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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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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 Eleven ADHD adolescents and twelve age-matched controls were recruited through
13 newspaper ads, posters and a university website. ADHD diagnosis was confirmed by
14 medical practitioners according to DSM-IV criteria. Blood, dietary intake information as
15 well as 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 abnormal essential
25 fatty acid profiles that are often observed in younger children and distinctly different
26 from normal controls of similar age. Further these red blood cell fatty acid differences
27 are not explained by differences in intake. This suggests that there are metabolic
28 differences in fatty acid handling between ADHD adolescents and normal controls. The
29 value of omega-3 supplements to improve fatty acid profiles and possibly behaviours
30 associated 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” [1,2]. The American Psychiatric Association estimates that 3-5% of
6 school aged children have ADHD (DSM-IV), while other sources report higher
7 prevalence rates ranging from 5-13% [3-6,6]. ADHD is the most common psychiatric
8 disorder in children and is diagnosed in males two to nine times as often as in females.
9 ADHD shows high comorbidity with several other conditions including learning
10 differences, oppositional defiance disorder (ODD), obsessive compulsive disorder (OCD)
11 and depression[7,8] For up to 60% of these children, ADHD symptoms and difficulties
12 will persist into adulthood [9,10]

13 The cause of ADHD is generally acknowledged to be multifactorial, involving both
14 biological and environmental influence [2,11]. In the past two decades, there has been an
15 increasing focus particularly on the effects of diet in hyperactivity in children.
16 Researchers have reported that various aspects of a child’s diet including food additives,
17 refined sugars, food allergies, minerals and fatty acid metabolism may have adverse
18 effects on behaviour[7,12,13]. While there is no definitive proof that any of these is
19 responsible for the spectrum of ADHD symptoms, there is a compelling argument for a
20 role for long-chain polyunsaturated fatty acids.

21 The processes of elongation and desaturation occur mainly in the liver, but also in the
22 central nervous system, placenta, glial tissue and choroid plexus vasculature[14]. Within
23 the brain, four fatty acids are particularly important; dihomogammalinolenic acid (20:3n-
24 6, DGLA), arachidonic acid (20:4n-6, AA), eicosapentaenoic acid (20:5n-3, EPA) and
25 docosahexaenoic acid (22:6n-3). AA and DHA play a major structural role in neuronal
26 membranes and make up 20% of the dry mass of the brain[11]. In addition the
27 eicosanoid and other fatty acid metabolites of various LC-PUFAs, though at much lower
28 concentrations, could play important roles in brain function [15-19]. EPA and DGLA
29 play a more minor structural role but are also crucial for normal brain function. Since
30 optimal requirements are not fully known, definitive dietary reference intakes (DRIs) for
31 the omega-3 and omega-6 fatty acids have not yet been determined[20]. However, Petrie

1 and colleagues published recommendations for adequate intake (AI) for boys 9-16 yr as
2 12-16 g linoleic acid (LA)/d and 1.2-1.6 g α -linolenic acid (ALA)/d. For girls the
3 corresponding amounts were 10-11 LA g/d and 1.0-1.1 ALA g/d[21]. In order to ensure
4 the best biological functions, Bjerve suggests an intake of 900mg/d EPA and 400 mg/d
5 DHA[22].

6 A number of the physical and behavioural symptoms of essential fatty acid
7 deficiency mimic some of the symptoms described in typical ADHA patients, therefore it
8 is conceivable, that either dietary deficiency of omega-3 fatty acids, or altered metabolic
9 handling of these fatty acids, could contribute to the abnormalities observed in those
10 affected by ADHD. Several studies have examined fatty acid status in patients with
11 ADHD, but only recently have researchers begun to examine efficacy of high dose
12 supplementation on ADHD behaviours [13,23-25].

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

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2 **Experimental Methods**

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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 anxiety²⁹.

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 $p < 0.05$, using a two-tailed test. All statistical
2 analysis was performed using SPSS 10.0 for Windows Student Version. Statistical
3 analyses performed included independent samples t-test for means comparisons and bi-
4 variate Pearson's correlations. Data is reported as mean \pm standard deviation (SD), and
5 in some cases, followed by the range of values in parentheses. All data were normally
6 distributed and the differences in SDs between groups was not significantly different.

7 **RESULTS**

8 Subject characteristics at visit #1 are given in Table 1. There were no significant
9 differences between the groups for age and sex characteristics or anthropometric measures.

10 A health questionnaire was filled out at the start of the study by the parent/guardian for
11 each subject. The questionnaire collected information regarding ADHD diagnosis,
12 medication use, co-morbid disorders/conditions, vitamin/supplement use, family history
13 of ADHD, allergies, duration of breastfeeding and prevalence of fatty acid deficiency
14 symptoms. Six of eleven subjects in the ADHD group were taking medications (55%),
15 five of eleven presented with a co-morbid learning disorder (45%), and eight of eleven
16 reported a history of ADHD within the family (73%). These three variables were
17 significantly different from the control group with $p=0.016$, $p=0.016$ and $p=0.001$,
18 respectively.

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

28 *Behaviour Assessment*

29 Analysis of the Conners' Parent Rating Scales Long Version (CPRS:L) revealed several
30 significant differences between the ADHD and control groups (Table 2). When
31 compared to the control group, the ADHD group presented with significantly higher

1 mean T scores on ten of the fourteen scales included in the assessment ($p < 0.05$) (Figure
2 1). These included measures for oppositional behaviours, cognitive problems and
3 inattention, restlessness and impulsivity, hyperactivity, emotional lability and overall
4 problematic behaviour. Children were also identified as 'at risk' through scores on the
5 ADHD index, while also being assessed on scales directly related to DSM-IV criteria
6 including inattentive, hyperactive-impulsive and total DSM scores.

7

8 *Diet Analysis*

9 Analysis of seven-day dietary records from subjects (ADHD $n=11$, Control $n=8$)
10 revealed several differences between intake patterns (Table 3, not all data is shown). The
11 ADHD group consumed significantly more calories (2652 ± 458 vs. 2051 ± 407 ,
12 $p=0.009$), more protein ($91 \pm 19\text{g}$ vs. $73 \pm 17\text{g}$, $p=0.049$) and more carbohydrates ($357 \pm$
13 68g vs. $271 \pm 60\text{g}$, $p=0.011$) per day when compared to the control group. The ADHD
14 group also consumed significantly more total fat ($99 \pm 22\text{g}$ vs. $77 \pm 14\text{g}$, $p=0.013$),
15 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. $1.3 \pm$
16 0.7g , $p=0.038$) per day. There was also a trend toward higher monounsaturated fat
17 consumption in the ADHD group ($31 \pm 9\text{g}$ vs. $24 \pm 6\text{g}$), however this difference was not
18 significance ($p=0.077$). There were no significant differences in mean consumption of
19 total n-3 fatty acids, ALA, EPA or DHA between the two groups. Furthermore, there
20 were no differences in mean consumption of total n-6 fatty acids, LA or AA. Calculated
21 n-6: n-3 ratios revealed values of 9.47 in the ADHD group and 9.03 in the control group.
22 These ratios were not significantly different from one another.

23 The ADHD group consumed significantly greater amounts of vitamin B1 ($1.84 \pm$
24 0.34mg 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 ($21.66 \pm$
25 7.04mg vs. $12.52 \pm 3.43\text{mg}$) and sodium ($4001 \pm 931\text{mg}$ vs. $3119 \pm 756\text{mg}$) in their diets
26 when compared to controls ($p < 0.05$). There were also trends toward increased calcium
27 and zinc intake in the ADHD group when compared to the control group however, these
28 differences were not significant.

29 Pearson correlations between diet variables and CPRS:L scale rating revealed
30 several positive and significant relationships. Total energy intake was positively
31 correlated with scores for oppositional and hyperactive behaviours ($p < 0.01$), in addition

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

11 *Fatty Acid Analysis*

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

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

30 There were several significant correlations between specific red blood cell
31 parameters and Conners' scale ratings. Red blood cell DHA content was negatively

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

9 **Discussion**

10 Most clinical studies have focused on fatty acid and other abnormalities associated
11 with ADHD in children (6-12 years) and adult populations (18-65 years) [25-28]. One
12 recent study used a slightly older population where the average age of subjects was 11
13 years [24]and in the current study our subjects had a mean age of 14 years. The primary
14 objective of this study was to determine whether abnormalities typically observed in
15 younger and older populations are also observed in adolescents with ADHD when
16 compared to controls.

17 The Conners' Parent Rating Scale (CPRS:L) was utilized for behaviour
18 assessment in this study. The CPRS:L is well respected and has been used widely among
19 studies assessing ADHD behaviours in children [28-32]. The Conners' scale used
20 provides an appropriate instrument for measuring behaviours in youths aged 3 to 17 and
21 conveys information that corresponds to the official ADHD criteria in the DSM-IV[33].
22 In general, T-scores of 65 and above are usually taken to indicate a clinically significant
23 problem. In this study, the ADHD group presented with significantly higher mean T-
24 scores than the control group on ten of the fourteen scales on the CPRS:L. This
25 significant difference was expected as individuals were assigned to the ADHD group
26 based on whether or not they had been previously diagnosed by a physician as having the
27 disorder. It is interesting to note however, that the mean T-scores for the ADHD group
28 did not reach 65 or above on 4 of the 14 measures. Furthermore, there were subjects
29 within the ADHD group who did not present with 65 or higher on any of the scales.
30 Perhaps, these particular individuals have become better able to cope with their
31 symptoms over time, which contributed to the presence of less problematic behaviours.

1 It is also possible that some of these individuals were misdiagnosed with ADHD or “grew
2 out of it”. Regardless, had these potentially misdiagnosed individuals not been in the
3 ADHD group here, it is possible that there may have been more significant differences
4 between the groups. There were also subjects in the control group who had multiple
5 scales with T-scores of 65 or more. To this effect, one subject in the control group
6 presented with T-scores greater than 65 on 11 of 14 scales, the fifth most out of all 23
7 subjects in the study. This same individual was subsequently diagnosed with ADHD
8 shortly after this study ended. As such, all of the data relating to this subject was
9 transferred to the ADHD data pool.

10 As a group, the ADHD subjects in this study presented with mean T-scores that
11 are comparable to other studies [27-32], although slightly higher on most scales. The
12 higher T-scores in the current study may simply reflect the fact that similar behaviours in
13 a nine-year old would be given a much higher T score, if exhibited by a 13-year old.
14 The behaviour assessment performed here identified that the ADHD group had higher
15 mean T-scores than controls on over seventy-five percent of the Conners’ scales,
16 indicating that problematic behaviours persist into adolescence for the majority of these
17 individuals. The Conners’ Parent Rating Scale appears to be an extremely reliable tool
18 when comparing ADHD behaviours among individuals of different gender and age.

19 The red blood cell phospholipid fatty acid composition data reported here, indicates
20 that adolescent ADHD subjects have lower total omega-3 fatty acids, lower DHA levels
21 and higher linoleic acid levels when compared to age-matched control subjects. The
22 absolute values and degree of difference between groups are similar to those reported
23 elsewhere in studies of younger children and adults with and without ADHD[12,30,32,34]
24 However, we did not see several differences reported by Stevens, which included lower
25 AA and adrenic acid levels, and higher docosapentaenoic acid levels in ADHD subjects
26 when compared to controls[29]. Stevens did report lower DHA levels in the ADHD
27 group but this was not statistically significant in their study[29]. In 2004, Chen and
28 colleagues reported significantly lower levels of AA, LA, DHA and total n-3 in red blood
29 cell phospholipids of young children with ADHD when compared to controls[12].
30 Similarly, when fatty acid composition of red blood cells were compared in an adult
31 population, Young and her colleagues reported significantly lower DHA and significantly

1 higher total n-6 fatty acids among other measures, in the individuals with ADHD[34].
2 Thus, while there are some differences in the specific fatty acid changes, the data we
3 report here for adolescents indicates that childhood patterns persist through adolescence
4 and into adulthood with few alterations. In several other studies, authors have reported
5 higher AA/EPA ratios in subjects with ADHD. In our study there was a numerical
6 difference but this was not statistically different; a larger sample size may have been able
7 to pick up this difference as the standard deviation for this estimate was quite large. If
8 indeed the levels of AA and EPA are not different between groups, this would suggest
9 that delta-5 desaturase activity may be normal in the ADHD subjects. Rather, the fact
10 that DHA levels were significantly lower in ADHD subjects may suggest a higher rate of
11 oxidation of this fatty acid in these patients. None-the-less, a lower level of circulating
12 DHA may indicate lower levels in the brain as well.

13 Two previous studies reported dietary patterns in children with ADHD using three
14 day diet records[12,29]. Using a complete seven day diet record, we examined the
15 intake patterns of our adolescent subjects. We demonstrate that ADHD subjects
16 consumed higher levels of at least 10 different nutrients, than their control counterparts.
17 This included 25% more energy, and more grams of carbohydrate, total fat, omega-6 fatty
18 acids and trans fatty acids in addition to others. Stevens reported significantly higher
19 intakes of total fat (g) and polyunsaturated fatty acids (g) in ADHD subjects when
20 compared to control subjects[29]. In 2004, Chen and colleagues reported significantly
21 increased iron (mg) and vitamin C (mg) consumption in subjects with ADHD versus
22 controls[12]. As has been previously proposed, it is possible that over-ingestion of fats
23 could interfere with the conversion of parent essential fatty acids (EFAs) to long-
24 chain(LC)-PUFAs[11]. Despite the many dietary differences between our subject groups,
25 they did not differ in either total omega-3 fatty acid consumption, or consumption of the
26 LC-PUFAs. Thus, it is unlikely that dietary differences in omega-3 consumption can
27 explain the significant differences in red blood cell membrane fatty acids observed in the
28 ADHD subjects herein.

29 Whatever the reason for lower levels of DHA in ADHD subjects or abnormal LC-
30 PUFA status, an important question is whether this pattern can be normalized through
31 dietary intervention or supplementation. Early supplementation trials with modest levels

1 (eg. 300-500 mg DHA/day) of LC-PUFAs were largely neutral or showed only modest
2 improvement in biochemical or behavioural parameters in ADHD subjects[27,28,35,36].
3 However, two recent studies using very high levels of long chain omega-3 PUFAs
4 suggest that the plasma fatty acid abnormalities can be reversed and that there may be
5 significant impact on behavioural parameters [24,25]. Germano and coworkers examined
6 the effects of 0.23 g/kg body weight/day, fish-oil distillate containing a 2:1 ratio of
7 EPA:DHA in 16 ADHD and 31 control children after an 8-week supplementation
8 period[25]. The ADHD group had a significantly higher AA:EPA ratio than the controls
9 at time zero and the supplementation decreased both ratios substantially so that there was
10 no difference between the groups after 8 weeks supplementation. They also reported
11 significant improvements in Inattention and Hyperactivity using the Conner's short-
12 version inventory [25]. Sorgi and colleagues examined the effects of a high dose
13 (initially 16.2g) EPA/DHA concentrate on nine ADHD children over 8 weeks with
14 dosage adjustment at week 4 depending on AA:EPA ratio [24]. They showed a similar,
15 dramatic decrease in the AA:EPA ratio in the subjects and improvements using the
16 ADHD Symptom Checklist 4, and the short version of the Conners' Parents Rating
17 Scale{Sorgi, 2007 2845 /id. These two latest studies suggest that fatty acid
18 supplementation at high levels may be necessary to normalize blood fatty acids, and by
19 assumption brain fatty acids, to achieve improvements in ADHD symptomology. Clearly
20 additional large scale interventions are now warranted.

21 Regardless of diagnosis in the current study, dietary fat intake was significantly and
22 positively correlated with scores on five of the Conners' scales, positively correlated with
23 total n-6 red blood cell content and negatively correlated with total n-3 red blood cell
24 content. Also, as Chen and colleagues have reported, there was a significantly higher
25 intake of iron in the ADHD group{Chen, 2004 2816 /id}. In the present study, iron
26 intake also displayed a significant and positive correlation to scores on eight of the
27 Conners' scales. It is possible that iron levels in children and adolescents have an effect
28 on behaviours and should therefore be investigated in future studies. Reports have also
29 indicated that children with ADHD may be deficient in magnesium[7,37]. However, in
30 the present study, ADHD subjects reported slightly higher (but not significant) intakes of
31 magnesium when compared to controls. Previous research had also suggested lower

1 intakes of tyrosine, tryptophan and phenylalanine in individuals with ADHD compared to
2 controls[38] however, this was not the case in the current study where again, if anything
3 intakes for these amino acids were higher in ADHD subjects. From these data, a clear
4 role for minerals and amino acids in ADHD behaviours can not be established and will
5 require further investigation.

6

7 **Conclusions**

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

21

22

- 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

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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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32

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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 ** $p < 0.01$, * $p < 0.04$.

11

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

14

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 $r = .496$, $p = 0.015$.

17

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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Table 1: Baseline subject characteristics and anthropometric measurements

Variable	ADHD Group (n=11) Mean ± SD	Control Group (n=12) Mean ± SD
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 ²)	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**

Conners' Rating Scale: Measurement	ADHD Group (n=11) Mean T Score ± SD (range)	Control Group (n=12) Mean T Score ± SD (range)
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**

Dietary Variable	ADHD Group (n=11)	Control Group (n=8)	p value
Total Energy (kcal)	2651.0 ± 458.1 *	2051.4 ± 407.0	0.009
Protein (g)	90.9 ± 19.0 *	73.0 ± 17.0	0.049
Carbohydrates (g)	356.9 ± 68.1 *	271.1 ± 59.6	0.011
Total Fat (g)	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
Total omega-3 (g)	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
Total omega-6 (g)	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
omega-6: omega 3	9.47	9.03	NS
Vitamin B1 (mg)	1.8 ± 0.3 *	1.4 ± 0.4	0.019
Vitamin B2 (mg)	2.3 ± 0.7 *	1.5 ± 0.4	0.01
Iron (mg)	21.7 ± 7.0 *	12.5 ± 3.4	0.002
Sodium (mg)	4001 ± 931*	3119 ± 755	0.042
Calcium (mg)	1050 ± 282	798 ± 239	NS
Zinc (mg)	13 ± 5	9 ± 3	NS

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

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

	ADHD (n=11)	Control (n=12)
Total omega-3 Fatty Acids	5.79 ± 1.39 †	7.42 ± 1.64
Alpha-Linolenic Acid (18:3 n-3)	0.16 ± 0.07	0.13 ± 0.09
Stearidonic Acid (18:4 n-3)	0.07 ± 0.04	0.07 ± 0.04
20:3 n-3	0.02 ± 0.01	0.02 ± 0.01
Eicosatetraenoic Acid (20:4 n-3)	0.05 ± 0.05	0.05 ± 0.04
Eicosapentaenoic Acid (20:5 n-3)	0.51 ± 0.21	0.64 ± 0.24
Docosapentaenoic Acid (22:5 n-3)	1.88 ± 0.71	2.03 ± 0.56
Docosahexaenoic Acid (22:6 n-3)	3.12 ± 0.75 †	4.39 ± 1.34
Total omega-6 Fatty Acids	33.33 ± 1.83	32.51 ± 1.59
Linoleic Acid (18:2 n-6)	13.26 ± 0.95 *	12.02 ± 2.14
Gamma-Linolenic Acid (18:3 n-6)	0.05 ± 0.03	0.06 ± 0.03
Eicosadienoic Acid (20:2 n-6)	0.15 ± 0.07	0.14 ± 0.09
Dihomo-gamma-linolenic Acid (20:3 n-6)	1.69 ± 0.20 *	1.84 ± 0.51
Arachidonic Acid (20:4 n-6)	14.51 ± 1.67	14.73 ± 1.48
Docosadienoic Acid (22:2 n-6)	0.05 ± 0.03	0.04 ± 0.06
Adrenic Acid (22:4 n-6)	3.61 ± 1.33	3.63 ± 0.72
Docosapentanoic Acid (22:5 n-6)	0.03 ± 0.03	0.07 ± 0.01
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 Saturated Fatty Acids	39.38 ± 1.79	39.26 ± 1.16
Total Monounsaturated Fatty Acids	21.51 ± 1.15	20.81 ± 1.15
Total Polyunsaturated Fatty Acids	39.11 ± 2.48	39.93 ± 1.76

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3 Values are means +/- standard deviation and given as a mol % of total fatty acids. †

4 Significant difference between groups (p<0.02), * Difference between groups (p≤0.095)

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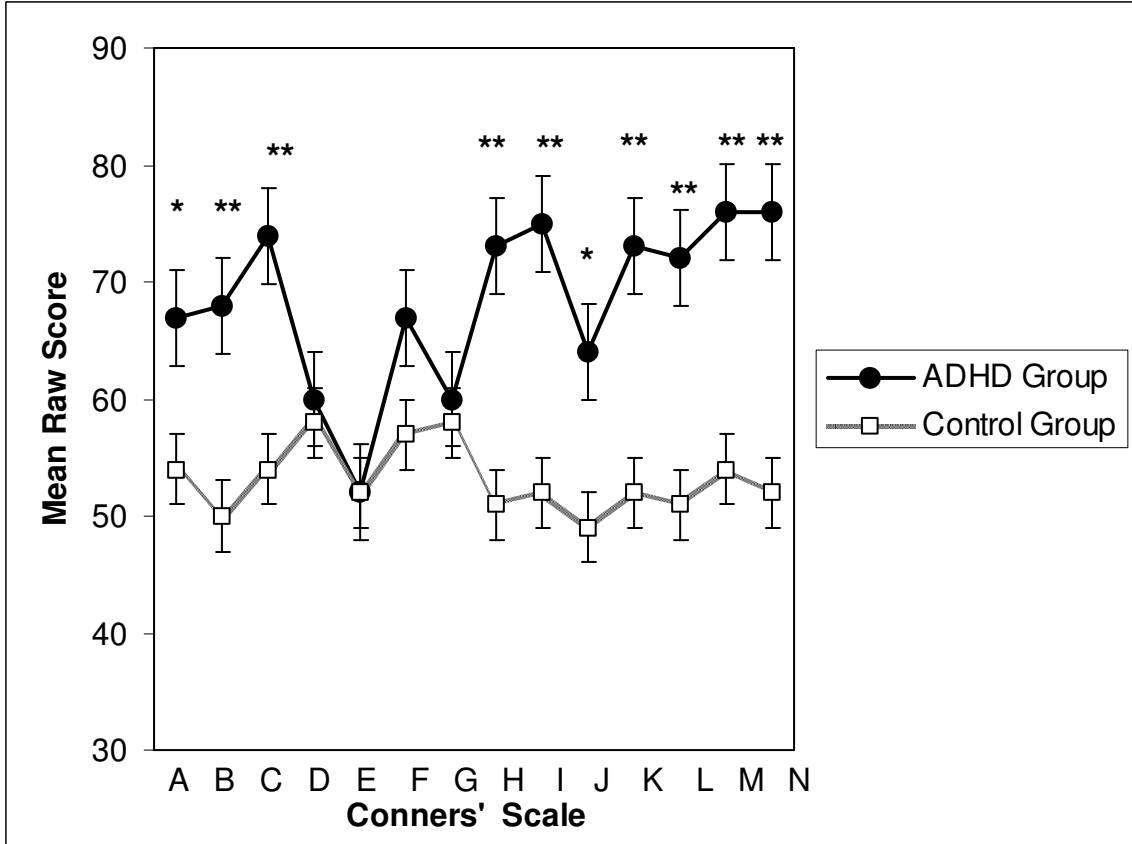


Figure 1.

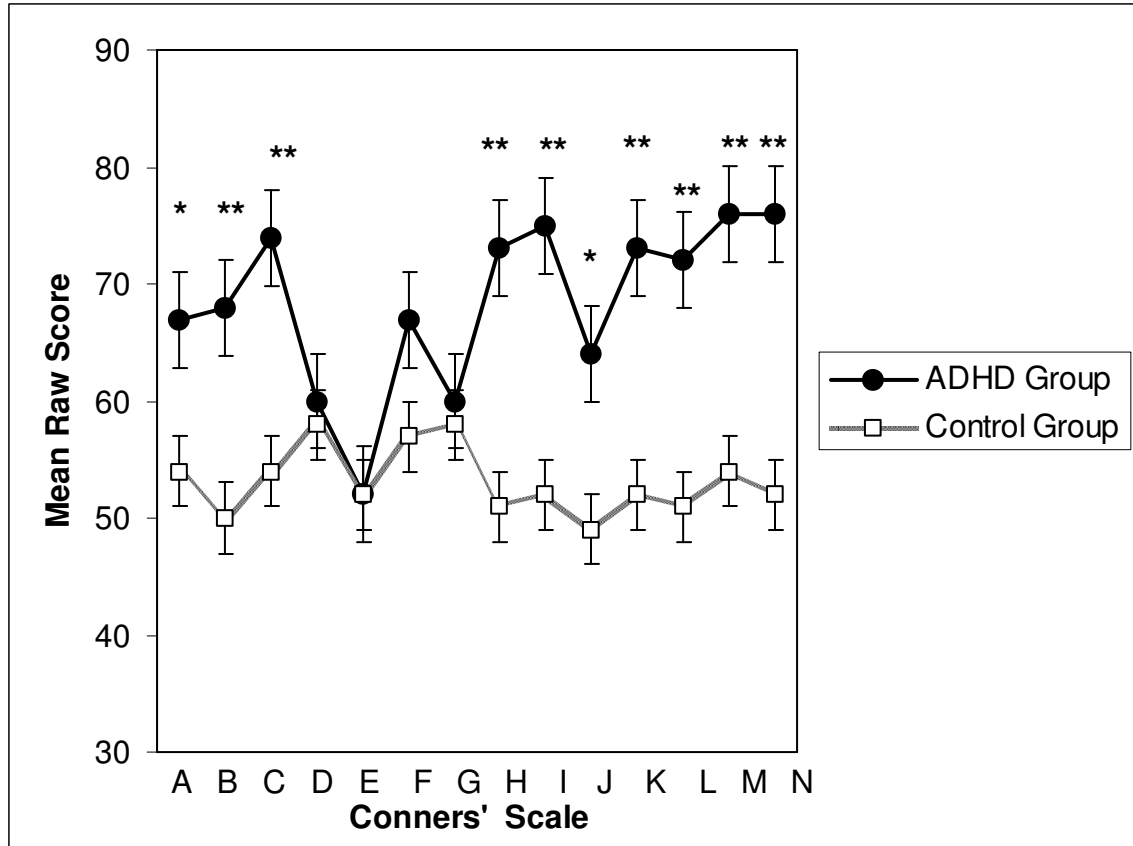


Figure 2.

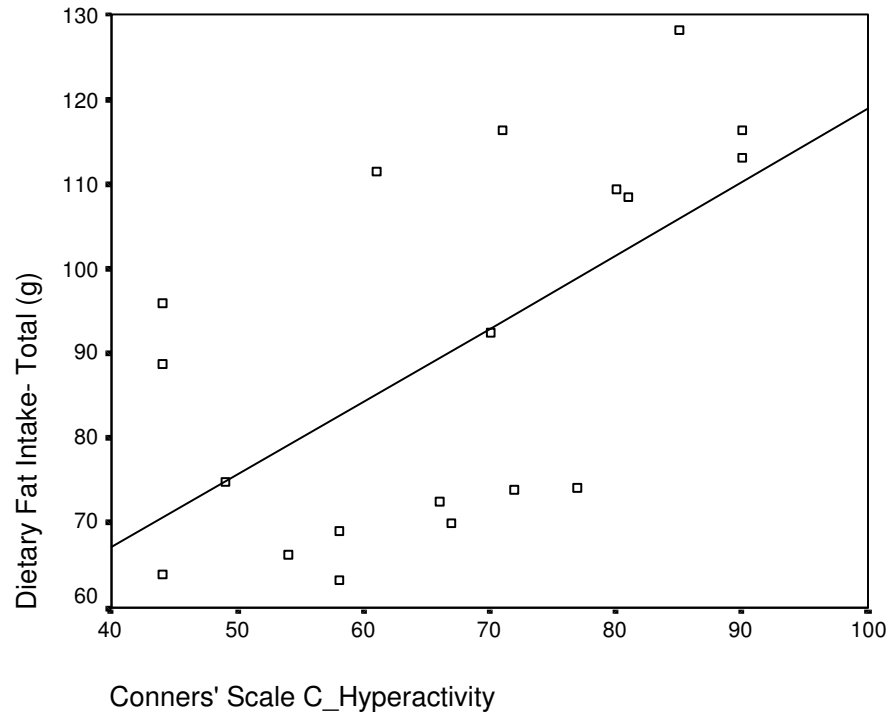


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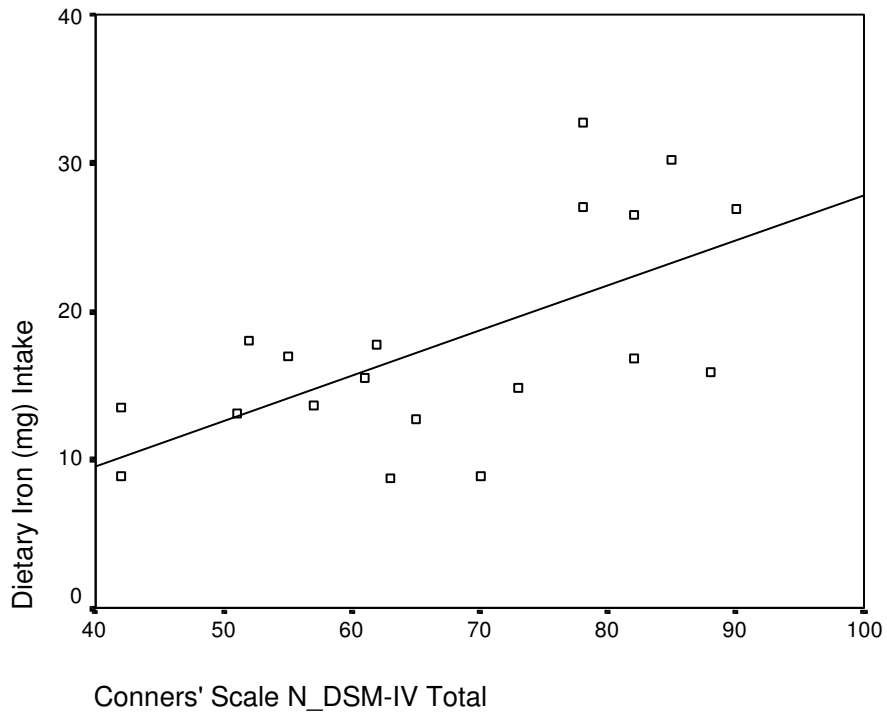


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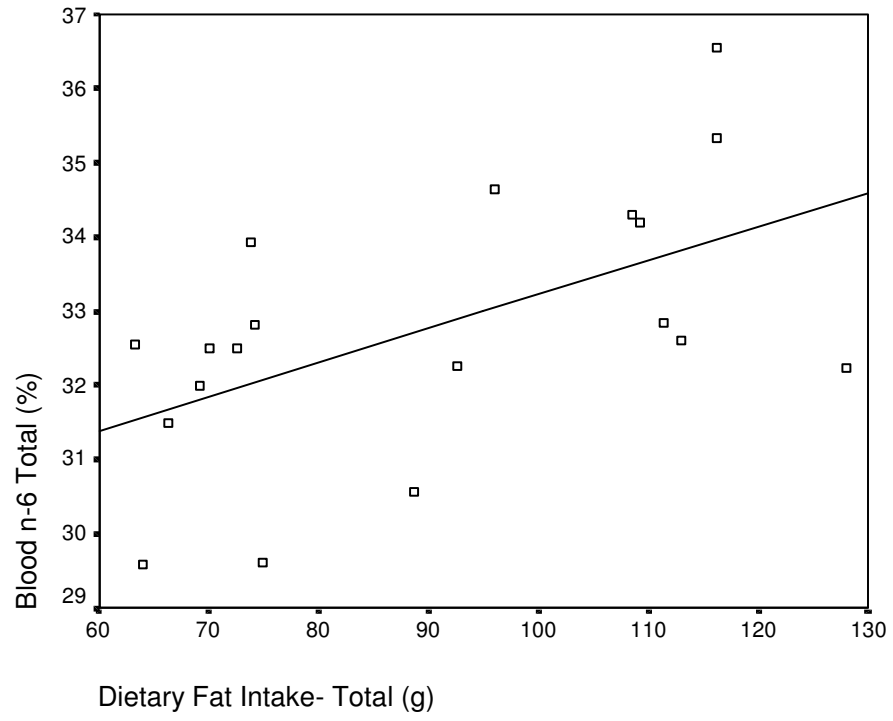


Figure 5.

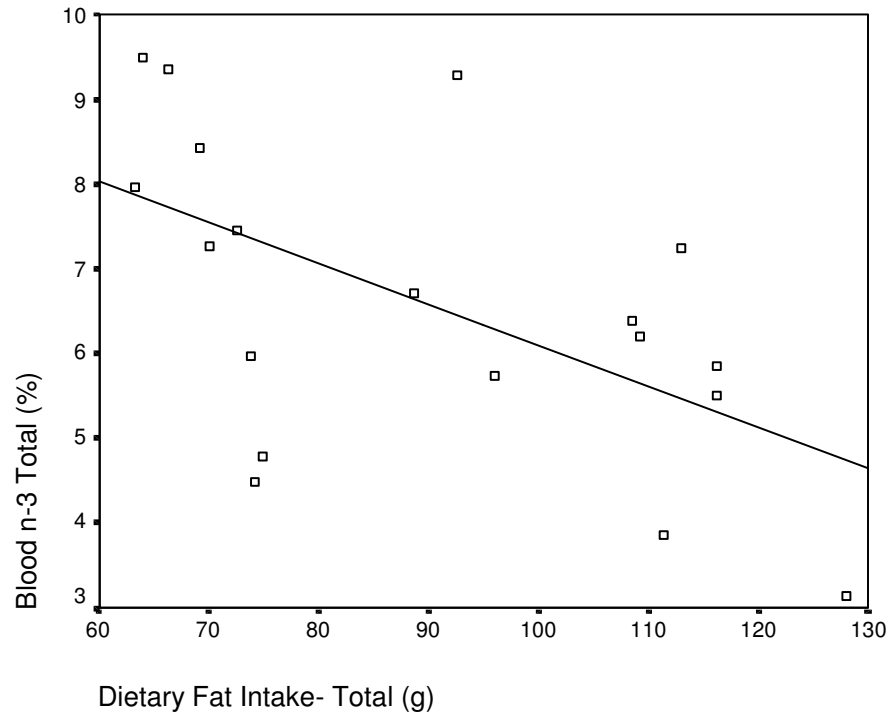


Figure 6.

