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10 Fatty acid status and behavioural symptoms of Attention Deficit Hyperactivity Disorder  
11 in adolescents: A case-control study

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25 Running Title: Omega-3 fatty acids in ADHD

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27 Key words: omega-3 fatty acids, Conners' testing, dietary intake, alternative therapy

1 Abstract

2 Background:

3 Most studies of Attention-deficit hyperactivity disorder (ADHD) have focused on either  
4 young children or older adults. The current study compared 11 ADHD adolescents with  
5 12 age-matched controls. The purpose was to examine differences in dietary intake,  
6 particularly of essential fatty acids, and determine whether this could explain the typical  
7 abnormalities in red blood cell fatty acids observed in previous studies of young children.  
8 A secondary purpose was to determine if there were relationships between circulating  
9 concentrations of essential fatty acids and specific ADHD behaviours as measured by the  
10 Conners' Parent Rating Scale (CPRS-L).

11 Methods:

12 ADHD adolescents and age-matched controls were recruited through newspaper adds,  
13 posters and a university website. ADHD diagnosis was confirmed by medical  
14 practitioners according to DSM-IV criteria. Blood, dietary intake information as well as  
15 behavioural assessments were completed.

16 Results:

17 Results showed that ADHD adolescents consumed more energy and fat than controls but  
18 had similar anthropometry. ADHD children consumed equivalent amounts of omega-3  
19 and omega-6 fatty acids to controls, however they had significantly lower levels of  
20 docosahexaenoic acid (DHA, 22:6n-3) and total omega-3 fatty acids, higher omega-6  
21 fatty acids and a lower ratio of n-3:n-6 fatty acids than control subjects. In addition, low  
22 omega-3 status correlated with higher scores on several Conners' behavioural scales.

23 Conclusions:

24 These data suggest that adolescents with ADHD, continue to display essential fatty acid  
25 profiles that are often observed in younger children and distinctly different from normal  
26 controls of similar age. Further these red blood cell fatty acid differences are not  
27 explained by differences in intake. This suggests that there are metabolic differences in  
28 fatty acid handling between ADHD adolescents and normal controls. The value of  
29 omega-3 supplements to improve fatty acid profiles and possibly behaviours associated  
30 with ADHD, need to be examined.

## 1 Background

2 Attention deficit hyperactivity disorder (ADHD) is primarily characterized by a  
3 “persistent pattern of inattention and/or hyperactivity-impulsivity that is more frequent  
4 and severe than is typically observed in individuals at a comparable level of  
5 development”<sup>1,2</sup>. The American Psychiatric Association estimates that 3-5% of school  
6 aged children have ADHD<sup>1</sup>, while other sources report higher prevalence rates ranging  
7 from 5-13%<sup>3-9</sup>. ADHD is the most common psychiatric disorder in children and is  
8 diagnosed in males two to nine times as often as in females. ADHD shows high  
9 comorbidity with several other conditions including learning differences, oppositional  
10 defiance disorder (ODD), obsessive compulsive disorder (OCD) and depression<sup>10,11</sup>. For  
11 up to 60% of these children, ADHD symptoms and difficulties will persist into adulthood  
12<sup>12,13</sup>.

13 The cause of ADHD is generally acknowledged to be multifactorial, involving both  
14 biological and environmental influence<sup>2,14</sup>. Increasingly, attention is being focussed on  
15 the clinical heterogeneity of the disorder, with the aims of identifying both core and  
16 associated properties, while clarifying the relationship to other disorders<sup>14</sup>. Genetics,  
17 dopamine neurotransmission, abnormalities in brain structure and several environmental  
18 factors have all been noted as playing a potential role in the development of the disorder  
19<sup>7</sup>.

20 Despite the demonstrated efficacy of stimulant medications<sup>2,7,15</sup> in many children  
21 and adults with a diagnosis of ADHD, none of these strategies is curative and a very large  
22 proportion of the community of sufferers and their families continue to look for  
23 alternative treatments. In the past two decades, there has been an increasing focus  
24 particularly on the effects of diet in hyperactivity in children. Researchers have reported  
25 that various aspects of a child’s diet including food additives, refined sugars, food  
26 allergies, minerals and fatty acid metabolism may have adverse effects on behaviour<sup>10</sup>.  
27 While there is no definitive proof that any of these is responsible for the spectrum of  
28 ADHD symptoms, there is a compelling argument for a role for long-chain  
29 polyunsaturated fatty acids.

30 The processes of elongation and desaturation occur mainly in the liver, but also in the  
31 central nervous system, placenta, glial tissue and choroid plexus vasculature<sup>16</sup>. Within

1 the brain, four fatty acids are particularly important; dihomogammalinolenic acid (20:3n-  
2 6, DGLA), arachidonic acid (20:4n-6, AA), eicosapentaenoic acid (20:5n-3, EPA) and  
3 docosahexaenoic acid (22:6n-3). AA and DHA play a major structural role in neuronal  
4 membranes and make up 20% of the dry mass of the brain <sup>14</sup>. EPA and DGLA play a  
5 more minor structural role but are also crucial for normal brain function. Since optimal  
6 requirements are not fully known, definitive dietary reference intakes (DRIs) for the  
7 omega-3 and omega-6 fatty acids have not yet been determined <sup>17</sup>. However, Petrie and  
8 colleagues published recommendations for adequate intake (AI) for boys 9-16 yr as 12-16  
9 g linoleic acid (LA)/d and 1.2-1.6 g  $\alpha$ -linolenic acid (ALA)/d. For girls the  
10 corresponding amounts were 10-11 LA g/d and 1.0-1.1 ALA g/d <sup>18</sup>. In order to ensure  
11 the best biological functions, Bjerve suggests an intake of 900mg/d EPA and 400 mg/d  
12 DHA <sup>19</sup>.

13 A number of the physical and behavioural symptoms of essential fatty acid  
14 deficiency mimic some of the symptoms described in typical ADHA patients, therefore it  
15 is conceivable, that either dietary deficiency of omega-3 fatty acids, or altered metabolic  
16 handling of these fatty acids, could contribute to the abnormalities observed in those  
17 affected by ADHD. Several studies since the early 1980's have attempted to demonstrate  
18 abnormal fatty acid profiles in children or adults with ADHD <sup>20</sup>. In addition, several  
19 intervention trials with mixtures of nutrients including omega-3 and/or omega-6 fatty  
20 acids have been attempted. The outcomes however, have been highly variable with some  
21 showing improvement in behaviours and others no changes at all despite substantive  
22 alterations in blood fatty acid levels <sup>20-28</sup>. To date, no studies have specifically  
23 investigated adolescent populations for the relationship between fatty acid status and  
24 ADHD associated behaviours.

25 The overall purpose of the present study was to compare several parameters in an  
26 adolescent ADHD population versus an adolescent control population. Parameters  
27 included comparisons of dietary patterns between the groups based on 7-day diet records  
28 and analysis of red blood cell fatty acid composition and serum hormone levels as well as  
29 the frequency of symptoms of fatty acid deficiency and ADHD-associated behaviours in  
30 both populations.

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2 **Experimental Methods**

3

4 *Subject Selection*

5 This study was approved by the Research Ethics Board at the University of Guelph.

6 Adolescent males and females aged ten to sixteen years were recruited from the City of  
7 Guelph and surrounding area starting in March 2004 via flyers and local advertisements.

8 Approximately 45 parents and/or subjects contacted the study coordinator for further  
9 information regarding the study protocol. Of these, 23 subjects and their families agreed  
10 to participate and gave informed consent. The main reason for declining to participate  
11 was a result of the child or adolescent's refusal to have blood taken.

12 In all there were 11 subjects with a confirmed physician diagnosis of ADHD according to  
13 DSM-IV criterion and 12 control subjects without a diagnosis of ADHD that acted as  
14 participants. One subject in the control group completed study visit #1 before dropping  
15 out of the study due to lack of interest and three subjects in the control group participated  
16 in a single visit, condensed study protocol, due to time constraints. The remaining 19  
17 subjects participated in the entire protocol.

18 *Experimental Protocol*

19 All study visits occurred at the Human Nutraceutical Research Unit. All subject visits  
20 were scheduled in advance via email or telephone and occurred on weekday mornings  
21 between the hours of 7 and 11am. Study visits for male and pre-menstrual female  
22 subjects were coordinated on the basis of convenience for the participants. Study visits  
23 for female subjects who had begun menstruation were coordinated to occur on or as close  
24 menstrual cycle day 5. It was mandatory that a parent or guardian be on campus with the  
25 subject for visit one. All subjects were required to fast for a period of at least 8 h prior to  
26 study visits and were allowed to consume water only during this period.

27 *Study Visit #1*

28 Following introductions, the subject and guardian were invited to sit with the study  
29 coordinator to go through experimental details and protocols and sign a consent form.

30 Following this, a Subject Health Questionnaire and Conners' Parent Rating Scale  
31 (CPRS:L) were explained and presented to the guardian for completion. During this visit,

1 a variety of baseline measurements were taken for each subject. Height (without shoes)  
2 was measured in centimeters (cm) and weight (kg) in light clothing recorded (Acculab  
3 SV-100, Haverhill, MA). Following a 5 min rest period, blood pressure and pulse were  
4 measured using a battery operated portable machine (Lifesource, Milpitas, CA) and were  
5 recorded in systole over diastole and beats per minute (bpm), respectively. Next, body  
6 composition was estimated using bioelectrical impedance analysis (BIA, BodyStat 1500,  
7 British Isles). Following body composition measures, a fasting venous blood sample was  
8 taken for further analysis (four untreated, four in heparinized tubes, Vacutainer, Becton  
9 Dickinson, NJ). Following blood collection, subjects were provided with unlimited  
10 beverages and a small breakfast.

11 The purpose and protocol for the completion of the 7-day dietary record was then  
12 explained to both the subjects and their guardians. Subjects were instructed to keep their  
13 usual dietary habits and the importance of accurate completion was stressed. Subjects  
14 were given a study package to take home with them, which included 5 two-sided dietary  
15 record sheets, written explanation and details of how to complete them, and reference  
16 sheets for common food sizes. Before leaving a tentative return date was scheduled for  
17 4-6 weeks later.

### 18 *Behaviour Assessment*

19 The revised Conners' Parent Rating Scale long version (CPRS-R:L) was administered  
20 during visit #1 of the study for all subjects and completed by the attending parent or  
21 guardian. Assessment of 80 common problems was based on the child's behaviour in the  
22 preceding month and they were asked to circle the best answer for each item. All parent-  
23 rating scores were converted to T-scores through the use of the CPRS:L sheet for score  
24 profiling. The scale was used to assess not only ADHD, but also problems with conduct,  
25 cognition, family relationships, emotional issues, anger management, and/or anxiety<sup>29</sup>.

### 26 *Study Visit #2*

27 Four to six weeks following the first visit, a second study visit was coordinated. Subjects  
28 were required to fast for a period of at least 8 hours as with visit one, and the four tubes  
29 of blood were taken as close to arrival time as possible. Subjects were again provided  
30 with a snack following blood collection. Following this, the dietary record sheets were  
31 collected and the distribution and access to the study results were explained to all subjects

1 and their guardian. Prior to departure, the subject was presented with a \$25 gift  
2 certificate for participation. Subjects who participated in the condensed study protocol,  
3 which included one visit only, were presented with a \$15 gift certificate. All study visits  
4 occurred between June 2004 and August 2005.

#### 5 *Diet Analysis*

6 All subjects recorded their daily diet consumption for seven consecutive days between  
7 study visit 1 and 2. On study visit 2 these pages were submitted to the investigator. Diet  
8 analysis was performed using the computer-based program Food Processor© (Version  
9 7.11, ESHA Research, Oregon). All food and beverage items ingested over the seven day  
10 period were entered into the program, and nutrition values were averaged to a per day  
11 basis.

#### 12 *Blood Analysis*

13 Following collection, the red top tubes were placed at room temperature for at least 15  
14 min to allow clotting. Heparinized tubes were immediately placed on ice. All tubes were  
15 centrifuged for 20 min at 500 X g. The serum and plasma fractions were collected by  
16 pipette and deposited into labeled aliquot tubes for storage. Red blood cell fractions were  
17 prepared following removal of plasma and white blood cells from sodium heparinized  
18 samples and tubes were filled to the top with 0.9% sodium chloride saline solution  
19 (Abbott Laboratories Ltd., Quebec). The contents were mixed well by inversion and the  
20 tube was then centrifuged for 20 min at 500 X g. Following this wash, the upper saline  
21 layer was discarded and the red blood cell fraction was collected and pipetted into labeled  
22 aliquot tubes. All aliquot tubes were stored at -20 degrees for a maximum of 5 days and  
23 were then transferred to a -80 freezer until analysis.

#### 24 *Red blood cell fatty acid analysis*

25 Following chloroform:methanol extraction, phospholipids were separated from other  
26 lipids by thin layer chromatography and methylated fatty acids determined by an outside  
27 commercial laboratory (Lipid Analytical Laboratories, Guelph, ON). Fatty acids were  
28 measured in RBC blood samples from both study visits 1 and 2, when available, and  
29 values were averaged for the purpose of statistical analyses. Fatty acid values are  
30 presented as percentage of molecular weight.

#### 31 *Statistical Analysis*

1 Statistical significance was accepted at  $p < 0.05$ . All statistical analysis was performed  
2 using SPSS 10.0 for Windows Student Version. Statistical analyses performed included  
3 independent samples t-test for means comparisons and bi-variate Pearson's correlations.  
4 Data is reported as mean  $\pm$  standard deviation, and in some cases, followed by the range  
5 of values in parentheses.

## 6 **RESULTS**

7 Subject characteristics at visit #1 are given in Table 1. There were no significant  
8 differences between the groups for age and sex characteristics or anthropometric measures.  
9 A health questionnaire was filled out at the start of the study by the parent/guardian for  
10 each subject. The questionnaire collected information regarding ADHD diagnosis,  
11 medication use, co-morbid disorders/conditions, vitamin/supplement use, family history  
12 of ADHD, allergies, duration of breastfeeding and prevalence of fatty acid deficiency  
13 symptoms. Six of eleven subjects in the ADHD group were taking medications (55%),  
14 five of eleven presented with a co-morbid learning disorder (45%), and eight of eleven  
15 reported a history of ADHD within the family (73%). These three variables were  
16 significantly different from the control group with  $p = 0.016$ ,  $p = 0.016$  and  $p = 0.001$ ,  
17 respectively.

18 The duration of breastfeeding in infancy was also reported. Subjects in the ADHD group  
19 were breastfed an average of  $4.14 \pm 2.6$  (0-7) months, whereas subjects in the control  
20 group were breastfed an average of  $10.4 \pm 10.9$  (3-39) months. With respect to  
21 symptoms of fatty acid deficiency, subjects in the ADHD group reported an average of  
22  $2.4 \pm 3.5$  (0-9) symptoms versus  $1.6 \pm 1.6$  (0-4) symptoms in the control group. Neither  
23 of these differences between groups was significant ( $p = 0.077$  and  $p = 0.461$ , respectively).  
24 The ADHD group was also more likely to have allergies (27% vs. 8%) and less likely to  
25 be taking vitamins (18% vs. 50%) when compared to the control group. Again, neither of  
26 these differences were significant.

### 27 *Behaviour Assessment*

28 Analysis of the Conners' Parent Rating Scales Long Version (CPRS:L) revealed several  
29 significant differences between the ADHD and control groups (Table 2). When  
30 compared to the control group, the ADHD group presented with significantly higher  
31 mean T scores on ten of the fourteen scales included in the assessment ( $p < 0.05$ ) (Figure

1) These included measures for oppositional behaviours, cognitive problems and inattention, restlessness and impulsivity, hyperactivity, emotional lability and overall problematic behaviour. Children were also identified as 'at risk' through scores on the ADHD index, while also being assessed on scales directly related to DSM-IV criteria including inattentive, hyperactive-impulsive and total DSM scores.

### *Diet Analysis*

Analysis of seven-day dietary records from subjects (ADHD n=11, Control n=8) revealed several differences between intake patterns (Table 3, not all data is shown). The ADHD group consumed significantly more calories ( $2652 \pm 458$  vs.  $2051 \pm 407$ ,  $p=0.009$ ), more protein ( $91 \pm 19\text{g}$  vs.  $73 \pm 17\text{g}$ ,  $p=0.049$ ) and more carbohydrates ( $357 \pm 68\text{g}$  vs.  $271 \pm 60\text{g}$ ,  $p=0.011$ ) per day when compared to the control group. The ADHD group also consumed significantly more total fat ( $99 \pm 22\text{g}$  vs.  $77 \pm 14\text{g}$ ,  $p=0.013$ ), saturated fats ( $38 \pm 9\text{g}$  vs.  $28 \pm 8\text{g}$ ,  $p=0.027$ ) and trans fatty acids ( $3.6 \pm 3.1\text{g}$  vs.  $1.3 \pm 0.7\text{g}$ ,  $p=0.038$ ) per day. There was also a trend toward higher monounsaturated fat consumption in the ADHD group ( $31 \pm 9\text{g}$  vs.  $24 \pm 6\text{g}$ ), however this difference was not significant ( $p=0.077$ ). There were no significant differences in mean consumption of total n-3 fatty acids, ALA, EPA or DHA between the two groups. Furthermore, there were no differences in mean consumption of total n-6 fatty acids, LA or AA. Calculated n-6: n-3 ratios revealed values of 9.47 in the ADHD group and 9.03 in the control group. These ratios were not significantly different from one another.

The ADHD group consumed significantly greater amounts of vitamin B1 ( $1.84 \pm 0.34\text{mg}$  vs.  $1.42 \pm 0.37\text{mg}$ ), vitamin B2 ( $2.34 \pm 0.70\text{mg}$  vs.  $1.54 \pm 0.36\text{mg}$ ), iron ( $21.66 \pm 7.04\text{mg}$  vs.  $12.52 \pm 3.43\text{mg}$ ) and sodium ( $4001 \pm 931\text{mg}$  vs.  $3119 \pm 756\text{mg}$ ) in their diets when compared to controls ( $p<0.05$ ). There were also trends toward increased calcium and zinc intake in the ADHD group when compared to the control group however, these differences were not significant.

Pearson correlations between diet variables and CPRS:L scale rating revealed several positive and significant relationships. Total energy intake was positively correlated with scores for oppositional and hyperactive behaviours ( $p<0.01$ ), in addition to restlessness, problematic behaviour and DSM-IV total ( $p<0.05$ ). Saturated fat and

1 total fat intakes displayed a significant positive correlation to scales measuring  
2 oppositional, hyperactive (Figure 2) and problematic behaviours ( $p < 0.01$ ), as well as  
3 DSM-IV total score ( $p < 0.05$ ). Iron intake was also positively correlated with the  
4 cognitive problem, DSM-IV inattentive, DSM-IV total (Figure 3), problematic behaviour  
5 and restlessness scales ( $p < 0.01$ ), in addition to the oppositional, hyperactivity and ADHD  
6 index scales ( $p < 0.05$ ). Finally, intake of sodium was positively correlated with the  
7 hyperactivity and restlessness scales ( $p < 0.01$ ), and with the oppositional, problematic  
8 behaviour, ADHD index, DSM inattentive and DSM total scales ( $p < 0.05$ ).

### 9 10 *Fatty Acid Analysis*

11 Lipid analysis of red blood cell samples was performed and all fatty acids were reported  
12 as a percentage of molecular weight (Table 4, not all data is shown). When compared to  
13 controls, the ADHD group presented with significantly lower DHA ( $3.12 \pm 0.75$  vs.  $4.39$   
14  $\pm 1.34$ ,  $p = 0.012$ ) and total n-3 fatty acids ( $5.79 \pm 1.39$  vs.  $7.42 \pm 1.64$ ,  $p = 0.018$ ). The  
15 ratio of n-3 fatty acids to n-6 fatty acids was also significantly lower in the ADHD group  
16 ( $0.17 \pm 0.04$  vs.  $0.23 \pm 0.06$ ,  $p = 0.017$ ). The ADHD group also presented with elevated  
17 LA ( $13.26 \pm 0.95$  vs.  $12.02 \pm 2.14$ ), total n-6 ( $33.33 \pm 1.83$  vs.  $32.51 \pm 1.59$ ) and total  
18 saturated fatty acid levels when compared to the control group however; none of these  
19 latter differences were significant.

20 Analysis of the relationship between red blood cell content and diet variables  
21 identified multiple significant correlations. Total caloric intake was positively correlated  
22 with total n-6 red blood cell content ( $r = .451$ ,  $p = 0.026$ ) and negatively correlated with  
23 DHA ( $r = -.491$ ,  $p = 0.016$ ), total n-3 ( $r = -.509$ ,  $p = 0.013$ ) and the n-3: n-6 ratio ( $r = -.544$ ,  
24  $p = 0.008$ ). Similarly, total dietary fat intake was positively correlated with total n-6 red  
25 blood cell content ( $r = .552$ ,  $p = 0.007$ ) (Figure 4) and negatively correlated with DHA ( $r =$   
26  $-.532$ ,  $p = 0.010$ ), total n-3 ( $r = -.570$ ,  $p = 0.005$ ) (Figure 5) and the n-3: n-6 ratio ( $r = -.609$ ,  
27  $p = 0.003$ ). Saturated fat and sodium intakes displayed similar trends with respect to  
28 correlation to blood fatty acid parameters.

29 There were several significant correlations between specific red blood cell  
30 parameters and Conners' scale ratings. Red blood cell DHA content was negatively  
31 correlated with scale ratings for oppositional behaviour, hyperactivity, cognitive

1 problems, restlessness, problematic behaviour, DSM-IV inattention and DSM-IV total  
2 ( $p<0.05$ ) (Figure 6). Total n-3 content was negatively correlated with the Conners' scale  
3 for restlessness ( $p<0.05$ ) and the n-3: n-6 ratio was negatively related to the oppositional,  
4 restlessness and problematic behaviour scales ( $p<0.05$ ). Finally, total red blood cell n-6  
5 content was positively correlated with the oppositional, restlessness, problematic  
6 behaviour, DSM-IV inattentive, DSM-IV total and ADHD index scales ( $p<0.05$ ).

7

## 8 **Discussion**

9 To date, clinical studies have focused on fatty acid and other abnormalities  
10 associated with ADHD in children (6-12 years) and adult populations (18-65 years)<sup>20-</sup>  
11 <sup>28,30,31</sup>. Until the current study, there was a clear gap in the knowledge with respect to  
12 individuals aged 12-18 years. We were interested in whether puberty and adolescence  
13 have any effect on differences that have been identified between individuals with and  
14 without ADHD in other populations. Here, characteristics and parameters were  
15 compared among adolescent individuals between the ages of 10 and 16 years, with a  
16 mean age of 14 years. The primary objective of this study was to determine whether  
17 abnormalities of red blood cell fatty acids are present in adolescents with ADHD when  
18 compared to controls. Health status, behaviour, and dietary patterns were also compared  
19 between the groups.

20 The red blood cell phospholipid fatty acid composition data reported here, indicates  
21 that adolescent ADHD subjects have lower total omega-3 fatty acids, lower DHA levels  
22 and higher linoleic acid levels when compared to age-matched control subjects. The  
23 absolute values and degree of difference between groups are similar to those reported  
24 elsewhere in studies of younger children and adults with and without ADHD<sup>21,24,26,28</sup>.  
25 However we did not see several differences reported by Stevens, which included lower  
26 AA and adrenic acid levels, and higher docosapentaenoic acid levels in ADHD subjects  
27 when compared to controls<sup>21</sup>. Stevens did report lower DHA levels in the ADHD group  
28 but this was not statistically significant in their study<sup>21</sup>. In 2004, Chen and colleagues  
29 reported significantly lower levels of AA, LA, DHA and total n-3 in red blood cell  
30 phospholipids of young children with ADHD when compared to controls<sup>28</sup>. Similarly,  
31 when fatty acid composition of red blood cells were compared in an adult population,

1 Young and her colleagues reported significantly lower DHA and significantly higher total  
2 n-6 fatty acids among other measures, in the individuals with ADHD <sup>26</sup>. Thus, while  
3 there are some differences in the specific fatty acid changes, the data we report here for  
4 adolescents indicates that childhood patterns persist through adolescence and into  
5 adulthood with few alterations.

6 Two previous studies reported dietary patterns in children with ADHD using three  
7 day diet records <sup>21,28</sup>. Using a complete seven day diet record, we examined the intake  
8 patterns of our adolescent subjects. We demonstrate that ADHD subjects consumed  
9 higher levels of at least 10 different nutrients, than their control counterparts. This  
10 included 25% more energy, and more grams of carbohydrate, total fat, omega-6 fatty  
11 acids and trans fatty acids in addition to others. Stevens reported significantly higher  
12 intakes of total fat (g) and polyunsaturated fatty acids (g) in ADHD subjects when  
13 compared to control subjects <sup>21</sup>. In 2004, Chen and colleagues reported significantly  
14 increased iron (mg) and vitamin C (mg) consumption in subjects with ADHD versus  
15 controls <sup>28</sup>. As has been previously proposed, it is possible that over-ingestion of fats  
16 could interfere with the conversion of parent essential fatty acids (EFAs) to long-  
17 chain(LC)-PUFAs <sup>14</sup>. Despite the many dietary differences between our subject groups,  
18 they did not differ in either total omega-3 fatty acid consumption, or consumption of the  
19 LC-PUFAs. Thus, it is unlikely that dietary differences in omega-3 consumption can  
20 explain the significant differences in red blood cell membrane fatty acids observed in the  
21 ADHD subjects herein.

22 A possible explanation for the deficiencies seen here could be an inferior ability to  
23 convert the 18-carbon precursor ALA to LC-PUFAs such as DHA. Decreased  
24 concentrations of DHA could negatively affect brain function through alterations in  
25 membrane fluidity and transport, for example <sup>22,32</sup>. Animal studies have shown that rats  
26 with n-3 PUFA deficiency display decreased levels of endogenous dopamine and  
27 decreased D2 receptors in the frontal cortex <sup>33,34</sup>. Dopamine is known to act as a  
28 modulator of attention, motivation and emotion and, as such, has been a target of several  
29 pharmaceuticals aimed at normalizing ADHD behaviours. Since a decrease in LC-  
30 PUFAs may be directly linked to dopamine concentrations in the frontal cortex, a  
31 decreased availability of dopamine could be resulting in ADHD symptoms. It may also

1 be possible that there is a deficiency in enzymes critical for fatty acid metabolism such as  
2 phospholipase-A2 (PLA2), or the desaturase and elongase enzyme series.

3 As official dietary reference intakes (DRIs) do not exist, it can be difficult for  
4 individuals to know what levels to strive toward when consuming EFAs in the diet. In  
5 comparison with the recommended adequate intakes (AIs) that do exist in the literature,  
6 both subject groups in the present study consumed much less than the suggested target  
7 amounts of AA, ALA, EPA and DHA in their diets<sup>18,19,35</sup>. Daily intakes of LA and ALA  
8 were also considerably lower than those reported by Chen and colleagues, however, total  
9 PUFA intake appears to be comparable to values reported by Stevens and colleagues<sup>21,28</sup>.  
10 Despite the substantial deficit in the intake of individual omega fatty acids here, the ratios  
11 of n-6: n-3 fatty acids for the groups were within the recommended target range  
12 according to the WHO<sup>36</sup>.

13  
14 Regardless of diagnosis in the current study, dietary fat intake was significantly and  
15 positively correlated with scores on five of the Conners' scales, positively correlated with  
16 total n-6 red blood cell content and negatively correlated with total n-3 red blood cell  
17 content. Also, as Chen and colleagues have reported, there was a significantly higher  
18 intake of iron in the ADHD group<sup>28</sup>. These findings conflict with reports that indicate  
19 iron deficiency as a possible contributor to ADHD and other problematic behaviours such  
20 as inattention and increased activity levels in children<sup>37</sup>. In the present study, iron intake  
21 also displayed a significant and positive correlation to scores on eight of the Conners'  
22 scales. It is possible that iron levels in children and adolescents have an effect on  
23 behaviours and should therefore be investigated in future studies. Reports have also  
24 indicated that children with ADHD may be deficient in magnesium<sup>10,38</sup>. However, in the  
25 present study, ADHD subjects reported slightly higher (but not significant) intakes of  
26 magnesium when compared to controls. Previous research had also suggested lower  
27 intakes of tyrosine, tryptophan and phenylalanine in individuals with ADHD compared to  
28 controls<sup>39</sup> however, this was not the case in the current study where again, if anything  
29 intakes for these amino acids were higher in ADHD subjects. From these data, a clear  
30 role for minerals and amino acids in ADHD behaviours can not be established and will  
31 require further investigation.

1  
2           The earliest of fatty acid supplementation studies in individuals with ADHD  
3 utilized the n-6 fatty acid based supplementation efamol. These two clinical trials  
4 performed in the late 1980s reported that there was minimal or no improvement in  
5 behaviour when children with ADHD were supplemented with n-6 fatty acids<sup>30,40</sup>. It  
6 was after these trials that the scientific community acknowledged the importance of n-3  
7 fatty acids in the brain and their potential to produce favourable results with  
8 supplementation. Since this time, three studies have supplemented their subjects with n-3  
9 fatty acids. However, the results have been conflicting. In 2001, Voigt and colleagues  
10 reported no significant improvement in related behaviours and symptoms in children with  
11 ADHD when they were supplemented with 345mg/day DHA for 4 months<sup>31</sup>. Although  
12 the subject number would seem to be sufficient (n=63), it is likely that the DHA dosage  
13 was simply too low to realize an effect. As reported in the literature review, it has been  
14 recommended that in order to ensure one's best biological function, at least 400mg/day of  
15 DHA be consumed<sup>19</sup>. However, when Hirayama and colleagues fed DHA-containing  
16 foods providing just over 500mg/day DHA to children with ADHD, they too were unable  
17 to document a significant improvement in ADHD-related symptoms<sup>25</sup>. It should be  
18 noted however, that only twenty subjects were supplemented for this shortened period of  
19 2 months<sup>25</sup>. It may be possible that a longer supplementation period, with more than  
20 500mg/day DHA, in a larger subject group, could be successful in attenuating ADHD  
21 behaviours in affected individuals.

22  
23           There were no significant differences between ADHD and control subjects with  
24 respect to baseline measurements. Previous studies have indicated that children with  
25 ADHD display significantly more symptoms of fatty acid deficiency including polyuria,  
26 polydipsia and dry skin<sup>21,41</sup>. Data from the health questionnaire used here did indicate  
27 that adolescents with ADHD had more symptoms of fatty acid deficiency, were more  
28 likely to have allergies and were less likely to be taking vitamins. However, these  
29 differences were not significant, and therefore the strength of their potential links to  
30 ADHD-related behaviours remains unknown. It is acknowledged by the authors that the  
31 current study may not have been sufficiently powered to reveal these differences.

1           The importance of exclusive breastfeeding for the first six months of infancy has  
2 been implicated by multiple pediatric associations in Canada (Canadian Institutes of  
3 Health, 2005; Canadian Pediatric Society, 2005)<sup>42,43</sup>. Breast milk provides an abundant  
4 supply of EFAs which lead to optimal brain development in infants<sup>43</sup>. It is interesting to  
5 note that in the present study individuals in the ADHD group were breastfed for an  
6 average of just more than four months compared to more than ten months in the control  
7 group. Although this difference between groups was not significant, it is still unknown if  
8 there is a direct and conclusive link between duration of breastfeeding in infancy and  
9 development of ADHD in childhood.

10           The etiology of ADHD remains unknown. It is generally acknowledged to be  
11 multifactorial, involving both biological and environmental influence<sup>2,14</sup>. Population  
12 studies have indicated that attention, conduct and emotional problems tend to cluster  
13 within families<sup>44</sup> and heritability of ADHD has been estimated by one group to be 76%<sup>45</sup>.  
14 Similar to this estimate, in the present study, 73% of adolescents with ADHD had a close  
15 family member with suspected or diagnosed ADHD. The data presented here provides  
16 strong support for possible genetic influence on ADHD.

17           It has been reported that 25-30% of children with ADHD have a learning  
18 disability and many may also have speech and language disorders<sup>46</sup>. In the present study,  
19 45% of subjects in the ADHD group had co-morbid disorders, the majority of which were  
20 related to language difficulties. Whether or not the presence of these additional  
21 conditions has confounded any of the comparative data in individuals with ADHD is  
22 unknown.

23           The Conners' Parent Rating Scale (CPRS:L) was utilized for behaviour  
24 assessment in this study. The CPRS:L is well respected and has been used widely among  
25 studies assessing ADHD behaviours in children<sup>21-24,31</sup>. The Conners' scale used provides  
26 an appropriate instrument for measuring behaviours in youths aged 3 to 17 and conveys  
27 information that corresponds to the official ADHD criteria in the DSM-IV<sup>29</sup>. Normative  
28 data for the scale comes from a large community-based sample of parents, teachers,  
29 children, and adolescents collected throughout the United States and Canada from 1993  
30 to 1996<sup>29</sup>. All scores are assessed based on the sex and age of the subject and are then  
31 converted to T-scores using the Conners' profile for scoring. In general, T-scores of 65

1 and above are usually taken to indicate a clinically significant problem <sup>29</sup>. In this study,  
2 the ADHD group presented with significantly higher mean T-scores than the control  
3 group on ten of the fourteen scales on the CPRS:L. This significant difference was  
4 expected as individuals were assigned to the ADHD group based on whether or not they  
5 had been previously diagnosed by a physician as having the disorder. It is interesting to  
6 note however, that the mean T-scores for the ADHD group did not reach 65 or above on  
7 4 of the 14 measures. Furthermore, there were subjects within the ADHD group who did  
8 not present with 65 or higher on any of the scales. Perhaps, these particular individuals  
9 have become better able to cope with their symptoms over time, which contributed to the  
10 presence of less problematic behaviours. It is also possible that some of these individuals  
11 were misdiagnosed with ADHD or “grew out of it”. After all, there have been allegations  
12 that ADHD is an over diagnosed and over medicated disorder by particular interest  
13 groups in the media <sup>12</sup>. Regardless, had these potentially misdiagnosed individuals not  
14 been in the ADHD group here, it is possible that there may have been more significant  
15 differences between the groups. There were also subjects in the control group who had  
16 multiple scales with T-scores of 65 or more. To this effect, one subject in the control  
17 group presented with T-scores greater than 65 on 11 of 14 scales, the fifth most out of all  
18 23 subjects in the study. This same individual was subsequently diagnosed with ADHD  
19 shortly after this study ended. As such, all of the data relating to this subject was  
20 transferred to the ADHD data pool.

21 As a group, the ADHD subjects in this study presented with mean T-scores that  
22 are comparable to other studies <sup>21-24,30,31</sup>, although slightly higher on most scales. This  
23 slight increase may simply reflect the fact that similar behaviours in a nine-year old  
24 would be given a much higher T score, if exhibited by a 13-year old. The behaviour  
25 assessment performed here identified that the ADHD group had higher mean T-scores  
26 than controls on over seventy-five percent of the Conners’ scales, indicating that  
27 problematic behaviours persist into adolescence for the majority of these individuals. The  
28 Conners’ Parent Rating Scale appears to be an extremely reliable tool when comparing  
29 ADHD behaviours among individuals of different gender and age.

30 Richardson and Puri were the first group to supplement with a combination of n-3  
31 and n-6 fatty acids in children and reported a significant benefit in alleviating symptoms

1 and learning difficulties <sup>23</sup>. As promising as these results appear at first glance, they  
2 should be interpreted with some caution. Although the 41 subjects in this study did  
3 present with ADHD-related symptoms and learning difficulties, they had not been  
4 professionally diagnosed with ADHD to qualify as participants <sup>23</sup>. The results attained  
5 here with a combination of fatty acids (including 480mg/day DHA) and vitamins for a  
6 period of 3 months, might warrant a similar design to be tested in individuals who have  
7 been clinically diagnosed with ADHD. Whether or not a similar outcome would result is  
8 unknown, but if so, could provide great hope for the future.

9         Studies like that administered by Harding and colleagues in 2002 also serve to  
10 support a potential positive impact with a combination supplement. They reported  
11 comparable efficacy to methylphenidate (Ritalin) with a formulated supplement  
12 containing fatty acids (EPA, DHA and GLA), amino acids and other constituents in  
13 response to the specific nutrient profiles of individuals with ADHD <sup>2</sup>. Although this  
14 study did not have a placebo group, and the groups were small (n=10 per group), they  
15 analyzed effects using a variety of behavioural and performance tests before and after  
16 supplementation or medication for four weeks following ADHD diagnosis <sup>23</sup>. Since  
17 parents were responsible for choosing the group their child would participate in here, it is  
18 possible that ratings given by them could have underestimated problematic behaviours, as  
19 they may have been very hopeful to see an effect. Furthermore, since supplementation  
20 involved over thirty different constituents, it is impossible to identify any one constituent  
21 or combination thereof as being responsible for the positive effect. Nonetheless, it  
22 appears that mixed supplementation may warrant further attention.

23         Hormones present during puberty were analyzed as a pilot arm in the present  
24 study. Analysis of serum estradiol in female subjects and serum testosterone in male  
25 subjects revealed no significant differences between individuals with ADHD and controls.  
26 However, there were trends toward decreased serum estradiol content in females with  
27 ADHD, and increased serum testosterone in males with ADHD. Again the small subject  
28 number and gender imbalance in this study did not provide the necessary power to  
29 properly assess this outcome. Since these hormones are in abundance throughout  
30 puberty, it is possible that abnormal levels could contribute to problematic behaviours.  
31 Multiple researchers have noted that abnormalities in hormone levels may be playing a

1 role in the manifestations of ADHD, however the data is inconclusive at this time <sup>47-49</sup>.  
2 Therefore, hormone levels in larger groups of individuals with ADHD, including  
3 adolescents, should be investigated in order to refute or support the data reported here.  
4

## 5 **Conclusions**

6 This study investigated differences between adolescent populations with and without  
7 ADHD with respect to a variety of measures. Despite the small sample size and an  
8 imbalance of genders within the groups, several significant differences were reported  
9 with respect to overall health, dietary patterns and red blood cell fatty acid compositions.  
10 Adolescents with ADHD appear to consume diets more rich in total energy, in addition to  
11 specific fats, minerals and other constituents. Although there was no difference in the  
12 dietary consumption of n-3 or n-6 fatty acids, adolescents with ADHD did present with  
13 significantly lower levels of DHA, total n-3 fatty acids and a lower n-3: n-6 ratio in red  
14 blood cell phospholipids. Abnormalities in fatty acid profile were also positively  
15 correlated with higher ratings on the Conner's scales. Further research is required to  
16 determine the mechanisms by which these fatty acid anomalies occur, and whether  
17 supplementation for a longer period of time with higher concentrations of fatty acids will  
18 positively influence ADHD behaviours in patients of all ages.

19  
20

- 1 List of Abbreviations
- 2 AA – arachidonic acid
- 3 ADHD- Attention Deficit Hyperactivity Disorder
- 4 ALA – alpha linoleic acid
- 5 BIA – bioelectrical impedance analysis
- 6 CPRS-L – Conner’s Parent Rating Scale – Long form
- 7 DHA – docosahexaenoic acid
- 8 EFA – essential fatty acid
- 9 EPA – eicosapentaenoic acid
- 10 GLA – gamma linoleic acid
- 11 LA – linoleic acid
- 12 LC-PUFA – long chain polyunsaturated fatty acid
- 13 PUFA – polyunsaturated fatty acid
- 14 RBC – red blood cell
- 15 WHO – World Health Organization

16  
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18 **Competing Interests:**

19 The authors declare that they have no competing interests.

20

21 **Author’s Contributions:**

22 Kelly Meckling conceived of the original idea and aided with the experimental design,  
23 writing the final manuscript, data interpretation and provided funding for the study.

24 Caroline Cutler carried out the initial pilot studies on the first few subjects and did much  
25 of the background work for the study. Ashley Colter carried out the all of the  
26 subject/parent interviews, collection of biological and behavioural data and all  
27 subsequent analysis and assisted with writing of all versions of the manuscript.

28

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1 References

- 2
- 3 1. American Psychiatric Association: **Attention deficit hyperactivity disorder**. In  
4 *Diagnostic and Statistical Manual of Mental Disorders*, 4<sup>th</sup> ed., Washington, DC,  
5 1994:78-85.
  - 6 2. Harding KL, Judah RD, Gant CE: **Outcome-based comparison of Ritalin versus**  
7 **food-supplement treatment treated children with AD/HD**. *Altern Med Rev*  
8 2003, **8**:319-330.
  - 9 3. Scahill L and Schwab-Stone M : **Epidemiology of ADHD in school-age**  
10 **children**. *Child Adolesc Psychiatr Clin N Am* 2000, **9**:541-555.
  - 11 4. Boyle MH, Offord DR, Racine Y, Sandford M, Szatmari P, Fleming JE:  
12 **Evaluation of the original Ontario child health study**. *Can J Psychiatry* 1993,  
13 **38**:397-405.
  - 14 5. Breton JJ, Bergeron L, Valla JP, Berthiaume C, Gaudet N, Lambert J, St-Georges,  
15 M, Houde L, Lepine S: **Quebec child mental health survey: Prevalence of**  
16 **DSM-III-R mental health disorders**. *J Child Psychol Psychiatry* 1999, **40**:375-  
17 384.
  - 18 6. Rowland AS, Lesesne CA, Abramowitz AJ: **The epidemiology of attention-**  
19 **deficit/hyperactivity disorder (ADHD): A public health view**. *Ment Retard*  
20 *Dev Disabil Res Rev* 2002, **8**:162-170.
  - 21 7. Biederman J and Faraone SV: **Attention-deficit hyperactivity disorder**. *Lancet*  
22 2005, **366**:237-248.
  - 23 8. Richardson AJ and Ross MA: **Fatty acid metabolism in neurodevelopmental**  
24 **disorder: A new perspective on associations between attention-**  
25 **deficit/hyperactivity disorder, dyslexia, dyspraxia and the autistic spectrum**.  
26 *Prostaglandins Leukot Essent Fatty Acids* 2000, **63**:1-9.
  - 27 9. Goldman LS, Genel M, Bezman RJ, Slanetz PJ: **Diagnosis and treatment of**  
28 **attention-deficit/hyperactivity disorder in children and adolescents**. *JAMA*  
29 1998, **279**:1100-1107.
  - 30 10. Kidd PM: **Attention deficit/hyperactivity disorder (ADHD) in children:**  
31 **Rationale for its integrative management**. *Altern Med Rev* 2000, **5**:402-428.
  - 32 11. Andersen SL and Teicher MH: **Sex differences in dopamine receptors and**  
33 **their relevance to ADHD**. *Neurosci Biobehav Rev* 2000, **24**:137-141.
  - 34 12. Faraone SV: **The scientific foundation for understanding attention-**  
35 **deficit/hyperactivity disorder as a valid psychiatric disorder**. *Eur Child*  
36 *Adolesc Psychiatry* 2005, **14**:1-10.
  - 37 13. Biederman J, Mick E, Faraone SV: **Age-dependent decline of symptoms of**  
38 **attention deficit hyperactivity disorder: Impact of remission definition and**  
39 **symptom type**. *Am J Psychiatry* 2000, **157**:816-818.
  - 40 14. Richardson AJ and Puri BK: **The potential role of fatty acids in attention-**  
41 **deficit/hyperactivity disorder**. *Prostaglandins Leukot Essent Fatty Acids* 2000,  
42 **63**:79-87.
  - 43 15. Spencer T, Biederman J, Wilens T, Harding W, O'Donnell, D, Griffin S:  
44 **Pharmacotherapy of attention-deficit hyperactivity disorder across the life**  
45 **cycle**. *J Am Acad Child Adolesc Psychiatry* 1996, **35**:409-432.

- 1 16. Uauy R, Mena P, Rojas C: **Essential fatty acids in early life: Structural and**  
2 **functional role.** *Proc Nutr Soc* 2000, **59**:3-15.
- 3 17. Simopoulos AP, Leaf A, Salem Jr. N: **Workshop statement on the essentiality**  
4 **of and recommended dietary intakes for omega-6 and omega-3 fatty acids.**  
5 *Prostaglandins Leukot Essent Fatty Acids* 2000, **63**:119-121.
- 6 18. Petrie HJ, Stover EA, Horswill CA: **Nutritional concerns for the child and**  
7 **adolescent competitor.** *Nutrition* 2004, **20**:620-631.
- 8 19. Bjerve KS: **Omega 3 fatty acid deficiency in man: Implications for the**  
9 **requirement of alpha-linolenic acid and long-chain omega 3 fatty acids.**  
10 *World Rev Nutr Diet* 1991, **66**:133-142.
- 11 20. Mitchell EA, Aman MG, Turbott SH: **Clinical characteristics and serum**  
12 **essential fatty acid levels in hyperactive children.** *Clin Pediatr* 1987, **26**:406-  
13 411.
- 14 21. Stevens LJ, Zentall SS, Deck JL, Abate, ML, Watkins, BA, Lipp SR, Burgess JR:  
15 **Essential fatty acid metabolism in boys with attention deficit hyperactivity**  
16 **disorder.** *Am J Clin Nutr* 1995, **62**:761-768.
- 17 22. Stevens LJ, Zentall SS, Abate ML, Kuczek T, Burgess JR: **Omega-3 fatty acids**  
18 **in boys with behavior, learning, and health problems.** *Physiol Behav* 1996,  
19 **59**:915-920.
- 20 23. Richardson AJ and Puri BK: **A randomized double-blind, placebo-controlled**  
21 **study of the effects of supplementation with highly unsaturated fatty acids on**  
22 **ADHD-related symptoms in children with specific learning difficulties.** *Prog*  
23 *Neuropsychopharmacol Biol Psychiatry* 2002, **26**:233-239.
- 24 24. Stevens LJ, Zhang W, Peck L, Kuczek T, Grevstad N, Mahom A, Zentall SS,  
25 Arnold LE & Burgess JR: **EFA supplementation in children with inattention,**  
26 **hyperactivity, and other disruptive behaviors.** *Lipids* 2003, **38**:1007-1021.
- 27 25. Hirayama S, Hamazaki T, Terasawa K: **Effect of docosahexaenoic acid-**  
28 **containing food administration on symptoms of attention-**  
29 **deficit/hyperactivity disorder - a placebo-controlled double-blind study.** *Eur J*  
30 *Clin Nutr* 2004, **58**:467-473.
- 31 26. Young GS, Maharaj NJ, Conquer JA: **Blood phospholipid fatty acid analysis of**  
32 **adults with and without attention deficit/hyperactivity disorder.** *Lipids* 2004,  
33 **39**:117-123.
- 34 27. Young GS, Conquer JA, Thomas R: **Effect of randomized supplementation**  
35 **with high dose, flax or fish oil on serum phospholipid fatty acid levels in**  
36 **adults with attention deficit hyperactivity disorder.** *Reprod Nutr Dev* 2005,  
37 **45**:549-558.
- 38 28. Chen JR, Hsu SF, Hsu CD, Hwang LH, Yang SC: **Dietary patterns and blood**  
39 **fatty acid composition in children with attention-deficit hyperactivity**  
40 **disorder in Taiwan.** *J Nutr Biochem* 2004, **15**:467-472.
- 41 29. Conners CK: *Conners' Rating Scales - Revised: Technical Manual.* Toronto:  
42 Multi-Health Systems. 1997.
- 43 30. Arnold LE, Kleykamp D, Votolato NA, Taylor WA, Kontras, SB, Tobin K:  
44 **Gamma-linoleic acid for attention-deficit hyperactivity disorder: placebo-**  
45 **controlled comparison to D-amphetamine.** *Biol Psychiatry* 1989, **25**:222-228.

- 1 31. Voigt RG, Llorente AM, Jensen CL, Fraley JK, Berretta MC, Heird WC: (2001)  
2 **A randomized, double-blind, placebo-controlled trial of docosahexaenoic**  
3 **acid supplementation in children with attention-deficit/hyperactivity**  
4 **disorder.** *J Pediatr* 2001, **139**:189-196.
- 5 32. Fontani G, Corraleschi A, Felici F, Alfatti, S, Migliorini, Lodi L: **Cognitive and**  
6 **physiological effects of Omega-3 polyunsaturated fatty acid supplementation**  
7 **in healthy subjects.** *Eur J Clin Invest* 2005, **35**:691-699.
- 8 33. Zimmer L, Delpal S, Guilloteau D, Aioun J, Durand G, Chalon S: **Chronic n-3**  
9 **polyunsaturated fatty acid deficiency alters dopamine vesicle density in the**  
10 **rat frontal cortex.** *Neurosci Lett* 2000, **284**:25-28.
- 11 34. Delion S, Chalon S, Guilloteau D, Besnard JC, Durand G: **Alpha-linolenic**  
12 **dietary deficiency alters age-related changes of dopaminergic and**  
13 **serotonergic neurotransmission in the rat frontal cortex.** *J Neurochem* 1996,  
14 **66**:1582-1591.
- 15 35. United States Institute of Medicine: **Dietary reference intakes for Energy,**  
16 **Carbohydrate, Fiber, Fatty Acids, Cholesterol, Protein and Amino Acids.**  
17 <http://www.iom.edu/CMS/3788/21370.aspx> (accessed November 2005).
- 18 36. World Health Organization : **Population nutrient intake goals for preventing**  
19 **diet-related chronic diseases.**  
20 <http://www.who.int/dietphysicalactivity/publications/trs916/en/gsfao/overall.pdf>  
21 (accessed November 2005).
- 22 37. Murray MT and Pizzorno JT: *Encyclopedia of Natural Medicine*, 2nd ed., pp.  
23 946. Roseville, CA: Prima Publishing. 1998:946.
- 24 38. Kozielc T smf Starobrat-Hermelin B: **Assessment of magnesium levels in**  
25 **children with attention deficit hyperactivity disorder (ADHD).** *Magnes Res*  
26 1997, **10**:143-148.
- 27 39. Bornstein RA, Baker GB, Carroll A, King G, Wong JT, Douglass AB: **Plasma**  
28 **amino acids in attention deficit disorder.** *Psychiatry Res* 1990, **33**:301-306.
- 29 40. Aman MG, Mitchell EA, Turbott SH: **The effects of essential fatty acid**  
30 **supplementation by efamol in hyperactive children.** *J Abnorm Child Psychol*  
31 1987, **15**:75-90.
- 32 41. Richardson AJ, Calvin DM, Clisby C, Schoenheimer DR, Montgomery P, Hall JA,  
33 Hebb G, Westwood E, Talcott JB, Stein JF: (2000) **Fatty acid deficiency signs**  
34 **predict the severity of reading and related difficulties in dyslexic children.**  
35 *Prostaglandins Leukot Essent Fatty Acids* 2000, **63**:69-74.
- 36 42. **Canadian Pediatric Society: Breastfeeding.**  
37 <http://www.caringforkids.cps.ca/babie/breastfeeding.htm> (accessed November  
38 2005).
- 39 43. **Canadian Institutes of Health/Child & Family Canada: Supporting**  
40 **breastfeeding in childcare.** [http://www.cfc-efc.ca/docs/eccf/rs057\\_en.htm](http://www.cfc-efc.ca/docs/eccf/rs057_en.htm)  
41 (accessed November 2005).
- 42 44. Szatmari P, Boyle MH, Offord DR: **Familial aggregation of emotional and**  
43 **behavioural problems of childhood in the general population.** *Am J Psychiatry*  
44 1993, **150**:1398-1403.

- 1 45. Faraone SV, Perlis RH, Doyle AE, Smoller JW, Goralnick JJ, Holmgren MA,  
2 Sklar P: (2005) **Molecular genetics of attention-deficit/hyperactivity disorder.**  
3 *Biol Psychiatry* 2005, **57**:1313-1323.
- 4 46. Greenberg S: Ontario **Association of Peadiatricians - Attention deficit**  
5 **hyperactivity disorder.** <http://www.utoronto.ca/kids/index.htm#topics> (accessed  
6 September 2005).
- 7 47. Scerbo AS and Kolko DJ: **Salivary testosterone and cortisol in disruptive**  
8 **children: Relationship to aggressive, hyperactive, and internalizing behaviors.**  
9 *J Am Acad Child Adolesc Psychiatry* 1994, **33**:1174-1184.
- 10 48. Quinn P: **Treating adolescent girls and women with ADHD: gender-specific**  
11 **issues.** *J Clin Psychol* 2005, **61**:579-587.
- 12 49. Sawada H and Shimohama S: **Neuroprotective effects of estradiol in**  
13 **mesencephalic dopaminergic neurons.** *Neurosci Biobehav Rev* 2000, **24**:143-  
14 147.  
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1 Figure Legends:

2 **Figure 1.** Conners' Parent Rating Scale (CPRS:L) mean T scores (with mean standard  
3 error) for ADHD group versus controls. CPRS:L scales; A: Oppositional, B: Cognitive  
4 Problems/Inattention, C: Hyperactivity, D: Anxious-Shy, E: Perfectionism, F: Social  
5 Problems, G: Psychosomatic, H: Conners' ADHD Index, I: CGI Restlessness-Impulsive,  
6 J: CGI Emotional Lability, K: CGI Total, L: DSM-IV Inattentive, M: DSM-IV  
7 Hyperactive-Impulsive, N: DSM-IV Total.

8 ADHD group presented with significantly higher mean raw scores on Scales A-C and H-  
9 N, for a total of 10/14 scales.

10 Significantly different from control group \*\*  $p < 0.01$ , \*  $p < 0.04$ .

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12 **Figure 2.** Scatterplot of dietary fat intake (y-axis) and mean raw score on Conners'  
13 hyperactivity scale (x-axis). Significant positive correlation  $r = .606$ ,  $p = 0.003$ .

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15 **Figure 3.** Scatterplot of dietary iron intake (y-axis) and mean raw score on Conners'  
16 DSM-IV total scale (x-axis). Significant positive correlation  $r = .496$ ,  $p = 0.015$ .

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18 **Figure 4.** Scatterplot of red blood cell total n-6 content (y-axis) and dietary fat intake (x-  
19 axis). Significant positive correlation  $r = .552$ ,  $p = 0.007$ .

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21 **Figure 5.** Scatterplot of red blood cell total n-3 content (y-axis) and dietary fat intake.  
22 Significant negative correlation  $r = -.570$ ,  $p = 0.005$ .

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24 **Figure 6.** Scatterplot of red blood cell DHA content (y-axis) and Conners' DSM-IV total  
25 score (x-axis). Significant negative correlation  $r = -.378$ ,  $p = 0.037$ .

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4 **Table 1: Baseline subject characteristics and anthropometric measurements**

<b>Variable</b>	<b>ADHD Group (n=11) Mean ± SD</b>	<b>Control Group (n=12) Mean ± SD</b>
Sex, n male/female	9/2	6/6
Age, years ± SD	13.6 ± 2.2	14.2 ± 1.9
Age range, years	10.4-16.4	11.3-16.6
Height (cm)	163.5 ± 12.2	160.5 ± 8.4
Weight (kg)	52.7 ± 15.5	49.5 ± 12.2
BMI (kg/m <sup>2</sup> )	19.3 ± 3.8	19.0 ± 3.2
Lean weight (kg)	43.6 ± 16.4	38.3 ± 8.4
% lean mass	80.6	77.7
Fat weight (kg)	9.1 ± 3.6	11.2 ± 4.8
% fat mass	19.4	22.3
Systolic blood pressure (mmHg)	107.0 ± 17.7	109.6 ± 9.7
Diastolic blood pressure (mmHg)	66.6 ± 8.2	66.5 ± 7.1
Heart Rate (bpm)	66.5 ± 8.7	72.8 ± 14.4

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3 **Table 2: Behaviour Assessment Data**

<b>Conners' Rating Scale: Measurement</b>	<b>ADHD Group (n=11) Mean T Score ± SD (range)</b>	<b>Control Group (n=12) Mean T Score ± SD (range)</b>
A: Oppositional	66.6 ± 15.8 (44-90) *	53.8 ± 8.3 (39-66)
B: Cognitive Problems/Inattention	68.0 ± 9.7 (56-84) **	50.1 ± 7.5 (43-66)
C: Hyperactivity	73.8 ± 12.8 (49-90) **	53.8 ± 10.6 (44-77)
D: Anxious-Shy	59.8 ± 17.6 (42-90)	58.3 ± 14.4 (42-90)
E: Perfectionism	51.9 ± 14.5 (40-82)	51.6 ± 9.9 (41-70)
F: Social Problems	67.4 ± 15.7 (45-90)	56.9 ± 16.8 (45-90)
G: Psychosomatic	59.6 ± 14.7 (42-90)	57.9 ± 13.4 (42-83)
H: Conners' ADHD Index	73.2 ± 10.6 (55-90) **	51.2 ± 10.8 (42-79)
I: CGI Restless-Impulsive	74.7 ± 11.7 (54-89) **	52.4 ± 8.6 (43-70)
J: CGI Emotional Lability	64.1 ± 19.5 (42-90) *	49.3 ± 7.9 (41-65)
K: CGI Total	72.6 ± 13.5 (51-90) **	51.8 ± 9.1 (42-69)
L: DSM-IV Inattentive	71.6 ± 10.0 (58-90) **	50.7 ± 7.8 (43-69)
M: DSM-IV Hyperactive-Impulsive	76.0 ± 14.1 (48-90) **	53.7 ± 10.8 (43-71)
N: DSM-IV Total	76.2 ± 11.3 (55-90) **	52.1 ± 9.0 (42-70)

4 Significantly different from control subjects \*\* p<0.01, \* p<0.04

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2 **Table 3: Diet Record Analysis Data**

<b>Dietary Variable</b>	<b>ADHD Group (n=11)</b>	<b>Control Group (n=8)</b>	<b>p value</b>
<b>Total Energy (kcal)</b>	2651.0 ± 458.1 *	2051.4 ± 407.0	0.009
<b>Protein (g)</b>	90.9 ± 19.0 *	73.0 ± 17.0	0.049
<b>Carbohydrates (g)</b>	356.9 ± 68.1 *	271.1 ± 59.6	0.011
<b>Total Fat (g)</b>	99.4 ± 21.8 *	76.7 ± 13.6	0.013
Saturated Fat (g)	38.1 ± 9.1 *	28.3 ± 8.2	0.027
Monounsaturated Fat (g)	30.9 ± 8.6	24.1 ± 6.4	NS
Polyunsaturated Fat (g)	10.8 ± 4.0	9.7 ± 4.3	NS
<b>Total omega-3 (g)</b>	0.72 ± 0.31	0.76 ± 0.37	NS
Alpha Linolenic Acid (g)	0.66 ± 0.28	0.71 ± 0.36	NS
Eicosapentaenoic Acid (mg)	22 ± 54	14 ± 17	NS
Docosahexaenoic Acid (mg)	31 ± 53	39 ± 47	NS
<b>Total omega-6 (g)</b>	6.82 ± 3.79	6.86 ± 3.85	NS
Linoleic Acid (g)	6.78 ± 3.79	6.81 ± 3.84	NS
Arachidonic Acid (mg)	38 ± 19	51 ± 35	NS
<b>omega-6: omega 3</b>	9.47	9.03	NS
<b>Vitamin B1 (mg)</b>	1.8 ± 0.3 *	1.4 ± 0.4	0.019
<b>Vitamin B2 (mg)</b>	2.3 ± 0.7 *	1.5 ± 0.4	0.01
<b>Iron (mg)</b>	21.7 ± 7.0 *	12.5 ± 3.4	0.002
<b>Sodium (mg)</b>	4001 ± 931*	3119 ± 755	0.042
<b>Calcium (mg)</b>	1050 ± 282	798 ± 239	NS
<b>Zinc (mg)</b>	13 ± 5	9 ± 3	NS

3 Data represents daily consumption as averaged from 7-day diet records. Values are reported as  
4 mean ± SD. \* Significantly different from control group (p<0.05)

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1 **Table 4: Red Blood Cell Fatty Acid Analysis Data**

<b>Fatty Acid</b>	<b>ADHD Group (n=11) Mean ± SD</b>	<b>Control Group (n=12) Mean ± SD</b>
<b>Total omega-3 Fatty Acids</b>	5.79 ± 1.39 **	7.42 ± 1.64
Alpha-Linolenic Acid (18:3 n-3)	0.16 ± 0.07	0.13 ± 0.09
Eicosapentaenoic Acid (20:5 n-3)	0.51 ± 0.21	0.64 ± 0.24
Docosahexaenoic Acid (22:6 n-3)	3.12 ± 0.75 **	4.39 ± 1.34
<b>Total omega-6 Fatty Acids</b>	33.33 ± 1.83	32.51 ± 1.59
Linoleic Acid (18:2 n-6)	13.26 ± 0.95 *	12.02 ± 2.14
Gamma-Linoleic Acid (18:3 n-6)	4.54 ± 2.62	6.08 ± 2.91
Arachidonic Acid (20:4 n-6)	14.51 ± 1.67	14.73 ± 1.48
n-3: n-6 Ratio	0.17 ± 0.04 **	0.23 ± 0.06
n-6: n-3 Ratio	5.86 ± 2.04 *	4.64 ± 1.27
AA/EPA	31.61 ± 8.91	26.04 ± 10.27
Total Saturates	39.38 ± 1.79	39.26 ± 1.16
Total Monounsaturates	21.51 ± 1.15	20.81 ± 1.15
Total Polyunsaturates	39.11 ± 2.48	39.93 ± 1.76

2 Fatty acids reported as % molecular weight. Significantly different from control group \*\* p<0.02, \*  
3 p≤0.095.

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Figure 1.

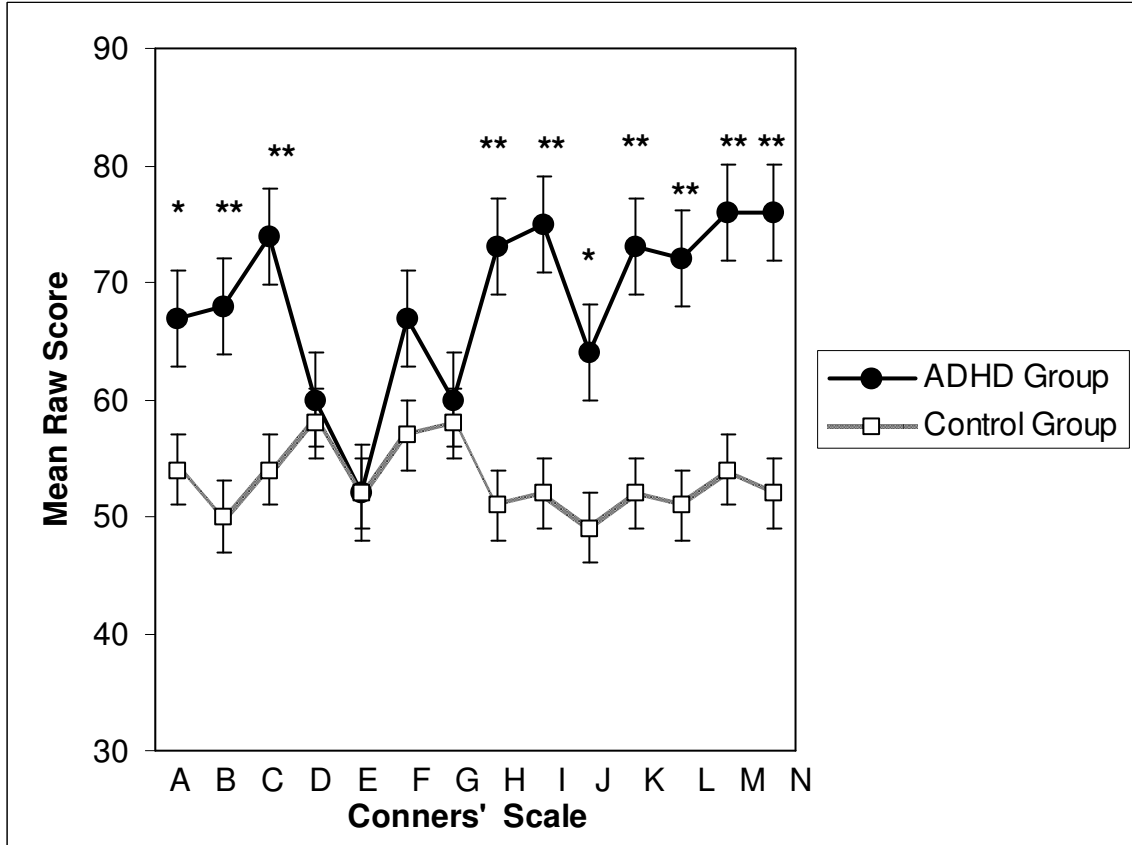




Figure 2.

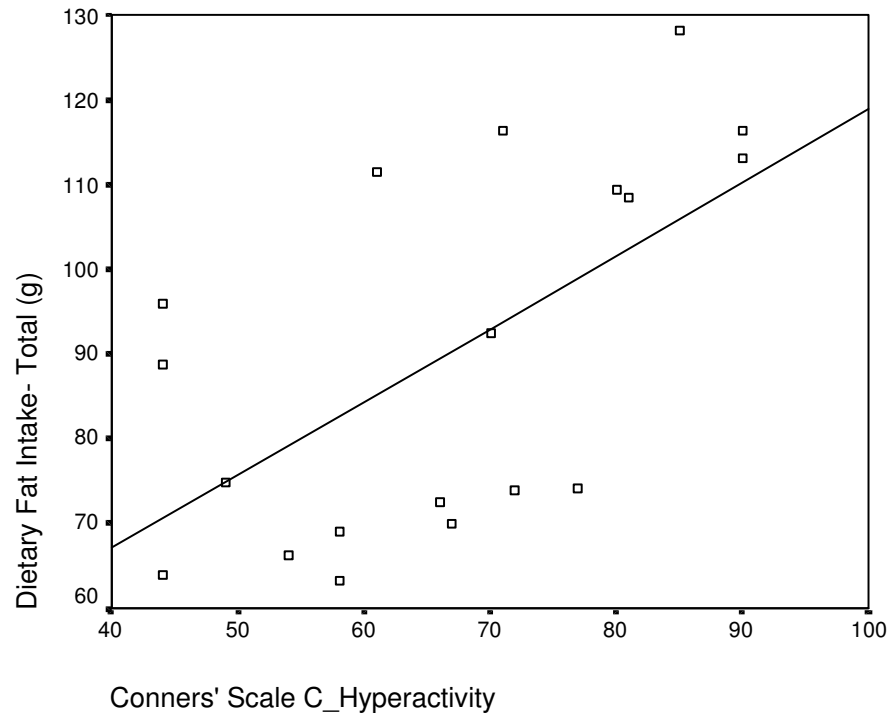




Figure 3.

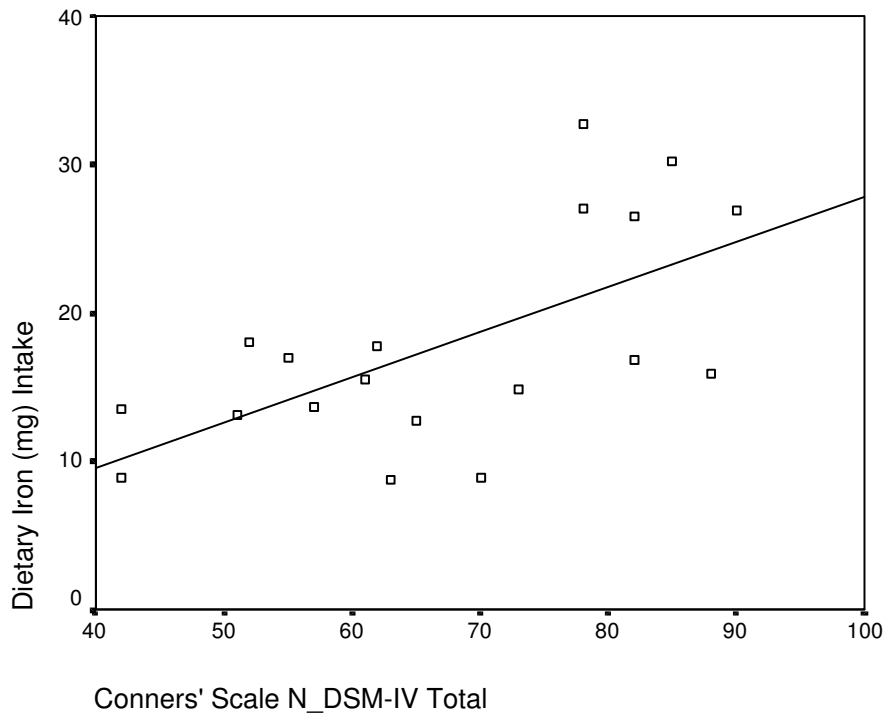


Figure 4.

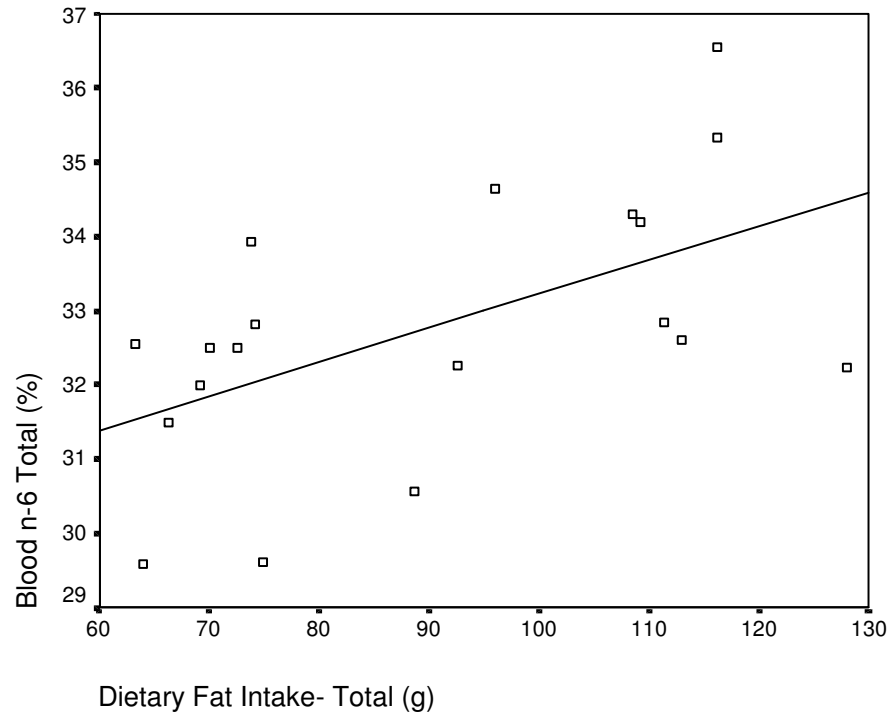


Figure 5.

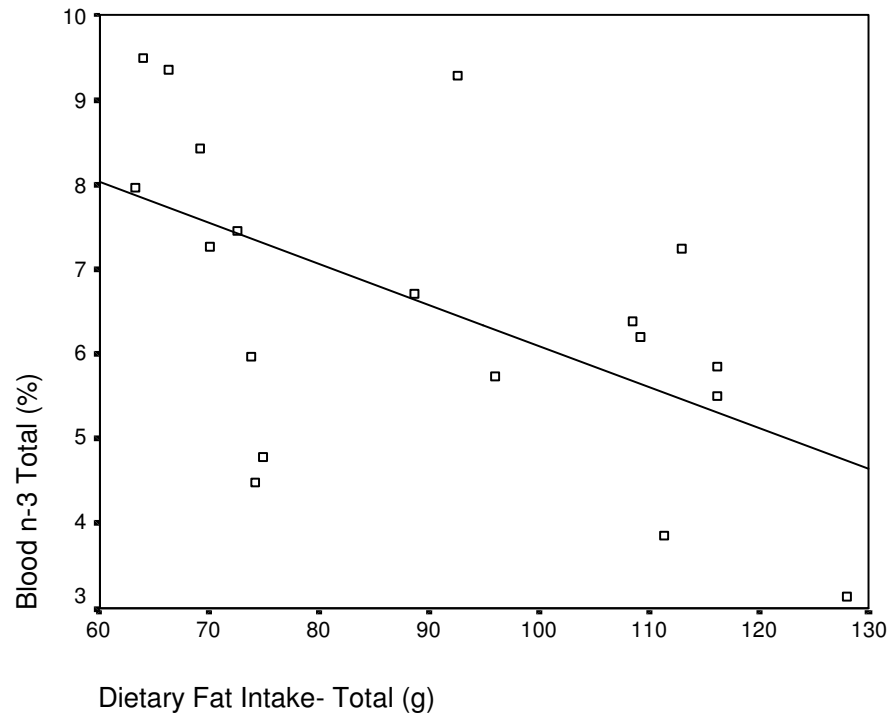


Figure 6.

