results observed could be explained, especially for adolescents, by the tendency of them to experience emotional distress to crave fat-rich snack foods as a self-soothing strategy. Therefore, the results found could be more reflective of adolescent dietary preferences and cravings rather than nutritional factors alone. Also, it has been observed that a healthy diet is related to better family functioning [39] and given that families of children and adolescents with ADHD are more likely to face parenting challenges, it is possible that the relationship between a “unhealthy” dietary pattern and ADHD diagnosis is mediated by poor family functioning [24].

Despite the fact that conclusions of these studies are challenging, we cannot justify that a poor dietary choice is the responsible for ADHD. The idea that dietary factors are the exclusive and sufficient explanation for childhood behavioral problems may place a barrier in the way of access to appropriate evidence-based assessment and treatment – so placing the child at unnecessary risk [40]. Further studies are necessary to understand the role that the dietary pattern has in this disorder and to know which dietary approaches can benefit the ADHD symptomatology.

1.2. Restrictive dietary treatments for ADHD

There are mainly two dietary treatments for ADHD, which have been tested in repeated, randomized controlled trials: the artificial food colors elimination (AFCE) and the restricted elimination diets (RED).

1.2.1. Artificial food colorants elimination (AFCE)

The research within artificial food colorants and other additives began in the 1970s. Dr. Benjamin Feingold proposed a new diet called the "Kaiser Permanente diet" also known as the "K-P diet" or the Feingold diet. It was hypothesized that the hyperactivity and learning problems observed in certain schoolchildren were due to the ingestion of certain foods and food additives [41].

The K-P diet removed all foods containing artificial food colors and flavorings and certain preservatives and also food which naturally contain salicylates (Table 3). It was very popular during the 70s and 80s, although it received repeated criticism because solid scientific studies demonstrating its efficacy were very scarce [41] and subsequently support from professionals waned. The "K-P diet" is not longer used, but some of the recommendations, including the elimination of artificial colors, are still being applied. Indeed, two recent meta-analyses carried out concluded that artificial food colorants have small, but statistically significant adverse effect on ADHD symptoms in some children [38,42], even though the conclusions were based on studies of limited quality, as the authors themselves pointed out.

Table 3. Dietary guidelines of the "Kaiser Permanente diet".

- To avoid all food, medications, and cosmetic which may contain artificial colors and flavors.
- To avoid all food that may contain preservatives such BHA, BHT, TBHQ and sodium benzoate[a]
- To avoid foods that naturally contain salicylates: almonds, apples, peaches, apricots, nectarines, cherries, grapes, raisins, oranges, plums, tomatoes, cucumber, coffee and tea.

[a] Those preservatives were later added to the list. Abbreviations: BHA, buttylated hydroxyanisole; BHT, butylated hydroxytoluene; TBHQ, tertiary butylhydroquinone.

In the same direction, Stevenson *et al.* [43] concluded that the artificial food color elimination is a potentially valuable treatment for ADHD but its effect size remains uncertain, as does the type of child for whom it is likely to be efficacious. The authors added the urgent need for studies using more redefined methodologies with blind evaluation to unselected samples of children with ADHD and also the concern that some studies of food colorings and additives were undertaken some time ago, so the findings could be no clear as diet and food products have changed markedly.

The possible mechanisms by which the food colorants and other additives may trigger symptoms are not well understood [44]. Therefore, the controversy about the hypothesis that certain food colorants and additives mainly may cause hyperactivity and inattention in children both ADHD diagnosed or without this disorder is still open. Some authors strongly affirm that these additives do not cause ADHD [2,45], relaying in the fact that the symptomatology of ADHD is different from those induced by artificial coloring [21,44]. The last ones have been associated with more irritability and insomnia than restlessness and inattention.

In 2007, a study funded by the Food Safety Agency (FSA) from UK and conducted by McCann *et al.* [21] had a high impact on the public opinion. The authors provided statistically evidence on the relationship between the consumption of certain mixtures of artificial food colorings (tartrazine, quinoline yellow, sunset yellow, azorubine, cochineal red and allura red) and an artificial preservative (sodium benzoate) and the increase of the hyperactivity in children of 3 years and also in children from 8 to 9 years. In view of these results, the FSA recommended to parents with hyperactive children to consider limiting the intake of these colorants and preservatives. The study, however, has certain methodology weaknesses, as the authors themselves recognized in their publication. The changes observed in the hyperactivity children were very small relative to the inter-individual variation, while the changes in behavior were not evident in all the studied children. Furthermore, it was not possible to extrapolate the study findings to each single additive, which was in the mixture assayed. Moreover, information about the possible biological mechanisms was not provided.

While neither the EFSA (European Food Safety Agency) nor the European Commission have issued any cautious recommendation, nowadays, in the European Union, is required on the food packaging the following warning *"This product may have adverse effect on activity and attention in children"* when sunset yellow (E110), quinoline yellow (E104), azorubine/carmoisine (E122), allura red AC (E129), tartrazine (E102) and cochineal natural red (E124) are employed in foods and beverages.

There is a consensus in the scientific community about the need for more studies on the association between artificial colorings and hyperactivity and ADHD. It is required some caution before advising a complete restriction of foods containing these colorings. The imposition of a diet completely free of artificial colorings should not be done until a reliable methodology is developed to identify which colorant or colorants may be responsible, and who is really sensitive to these compounds, given the inter-individual variation observed.

1.2.2. Restricted elimination diets (RED) or few foods diets

A restricted elimination diet (also called oligoantigenic) removes most foods that may have antigenic or allergenic potential, such as milk and dairy products, eggs, nuts and some fruits, among others. It is thought that ADHD may be, in some children, a hypersensitivity reaction to certain foods [41,45,46]. Therefore, according to this allergic hypothesis, there would be foods that induce high levels of IgG, leading to a relapse in ADHD child behavior, while the intake of those that does not induce IgG or very low levels of them, would not cause a recurrence in ADHD symptoms [32,47]. While interesting the hypothesis, it has not yet been fully demonstrated. Pelsser *et al.* [45] carried out a study about restricted elimination diets with uncertain results. They did conclude that the children who responded to the dietary intervention, independently of whether IgG levels were high or low, showed a decrease of 20.8 points on the ADHD rating scale (ADHD Rating Scale) and 11.6 points on the Conners Scale (Conner's Score). However, the determination of IgG levels was not useful, since the levels of IgG and symptoms of ADHD were totally independent.

More recently a meta-analysis on ADHD, restriction diet and food color additives has been published [42], concluding that a restriction diet benefits some children with ADHD since it reduces ADHD symptoms; however, the authors themselves strongly recommended a renewed investigation of diet and ADHD. From a practical point of view, the restricted elimination diets are very difficult to follow, both for ADHD patients and for the families. Moreover, children and adolescents who are prescribed to follow a different diet than their friends may influence in their behavior, creating unnecessary stress situations [19,20].

To summarize, restricted elimination diets may be beneficial, but large-scale studies are needed, using blind assessment, and including assessment of long-term outcome. On the other hand, artificial food color elimination is a potentially valuable treatment but its effect size remains uncertain, as does the type of child for whom it is likely to be efficacious. Three recommendations have been suggested for the design of future studies: 1) To

have a sample of children with ADHD who have not been selected on the basis of previous responses to food constituents, 2) To include observations of the children´s behavior by a reporter who is truly blind as to dietary treatment, and 3) to control for nonspecific treatment effects [38,43].

2. The role of physical activity on ADHD

It is well established that physical activity (PA) has positive effects on mental health in both clinical and nonclinical populations. In the last decade, several studies have been addressed to study the potential benefits of exercise in children diagnosed with ADHD. The evidence suggests that physical exercise may have benefits in behavioral, neurocognitive, and scholastic performance [27,47,48] and in inhibitory control [47,49]. The etiology of ADHD and the putative mechanisms by which PA impacts cognitive performance suggest that PA might be particularly beneficial for ADHD individuals [27].

Pontifex *et al.* [48] concluded that moderately intense aerobic exercise might have positive implications for aspects of neurocognitive function and inhibitory control in children with ADHD, improving their school performance (Fig. 1). The children could better focus and were less distracted after a quick workout. Moreover, it seems that this type of exercise produces enhancements in reading and in mathematics [49]. This is interesting because children with ADHD have usually more learning problems in these two areas. On the other hand, moderate exercise sessions in ADHD's children have led to improvements in behavior and attention, but no relationship with academic performance has been found [50].

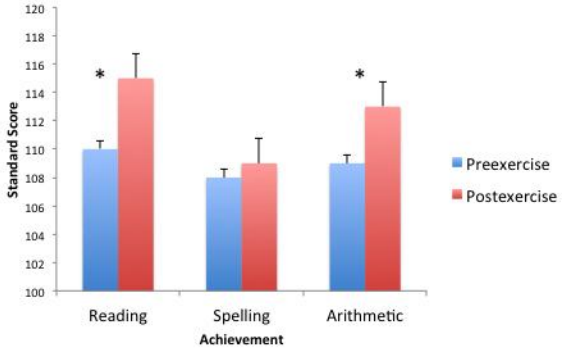

Figure 1. Mean (+SE) standard score for each session on each of the three academic performance tests done. Bars with * are statistically different (adapted from [48]).

Other authors have investigated if the beneficial effects of short moderate PA are also helpful when exercise is carried out for longer periods of time. Thus, Verret *et al.* [50] demonstrated that, in addition to producing improvements in strength and motor skills, exercise showed a positive influence on the behavior and attention of ADHD's children. Also, the work done by Smith *et al.* [47] suggested that after 26 min of continuous moderate-to-vigorous physical activity daily over eight school weeks offered benefits to motor, cognitive, social, and behavioral functioning in young people exhibiting ADHD symptoms. Both authors [47,48] pointed out that the benefits of PA would act on the inhibitory control and the executive function. ADHD appears to have a strong impact on executive function, where processes related to learning and behavior are altered. Although it is not clear which elements are regulated by the executive function, it is believed to be related to cognitive processes such as memory, emotional control, activation, arousal, effort, organization and planning tasks [51].

On the other hand, the majority of exercise and cognition research has primarily focused on aerobic exercise but it is also important to consider forms of coordinative exercise, which includes exercises involving motor coordination and cognitive training. Yu-Kai Chang *et al.* [52] demonstrated that an aquatic exercise intervention, which involves both aerobic and coordinative exercises, influences positively on the restraint inhibition component of behavioral inhibition in children with ADHD.

Research aimed to investigate the influence of PA in children with ADHD under drug therapy, such as methylphenidate, has also been carried out [53], demonstrating that the PA had a positive impact in ADHD symptomatology when medication was present, too. It was found that besides improving strength and motor skills, PA positively influences behavior and cognitive function such as attention in children under medication. Although there is limited research about how drugs affect the motor skills and physical lifestyle of children with ADHD, most of the studies agree that motor skills are improved as a result of PA [50]. In order to add support to those outcomes, future research should include greater executive functions assessment. Moreover, follow-up and additive effects of others therapies should be explored.

Children with ADHD might have lower participation in sport activities, because of their mood liability, disciplinary problems, poor self-esteem, anxiety and inattention. However, research evidence has showed that ADHD children who participated in three or more sports present fewer anxiety or depression symptoms than did those who participated in fewer than three sports. Another aspect worth to comment is the positive influence that PA has showed also in aerobic function, flexibility and cardiovascular fitness,

since children with ADHD have lower levels of them compared with typically developed children. Thus, when compared only between ADHD children the ADHD training group demonstrated more favorable levels of aerobic function and flexibility than the ADHD no training group after the PA intervention [54].

All these findings related to PA and improvement in ADHD symptomatology would support the hypothesis that the pathophysiology of ADHD is related with inadequate levels of certain neurotransmitters [55] such as serotonin, dopamine, and noradrenaline. PA increased the levels of these three neurotransmitters in the prefrontal cortex, which seems to be crucial for the attention and inhibitory control [51]. Individuals with ADHD might have neurochemical and neuroanatomical anomalies in this brain region, which could lead to neurotransmitter deficits and originating some of the cognitive problems related to the ADHD.

As a summary, the research done up to now seems to support the fact that physical exercise could establish itself as an adjunct treatment for people with ADHD [28,47,48,50]. It is recommended that clinicians, parents and teachers work together monitoring the participation of these children in physical and sports activities to help them improve their motor skills performance (Fig. 2).

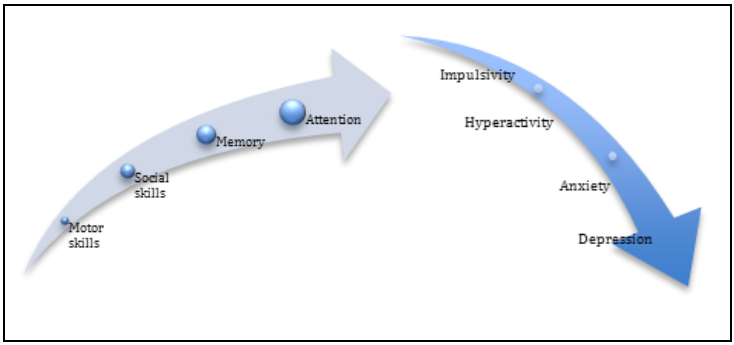

Figure 2. Effects of exercise on ADHD children behavior.

3. Conclusions

The cause of ADHD is multifactorial, with a certain influence of the environmental factors, such as diet and physical activity. The multimodal treatment is recommended by principal clinical practice guidelines for ADHD and includes psychological, psycoeducational and pharmacologic

treatment. Nevertheless, the review of the published scientific evidence indicated that several dietary strategies and physical activity might also help to improve the quality of life of children and adolescents with ADHD. The main recommendation is to educate families and children: 1) to have healthy eating habits with a balanced diet, avoiding excessive consumption of saturated fats and simple sugars, foods with artificial colorants, a good consumption of fish, nuts and seeds, all of them rich in omega 3 and 2) to introduce into the routine of children and adolescents daily physical activity, adapted to their preferences and needs.

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References

1. Cortese, S., Angriman, M., Lecendreux, M. 2012. *Expert Rev. Neurother.*, 12, 1227.
2. Biederman, J., Faraone, S.V. 2005. *Lancet*, 366, 237.
3. Konofal, E., Lecendreux, M., Deron, J., Marchand, M., Cortese, S., Zaïm, M., Mouren, M. C., Arnulf, I. 2008. *Pediatr. Neurol.*, 38, 20.
4. Bilici, M., Yildirim, F., Kandil, S., Bekaroğlu, M., Yildirmiş, S, Değer, O., Ulgen, M., Yildiran, A., Aksu, H. 2004. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 28, 181.
5. Akhondzadeh, S., Mohammadi, M., Khademi, M. 2004. *BMC Psych.*, 6, 1.
6. Bloch, M. H., Qawasmi, A. 2011. *J. Am. Acad. Child Adolesc. Psychiatry*, 50, 991.
7. Gillies, D., Taylor, F., Gray, C., O'Brien, L., D'Abrew, N. 2012. *Cochrane Database Syst. Rev.*, 12, CD006726.
8. Colter, A. L., Cutler, C., Meckling, K. A. 2008. *Nutr. J.*, 7, 8.
9. Transler, C., Eilander, A., Mitchell, S., van de Meer, N. 2010. *J. Atten. Disord.*, 14, 232.
10. Widenhorn-Müller, K., Schwanda, S., Scholz, E., Spitzer, M., Bode, H. 2014. *Prostaglandins Leukot. Essent. Fat. Acids*, 91, 49.
11. Konofal, E., Lecendreux, M., Arnulf, I., Mouren, M.-C. 2004. *Arch. Pediatr. Adolesc. Med.*, 158, 1113.
12. Oner, O., Oner, P., Bozkurt, O. H., Odabas, E., Keser, N., Karadag, H., Kizilgün, M. 2010. *Child Psychiatry Hum. Dev.*, 41, 441.
13. Donfrancesco, R., Parisi, P., Vanacore, N., Martines, F., Sargentini, V., Cortese, S. 2013. *J. Atten. Disord.*, 17, 347.

14. Arnold, L. E., Disilvestro, R. A., Bozzolo, D., Bozzolo, H., Crowl, L., Fernandez, S., Ramadan, Y., Thompson, S., Mo, X., Abedl-Rasoul, M., Joseph, E. 2011. *J. Child Adolesc. Psychopharmacol.*, 21, 1.
15. Kozielec, T., Starobrat-Hermelin, B. 1997. *Magnes. Res.*, 10, 143.
16. Mousain-Bosc, M., Roche, M., Rapin, J., Bali, J.-P. 2004. *J. Am. Coll. Nutr.*, 23, 545S.
17. Huss, M., Völp, A., Stauss-Grabo, M. 2010. *Lipids Health Dis.*, 9, 105 .
18. Schab, D. W., Trinh, N.-H. T. 2004. *J. Dev. Behav. Pediatr.*, 25, 423.
19. Stevens, L. J., Burgess, J. R., Stochelski, M. A., Kuczek, T. 2014. *Clin. Pediatr.*, 53, 133.
20. Kanarek, R. B. 2011. *Nutr. Rev.*, 69, 385.
21. McCann, D., Barret, A., Cooper, A., Crumpler, D., Dalen, L., Grimshaw, K., Kitchin, E., Lok, K., Porteous, L., Prince, E., Sonuga-Barke, E., Warner, J. O., Stevenson, J. 2007. *Lancet*, 370, 1560.
22. Blunden, S. L., Milte, C. M., Sinn, N. 2011. *J. Child Health Care*, 15, 14.
23. Azadbakht, L., Esmaillzadeh, A. 2012. *Nutrition*, 28, 242.
24. Howard, A. L., Robinson, M., Smith, G. J., Ambrosini, G. L., Piek, J. P., Oddy, W. H. 2011. *J. Atten. Disord.*, 15, 403.
25. Woo, H. D., Kim, D. W., Hong, Y.-S., Kim, Y.-M., Seo, J.-H., Choe, B. M., Park, J. H., Kang, J.-W., Yoo, J.-H., Chueh, H. W., Lee, J. H., Kwak, M. J., Kim, J. 2014. *Nutrients*, 6, 1539.
26. Grassmann, V., Alves, M. V., Santos-Galduróz, R. F., Galduróz, J. C. F. *J. Atten. Disord.*, 2014, 1087054714526041.
27. Gapin, J. I., Labban, J. D., Etnier, J. L. 2011. *Prev. Med. (Baltim).*, 52, S70.
28. Wigal, S. B., Emmerson, N., Gehricke, J.-G., Galassetti, P. 2013. *J. Atten. Disord.*, 17, 279.
29. Berwid, O. G., Halperin, J. M. 2012. *Curr. Psychiatry Rep.*, 14, 543.
30. Arnsten, A. F. T. 2009. *CNS Drugs*, 23, 33.
31. Faraone, S. V, Biederman, J. 1998. *Biol. Psychiatry*, 44, 951.
32. National Institute of Mental Health. Attention Deficit Hyperactivity Disorder (ADHD) Publication. at <http://www.nimh.nih.gov/health/publications/attention-deficit-hyperactivity-disorder/adhd_booklet_cl508.pdf>
33. Heilskov Rytter, M. J., Borup Andersen, L. B., Houmann, T., Bilenberg, N., Hvolby, A., Mølgaard, C. 2014. *Nord. J. Psychiatry*, 69, 1.
34. Van Egmond-Fröhlich, A. W. A, Weghuber, D., de Zwaan, M. 2012. *PLoS One*, 7, e4978.
35. Park, S., Cho, S.-C., Hong, Y.-C., Oh, S-Y., Kim, J.-W., Shin, M.-S., Kim, B.-N., Yoo, H.-J., Cho, Y.-H., Bhang, S.-Y. 2012. *Psychiatry Res.*, 198, 468.
36. Liu, J., He, P., Li, L., Shen, T., Wu, M., Hu, J., Zhuang, Y., Yin, J., He, G. *2014. Wei Sheng Yan Jiu*, 43, 235.
37. Ghanizadeh, A., Haddad, B. 2015. *Ann. Gen. Psychiatry*, 14, 1.
38. Sonuga-Barke, E. J., Brandeis, D., Cortese, S., Daley, D., Ferrin, M., Holtmann, M., Stevenson, J., Danckaerts, M., van der Oord, S., Döpfner, M., Dittmann, R. W., Simonoff, E., Zuddas, A., Banaschewski, T., Buitelaar, J., Coghill, D., Hollis,

C., Konofal, E., Lecendreux, M., Wong, I. C., Sergeant, J. European ADHD Guidelines Group. 2013. *Am. J. Psychiatry*, 170, 275.
39. Ambrosini, G. L., Oddy, W. H., Robinson, M., O´Sullivan, T. A., Hands, B. P., de Klerk, N. H., Silburn, S. R., Zubrick, S. R., Kendall, G. E., Stanley, F. J., Beilin, L. J. 2009. *Public Health Nutr.*, 12, 1807.
40. Sonuga-Barke, E. J. S. 2015. *J. Child Psychol. Psychiatry*, 56, 497.
41. Stevens, L. J., Kuczek, T., Burgess, J. R., Hurt, E., Arnold, L. E. 2011. *Clin. Pediatr.*, 50, 279.
42. Nigg, J. T., Lewis, K., Edinger, T., Falk, M. 2012. *J. Am. Acad. Child Adolesc. Psychiatry*, 51, 86.
43. Stevenson, J., Buitelaar, J., Cortese, S., Ferrin, M., Konofal, E., Lecendreux, M., Simonoff, E., Wong, I. C., Sonuga-Barke, E. 2014. *J. Child Psychol. Psychiatry.*, 55, 416.
44. Rowe, K.S., Rowe, K. J. 1994. *J. Pediatr.*, 125, 691.
45. Pelsser, L. M., Frankena, K., Toorman, J., Savelkoul, H. F., Dubois, A. E., Pereira, R. R., Haagen, T. A., Rommelse, N. N., Buitelaar, J. K. 2011. *Lancet*, 377, 494.
46. Millichap, J. G., Yee, M. M. 2012. *Pediatrics*, 129, 330.
47. Smith, A. L., Hoza, B., Linnea, K., McQuade, J. D., Tomb, M., Vaughn, A. J., Shoulberg, E. K., Hook, H. 2013. *J. Atten. Disord.*, 17, 70.
48. Pontifex, M. B., Saliba, B. J., Raine, L. B., Picchietti, D. L., Hillman, C. H. 2013. *J. Pediatr.*, 162, 543.
49. Hillman, C. H., Pontifex, M. B., Castelli, D. M., Khan, N. A., Raine, L. B., Scudder, M. R., Drollette, E. S., Moore, R. D., Wu, C. T., Kamijo, K. 2014. *Pediatrics*, 134, e1063.
50. Verret, C., Guay, M.-C., Berthiaume, C., Gardiner, P. & Béliveau, L 2012. *J. Atten. Disord.*, 16, 71.
51. Carriedo, A. 2014. *J. Sport Heal. Res.*, 6, 47.
52. Chang, Y.-K., Liu, S., Yu, H.-H., Lee, Y.-H. 2012. *Arch. Clin. Neuropsychol.*, 27, 225.
53. Verret, C., Gardiner, P., Béliveau, L. 2010. *Adapt. Phys. Activ. Q.*, 27, 337.
54. Pan, C.-Y., Chang, Y.-K., Tsai, C.-L., Chu, C.-H., Cheng, Y.-W., Sung, M.-C. 2014. *J. Atten. Disord.*,1087054714533192.
55. Volkow, N. D., Wang, G.-J., Newcorn, J. H., Kollins, S. H., Wigal, T. L., Telang, F., Fowler, J. S., Goldstein, R. Z., Klein, N., Logan, J., Wong, C., Swanson, J. M. 2011. *Mol. Psychiatry* 16, 1147.