OMEGA-3 FATTY ACIDS AND COGNITIVE OUTCOMES IN SOLDIERS DEPLOYED TO COMBAT AREAS

by

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B.S., University of Minnesota, 1989
M.S., University of Nevada, Reno, 2000

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Human Nutrition
College of Human Ecology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2011

Abstract

Psychological problems and human error are leading causes of death and disability among military service members. Strategies to improve the psychological health and cognitive performance of those in the military are much needed. Recent advances in neuroscience suggest that omega-3 fatty acids may play an important role in the psychological well-being of those in the military. The purpose of this research was to explore the relationship between omega-3 status and psychological outcome variables among soldiers deploying to combat. Data collection was preceded by the development and reliability testing of a novel food frequency questionnaire (FFQ) designed to capture intake from contemporary sources of omega-3 fatty acids including functional foods and supplements. Based on the instrument assessment study (Chapter 2) conducted among university students ($n = 165$), this FFQ appears to be a comprehensive and reliable ($n = 54$, $\rho = 0.86$, $p < 0.001$) instrument for measuring docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) intakes in young adults. As described in Chapter 3, intake of EPA + DHA as estimated by the FFQ was positively correlated ($r = 0.39$, $p < 0.001$) with biomarker measurements of omega-3 status.

Primary data were obtained from a volunteer sample of soldiers ($n = 272$) scheduled for deployment to Iraq. Preliminarily analyses revealed relationships between attention deficit hyperactivity disorder (ADHD) screening scores and psychological outcome variables (Chapter 4). Primary analyses (Chapter 5) indicated intake of EPA + DHA was not significantly correlated with mood, nor were omega-3 exposure variables correlated with cognitive performance based on the required p value (< 0.001) calculated using the Bonferroni correction for multiple tests. Among participants with EPA + DHA intakes at or below the median, omega-3 HUFA was related ($p \leq 0.002$) to happiness ($\beta = -0.46$), depression ($\beta = 0.44$), and fatigue ($\beta = 0.43$). Although exploratory in nature, the results of this study suggest a relationship between omega-3 fatty acids and mood. Given the current concerns regarding the psychological health of those in the military, additional research is warranted.

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Lastly, I would like to thank the military leadership and the soldiers for their support and participation.

Dedication

To the men and women who have so proudly served in defense of our nation. May we always remember your sacrifices.

Chapter 1 - Introduction

As US military operations continue in Afghanistan and Iraq, the toll on the psychological health of those in the military has grown. Depression, posttraumatic stress disorder (PTSD) and traumatic brain injury (TBI) are serious health problems common among soldiers returning from deployments in Afghanistan and Iraq (1, 2). Rising suicide rates among soldiers and veterans have also raised concerns (3). At the same time, and despite remarkable technological advances, human cognitive factors remain a major limiting factor in current military operations (4, 5). The operational limitations imposed by human factors are well evidenced by the fact that human error accounts for approximately 80-85% of all accidents in the military (5).

Taken together, psychological health problems and human error are leading causes of death and disability among service members. Strategies to improve the psychological health and cognitive performance of those in the military are much needed. The growing concerns about the psychological health of service members, combined with recent advances in neuroscience, have led researchers and clinicians to believe that omega-3 fatty acids may be an important new discovery in military health. Research regarding the role of omega-3 fatty acids during pregnancy and infancy is well established (6). Similarly, research into the relationship between cognitive health and omega-3 fatty acids is plentiful (7). However, until very recently (8), the question of whether omega-3 fatty acids could be of benefit to military service members was not considered. Despite the vast potential, very little research has been conducted to explore the role of omega-3 fatty acids in military populations (8).

Omega-3 fatty acids are naturally occurring fats found in a variety of foods such as canola oil, eggs, and fish. They are also ingested in the form of supplements and as constituents of omega-3 enriched functional foods. In the US, the estimated median dietary intake of the long-chain omega-3 fatty acids (9) is below optimal levels (10). However, most large government nutrition studies (e.g. NHANES) are not inclusive of the military, and in a recent study of dietary supplement use in the military (11), fish oil was reported as "other" thus preventing the establishment of any firm conclusions about omega-3 supplement use in this population. US military service members consume food from a variety of military-affiliated (dining facilities, commissary, feeding rations) and non-military-affiliated (e.g. grocery stores, restaurants, convenience stores) sources. With regard to omega-3 fatty acids, the food environment is rapidly changing. Omega-3 enriched products such as dairy foods and juices are continually being added to the market. Sushi kiosks are appearing in both grocery stores and military commissaries, and omega-3 supplement use is common. Although military feeding rations are relatively low in omega-3 fatty acids (12), they comprise only one part of the picture and it would be incorrect to make assumptions regarding intake or omega-3 status based solely on rations.

The purpose of this dissertation was to explore the relationship between omega-3 status and psychological outcome variables among soldiers deploying to combat. Measures of omega-3 status included: a) eicosapentaenoic aid (EPA) + docosahexaenoic acid (DHA) intake, b) the percent of omega-3 highly unsaturated fatty acids (HUFA) in total HUFA of whole blood (13), and c) the percent of DHA in total fatty acids of whole blood. Psychological outcomes included cognitive performance and mood as assessed using the Combat Stress Assessment (CSA) (14), the Controlled Oral Word Association Test (COWT) (15), and the Hopkins Verbal Learning Test-Revised (HVLT-R) (16). To control for intervening variables, data were collected regarding demographic characteristics, height, weight, alcohol use, smoking status, and prior military deployments. Because of the association between attention deficient hyperactivity disorder (ADHD) and psychological outcomes (17-19), as well as the association between ADHD and long-chain polyunsaturated fatty acids (20, 21), the World Health Organization Adult ADHD Self-Report Scale (ASRS) Screener (22) was utilized in order to explore the relationship between reported frequency of ADHD symptoms and outcome variables.

An exhaustive search of the literature and a query of the American Dietetic Association Research Dietetic Practice Group members failed to produce a FFQ (food frequency questionnaire) designed specifically to measure EPA and DHA intake in US adults. Therefore, this research project began with the development and reliability testing of an appropriate FFQ. Using expert feedback and the literature as a guide, modifications were made to a preexisting FFQ used to measure fish consumption, omega-3 rich foods, and supplements (23). Items to capture intake of EPA and DHA from functional foods, supplements, and contemporary sources (i.e. sushi) were included. Additional items to capture intake from non-marine sources such as eggs and poultry have also been added. The clarity, completeness, and repeatability of the questionnaire were evaluated, and a manuscript (Chapter 2) based on this work is currently under review. Chapter 3 describes the relationship between intake of EPA + DHA as estimated by the FFQ and biomarker measurement of omega-3 status.

Primary data were obtained from a volunteer sample of soldiers (n = 272) scheduled for deployment to Iraq. Participants were from one of two combat brigades. One was a brigade combat team (BCT) from the Texas National Guard and the other was an active duty brigade from Kansas. Data were collected between October 2009 and February 2010. Due to the operational constraints of the military, not all participants were able to complete all components of the study. As a result, subsamples were used to explore the relationship between omega-3 exposure variables and outcome variables.

Preliminarily analyses revealed relationships between attention deficit hyperactivity disorder (ADHD) screening scores and psychological outcome variables (Chapter 4). Results of the primary analyses (Chapter 5) indicate intake of EPA + DHA was not significantly correlated with mood, nor were omega-3 exposure variables correlated with cognitive performance based on the p value (≤ 0.001) corrected for multiple tests. However, among participants with EPA + DHA intakes at or below the median, omega-3 HUFA was related to scores on several of the mood scales.

Although not presented as a component of my dissertation, post-deployment data were collected from the returning soldiers. This data will be used to explore the relationship between omega-3 status and psychological outcomes following military deployment. It will also allow for the measurement of any observable changes in omega-3 status and outcomes variables.

Prior to the completion of this research, very little was known about the dietary intake of EPA and DHA in the military. Although exploratory in nature, the results presented here appear promising and will provide a foundation for future research. In addition to being a concern for those in the US military, psychological well-being is an important health concern in other populations as well. Each year in the US an estimated 1.4 million individuals sustain a TBI (24). Age-related cognitive health issues are also a major public health concern. With regard to Alzheimer's disease alone, the prevalence rate is 19% for 75-84 year olds and 42% for those 85 years old and older (25). In this regard, the results presented here are relevant to the general population and advance the body of knowledge related to the relationship between omega-3 status and psychological well-being.

Disclosures

The research conducted for this dissertation was approved by the Kansas State University Institution Review Board (IRB; Appendix A). In addition, prior to beginning data collection with the military, the study protocol and numerous supporting documents were submitted to the Office of the Surgeon General, Army Human Research Protections Office for review. The support documents included a letter certifying scientific review had taken place, the assurance and IRB registration information for the Kansas State University IRB, copies of the IRB approval letter and the informed consent document, verification that all key research team members had completed human research ethics training, a statement from the Military Affairs Office at Kansas State University, and a letter from the University Research Compliance Office stating that Kansas State University and the IRB have agreed to comply with all applicable federal, Department of Defense and Army regulations for the protection of human research subjects.

In addition, approval was obtained from the Texas National Guard leadership as well as from the commanders of each of the combat brigades to which the soldiers belonged.

It should also be noted that the opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the US Army and/or the US Department of Defense.

References

1. Milliken CS, Auchterlonie JL, Hoge CW. Longitudinal assessment of mental health problems among active and reserve component soldiers returning from the Iraq war. JAMA. 2007;298(18):2141-8.
2. Schneiderman AI, Braver ER, Kang HK. Understanding sequelae of injury mechanisms and mild traumatic brain injury incurred during the conflicts in Iraq and Afghanistan: persistent postconcussive symptoms and posttraumatic stress disorder. Am J Epidemiol. 2008;167(12):1446-52.
3. Kuehn BM. Military probes epidemic of suicide: mental health issues remain prevalent. JAMA. 2010;304(13):1427, 9-30.
4. Wesensten N, Belenky, G, Balkin, T. Cognitive readiness in network-centric operations. Parameters. 2005;Spring:94-105.
5. Warfighter Judgment and Decision Making. Military Operational Medicine Research Program 2008; Available from: https://www.momrp.org/cpa1.html.
6. Ryan AS, Astwood JD, Gautier S, Kuratko CN, Nelson EB, Salem N, Jr. Effects of long-chain polyunsaturated fatty acid supplementation on neurodevelopment in childhood: a review of human studies. Prostaglandins Leukot Essent Fatty Acids. 2010;82(4-6):305-14.
7. Solfrizzi V, Frisardi V, Capurso C, D'Introno A, Colacicco AM, Vendemiale G, et al. Dietary fatty acids in dementia and predementia syndromes: epidemiological evidence and possible underlying mechanisms. Ageing Res Rev. 2010;9(2):184-99.
8. Erdman J, Oria M, Pillsbury L, Editors; Committee on Nutrition, Trauma, and the Brain; Institute of Medicine. Nutrition and Traumatic Brain Injury: Improving Acute and Subacute Health Outcomes in Military Personnel: The National Academies Press; 2011.
9. Institute of Medicine. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids. Washington, DC: National Academies Press; 2005.
10. Kris-Etherton PM, Innis S, American Dietetic Association, Dietitians of Canada. Position of the American Dietetic Association and Dietitians of Canada: dietary fatty acids. J Am Diet Assoc. 2007;107(9):1599-611.

11. Lieberman HR, Stavinoha TB, McGraw SM, White A, Hadden LS, Marriott BP. Use of dietary supplements among active-duty US Army soldiers. Am J Clin Nutr. 2010;92(4):985-95.

12. Samueli Institute, Defence Advanced Research Projects Agency, and National Institute on Alcohol Abuse and Alcoholism. Nutritional Armor for the Warfighter: Can Omega-3 Fatty Acids Enhance Stress Resilience, Wellness, and Military Performance? (Workshop Day 2) http://videocast.nih.gov/summary.asp?file=15353

13. Stark KD. The percentage of n-3 highly unsaturated fatty acids in total HUFA as a biomarker for omega-3 fatty acid status in tissues. Lipids. 2008;43(1):45-53.

14. Elsmore TF, Reeves DL, Reeves AN. The ARES test system for palm OS handheld computers. Arch Clin Neuropsychol. 2007;22 Suppl 1:S135-44.

15. desRosiers G, Kavanagh D. Cognitive assessment in closed head injury: Stability, validity and parallel forms for two neuropsychological measures of recovery. International Journal of Clinical Neuropsychology. 1987;9(4):162-73.

16. Brandt J. The Hopkins Verbal Learning Test-Revised. Odessa, Fla: Psychological Assessment Resources (PAR); 1991.

17. Wilens TE, Faraone SV, Biederman J. Attention-deficit/hyperactivity disorder in adults. JAMA. 2004;292(5):619-23.

18. Fayyad J, De Graaf R, Kessler R, Alonso J, Angermeyer M, Demyttenaere K, et al. Cross-national prevalence and correlates of adult attention-deficit hyperactivity disorder. Br J Psychiatry. 2007;190:402-9.

19. Yudofsky SC, Hales RE, editor. Neuropsychiatry and Behavioral Neuroscience. Washington, DC: American Psychiatric Publishing; 2008.

20. Colter AL, Cutler C, Meckling KA. Fatty acid status and behavioural symptoms of attention deficit hyperactivity disorder in adolescents: a case-control study. Nutr J. 2008;7:8.

21. Antalis CJ, Stevens LJ, Campbell M, Pazdro R, Ericson K, Burgess JR. Omega-3 fatty acid status in attention-deficit/hyperactivity disorder. Prostaglandins Leukot Essent Fatty Acids. 2006;75(4-5):299-308.

22. Kessler RC, Adler LA, Gruber MJ, Sarawate CA, Spencer T, Van Brunt DL. Validity of the World Health Organization Adult ADHD Self-Report Scale (ASRS) Screener in a representative sample of health plan members. Int J Methods Psychiatr Res. 2007;16(2):52-65.

23. Mina K, Fritschi L, Knuiman M. A valid semiquantitative food frequency questionnaire to measure fish consumption. Eur J Clin Nutr. 2007;61(8):1023-31.

24. Langlois JA, Rutland-Brown W, KE T. Traumatic Brain Injury in the United States: Emergency Department Visits, Hospitalizations, and Deaths. 2006 May 30, 2011: Available from:
http://www.cdc.gov/ncipc/pub-res/tbi_in_us_04/tbi%20in%20the%20us_jan_2006.pdf.

25. Alzheimer's, Association. Every 72 seconds someone in America develops Alzheimer's.2007 May 30, 2011: Available from:
http://www.alz.org/national/documents/Report_2007FactsAndFigures.pdf.

Chapter 2 - Clarity, Completeness and Reliability of a Food Frequency Questionnaire to Measure Eicosapentaenoic Acid and Docosahexaenoic Acid Intakes in Young Adults

Now under revision as Hanson, J. A., Rosenkranz, R. R., Holcomb, C. A., Haub, M. D. Clarity, completeness and reliability of a food frequency questionnaire to measure eicosapentaenoic acid and docosahexaenoic acid intakes in young adults. J Human Nutr Dietetics. (formatted to meet journal guidelines)

Abstract

Background: Research on the health effects of omega-3 fatty acids is hindered by the lack of a tool specifically designed to measure intakes of long-chain omega-3 fatty acids in US populations. The purpose of this study was to assess the clarity, completeness, and reliability of a 14-item food frequency questionnaire (FFQ) designed to measure eicosapentaenoic aid (EPA) and docosahexaenoic acid (DHA). Methods: During May of 2009, college students (n = 165) completed the FFQ and provided feedback. Forty-five completed the questionnaire a second time allowing for the evaluation of test-retest reliability. Results: None of the students reported consuming a food rich in EPA or DHA that was not included in the FFQ. Uncertainty regarding omega-3 functional foods was listed as a reason by eight of the twelve who felt one or more of the questions were difficult to answer. Students reported that the amount of fish they ate changed based on the season (18%; n = 30), religious or cultural practices (20%; n = 33), and the school year (40%; n = 66). Overall instrument reliability (n = 54) was strong ($\rho = 0.86$, $p < 0.001$). Reliability for each of the non-functional food items ranged from moderate to strong ($\rho = 0.48$ to 0.86, $p < 0.001$). Conclusions: Overall reliability of the FFQ is good. Season, cultural and religious practices, and the school year appear to influence fish consumption and should be considered when measuring omega-3 fatty acids. Unfamiliarity regarding omega-3 functional foods may be problematic in this population.

Introduction

The omega-3 fatty acids have become some of the most widely studied nutrients of the decade. Long-chain omega-3 fatty acids, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been linked to an array of favorable health outcomes in numerous studies (Hu et al., 2003, Schaefer et al., 2006, Wang et al., 2006, Hallahan et al., 2007, Dunstan et al., 2008, de Goede et al., 2010, Farzaneh-Far et al., 2010, Gopinath et al., 2010, Naqvi et al., 2010). Nonetheless, contradictory results have been reported (Brouwer et al., 2006, Rogers et al., 2008, Carney et al., 2009, van de Rest et al., 2009, Djousse et al., 2011), and the essentiality of these nutrients lacks consensus (International Society for the Study of Fatty Acids and Lipids, 2004, Institute of Medicine, 2005, Kris-Etherton et al., 2007, Harris et al., 2009, Kris-Etherton et al., 2009). Without a doubt, there is much work yet to be done before the issues surrounding the long-chain omega-3 fatty acids are resolved. One aspect of research that could benefit from additional study is the development of sound methodology to measure long-term intakes of EPA and DHA (Overby et al., 2009).

Seafood, particularly oily fish, is a rich source of EPA and DHA. However, US consumers ingest EPA and DHA from a variety of supplements and foods including eggs, poultry, milk, juices, vegetable oil spreads, and sushi. In fact, fish oil and other long-chain omega-3 supplements are the most commonly used non-vitamin, non-mineral supplements in the US (Barnes et al., 2008), and since 1988, roughly 2,200 foods containing EPA and DHA have been introduced worldwide (Ismail, 2009). Consumer research indicates almost one third of US adults have purchased a food, drink, or supplement containing omega-3 fatty acids (Mintel, 2008).

Thus far, measurements of omega-3 fatty acid intakes in US populations have not kept pace with consumer trends by taking into account all sources of long-chain omega-3 fatty acids. For example, one recently developed omega-3 food frequency questionnaire (FFQ) included sushi but did not include eggs, chicken, or functional foods (Sublette et al., 2011). Currently, a convenient and comprehensive instrument specifically designed to measure EPA and DHA intakes in US populations does not appear to exist. Therefore, a 14-item FFQ was developed to measure consumption of these fatty acids. This newly developed FFQ takes approximately five minutes to complete and was designed to prevent excessive participant burden while measuring the relationship between EPA and DHA intakes and variables related to cognition and mood among young adults. The purpose of this study was to estimate the performance of this FFQ among young adults. Questionnaire clarity, completeness, and reliability were evaluated, while factors related to the repeatability of the instrument were explored.

Materials and Methods

Sample

A self-administered survey containing both the FFQ and a set of instrument evaluation questions was administered to a mixed-gender convenience sample of college students in May of 2009. To assess the test-retest reliability of the FFQ, a subset of participants willing to complete the survey a second time was identified. These students were given postage-paid return envelopes and instructed to complete and return the second survey in two weeks. At four weeks and again at six weeks, students who had not completed and returned the second survey were emailed a note reminding them to return the survey. All surveys returned between two and eight weeks were included in the test-retest reliability analysis.

Students were awarded ten points (one percent of total points) for completion of the initial survey. An alternative assignment worth the same number of points was available for those who did not want to participate in the initial survey. Students who completed and returned the second FFQ were entered into a drawing in which three $25 retail gift cards were awarded. This protocol was approved by the Kansas State University Institutional Review Board (Appendix A), and all participants were required to provide written informed consent prior to participation.

Questionnaire

Using expert feedback and the literature as a guide, a semi-quantitative FFQ was developed by modifying an existing FFQ that had been validated in an Australian population (Mina et al., 2007). The original FFQ was condensed and modified to reflect the food choices available to consumers in Northeast Kansas. The newly developed FFQ (Appendix B) included seven items related to seafood consumption. Five of the seafood items were based on the seafood items found on the Fred Hutchinson Cancer Research Center General FFQ (2001). Two seafood items were added to measure intake from sushi and sardines. In all, the newly developed FFQ included 14 items to capture intake of EPA and DHA from: 1) seafood, 2) poultry and eggs, 3) omega-3 functional foods, and 4) dietary supplements.

Participants were instructed to indicate how often they ate each food item during the previous six months. Nine response options were possible, and the options ranged from “never” to “two or more per day.” Participants were also instructed to indicate their usual serving size relative to a medium size (e.g., four ounces or 113 grams) serving. Size choices were “small,” “medium,” and “large.” A small serving was one-half (0.5) the medium serving size, and a large serving was one-and-one-half (1.5) times the medium serving size. A photo of a plated four-ounce portion of fish was used to help participants increase the accuracy of their estimates. The omega-3 supplement usage was measured by asking participants to write in the type and normal daily amount of supplements taken.

For each item on the FFQ, an average daily intake of EPA + DHA was calculated based on the fatty acid content of the item and the frequency and potion size in which it was consumed. The EPA + DHA content for each item was determined using the US Department of Agriculture National Nutrient Database for Standard Reference, Release 22 (US Department of Agriculture, 2009). As several items listed more than one type of fish, item values were based on an average of the fatty acid content. When available, consumption pattern trends were used to calculate a weighted average (US Department of Agriculture, 2005). The EPA + DHA content for sushi was determined using the number of times each type was mentioned to calculate a weighted average based on the top five types of sushi mentioned. For supplements and items not listed in the US Department of Agriculture database, fatty acid content data were obtained either from the product labels or from product representatives. The average intakes from each item were then summed to give an overall total, as well as categorical totals for seafood, chicken and eggs, functional foods, and supplements.

In addition, a set of questions was developed to evaluate the clarity and completeness of the FFQ, as well as estimate the impact of seasonal, cultural, and religious factors on seafood consumption. This set of questions was included with the FFQ and included questions with nominal response choices (i.e., “yes” or “no”) as well as open-ended questions (i.e., “if yes, explain”).

Statistical Analyses

Data analyses were performed using Predictive Analytics Software (version 18, 2009, SPSS, Inc, Chicago, IL). As the purpose of this study was to assess the performance of the FFQ, outlier values were not excluded from the analyses. Frequencies and percentages were calculated for categorical data. Percentages were calculated based on the number of complete responses. Mean and median values for average daily EPA + DHA intakes were calculated for each item, for all four categories of items (i.e., seafood, chicken and eggs, functional foods, and supplements), and for the overall total. FFQs with missing responses were not included in the EPA + DHA calculations for the categories to which the missing response belonged, nor were they included in the calculation of total EPA + DHA. However, the responses to individual items from these surveys were retained during item analyses.

Because intake values for the fatty acids were not normally distributed, non-parametric statistical analyses were performed. Test-retest reliability was evaluated using Spearman rank order correlation coefficients to measure the correlation between the fatty acid intake values obtained from the initial FFQ with those obtained on the repeat FFQ. Cohen's kappa coefficient was used to measure test-retest agreement for omega-3 supplement use. Mann-Whitney U tests were conducted to explore the relationship between categorical variable responses and fatty acid intake.

Results

Initial Survey

The initial survey participation rate was 83.7% (n = 165). The EPA + DHA intakes were positively skewed with values clustering at the low end. Calculation of total EPA + DHA intakes was possible for 144 participants and ranged from zero to 2734 mg/day with a mean of 189 mg/day, a median of 87.9 mg/day, and a standard deviation of 346 mg/day. The contribution of each category to the mean was as follows: fish and seafood 65% (122 mg/day), dietary supplements 19% (36 mg/day), chicken and eggs 13% (24 mg/day), and omega-3 functional foods 3% (6 mg/day).

Twenty-nine percent of participants (n = 47) consumed at least one omega-3 functional food, 21.3% (n = 35) had eaten sushi, 12.2 % (n = 18) consumed seafood twice a week or more, and nine percent (n = 15) had taken an omega-3 supplement. Although sushi was consumed by a sizeable proportion of participants, the calculated EPA + DHA content for sushi (143 mg/serving) was relatively low compared to the average content of other seafood items (796 mg/serving). Sushi was consumed less often than any of the other seafood items except sardines and accounted for less than one percent (1 mg/day; 0.76%) of the EPA + DHA intake. The most commonly listed types of sushi were California rolls, which were listed 13 times and accounted for 34% of all types listed, and salmon, which was listed seven times and accounted for 18.4% of all types listed.

Forty percent of the participants indicated that the amount of fish they ate changes during the school year. Among those who said fish consumption changed during the school year, explanations varied. Comments such as “I eat it a lot in school year” and “mom hates [fish] and won’t let me cook it at home,” indicated consumption increased during the school year for some, while other comments such as “my family cooks more fish than the cafeteria,” and “when home my family eats a lot of the fish we catch” indicated fish consumption decreased during the school year. Nonetheless, the majority (61%) wrote in comments that indicated consumption increased while they were at home.

Twenty percent 20.1% (n = 33) of the students indicated that the amount of fish they ate changed throughout the year for religious or cultural reasons and 18% (n = 30) said fish consumption changed based on the season. All of the students who indicated that the amount of fish they ate changed throughout the year for religious or cultural reasons cited the Christian liturgical season of Lent as an explanation.

With regard to evaluation of the FFQ’s completeness, 3.0% (n = 5) said they consumed omega-3 foods not incorporated in the FFQ. The following four foods comprised the list of foods that were consumed but not incorporated into the FFQ: flaxseed, peanut butter, granola bars, and flatbread. With regard to clarity, 7.2% of the participants (n = 12) said they had trouble understanding or answering one or more questions. Of the 12 participants who identified clarity as a concern, eight cited issues related to the omega-3 functional foods. Select representative responses from those who experienced confusion regarding omega-3 functional foods are provided in Table 2.1. Mann-Whitney U tests were conducted to explore the relationship between seasonal, cultural, and school-related factors and omega-3 fatty acid intake from seafood. Students whose fish intake changed based on the season and those whose intake changed based on the school year had higher omega-3 intakes than those who intake did not change (see Table 2.2).

Retest Survey

Of those students who took part in the initial survey, 54 agreed to complete the FFQ a second time. Forty-five of those who agreed to complete the survey a second time did so, yielding a return rate of 83.3% for the repeat survey. Results of the test-retest reliability assessment of food items are presented in Table 2.3. Test-retest reliability correlation coefficients for each of the non-functional food items and the overall scale were significant and ranged from $\rho = 0.48$ to 0.86; $p < 0.001$. Correlation coefficients for each of the functional food items were low and/or non-significant. Test-retest agreement for omega-3 supplement use was high with 97.6% (n = 41) of participants providing the same answer on both FFQs (K = 0.844; $p < 0.001$).

Discussion

With a correlation coefficient of $\rho = 0.86$, overall test-retest reliability was good. Test-retest agreement for omega-3 supplement use was excellent with 97.6% (n = 41) of participants supplying the same answer on both questionnaires (K = 0.844; p <0 .001). The FFQ appears to contain a comprehensive list of food items for this sample of the population, as the foods listed in response to the completeness evaluation question were not rich in EPA or DHA. Nonetheless, participant feedback and the decreased test-retest coefficients for these foods, suggest unfamiliarity regarding omega-3 functional foods may be problematic when measuring intakes from these food sources. This finding substantiates earlier consumer research in which 26% of respondents said that they were uncertain whether or not they had purchased a food, drink, or supplement that contained omega-3 fatty acids (Mintel, 2008). Even though the contribution of functional foods was only three percent of the total EPA + DHA intake, additional work is needed to improve the reliability of the FFQ items related to these foods. Using product names and pictures, as well as having participants specify the name of the products they consume, may improve the performance of the FFQ.

A sizeable proportion of the participants indicated their fish consumption changed throughout the year due to cultural, seasonal or school-related factors. Participants who indicated their fish consumption changed based on the season and those who said it changed based on the school year both had significantly higher intakes of omega-3 fatty acids from seafood. Although the relationship between these factors cannot be fully elucidated from this study, these results reinforce the importance of taking seasonal factors into consideration when measuring EPA + DHA intake.

The positive skewing of EPA + DHA intake has been reported in other omega-3 fatty acid studies conducted in the US (Farzaneh-Far et al., 2010, Sublette et al., 2011). A large proportion (87.2%) of the participants consumed fish less often than the commonly recommended frequency of twice per week (United Kingdom Scientific Advisory Committee on Nutrition and Committee on Toxicity, 2004, Lichtenstein et al., 2006, American Diabetes Association, 2007, Kris-Etherton et al., 2007). An even larger proportion (94.3%) of participants had EPA + DHA intakes below 500 mg/day which is the lower-bound threshold of what is generally considered to be the ideal intake level (International Society for the Study of Fatty Acids and Lipids, 2004, Kris-Etherton et al., 2007). Nonetheless, mean intake of EPA + DHA was higher than that previously reported in studies conducted through the year 2000 (Allison et al., 1999, Ervin et al., 2004). Unfortunately, there are few current studies to use for comparison. That said, EPA + DHA intake in the present study was lower than that reported in a study published in the year 2006 in which the EPA + DHA intake in cardiac patients was reported as 280 mg/day based on three 24-hour food recalls (Ritter-Gooder et al., 2006). Conversely, results in the present study are comparable to those obtained in the years 2006-2008 from adults in the greater metropolitan New York City area (Sublette et al., 2011). In that study, which utilized a FFQ and did not include supplement users or collect intake from functional foods, eggs, or poultry, mean intakes among healthy adults were 31 mg/day for EPA and 55 mg/day for DHA. In the present study, mean intakes of DHA and EPA were 56 mg/day and 65 mg/day, respectively, once the contribution from supplements, functional foods, poultry, and eggs were excluded.

Of clinical relevance is the finding that roughly one in ten participants reported omega-3 supplement use. This finding reinforces the importance of addressing supplement usage during dietary assessments. In addition, the reporting of omega-3 supplement appears to be reliable, and to the best of our knowledge, this is the first time that the reliability of omega-3 supplement reporting has been measured.

Although reproducibility evaluations are useful, and high test-retest correlations are desirable, reproducibility evaluations alone do not constitute a sufficient instrument evaluation (Willet, 1998). Modifications to improve the clarity of the omega-3 functional food items and subsequent validation are still needed. Since FFQs have a tendency to overestimate absolute intake, this instrument would be best suited for studies designed to rank participants based on their relative intake of EPA + DHA. In addition, because this study was conducted among college students in Kansas, the results are not necessarily generalizable to populations from different geographic locations or with lower literacy levels.

Conclusions

Currently, an up-to-date and convenient method to assess EPA and DHA intakes in US adult populations does not appear to exist. The FFQ evaluated in this study was developed to fill that void. The preliminary performance of this self-administered FFQ has been favorable. With additional development and subsequent validation, this FFQ has the potential to become an easy-to-administer instrument for ranking individuals based on their usual intake of EPA + DHA. Such an instrument could easily be adapted for other populations and will be key in understanding the relationship between intakes of long-chain omega-3 fatty acids and health outcomes.

References

Allison, D. B., Egan, S. K., Barraj, L. M., Caughman, C., Infante, M. & Heimbach, J. T. (1999) Estimated intakes of trans fatty and other fatty acids in the US population. J Am Diet Assoc, 99, 166-74; quiz 175-6.

American Diabetes Association (2007) Nutrition Recommendations and Interventions for Diabetes: a position statement of the American Diabetes Association. Diabetes Care, 30 Suppl 1, S48-65.

Barnes, P. M., Bloom, B. & Nahin, R. L. (2008) Complementary and alternative medicine use among adults and children: United States, 2007. Natl Health Stat Report, 1-23.

Brouwer, I. A., Heeringa, J., Geleijnse, J. M., Zock, P. L. & Witteman, J. C. (2006) Intake of very long-chain n-3 fatty acids from fish and incidence of atrial fibrillation. The Rotterdam Study. Am Heart J, 151, 857-62.

Carney, R. M., Freedland, K. E., Rubin, E. H., Rich, M. W., Steinmeyer, B. C. & Harris, W. S. (2009) Omega-3 augmentation of sertraline in treatment of depression in patients with coronary heart disease: a randomized controlled trial. JAMA, 302, 1651-7.

De Goede, J., Geleijnse, J. M., Boer, J. M., Kromhout, D. & Verschuren, W. M. (2010) Marine (n-3) fatty acids, fish consumption, and the 10-year risk of fatal and nonfatal coronary heart disease in a large population of Dutch adults with low fish intake. J Nutr, 140, 1023-8.

Djousse, L., Gaziano, J. M., Buring, J. E. & Lee, I. M. (2011) Dietary omega-3 fatty acids and fish consumption and risk of type 2 diabetes. Am J Clin Nutr, 93, 143-50.

Dunstan, J. A., Simmer, K., Dixon, G. & Prescott, S. L. (2008) Cognitive assessment of children at age 2(1/2) years after maternal fish oil supplementation in pregnancy: a randomised controlled trial. Arch Dis Child Fetal Neonatal Ed, 93, F45-50.

Ervin, R. B., Wright, J. D., Wang, C. Y. & Kennedy-Stephenson, J. (2004) Dietary intake of fats and fatty acids for the United States population: 1999-2000. Adv Data, 1-6.

Farzaneh-Far, R., Lin, J., Epel, E. S., Harris, W. S., Blackburn, E. H. & Whooley, M. A. (2010) Association of marine omega-3 fatty acid levels with telomeric aging in patients with coronary heart disease. JAMA, 303, 250-7.

Fred Hutchinson Cancer Research Center (2001) Food Frequency Questionnaires. http://www.fhcrc.org/science/shared_resources/nutrition/ffq; Accessed November 13, 2008

Gopinath, B., Flood, V. M., Rochtchina, E., Mcmahon, C. M. & Mitchell, P. (2010) Consumption of omega-3 fatty acids and fish and risk of age-related hearing loss. Am J Clin Nutr, 92, 416-21.

Hallahan, B., Hibbeln, J. R., Davis, J. M. & Garland, M. R. (2007) Omega-3 fatty acid supplementation in patients with recurrent self-harm. Single-centre double-blind randomised controlled trial. Br J Psychiatry, 190, 118-22.

Harris, W. S., Mozaffarian, D., Lefevre, M., Toner, C. D., Colombo, J., Cunnane, S. C., Holden, J. M., Klurfeld, D. M., Morris, M. C. & Whelan, J. (2009) Towards establishing dietary reference intakes for eicosapentaenoic and docosahexaenoic acids. J Nutr, 139, 804S-19S.

Hu, F. B., Cho, E., Rexrode, K. M., Albert, C. M. & Manson, J. E. (2003) Fish and long-chain omega-3 fatty acid intake and risk of coronary heart disease and total mortality in diabetic women. Circulation, 107, 1852-7.

Institute of Medicine (2005) Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids. Washington, DC, National Academies Press.

International Society for the Study of Fatty Acids and Lipids (2004). ISSFAL Policy Statement 3: Recommendations for intake of polyunsaturated fatty acids in healthy adults. Available at: http://www.issfal.org/index.php/component/content/23?task=view. Accessed January 20, 2011

Ismail, A. (2009) Omega 3s' potential in foods: as the market continues its growth credibility among consumers will be the key to future success. Nutraceuticals World.

Kris-Etherton, P. M., Grieger, J. A. & Etherton, T. D. (2009) Dietary reference intakes for DHA and EPA. Prostaglandins Leukot Essent Fatty Acids, 81, 99-104.

Kris-Etherton, P. M., Innis, S., American Dietetic Association & Dietitians of Canada. (2007) Position of the American Dietetic Association and Dietitians of Canada: dietary fatty acids. J Am Diet Assoc, 107, 1599-611.

Lichtenstein, A. H., Appel, L. J., Brands, M., Carnethon, M., Daniels, S., Franch, H. A., Franklin, B., Kris-Etherton, P., Harris, W. S., Howard, B., Karanja, N., Lefevre, M., Rudel, L., Sacks, F., Van Horn, L., Winston, M. & Wylie-Rosett, J. (2006) Diet and lifestyle recommendations revision 2006: a scientific statement from the American Heart Association Nutrition Committee. Circulation, 114, 82-96.

Mina, K., Fritschi, L. & Knuiman, M. (2007) A valid semiquantitative food frequency questionnaire to measure fish consumption. Eur J Clin Nutr, 61, 1023-31.

Mintel (2008) Omega-3 – US. London. Mintel International.

Naqvi, A. Z., Buettner, C., Phillips, R. S., Davis, R. B. & Mukamal, K. J. (2010) n-3 fatty acids and periodontitis in US adults. J Am Diet Assoc, 110, 1669-75.

Overby, N. C., Serra-Majem, L. & Andersen, L. F. (2009) Dietary assessment methods on n-3 fatty acid intake: a systematic review. Br J Nutr, 102 Suppl 1, S56-63.

Ritter-Gooder, P. K., Lewis, N. M., Heidal, K. B. & Eskridge, K. M. (2006) Validity and reliability of a quantitative food frequency questionnaire measuring n-3 fatty acid intakes in cardiac patients in the Midwest: a validation pilot study. J Am Diet Assoc, 106, 1251-5.

Rogers, P. J., Appleton, K. M., Kessler, D., Peters, T. J., Gunnell, D., Hayward, R. C., Heatherley, S. V., Christian, L. M., Mcnaughton, S. A. & Ness, A. R. (2008) No effect of n-3 long-chain polyunsaturated fatty acid (EPA and DHA) supplementation on depressed mood and cognitive function: a randomised controlled trial. Br J Nutr, 99, 421-31.

Schaefer, E. J., Bongard, V., Beiser, A. S., Lamon-Fava, S., Robins, S. J., Au, R., Tucker, K. L., Kyle, D. J., Wilson, P. W. & Wolf, P. A. (2006) Plasma phosphatidylcholine docosahexaenoic acid content and risk of dementia and Alzheimer disease: the Framingham Heart Study. Arch Neurol, 63, 1545-50.

Sublette, M. E., Segal-Isaacson, C. J., Cooper, T. B., Fekri, S., Vanegas, N., Galfalvy, H. C., Oquendo, M. A. & Mann, J. J. (2011) Validation of a food frequency questionnaire to assess intake of n-3 polyunsaturated fatty acids in subjects with and without major depressive disorder. J Am Diet Assoc, 111, 117-123 e1-2.

United Kingdom Scientific Advisory Committee on Nutrition & Committee on Toxicity (2004) Advice on Fish Consumption: Benefits & Risks. Norwich, UK, The Stationery Office. http://www.food.gov.uk/multimedia/pdfs/fishreport2004full.pdf; Accessed March 28, 2011.

United States Department of Agriculture, 2005 Dietary Guidelines Advisory Committee, Appendix G-2: Original Food Guide Pyramid Patterns and Description of USDA Analyses; High Omega-3 Fish Analysis; The Report of the Dietary Guidelines Advisory Committee on Dietary Guidelines for Americans, 2005. http://www.health.gov/dietaryguidelines/dga2005/report/HTML/G2_Analyses.htm#omegafish; Accessed March 27, 2010.

United States Department of Agriculture, Agriculture Research Service (2009) USDA National Nutrient Database for Standard Reference, Release 22. Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/nutrientdata; Accessed March 26-29, 2010; April 29, 2010; and May 17, 2010.

van de Rest, O., Spiro, A., III, Krall-Kaye, E., Geleijnse, J. M., De Groot, L. C. & Tucker, K. L. (2009) Intakes of (omega-3) fatty acids and fatty fish are not associated with cognitive performance and 6-year cognitive change in men participating in the Veterans Affairs Normative Aging Study. J Nutr, 139, 2329-36.

Wang, C., Harris, W. S., Chung, M., Lichtenstein, A. H., Balk, E. M., Kupelnick, B., Jordan, H. S. & Lau, J. (2006) n-3 fatty acids from fish or fish-oil supplements, but not alpha-linolenic acid, benefit cardiovascular disease outcomes in primary- and secondary-prevention studies: a systematic review. Am J Clin Nutr, 84, 5-17.

Willet, W. (1998) Nutritional Epidemiology, New York, NY Oxford Press.

Tables

Table 2.1 Selected responses from participants who experienced confusion regarding omega-3 functional foods

"How do I know what is omega-3 enriched?"
"I'm not aware of my omega 3 intake."
"Not sure whether or not I have eaten omega-3 enriched foods."
"Explain omega 3 enriched foods."
"I don't know if the stuff I eat is omega-3 enriched."
"I don't know if foods are enriched."

Table 2.2 Social and environmental factors and EPA + DHA intake from seafood

Factor	n	Median mg/day	Mean Rank	U	p[a]
Culture/ Religion					
Yes	29	58	84.90	1366	0.103
No	117	40	70.68		
Season					
Yes	25	66	96.24	919	0.002*
No	120	36	68.16		
School Year					
Yes	53	81	96.14	1264	<0.001**
No	93	23	60.60		

[a] Mann-Whitney U tests were used to determine significance

* ≤ 0.01; ** ≤ 0.001

Table 2.3 Test-retest correlation coefficients for EPA + DHA intakes measured using 14-item food frequency questionnaire

FFQ Items	n	Spearman's rho	p
Seafood			
Canned Tuna	44	.80	< 0.001
Fried Fish	43	.64	< 0.001
Fried Shellfish	44	.70	< 0.001
Sardines	41	.83	< 0.001
Baked White Fish	44	.76	< 0.001
Baked Dark/Oily Fish	43	.48	< 0.001
Sushi	43	.58	< 0.001
All Seafood	38	.84	< 0.001
Other Food			
Dark Chicken	43	.79	< 0.001
Eggs	45	.67	< 0.001
Dark Chicken & Eggs	43	.68	< 0.001
Functional Food			
Omega-3 eggs	44	.52	< 0.001
Omega-3 margarine	42	.42	0.005
Omega-3 milk	44	.17	0.267
Omega-3 juice	44	.14	0.360
All Functional Foods	42	.24	0.127
Supplements	42	.86	< 0.001
Total All Sources	31	.86	< 0.001

Chapter 3 - The Relationship between Omega-3 HUFA Score and Intake of Eicosapentaenoic and Docosahexaenoic Acids

Abstract

Background: Although positive health outcomes have been associated with biomarker and diet measurements of omega-3 fatty acids, correlations between dietary intake estimates and biomarkers have generally not been strong. This paper describes the observed relationship between intake of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) as determined by a food frequency questionnaire (FFQ) and the percent of omega-3 highly unsaturated fatty acids (HUFA) in total HUFA of whole blood. Highly unsaturated fatty acids are those fatty acids with 20 or more carbons and three or more double bonds. Methods: A volunteer sample of deploying soldiers ($n = 191$) provided blood samples, completed the FFQ, and answered demographic and health-related questions. Following regression analysis, log 10 transformation was performed on the omega-3 HUFA scores and the FFQ-based intakes of EPA + DHA to reduce skewness, to reduce the number of outliers, and to improve homoscedasticity of the residuals. Assignment to quintiles was made according to FFQ-based intake of EPA + DHA and omega-3 HUFA score. Results: The FFQ-based intake of EPA + DHA was positively correlated with omega-3 HUFA score ($r = 0.39$), as well as with DHA ($r = 0.33$), and omega-3 polyunsaturated fatty acids ($r = 0.24$). Omega-3 HUFA scores were higher ($p < 0.01$) among those who took an omega-3 supplement, among those who consumed sushi, among those who consumed omega-3 functional foods, and among those who ate seafood at least twice a week. Sixty-four percent of the participants were assigned to either the same or an adjacent quintile. Conclusion: The correlation of $r = 0.39$ achieved in this study is adequate and consistent with those that have been reported for other studies correlating estimated dietary intake and biomarker measures of omega-3 status.

Introduction

The relationship between omega-3 fatty acids and health outcomes has been the topic of numerous population-based studies (1, 2). Both biological markers of tissue status and estimated dietary intakes of omega-3 fatty acids have been linked to a wide variety of favorable health outcomes. An assortment of biological markers of omega-3 fatty acid status have been utilized in research (3), and many including whole blood docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (4), plasma phosphatidylcholine (PC) DHA (5), and erythrocyte membrane omega-3 fatty acids (6) have been associated with favorable health outcomes in population based studies. Similarly, a variety of dietary assessment methods have been used to determine omega-3 fatty acid intake, and a number of these methods including 24-hour dietary recall (7), diet history (8), and food frequency questionnaire (FFQ) (9-11) have also been associated with health benefits.

Despite the positive health outcomes associated with biomarker and estimated dietary intake measurements of omega-3 fatty acids, correlations between diet and biomarkers have generally not been strong (12). Individual responses to changes in omega-3 fatty acid intake vary dramatically (13), and factors such as gender (14), age (15, 16), BMI (15), and smoking status (16) are associated with differences in various omega-3 biomarkers. Recently, Plourde et al (17) detected differences in plasma omega-3 fatty acids based on allele variant of the apolipoprotein E gene. These influences suggest that the lack of strength between dietary assessment and biomarker measures may not be due in large part solely to methodological error and that each category of assessment may represent separate yet correlated variables. Taking this concept into consideration, the concurrent use of dietary intake and biomarker measures appears to be the better approach to understanding the relationship between omega-3 fatty acids and health outcomes. Yet, it appears that compared to the large number of studies which have employed a single method of determining omega-3 status, relatively few studies have simultaneously measured both dietary and tissue omega-3 fatty acids.

As part of a study designed to investigate the relationship between omega-3 fatty acids and psychological well-being among deploying soldiers, both dietary intake and tissue status of the long-chain omega-3 fatty acids were measured. Dietary intake of EPA + DHA was measured using a semi-quantitative 11-item FFQ. This instrument was designed to minimize participant burden while taking into account consumer trends and encompassing all major sources of long-chain omega-3 fatty acids currently being ingested by young adults in the US. Omega-3 tissue status was determined by measuring the percent of omega-3 highly unsaturated fatty acids (HUFA) in total HUFA of whole blood (18), herein referred to as the omega-3 HUFA score. Highly unsaturated fatty acids are defined as those fatty acids with 20 or more carbons and three or more double bonds. This method of determining omega-3 tissue status has the advantage of being minimally invasive, low cost, and conducive to off-site work. The purpose of this paper is to describe the observed relationship between dietary intake of EPA + DHA, as determined by the FFQ, and tissue omega-3 status, as determined by the omega-3 HUFA score.

Subjects and Methods

Subjects

A volunteer sample of soldiers (n = 191) scheduled for deployment to Iraq provided blood samples, completed the FFQ, and answered a set of demographic and health-related questions. Participants were from one of two combat brigades. One was an active duty brigade from Kansas and the other was a brigade combat team (BCT) from the Texas National Guard. Data collection took place October 2009 through February 2010. The participants completed the study approximately one month prior to deployment. It was made clear to all potential subjects that participation was strictly voluntary and was not part of their required military duty. The soldiers did not receive compensation for their participation. This protocol was approved by, and was conducted in accordance with the ethical standards of, the Kansas State University Institutional Review Board. After being provided a complete description of the study, all of the soldiers recruited for this study were required to provide written informed consent prior to participation.

EPA + DHA Intake

An 11-item FFQ was used to measure consumption of EPA + DHA during the preceding six-month period. This FFQ was self-administered and included items to capture intake of EPA and DHA from: 1) seafood, 2) poultry and eggs, 3), omega-3 functional foods, and 4) dietary supplements. Development and assessment of the FFQ is described in detail in Chapter 2.

Based upon the initial assessment study, this FFQ appears to be a comprehensive and reliable ($n = 54$, $\rho = 0.86$, $p < 0.001$) instrument for measuring EPA + DHA intakes in young adults. Consumer research (19), as well as participant feedback from the FFQ assessment study, suggest unfamiliarity regarding functional foods is problematic, as was evidenced by the low test-retest coefficient obtained for the functional food items ($n = 42$, $\rho = 0.24$, $p = 0.127$) during assessment. As a result of this finding, pictures and open-ended questions in which participants specified products by name were used to improve the instrument performance. In all, the FFQ contained 11 items to measure EPA + DHA. For the nine items related to seafood, egg, and poultry consumption, participants indicated their usual serving size and frequency of consumption by selecting from the response options provided. For the two items related to functional foods and supplements, participants were asked to specify the product and usual quantity consumed. Participants were also asked to specify the type of sushi they consume. The EPA + DHA content of each food item was determined using the US Department of Agriculture National Nutrient Database for Standard Reference, Release 22 (US Department of Agriculture, 2009) (20). For supplements and items not listed in the US Department of Agriculture database, the omega-3 content was obtained either from the product labels or from product representatives. Because the omega-3 information obtained from product labels and representatives was typically given as the total amount of EPA + DHA, intakes for the separate fatty acids from these sources could not be determined. As such, total intake (i.e., intake from all sources) could only be calculated as EPA + DHA.

Demographic and Health-Related Behaviors

A self-administered questionnaire was used to measure demographic characteristics, smoking status, alcohol use, height and weight.

Omega-3 HUFA Score

Non-fasting fingertip prick blood samples were collected on strips of chromatography paper treated with BHT and acetone as described previously (21). The samples were placed in cryogenic containers and allowed to dry overnight before being stored at -80°C until analysis. A one-step, direct transesterification fatty acid analysis accelerated by microwave irradiation was developed from Lepage & Roy (22) and used for the determination of fatty acid in filter paper blood sample. The reaction reagents included, 2 mL of methanol: hexane (4:1, v/v), 200 µl of acetyl chloride, and 10 µg of 22:3n-3 ethyl ester as internal standard per sample. The reagents were contained in a chemical safe vessel and reacted at 125°C for 5 minutes in a multimode microwave system (MARS, CEM Corporation, Matthews, NC). Afterwards, the samples were chilled in ice, neutralized by an addition of 5 mL of 6% Na_2CO_3 solution and centrifuged at 1,700 g x 4 minutes. The upper organic phase was collected and reduced to ~ 50 µl under nitrogen prior to being placed in an autosampler tray of gas chromatograph (GC). One to two µl of the reduced organic phase was injected into GC for data acquisition. A 7890A GC system (Agilent Technologies, Inc.; Santa Clara, CA) coupled with a fused-silica, narrow-bored DB-FFAP capillary column (15 m x 0.1 mm I.D. x 0.1 µm film thickness) was used for chromatographic separation of the fatty acid methyl esters (FAME) as reported previously (23). Data were expressed as the percentage of each fatty acid in total weight of the identified fatty acids in each sample (wt %).

Statistical Analysis.

Data analyses were performed using Predictive Analytics Software (version 18, 2009, SPSS, Inc, Chicago, IL). As the purpose of this study was to assess the relationship between the values obtained using the FFQ and the fingertip prick method, outlier values were not excluded from the analyses. Blank responses on the FFQ were quantified as zero. Mean, median, and standard deviations were calculated for continuous variables. Frequencies and percentages were calculated for categorical data. Means and percentages were calculated based on the number of complete responses. Pairwise exclusion was used to exclude cases only if they were missing data for a specific analysis. Statistical regression analysis using backwards deletion was conducted with omega-3 HUFA score as the dependent variable and FFQ-based intake of EPA + DHA, gender, age, smoking status, alcohol consumption, body mass index (BMI), and location as the independent variables. Following visual inspection of the scatter plot of the residuals between the obtained and the predicted omega-3 HUFA values (Figure 3.1), log 10 transformation was performed on omega-3 HUFA and intake of EPA + DHA to reduce skewness, to reduce the number of outliers, and to improve homoscedasticity of the residuals (Figure 3.2). To allow analysis inclusive of the individual fatty acids, log 10 transformations were also performed on these variables. Pearson's correlation coefficients were conducted to measure bi-variant correlations, and independent t-tests were conducted to explore the relationship between categorical variables and omega-3 HUFA scores as well as the FFQ-based intake of EPA + DHA. Except for removal of variables from the regression analysis which was based on a significance of $p \leq 0.10$, two-tailed tests and a significance level of $p \leq 0.05$ were used for all analyses.

Results

Sample Characteristics

The majority (65%; n = 124) of the participants were from the National Guard and 92% (n = 176) were male. Age in years ranged from 18 to 54 with a mean age of 29.3 (SD = 8.0). More than half (59%; n = 133) were married, and all but one had completed high school or an equivalent. Mean BMI was 27.5 kg/m^2 (SD = 4.0). Sixty-five percent identified themselves as non-Hispanic Caucasian. Compared to the National Guard, the Active Duty soldiers were younger, had a lower BMI, and were less likely to report regular daily alcohol consumption. Additional demographic and health related characteristics by service component are summarized in Tables 3.1 and 3.2.

FFQ

The FFQ-based intakes of EPA + DHA were positively skewed with values clustering at the low end. Total EPA + DHA intakes ranged from zero to 1918 mg/day with a mean of 301 mg/day, a median of 206 mg/day, and a standard deviation of 325 mg/day. The contribution of each category to the mean was as follows: fish and seafood 64.5% (194 mg/day), dietary supplements 22.9% (69 mg/day), chicken 4.3 % (13 mg/day), eggs 6.3 % (19 mg/day), and omega-3 functional foods 2.0% (6 mg/day).

Eighteen percent of participants (n = 35) consumed at least one omega-3 functional food, 35.6% (n = 68) had eaten sushi, 12.2 % (n = 18) consumed seafood twice a week or more, and 13.1% (n = 25) had taken an omega-3 supplement. Sushi was consumed by a sizeable proportion of participants, and accounted for three percent (10 mg/day; 0.76%) of the FFQ-based intake of EPA + DHA. The most commonly listed types of sushi included those types made with salmon and tuna. The FFQ-based intake of EPA + DHA was higher among the National Guard participants (Table 3.2) and among those 29 years old and older (Table 3.3).

Omega-3 HUFA Scores

The omega-3 HUFA scores ranged from 11.4% to 33.0% with a mean of 16.9% (SD 3.1) and a median of 16.5%. The omega-3 HUFA score did not differ significantly based on military service category (Table 3.2). Omega-3 HUFA score was positively correlated with DHA, EPA, and DPA (Table 3.5). The correlations between omega-3 HUFA score and arachidonic acid (AA), linoleic acid (LA), and α-linolenic acid (ALA) were not significant (Table 3.5). The correlation between omega-3 HUFA score and time to last meal was also not significant (Table 3.5). The omega-3 HUFA scores were lower among smokers, and those 28 years old and younger (Table 3.4).

Relationship between FFQ-Based Intake of EPA+ DHA and Omega-3 HUFA Scores

The FFQ-based intake of EPA + DHA was positively correlated with omega-3 HUFA score ($r = 0.39$), as well as with DHA, and omega-3 polyunsaturated fatty acids (PUFA) (Table 3.5). Omega-3 HUFA scores were higher among those who took an omega-3 supplement, among those who consumed sushi, among those who consumed omega-3 functional foods, and among those who ate seafood at least twice a week (Table 3.6).

The results of the regression analysis with omega-3 HUFA score as the dependent variable are presented in Table 3.7. Collinearity diagnostics indicated the assumption of non-collinearity was met for this analysis. Five of the independent variables (FFQ-based intake of EPA + DHA, alcohol consumption, smoking status, service component, and age) were retained while two (BMI and gender) were eliminated. Intake of EPA + DHA ($\beta = 0.38$) made the largest contribution to the model.

For both the FFQ-based intakes of EPA + DHA and the omega-3 HUFA scores, participants were ranked and assigned to quintiles with the lowest value being assigned a rank of one, and the lowest rank being assigned to quintile one. Agreement of quintile assignment was measured and is presented in Table 3.8. Sixty-four percent of the participants were assigned to either the same or an adjacent quintile.

Discussion

The correlation between the FFQ-based intakes of EPA + DHA and the omega-3 HUFA score is comparable to those that have been reported for the relationship between other dietary assessment and biomarker methods. In a review of dietary assessment methodologies (12), unadjusted correlations between biomarkers and FFQ estimates of individual omega-3 fatty acids ranged from 0.18 for EPA and plasma phospholipids to 0.58 for EPA and serum phospholipids. Sullivan et al (24) attained a correlation of 0.54 between FFQ estimates of omega-3 long-chain PUFAs and plasma fatty acids, while Lucus et al (25) obtained a correlation of 0.42 between FFQ estimates of EPA + DHA and red blood cell membrane measurements of the corresponding fatty acids.

The participants in the current study had lower omega-3 HUFA scores (16.9%) compared to the 20.2 % reported by Metherel et al (26) for young men and women from Ontario who were consuming 0.22% of energy as omega-3 polyunsaturated fat. The omega-3 HUFA score and the FFQ-based intake of EPA + DHA in the present study were also lower than the 28.8% omega-3 HUFA score and the 350 mg/day (SD 508) food record estimate for EPA + DHA reported by Fratesi et al (27) for older adults. Noteworthy in the study by Fratesi et al (27) is the fact that estimates of EPA + DHA based on duplicate food collection were significantly lower compared with estimates based on the dietary records, but estimates based on both methods were strongly correlated. This finding illustrates the inconsistencies typically encountered when attempting to quantify omega-3 intake using dietary assessment methods, and suggests dietary assessment techniques are best suited to studies in which the objective is to rank participants based on their relative intake.

There are few US studies by which to compare intake of EPA + DHA. Nonetheless, EPA + DHA intake in the present study was higher than that obtained from adults in the greater metropolitan New York City area during the years 2006-2008 (28). In that study, which utilized a FFQ and did not include supplement users or collect intake from functional foods, eggs, or poultry, mean EPA + DHA intake was 86 mg/day. In the present study, mean intake of EPA + DHA was 194 mg/day once the contribution from supplements, functional foods, poultry, and eggs were excluded.

Several limitations need to be considered with regard to the interpretation of the current results. This study was not designed to be a validation study, but rather an investigation into the relationship between omega-3 fatty acid status and psychological well-being among soldiers. As such, the variables included in this report were only a few of numerous (social, cognitive and psychological) variables measured. Given the average amount of time required to complete the study (~ 1 ½ hours) and the somewhat chaotic circumstances under which data were collected, it is unlikely that participants completed the questionnaires as diligently as they would have under more ideal conditions.

An additional limitation of the study was the fact that we did not control for energy intake, ALA intake, or intake of the omega-6 fatty acids. One participant who was consuming 3000 mg of flaxseed oil daily exemplified the disadvantage of not controlling for these additional dietary factors. Although the conversion of ALA to the longer chain fatty acids is limited (29), this individual who was taking flaxseed oil had a an estimated EPA + DHA intake of 480 mg/day and a percent n-3 HUFA score of 23.0 which was well above the mean and put him above the 95th percentile for omega-3 HUFA scores.

As was the case in our assessment study, measuring intake from omega-3 functional foods was problematic in the current study. Eighteen percent of the participants left the question regarding functional foods blank. Other participants wrote in the names of omega-3 functional food products (particularly yogurt) that had been removed from the market over the course of time and for which information pertaining to the omega-3 content was no longer available. Nonetheless, despite these issues, individuals who reported omega-3 functional food use had higher omega-3 HUFA scores.

Because this study was based on the self-reported behavior of a volunteer sample, it should be noted that the participants' dietary intake and health related behaviors may have differed from those of the non-participants. In addition, because this study was conducted among soldiers from Texas and Kansas the results are not necessarily generalizable to civilian populations or to populations from different geographic locations. In addition, it should be noted that the differences observed between military service category (e.g. FFQ-based intake of EPA + DHA) could have just as well been related to the inherent geographical differences that exist between the two groups.

In summary, the methods we employed minimized participant burden in a number of important aspects. Participants were not required to fast, the amount of blood collected was minimal, and the time spent completing the FFQ (approximately 5-10 minutes) was relatively short. We were able to use the fingertip prick method in an off-site setting and ship or transport the samples back to our lab without the need for refrigeration or temperature control, and by comparison to other biomarker methods, analysis of percent n-3 HUFA is relatively low cost. Given the constraints of the research setting, and the benefits of the methods we utilized, the correlation of 0.39 achieved in this study is adequate and consistent with those that have been reported for other studies correlating dietary estimates and biomarker measures of omega-3 status.

References

1. Kris-Etherton PM, Innis S, American Dietetic Association, Dietitians of Canada. Position of the American Dietetic Association and Dietitians of Canada: dietary fatty acids. J Am Diet Assoc. 2007;107(9):1599-611.

2. Huang TL. Omega-3 fatty acids, cognitive decline, and Alzheimer's disease: a critical review and evaluation of the literature. J Alzheimer's Dis. 2010;21(3):673-90.

3. Fekete K, Marosvolgyi T, Jakobik V, Decsi T. Methods of assessment of n-3 long-chain polyunsaturated fatty acid status in humans: a systematic review. Am J Clin Nutr. 2009;89(6):2070S-84S.

4. Farzaneh-Far R, Lin J, Epel ES, Harris WS, Blackburn EH, Whooley MA. Association of marine omega-3 fatty acid levels with telomeric aging in patients with coronary heart disease. JAMA. 2010;303(3):250-7.

5. Schaefer EJ, Bongard V, Beiser AS, Lamon-Fava S, Robins SJ, Au R, et al. Plasma phosphatidylcholine docosahexaenoic acid content and risk of dementia and Alzheimer disease: the Framingham Heart Study. Arch Neurol. 2006;63(11):1545-50.

6. Thorseng T, Witte DR, Vistisen D, Borch-Johnsen K, Bjerregaard P, Jorgensen ME. The association between n-3 fatty acids in erythrocyte membranes and insulin resistance: the Inuit Health in Transition Study. Int J Circumpolar Health. 2009;68(4):327-36.

7. Naqvi AZ, Buettner C, Phillips RS, Davis RB, Mukamal KJ. n-3 fatty acids and periodontitis in US adults. J Am Diet Assoc. 2010;110(11):1669-75.

8. van Gelder BM, Tijhuis M, Kalmijn S, Kromhout D. Fish consumption, n-3 fatty acids, and subsequent 5-y cognitive decline in elderly men: the Zutphen Elderly Study. Am J Clin Nutr. 2007;85(4):1142-7.

9. Gopinath B, Flood VM, Rochtchina E, McMahon CM, Mitchell P. Consumption of omega-3 fatty acids and fish and risk of age-related hearing loss. Am J Clin Nutr. 2010;92(2):416-21.

10. Golding J, Steer C, Emmett P, Davis JM, Hibbeln JR. High levels of depressive symptoms in pregnancy with low omega-3 fatty acid intake from fish. Epidemiology. 2009;20(4):598-603.

11. de Goede J, Geleijnse JM, Boer JM, Kromhout D, Verschuren WM. Marine (n-3) fatty acids, fish consumption, and the 10-year risk of fatal and nonfatal coronary heart disease in a large population of Dutch adults with low fish intake. J Nutr. 2010;140(5):1023-8.

12. Overby NC, Serra-Majem L, Andersen LF. Dietary assessment methods on n-3 fatty acid intake: a systematic review. Br J Nutr. 2009;102 Suppl 1:S56-63.

13. Kohler A, Bittner D, Low A, von Schacky C. Effects of a convenience drink fortified with n-3 fatty acids on the n-3 index. Br J Nutr. 2010;104(5):729-36.

14. Childs CE, Romeu-Nadal M, Burdge GC, Calder PC. Gender differences in the n-3 fatty acid content of tissues. Proc Nutr Soc. 2008;67(1):19-27.

15. Sands SA, Reid KJ, Windsor SL, Harris WS. The impact of age, body mass index, and fish intake on the EPA and DHA content of human erythrocytes. Lipids. 2005;40(4):343-7.

16. Block RC, Harris WS, Pottala JV. Determinants of Blood Cell Omega-3 Fatty Acid Content. Open Biomark J. 2008;1:1-6.

17. Plourde M, Vohl MC, Vandal M, Couture P, Lemieux S, Cunnane SC. Plasma n-3 fatty acid response to an n-3 fatty acid supplement is modulated by apoE epsilon4 but not by the common PPAR-alpha L162V polymorphism in men. Br J Nutr. 2009;102(8):1121-4.

18. Stark KD. The percentage of n-3 highly unsaturated fatty acids in total HUFA as a biomarker for omega-3 fatty acid status in tissues. Lipids. 2008;43(1):45-53.

19. Mintel. Omega-3 – US. London: 2008.

20. USDA National Nutrient Database for Standard Reference, Release 22 [database on the Internet]. 2009 [cited March 26-29, 2010; April 29, 2010; and May 17, 2010]. Available from: http://www.ars.usda.gov/nutrientdata.

21. Ichihar K, Waku K, Yamaguchi C, Saito K, Shibahara A, Miyatani S, et al. A convenient method for determination of the C20-22 PUFA composition of glycerolipids in blood and breast milk. Lipids. 2002;37(5):523-6.

22. Lepage G, Roy CC. Direct transesterification of all classes of lipids in a one-step reaction. J Lipid Res. 1986;27(1):114-20.

23. Masood A, Stark KD, Salem N, Jr. A simplified and efficient method for the analysis of fatty acid methyl esters suitable for large clinical studies. J Lipid Res. 2005;46(10):2299-305.

24. Sullivan BL, Williams PG, Meyer BJ. Biomarker validation of a long-chain omega-3 polyunsaturated fatty acid food frequency questionnaire. Lipids. 2006;41(9):845-50.

25. Lucas M, Asselin G, Merette C, Poulin MJ, Dodin S. Validation of an FFQ for evaluation of EPA and DHA intake. Public Health Nutr. 2009;12(10):1783-90.

26. Metherel AH, Armstrong JM, Patterson AC, Stark KD. Assessment of blood measures of n-3 polyunsaturated fatty acids with acute fish oil supplementation and washout in men and women. Prostaglandins Leukot Essent Fatty Acids. 2009;81(1):23-9.

27. Fratesi JA, Hogg RC, Young-Newton GS, Patterson AC, Charkhzarin P, Block Thomas K, et al. Direct quantitation of omega-3 fatty acid intake of Canadian residents of a long-term care facility. Appl Physiol Nutr Metab. 2009;34(1):1-9.

28. Sublette ME, Segal-Isaacson CJ, Cooper TB, Fekri S, Vanegas N, Galfalvy HC, et al. Validation of a food frequency questionnaire to assess intake of n-3 polyunsaturated fatty acids in subjects with and without major depressive disorder. J Am Diet Assoc. 2011;111(1):117-23 e1-2.

29. Burdge GC, Calder PC. Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. Reprod Nutr Dev. 2005;45(5):581-97.

Figures and Tables

Figure 3.1 Scatter plot of predicted values and residuals from regression analysis using backwards deletion with omega-3 HUFA score as the dependent variable and FFQ-based intake of EPA + DHA, gender, age, smoking status, alcohol consumption, body mass index (BMI), and location as the independent variable

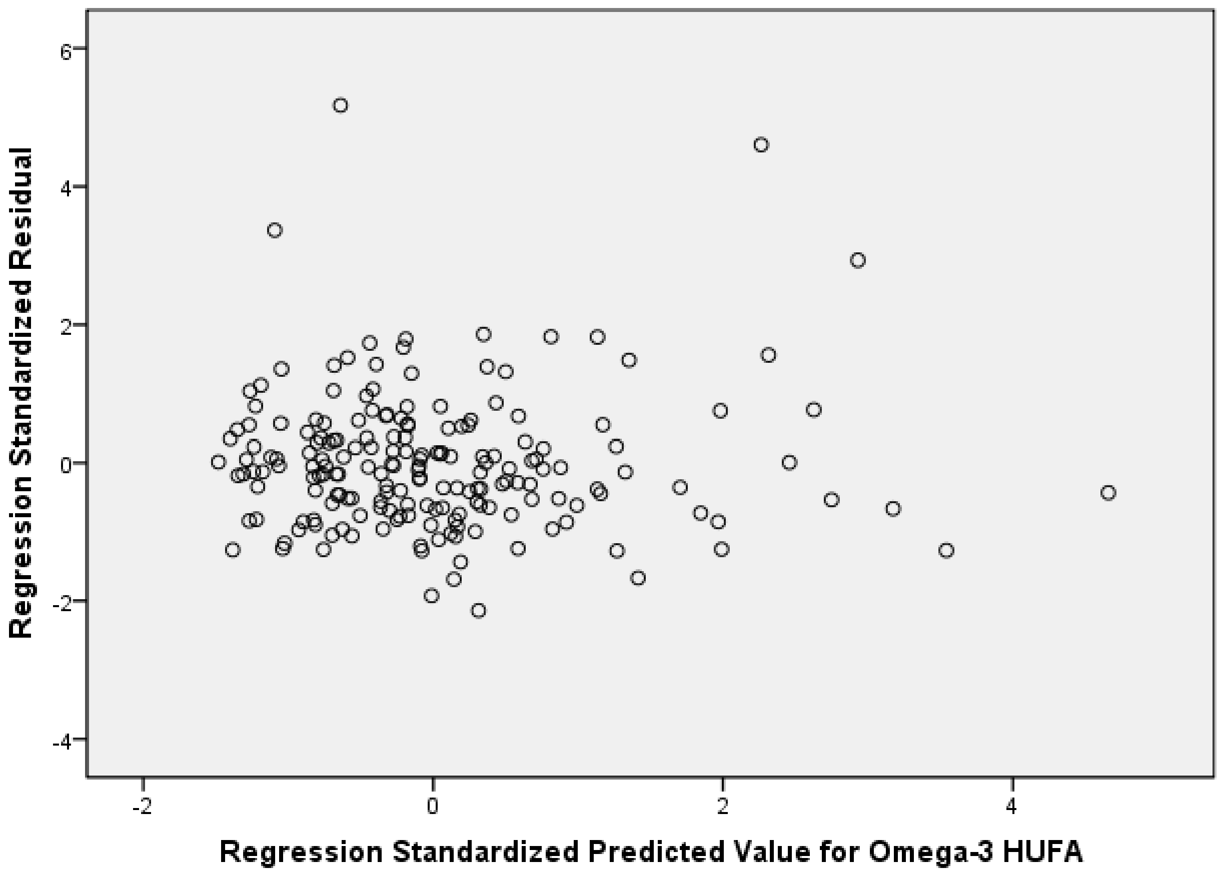

Figure 3.2 Scatter plot of predicted values and residuals from regression analysis using backwards deletion with log 10 omega-3 HUFA score as the dependent variable and log 10 FFQ-based intake of EPA + DHA, gender, age, smoking status, alcohol consumption, body mass index (BMI), and location as the independent variables

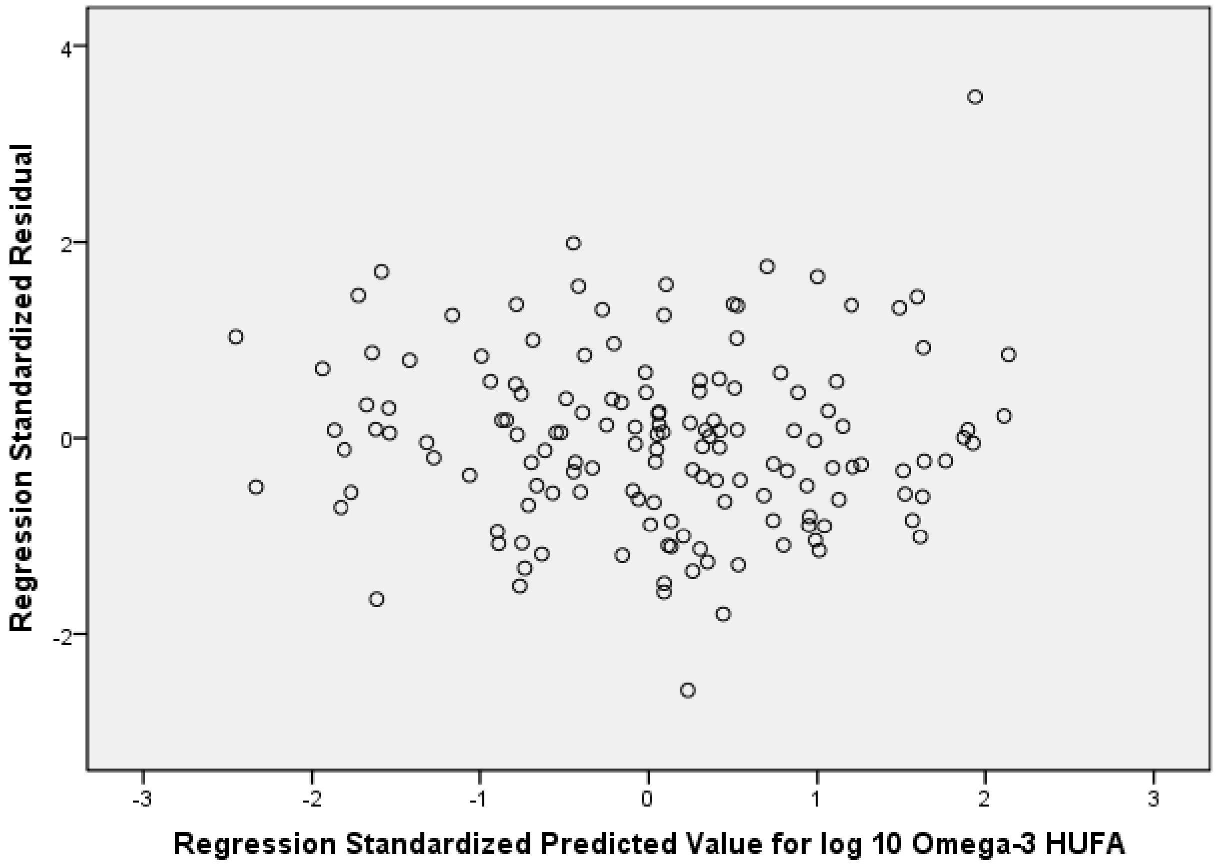

Table 3.1 Characteristics by military service category

Characteristic	National Gaurd		Active Duty		χ^2	p
	n	%	n	%		
Gender						
Male	116	93.5	60	89.5	0.96	0.327
Female	8	6.5	7	10.5		
Marital Status						
Married	70	56.5	43	64.2	1.08	0.300
Single, divorced, seperated	54	43.5	24	35.8		
Education						
Less than college degree	92	74.2	55	82.1	1.53	0.216
College degree	32	25.8	12	17.9		
Race/Ethnic Category						
Non-Hispanic Caucasian	76	61.3	48	71.6	2.05	0.153
All other	48	38.7	19	28.4		
Alcohol Use*						
Less than one drink/day	79	69.2	53	79.1	4.53	0.033
One or more drinks/day	44	35.8	14	20.9		
Smoking Status						
Non or ex-smoker	85	68.5	42	62.7	0.67	0.671
Current smoker	39	31.5	25	37.3		

*Groups differ, $p \leq 0.05$

Table 3.2 Additional characteristics by military service category

Characteristic	National Guard			Active Duty					
	n	mean	SD	n	mean	SD	df[c]	t	p[c]
Age*	123	30.5	8.7	67	27.2	6.1	176.5	3.12	0.003
BMI*	123	27.9	4.2	67	26.7	3.4	163.5	2.12	0.036
Intake EPA + DHA[a]*	124	2.33	0.42	67	2.14	0.54	109.3	2.58	0.011
Omega-3 HUFA score[b]	124	1.22	0.08	67	1.23	0.05	181.8	-0.86	0.390

[a]Log10 transformed value obtained using the FFQ

[b]Log10 transformed percent omega-3 highly unsaturated fatty acids (HUFA) in total HUFA of whole blood

[c]Equal variances not assumed

*Groups differ, $p \leq 0.05$

Table 3.3 Comparison of intake of EPA + DHA[a] by sample characteristics

Characteristics	n	df	Mean	SD	*t*	*p*
			Intake of EPA + DHA			
Gender	176	189	2.27	0.45	0.61	0.552
Male	15		2.17	0.65		
Female						
Age*						
28 or younger	104	188	2.20	0.46	-2.01	0.045
29 or older	86		2.34	0.48		
Marital Status						
Married	113	189	2.27	0.47	2.90	0.772
Single, divorced, separated	78		2.25	0.47		
Education						
Less than college degree	147	189	2.24	0.47	-1.32	0.188
College degree	44		2.35	0.47		
Ethnic Category						
Non-Hispanic Caucasian	124	189	2.27	0.51	0.08	0.935
All other	64		2.26	0.40		
Alcohol Use						
One or more drinks/day	132	188	2.24	0.50	-0.97	0.340
Less than one drink/day	58		2.31	0.39		
Smoking Status						
Never or former smoker	127	189	2.29	0.48	1.05	0.296
Current smoker	64		2.21	0.46		

[a]Log 10 transformed value obtained using the FFQ

*Groups differ, $p \leq 0.05$

Table 3.4 Comparison of omega-3 HUFA score[a] by sample characteristics

Characteristics	n	df	Mean	SD	t	p
			Omega-3 HUFA Score			
Gender						
Male	176	189	1.22	0.07	-2.63	0.793
Female	15		1.23	0.07		
Age*						
28 or younger	104	188	1.21	0.07	-2.55	0.012
29 or older	86		1.24	0.08		
Marital Status						
Married	113	189	1.23	0.07	1.47	0.142
Single, divorced, separated	78		1.21	0.07		
Education						
Less than college degree	147	189	1.22	0.07	-1.71	0.089
College degree	44		1.24	0.08		
Ethnic Category						
Non-Hispanic Caucasian	124	189	1.22	0.08	-0.77	0.444
All other	64		1.23	0.07		
Alcohol Use						
One or more drinks/day	132	188	1.22	0.07	0.030	0.976
Less than one drink/day	58		1.22	0.08		
Smoking Status*						
Never or former smoker	127	189	1.23	0.08	2.78	0.006
Current smoker	64		1.20	0.06		

[a]Log 10 transformed percent omega-3 highly unsaturated fatty acids (HUFA) in total HUFA of whole blood

*Groups differ, $p \leq 0.05$

Table 3.5 Correlations between omega-3 HUFA score[a], intake of EPA + DHA[b], time to last meal[c], and individual fatty acids[d]

	Omega-3 HUFA score	Omega-3 PUFA	DHA	EPA	DPA n-3	ALA	AA	LA
Omega-3 HUFA score	-	0.82**	0.62**	0.62**	0.29**	0.07	0.06	0.06
Intake EPA + DHA	0.39**	0.24**	0.33**	0.01	0.00	0.06	0.11	0.04
Time to last food	0.01	-0.05	0.08	0.08	0.11	-0.23**	0.12	-0.18*

[a]Log 10 transformed percent omega-3 highly unsaturated fatty acids (HUFA) in total HUFA of whole blood

[b]Log 10 transformed value obtained using the FFQ

[c]Time to last meal is self reported time in hours since last ingesting a food other than black coffee, diet soda, or water.

[d]Log 10 transformed value of the percent by weight of total fatty acids in whole blood

Pearson Correlation was used to determine significance

*$p \leq 0.05$; **$p \leq 0.01$

Table 3.6 Comparison of omega-3 HUFA score[a] by dietary practices

Dietary Practice	n	df	Mean	SD	*t*	*p*
Omega-3 Supplement Use*						
Yes	25	183	1.26	0.07	2.97	0.003
No	160		1.21	0.07		
Functional Food Use*						
Yes	35	155	1.24	0.08	2.62	0.010
No	122		1.21	0.07		
Sushi Consumption*						
Yes	68	188	1.24	0.08	3.02	0.003
No	122		1.21	0.07		
Consumed Seafood at Least Twice a Week*						
Yes	82	189	1.24	0.07	3.11	0.002
No	109		1.21	0.07		

[a]Log 10 transformed percent omega-3 highly unsaturated fatty acids (HUFA) in total HUFA of whole blood

*Groups differ, $p \leq 0.01$

Table 3.7 Results of regression analysis with omega-3 HUFA score[a] as dependent variable

Independent variables	*B*	SE *B*	β	t	F	p
Intake EPA + DHA[b]	0.060	0.012	0.382	5.07		<0.001
Alcohol	-0.004	0.003	-0.132	-1.70		0.092
Smoking	-0.022	0.012	-0.139	-1.86		0.065
Service Component	0.022	0.012	0.142	1.81		0.072
Age	0.001	0.001	0.158	2.05		0.042
Model					9.33	<0.001

[a]Log 10 transformed percent omega-3 highly unsaturated fatty acids (HUFA) in total HUFA of whole blood

[b]Log 10 transformed value obtained using the FFQ

Model R = 0.50

Table 3.8 Agreement of quintile assignment between EPA + DHA intakes[a] and omega-3 HUFA score[b]

EPA + DHA Intake Quintile	Intake EPA + DHA[a] vs. Omega-3 HUFA Score[b]		
	Same	Adjacent	Misclassified
1 (n = 39)	12	10	17
2 (n = 38)	12	15	11
3 (n = 38)	6	16	16
4 (n = 38)	12	16	10
5 (n = 38)	16	7	15
Total n (%)	58 (30%)	64 (34%)	69 (36 %)

[a]Log 10 transformed value obtained using the FFQ

[b]Log 10 transformed percent omega-3 highly unsaturated fatty acids (HUFA) in total HUFA of whole blood

Chapter 4 - Attention Deficit Hyperactivity Disorder Subtypes and their Relation to Cognitive Functioning, Mood States, and Combat Stress Symptomatology in Deploying U.S. Soldiers

Submission pending as Hanson JA, Haub MD, Junnila JL, Johnston DT, Nelson Goff, BS, Dretsch MN. Attention Deficit Hyperactivity Disorder Subtypes and their Relation to Cognitive Functioning, Mood States, and Combat Stress Symptomatology in Deploying U.S. Soldiers.

Abstract

Objective: There is a paucity of information about attention deficit hyperactivity disorder (ADHD) in the US military. This article describes the relationship between ADHD subtypes and neuropsychological function among deploying US soldiers. Methods: Soldiers (N = 260) scheduled for deployment to Iraq provided demographic information and completed the World Health Organization Adult ADHD Self-Report Scale (ASRS) Screener. Cognitive tests, a deployment health history questionnaire, and the PTSD (posttraumatic stress disorder) Checklist-Military Version (PCL-M) were completed by subsamples of available participants. Spearman correlation coefficients and Mann-Whitney U tests were used to explore the relationships among variables. Results: The prevalence of positive ASRS screens in the overall sample was 10.4 %. ASRS scores were correlated with avoidance ($N = 63$, $\rho = 0.37$, $p = 0.003$), hyperarousal ($N = 63$, $\rho = 0.25$, $p = 0.047$), and total ($N = 62$, $\rho = 0.33$, $p = 0.009$) PTSD scores, scores on the match-to-sample ($N = 110$, $\rho = -0.23$, $p = 0.014$) and emotional Stroop ($N = 108$, $\rho = -0.23$, $p = 0.016$) tasks, and all six mood (e.g., anger, anxiety) scale scores ($N = 110$; $\rho = -0.37$ to 0.43). Those with positive ADHD scores had both higher overall PTSD scores ($p = 0.011$) and PTSD avoidance scores ($p = 0.031$). Conclusions: The prevalence, functional difficulties, and co-morbidities of ADHD are similar among military and non-military populations. However, with regard to cognitive functioning, mood, and combat stress symptomatology, these novel findings suggest a differential pattern between subtypes of ADHD.

Introduction

With an estimated prevalence of roughly 4-5% (1, 2), adult attention deficient hyperactivity disorder (ADHD) is one of the most commonly occurring DSM-IV-TR disorders in the US. ADHD is characterized by problems with attending to external and internal stimuli, organizing and completing tasks, and controlling impulses, emotions, and behaviors (3). Based on the DSM-IV-TR, ADHD is comprised of three subtypes; 1) inattentive, 2) hyperactive/impulsive, and 3) combined. Whereas ADHD is a well-recognized disorder of childhood, adult ADHD has now come into the clinical spotlight (4). Although the neurobiological bases for ADHD is not fully understood, frontal system dysfunction as well as dopaminergic and noradrenergic deficits have been implicated (3).

Individuals with adult ADHD are more likely to experience diminished functioning and are also more likely to suffer from mood, substance abuse, and anxiety disorders (1, 2). Young motor vehicle drivers with ADHD face an increased likelihood of having a traffic accident as well as an increased likelihood of having their license suspended (5). Attention deficient hyperactivity disorder is associated with a higher incidence of depression and posttraumatic stress disorder (PTSD) (1), as well as substance abuse and dependency (6).

The consequences of the impaired neuropsychological functioning and increased co-morbidity associated with ADHD has important implications in combat scenarios. Although potentially waiverable, historically, a diagnosis of ADHD disqualified an individual from military service. In the year 2004, the Army standard for initial military service was modified. Under the new policy ADHD is no longer considered a disqualifying condition if the applicant has not taken medication for the condition within the last year, and can demonstrate passing academic performance (7). Soldiers may remain on Active Duty if they do not exhibit significant inattention or impulsivity traits (7). However, adult ADHD is often missed, undiagnosed, and may go unreported during military entrance examinations. An anecdotal report exists (8), but there is a scarcity of information regarding the prevalence of and factors associated with adult ADHD in the military. One study of military service members utilizing mental health services while in Iraq found that among those with a prior psychiatric diagnosis, the highest rate of relapse was among those with ADHD (9). Little else has been published on the topic. As part of a study designed to investigate the relationship between lifestyle factors and psychological resilience among deploying soldiers, we examined the relationship between inattention, hyperactivity/impulsiveness, mood, cognition, and PTSD symptoms.

Methods

Subjects

A volunteer sample of soldiers (N = 260) scheduled for deployment to Iraq provided demographic information and completed a set of questions related to ADHD. Participants were from one of two combat brigades from Kansas and Texas. Data collection occurred from October 2009 through February 2010. The participants completed the study roughly one month prior to deployment. The soldiers were not compensated for their participation. This protocol was approved by the Kansas State University Institutional Review Board. After being given a complete description of the study, all of the soldiers recruited for this study were required to provide written informed consent prior to participation.

Demographic and Health-Related Behaviors

A self-administered questionnaire was used to measure demographic characteristics, height, weight, smoking status, alcohol use, and caffeine consumption.

Inattention and Hyperactivity/Impulsiveness

Inattention and hyperactivity/impulsivity were measured using the World Health Organization Adult ADHD Self-Report Scale (ASRS) Screener (10). This screener was administered with the demographic and health-related behavior questions. Six questions (four inattention questions and two hyperactivity/impulsivity questions) comprise this validated self-administered tool. Numeric values ranging from zero to four were assigned to responses with zero assigned to the least frequent (i.e., "never") and four assigned to the most frequent (i.e., "always"). Using the zero to 24 scoring approach described by Kessler (10), a score of 14 or more on the ASRS was categorized as a positive screen. The ASRS has an alpha reliability coefficient of 0.77 and a receiver operating characteristic curve (area under the curve; AUC) of 0.90 (10).

Cognitive Performance and Mood

All neurocognitive tests and questionnaires were administered by trained research personnel. Cognitive performance was assessed using the following measures:

Combat Stress Assessment

The Combat Stress Assessment (CSA) (11) contains a battery of validated subtests (see below) of attention, memory, and executive functions administered on a handheld computer. The CSA has been used in prior studies and has been shown to be sensitive to neuropsychological functioning in soldiers (11) and civilians (12).

a) Sleep Scale. Participants rated their degree of alertness and sleepiness on a seven-step scale with the value of one being assigned to the most rested and alert state. Unlike the remaining CSA tasks listed below, sleep scale scores served as a control variable rather than an outcome variable.

b) Mood Scale. The seven mood categories measured include vigor, happiness, depression, anger, fatigue, anxiety, and restlessness. A total of 42 adjectives describing feelings (six for each scale) appeared on the screen and participants used zero-to-six visual analog scales to indicate how well each of these words represented their feelings. The lower the scale scores the less agreement indicated.

c) Pursuit Tracking. A moving target depicted as a bull's-eye moved across the screen and participants were instructed to follow the target across the screen using the stylus.

d) Simple Reaction Time. An asterisk appeared on the screen and participants responded as quickly as possible by tapping the asterisk.

e) Code Substitution. A key of symbols and numbers appeared on the screen. A symbol and number pair also appeared on the screen below the key. Respondents indicated whether or not the pair matched the key.

f) Procedural Reaction Time. Numbers appeared on the screen and participants responded by tapping the box that corresponded to each of the numbers.

g) Go-no-Go Reaction Time. An asterisk or a plus sign appeared and participants responded by either tapping the screen when the asterisk appeared or doing nothing when the plus sign appeared.

h) Match-to-Sample. A pattern appeared on the screen and then disappeared. Participants were then presented with two patterns and responded by tapping the pattern that matched the initial pattern.

i) Code Substitution Recall. A number and symbol pair appeared on the screen and participants indicated whether the pair matched based on the key they saw in the code substitution test.

j) Stroop. Words written in various colors appeared on the screen. Participants responded by tapping a box that matched the color of the word rather than the meaning of the word. Sub-set categories included congruent (color and meaning of word agreed), incongruent (color and meaning of word differed), neutral (nonsense words appeared), and emotional (combat related words such as "I.E.D" and "convoy" appeared).

Pursuit tracking task scores were recorded as percent of time on target. In contrast, for the tasks listed in the above section (d – j), a throughput score (average number of correct responses per minute) was used as a measure of performance.

Controlled Oral Word Association Test

The Controlled Oral Word Association Test (COWAT) (13), is a semantic fluency test in which participants had one minute to say as many words as possible that start with a given letter. This test measures verbal fluency and is sensitive to linguistic impairment and early mental decline (14) and has an alpha reliability coefficient of 0.88.

Hopkins Verbal Learning Test-Revised

The Hopkins Verbal Learning Test-Revised (HVLT-R) (15) is a test of memory of semantic categorical words. Participants received three learning trials, a delayed recall trial, and a delayed recognition trial. The test has been shown to be sensitive to cognitive decline in demented patients (16) and has an alpha reliability coefficient of 0.74.

The mean t-score based on age for trials one through three was used as the performance measure on the HVLT-R (17), whereas the total number of correct responses adjusted for gender and education was used as the measure of performance on the COWAT (18).

PTSD Symptoms and Deployment Health History

The US Department of Veterans Affairs Operation Iraqi Freedom/Operation Enduring Freedom Veterans Health Questionnaire was used to collect health information pertaining to prior military deployments (19). This questionnaire includes a validated traumatic brain injury (TBI) screen and the PTSD screen described below.

PTSD Checklist-Military Version (PCL-M)

The PTSD Checklist-Military Version (PCL-M) (20) is a 17-item self-report measure of the DSM-IV symptoms of PTSD. It has a test-retest reliability of 0.96, internal consistency (alpha coefficient) of 0.93 for B symptoms (i.e., re-experiencing), 0.92 for C symptoms (i.e., effortful avoidance), 0.92 for D symptoms (i.e., hyperarousal), and 0.97 for all 17 symptoms. An example of an item includes "Feeling very upset when something reminded you of a stressful military experience?"

Statistical Analysis

Data analyses were performed using Predictive Analytics Software (version 18, 2009, SPSS, Inc, Chicago, IL). Means and standard deviations were calculated for continuous variables. Frequencies and percentages were calculated for categorical data. Means and percentages were calculated based on the number of complete responses. Due to operational time constraints, not all participants were able to complete all components of the study. Pairwise exclusion was used to exclude cases only if they were missing data for a specific analysis. Demographic and health-related behavior responses were collapsed into meaningful categories to allow for comparison of ASRS, hyperactivity/impulsivity and inattention scores using Student's independent sample t-tests. For example, caffeine intake was categorized as 150 mg/day (content of approximately two cups of coffee) or less. Because many of the cognitive test scores, mood scales scores, and PCL-M scores were not normally distributed, non-parametric statistical analyses were performed in analysis of these scores. Spearman rank order correlation coefficients were used to measure the correlation between cognitive, mood, and PCL-M scores and ASRS scores. Mann-Whitney U tests were conducted to explore the relationship between

ASRS screen category and cognitive, mood, and PCL-M scores. Two-tailed tests and a significance level of $p \leq 0.05$ were used for all analyses.

Results

Sample Characteristics

Two hundred sixty participants provided demographic information and completed the ASRS. Age in years ranged from 18 to 56 with a mean age of 28.97 (N = 259; SD = 7.91). More than half (56%; N = 145) were married, and all but one had obtained a high school degree or higher. Mean body mass index was 27.41 kg/m^2 (N = 259; SD = 4.07). Additional demographic and health related characteristics are summarized in Table 4.1.

ASRS Scores

The ASRS scores ranged from 0 to 24 with a mean of 9.18 (SD = 3.40). The prevalence of positive ASRS screens was 10.4 % (N = 27). The ASRS scores were correlated with hyperactivity/impulsivity (N = 260, $\rho = 0.69$, $p < 0.001$) and inattention scores (N = 260, $\rho = 0.83$, $p < 0.001$) scores. Hyperactivity/impulsivity and inattention scores were also correlated (N = 260, $\rho = 0.20$, $p = 0.001$). The ASRS scores and hyperactivity/impulsivity scores differed among numerous demographic (Table 4.2) and health behavior categories (Table 4.3). The ASRS scores were higher among participants less than 28 years old, those who were not married, those who had not obtained a college degree, and those who identified themselves as non-Hispanic Caucasian. The ASRS scores were also higher among smokers, those who consumed alcohol daily, and those who consumed 150 mg or more of caffeine per day. Hyperactivity/impulsivity scores were higher among participants less than 28 years old, those who had not obtained a college degree, and those who identified themselves as non-Hispanic Caucasian. Hyperactivity/impulsivity scores were also higher among smokers, those who consumed alcohol daily, and those who consumed 150 mg or more of caffeine per day. Inattention scores did not differ among the demographic and health behavior categories.

ASRS Scores and Cognition

One hundred thirty participants completed the HVLT-R, 100 participants completed the COWAT, and 110 participants completed the CSA. The mean t-scores for trials one through three of the HVLT-R was 46.7 (SD = 8.2). For the COWAT, the mean number of correct responses adjusted for gender and education was 41.6 (SD = 9.8). The ASRS scores and inattention scores were negatively correlated with performance scores on several of the CSA cognitive tests (Table 4.4). Correlations between hyperactivity/impulsivity and cognitive test scores were not significant. Participants with positive ASRS screens had significantly lower performance scores for the procedural reaction time task (U = 283.5; p = 0.009), the go-no-go task (U = 319.5; p = 0.025) and the Stroop emotional task (U = 300.5; p = 0.018). The performance scores for the remaining tasks did not differ by ASRS screen category.

ASRS Scores and Mood

Mean mood scale scores ranged from 12.3 (SD = 13.8) for depression to 65.1 (SD = 17.2) for happiness. Scores for both anger and depression were positively skewed with 23% (N = 25) of participants obtaining a score of zero on the depression scale and 27% (N = 30) obtaining a score of zero on the anger scale. The ASRS, inattention, and hyperactivity/impulsivity scores were correlated with numerous mood scale scores (Table 4.4). Participants with positive ASRS screens had significantly higher restlessness scores (U = 298.5; p = .014). The mood scale scores for the remaining mood items did not differ significantly by ASRS screen category.

ASRS Scores, PCL-M Scores, and Deployment Health History

One hundred fifty-five participants provided information regarding prior deployments. Of these 47% (N = 73) had been on one or more previous deployment and 86% (N = 63) of those who had been on a previous deployment completed the PLC-M. Scores for the individual scale items ranged from 1.1 (SD = 0.3) for "trouble remembering important parts of a stressful military experience from the past," to 2.0 (SD = 1.0) for "trouble falling or staying asleep." PCL-M total scores ranged from 17 to 41 with a mean score of 24.9 (SD = 6.6). Total ASRS scores and hyperactivity/impulsivity scores were correlated with PCL-M scores (Table 4.4), and participants with positive ASRS screen had higher PCL-M avoidance subscale scores and higher total PCL-M scores (Table 4.5).

Among those who had been on previous deployments, 97% (N = 71) provided information regarding deployment related injuries. Of these, 47% (N = 34) indicated they sustained an injury from various causes (e.g., bullet, motor vehicle accident, fall, physical training) during deployment. Participants who reported a deployment related injury had significantly lower cognitive scores and significantly higher PCL-M re-experiencing scores (Table 4.6). Mood scores did not differ based on injury category. Among those reporting a deployment related injury, 50% (N = 17) reported symptoms consistent with mild (m)TBI, and among these 65% (N = 11) indicated they were currently experiencing problems that they thought might be related to a possible head injury or concussion (e.g., ringing in the ears, sleep problems, irritability).

Sleep Scale Scores

Of those participants who completed the sleep scale, 80% (N = 87) depicted themselves as something other than fully alert. Sleep scale scores were correlated with ASRS ($\rho = 0.26$, $p = 0.007$), inattention ($\rho = 0.29$, $p = 0.002$), happiness ($\rho = -0.58$, $p < 0.001$), depression ($\rho = 0.27$, $p = 0.005$), fatigue ($\rho = 0.54$, $p < 0.001$), restlessness ($\rho = 0.25$, $p = 0.009$), vigor ($\rho = -0.65$, $p < 0.001$), and match-to-sample throughput ($\rho = -0.20$, $p = 0.036$) scores. Among the participants who depicted themselves as fully awake and alert, ASRS correlated with PCL-M avoidance (N = 8, $\rho = 0.72$, $p = 0.044$) and PCL-M total (N = 8, $\rho = 0.93$, $p = 0.001$) scores. Among the participants who depicted themselves as something other than fully awake and alert, ASRS scores correlated with all of the mood scores (N = 87) with correlations ranging from $\rho = -0.40$ ($p < 0.001$) for happiness to $\rho = 0.46$ for restlessness ($p < 0 .001$). Among those not fully awake, ASRS scores also correlated with match to sample (N = 87, $\rho = -0.22$, $p = 0.040$), Stroop emotional (N = 85,$\rho = -0.23$, $p = 0.032$), PCL-M avoidance (N = 34, $\rho = 0.48$, $p = 0.004$), PCL-M hyperarousal (N = 34, $\rho = 0.38$, $p = 0.027$), and PCL-M total (N = 33, $\rho = 0.43$, $p = 0.012$) scores. Sleep scale scores did not differ based on injury category (See Table 4.6).

Discussion

The prevalence of positive ASRS screens in this sample (10.4%) was nearly identical to the prevalence rate (11.0 %) obtained in a representative sample of health plan members (10). For the most part, the relationships between demographic and health behavior characteristics and ASRS scores were consistent with what has been reported previously. ASRS scores were higher among non-Hispanic whites, and this finding corresponds with the results found in a nationally representative sample, in which non-Hispanic whites had higher estimated odds of ADHD (1). In addition, ASRS scores were lower among those with a college degree, and this is consistent with the findings from a multi-national study in which those with less than a college degree had higher estimated odds of ADHD (2). Although the incidence of ADHD has been found to be higher among males (1, 2), we did not detect a difference in ASRS scores based on gender. However, due to the relatively small number of female participants this finding should be interpreted with caution.

The positive correlations between ASRS scores and mood scale scores, as well the positive correlation between ASRS scores and PCL-M, are consistent with earlier studies in which the estimated odds of depression, anxiety, and PTSD were higher among those with ADHD (1). As reported previously in a study of U.S. service members returning from military deployments, "trouble falling or staying asleep" was the PCL-M item with the highest mean score (21).

The data collected from our subjective reports of ADHD was supplemented by behavioral findings that correlate with the inattention, hyperactivity/impulsivity, and combined subtypes. More specifically, while all three subtypes were correlated with mood symptom scores, the inattention subtype was most strongly associated with cognitive decrements in attention as assessed by the automated CSA, but not the traditional pencil-and-paper neuropsychological tests (i.e., HVLT and COWAT). Furthermore, PTSD symptom scores were only correlated with the hyperactivity/impulsivity and combined subtypes of ADHD, but not with inattention. These findings are of particular importance in that they might indicate that specific subtypes of ADHD are more vulnerable to developing PTSD symptoms when exposed to traumatic events as experienced in combat environments. Recent evidence (22) suggests a differential pattern of adjustment problems in early adulthood based on ADHD subtypes. However, these subtypes fluctuate across childhood development, and should therefore, not be considered discrete and stable categories. Further support for increased risk for psychopathology in hyperactivity/impulsivity subtype is revealed in a recent structural brain imaging study. Diffusion tensor imaging (DTI) is a magnetic resonance imaging technique based on the movement of water molecules, or anisotrophic diffusion, for measuring thickness of myelin sheaths and axons, distributions of directions, and density of white matter tracts (23). Konrad and colleagues (24) used DRI to show a correlation between impulsivity in adults with ADHD and frontal white matter tracts of the brain (e.g. orbitofrontal fibre tracts) that have also been implicated with PTSD (25, 26).

Our results did not show a correlation between reported injury and mood scores. However, reported injury was associated with lower cognitive scores, and higher PTSD re-experiencing scores. Although both mTBI and ADHD are strongly associated with similar attentional impairments, the small number of participants reporting symptoms or problems consistent with mTBI prevented meaningful analysis of this subgroup.

Limitations

It is important to consider several details with respect to the interpretation of the current results. This was a descriptive study, and as such, no attempt was made to establish a causal effect among the variables investigated. In addition, this study was based on the self-reported behavior and symptoms from a volunteer sample, and not clinical interviews. As such, screening instrument scores (not diagnosis of ADHD and PTSD) were used to explore the relationships among variables. Participants' performance and responses may have differed from those of non-respondents, and the stigma associated with mental health problem (15) may have resulted in reluctance to report negative feeling and behaviors. Although self-reported data are typically considered a limitation by researchers, reports indicate that the ASRS has adequate sensitivity (68.7%), excellent specificity (99.5%), excellent accuracy (97.9%), and good kappa scores (0.76) (27). Lastly, it should be pointed out that although measures were taken to minimize distractions, this study was not conducted in a controlled testing environment. Nevertheless, taken as a whole, this study adds to both the limited body of information available on ADHD in the military and the understanding of the potential influence adult ADHD may have in the performance and resilience of soldiers deploying to combat.

Conclusions

In the current study, ADHD symptom scores were associated with a number of demographic and health behavior characteristics, mood reports, and PTSD scores. The relationship between these variables is complex; however, the general magnitude of the correlations between hyperactivity/impulsivity scores with both mood and PTSD scores appear to be stronger than that between inattentive subtype of ADHD and cognitive outcomes. Particularly noteworthy is the fact that in this sample of deploying soldiers, mood was not related to prior deployment injury reports, whereas hyperactivity/impulsivity, cognition, and PTSD re-experiencing scores were. Research is needed to build upon the findings in the current study. Our results suggest the prevalence, functional difficulties, and co-morbidities of adult ADHD are similar among military and non-military populations (i.e. civilians). Given our findings and the current operational demands being placed on members of the U.S. military, it is imperative the relationship between these variables becomes better understood. In the interim, as has been suggested to health care providers in general (28), military health care providers should also consider the possibility of ADHD in adults with co-morbid conditions.

References

1. Kessler RC, Adler L, Barkley R, Biederman J, Conners CK, Demler O, Faraone SV, Greenhill LL, Howes, MJ, Secnik K, Spencer,T, Ustun, TB, Walters, EE, Zaslavsky, AM. The prevalence and correlates of adult ADHD in the United States: results from the National Comorbidity Survey Replication. Am J Psychiatry. 2006;163(4):716-23.

2. Fayyad J, De Graaf R, Kessler R, Alonso J, Angermeyer M, Demyttenaere K, De Girolamo G, Haro JM, Karam EG, Lara C, Lepine JP, Ormel J, Posada-Villa J, Zaslavsky AM, Jin R. Cross-national prevalence and correlates of adult attention-deficit hyperactivity disorder. Br J Psychiatry. 2007;190:402-9.

3. Yudofsky SC, Hales RE, editor. Neuropsychiatry and Behavioral Neuroscience. Washington, DC: American Psychiatric Publishing; 2008.

4. Mohundro BL, Wicker AM. Attention deficit hyperactivity disorder: More than a child's disease. Formulary. 2010;45(10):318-27.

5. Barkley RA, Cox D. A review of driving risks and impairments associated with attention-deficit/hyperactivity disorder and the effects of stimulant medication on driving performance. J Safety Res. 2007;38(1):113-28.

6. Lee SS, Humphreys KL, Flory K, Liu R, Glass K. Prospective association of childhood attention-deficit/hyperactivity disorder (ADHD) and substance use and abuse/dependence: A meta-analytic review. Clin Psychol Rev. 2011;31(3):328-41.

7. Rapid Action Revision (RAR), Army Regulation 40–501, Standards of Medical Fitness Washington, DC, August, 2010.

8. Friedman LS, Blaschke GS, Klam WP, Stein MT. ADHD, medication and the military service: a pediatrician's dilemma. J Dev Behav Pediatr. 2010;31(3 Suppl):S60-3.

9. Larson GE, Hammer PS, Conway TL, Schmied EA, Galarneau MR, Konoske P, Webb-Murphy JA, Schmitz KJ, Edwards N, Johnson, DC. Predeployment and in-theater diagnoses of American military personnel serving in Iraq. Psychiatr Serv. 2011;62(1):15-21.

10. Kessler RC, Adler LA, Gruber MJ, Sarawate CA, Spencer T, Van Brunt DL. Validity of the World Health Organization Adult ADHD Self-Report Scale (ASRS) Screener in a representative sample of health plan members. Int J Methods Psychiatr Res. 2007;16(2):52-65.

11. Elsmore TF, Reeves DL, Reeves AN. The ARES test system for palm OS handheld computers. Arch Clin Neuropsychol. 2007;22 Suppl 1:S135-44.

12. Lemaire JB, Wallace JE, Dinsmore K, Lewin AM, Ghali WA, Roberts D. Physician nutrition and cognition during work hours: effect of a nutrition based intervention. BMC Health Serv Res. 2010;10:241.

13. desRosiers G, Kavanagh D. Cognitive assessment in closed head injury: Stability, validity and parallel forms for two neuropsychological measures of recovery. International Journal of Clinical Neuropsychology. 1987;9(4):162-73.

14. Rodriguez-Aranda C, Martinussen M. Age-related differences in performance of phonemic verbal fluency measured by Controlled Oral Word Association Task (COWAT): a meta-analytic study. Dev Neuropsychol. 2006;30(2):697-717.

15. Brandt J. The Hopkins Verbal Learning Test-Revised. Odessa, Fla: Psychological Assessment Resources (PAR); 1991.

16. Hogervorst E, Combrinck M, Lapuerta P, Rue J, Swales K, Budge M. The Hopkins Verbal Learning Test and screening for dementia. Dement Geriatr Cogn Disord. 2002;13(1):13-20.

17. Benedict RHB, Brandt, Jason. Hopkins Verbal Learning Test-Revised/ Brief Visuospatial Memory Test-Revised. Lutz, FL: Psychological Assessment Resources; 2007.

18. Ruff RM, Light RH, Parker SB, Levin HS. Benton Controlled Oral Word Association Test: reliability and updated norms. Arch Clin Neuropsychol. 1996;11(4):329-38.

19. Schneiderman AI, Braver ER, Kang HK. Understanding sequelae of injury mechanisms and mild traumatic brain injury incurred during the conflicts in Iraq and Afghanistan: persistent postconcussive symptoms and posttraumatic stress disorder. Am J Epidemiol. 2008;167(12):1446-52.

20. Orsillo SM. Measures for acute stress disorder and posttraumatic stress disorder. In: Practitioner's Guide to Empirically based Measures of Anxiety, Antony MM & Orsillo SM, editors. Kluwer Academic/Plenum; 2001. p. 255-307.

21. McLay RN, Klam WP, Volkert SL. Insomnia is the most commonly reported symptom and predicts other symptoms of posttraumatic stress disorder in U.S. service members returning from military deployments. Mil Med. 2010;175(10):759-62.

22. Larsson H, Dilshad R, Lichtenstein P, Barker ED. Developmental trajectories of DSM-IV symptoms of attention-deficit/hyperactivity disorder: genetic effects, family risk and associated psychopathology. J Child Psychol Psychiatry; 2011.

23. Dretsch, M. Enhancing Operational Readiness through Neuroimaging: Mapping the Pathophysiology of Mild Traumatic Brain Injury in the U.S. Warfighter. Editors: Paul T. Bartone, Ross H. Pastel, & Mark A. Vaitkus. The 71F Advantage: Applying Research Psychology for Health and Performance Gains. National Defense University Press; 2010.

24. Konrad A, Dielentheis TF, El Masri D, Bayerl M, Fehr C, Gesierich T, Vucurevic G, Stoeter P, Winterer G. Disturbed structural connectivity is related to inattention and impulsivity in adult attention deficit hyperactivity disorder. Eur J Neurosci. 2010;31(5):912-9.

25. Jatzko A, Schmitt A, Kordon A, Braus DF. [Neuroimaging findings in posttraumatic stress disorder: review of the literature]. Fortschr Neurol Psychiatr. 2005;73(7):377-91.

26. Eckart C, Stoppel C, Kaufmann J, Tempelmann C, Hinrichs H, Elbert T, Heinze HJ, Kolassa IT. Structural alterations in lateral prefrontal, parietal and posterior midline regions of men with chronic posttraumatic stress disorder. J Psychiatry Neurosci. 2011;36(3):176-86.

27. Kessler RC, Adler L, Ames M, Demler O, Faroene S, Hiripi E, Howes MJ, Jin R, Secnik K, Spencer T, Ustun TB, Walters EE. The World Health Organization adult screening scale for use in the general population. Psychol Med, 2005;35(2):245-56.

28. Wilens TE, Faraone SV, Biederman J. Attention-deficit/hyperactivity disorder in adults. JAMA. 2004;292(5):619-23.

Tables

Table 4.1 Characteristics of sample

	Total Sample (N = 260)	
Characteristics	**N**	**percent**
Gender		
Male	236	90.8
Female	24	9.2
Ethnicity		
Caucasian	154	59.2
Hispanic	71	27.3
African American	21	8.1
Asian	6	2.3
Native American	3	1.2
Pacific Islander	1	0.4
Other	4	1.5
Smoking		
Never smoked	112	43.1
Current smoker	92	35.4
Former smoker	56	21.5
Alcohol Use		
None	93	35.9
Less than 1 drink/day	87	33.6
1-2 drinks/day	44	17.0
3 or more drinks/day	35	13.5

Table 4.2 Comparison of ASRS, hyperactivity/impulsivity and inattention scores by demographic characteristics

		ASRS				Hyperactivity/ Impulsivity				Inattention			
Characteristics	N	Mean	SD	*t*	*p*	Mean	SD	*t*	*p*	Mean	SD	*t*	*p*
Gender													
Male	236	9.2	3.4	0.27	0.785	4.2	1.8	-0.91	0.366	5.0	2.6	1.03	0.302
Female	24	9.0	3.5			4.6	2.2			4.4	1.9		
Age*													
Less than 28	136	9.7	3.4	2.61	0.010	4.7	1.8	3.72	<0.001	5.1	2.6	0.85	0.399
28 or older	123	8.6	3.3			3.8	1.8			4.8	2.4		
Marital Status*													
Single	115	9.7	3.6	2.23	0.027	4.5	1.8	1.92	0.056	5.2	2.7	1.62	0.107
Married	145	8.8	3.2			4.1	1.8			4.7	2.3		
Education*													
Less than college degree	203	9.3	3.4	0.90	0.372	4.4	1.8	2.06	0.041	4.9	2.5	-0.28	0.783
College degree	57	8.8	3.4			3.8	1.8			5.0	2.7		
Ethnic Category*													
Non-Hispanic Caucasian	154	9.7	3.3	2.95	0.004	4.6	1.8	3.14	0.002	5.1	2.5	1.70	0.091
All other	106	8.4	3.5			3.8	1.8			4.6	2.5		

*Groups differ, $p \leq 0.05$

Table 4.3 Comparison of ASRS, hyperactivity/impulsivity and inattention scores by health behavior characteristics

		ASRS				Hyperactivity/ Impulsivity				Inattention			
Characteristics	N	Mean	SD	*t*	*p*	Mean	SD	*t*	*p*	Mean	SD	*t*	*p*
Alcohol Use*													
Daily	79	9.9	3.6	2.39	0.018	4.6	1.8	2.10	0.037	5.3	2.8	1.70	0.091
Less frequently	180	8.9	3.3			4.1	1.8			4.7	2.4		
Smoking Status*													
Current or former	148	9.6	3.3	2.24	0.026	4.5	1.7	2.66	0.008	5.1	2.4	1.10	0.272
Never smoked	112	8.6	3.5			3.9	1.9			4.7	2.7		
Caffeine intake*													
Greater than 150 mg/day	85	9.8	3.3	2.04	0.042	4.8	1.7	3.32	0.001	5.0	2.5	0.44	0.663
150 mg/day or less	116	8.8	3.6			3.9	1.9			4.9	2.7		

*Groups differ, $p \leq 0.05$

Table 4.4 Correlation between ASRS scores and measures of cognition, mood, and frequency of PTSD symptoms

Scores	N	**ASRS**	**Inattention**	**Hyperactivity /Impulsivity**
Cognitive				
HVLT	130	-0.04	0.04	-0.08
COWA	100	-0.04	0.02	-0.06
Target Tracking	108	0.05	0.08	0.00
Simple Reaction	110	-0.02	-0.05	0.08
Code Substitution	110	-0.01	-0.07	0.04
Procedural Reaction	110	-0.10	-0.19*	0.06
Go-No-Go	110	-0.13	-0.21*	0.04
Match-to-sample	110	-0.23*	-0.25**	-0.10
Code Substitution Recall	109	0.08	-0.03	0.15
Stroop Congruent	109	-0.08	-0.19	0.01
Stroop Incongruent	109	-0.06	-0.15	0.03
Stroop Neutral	108	-0.17	-0.28**	-0.02
Stroop Emotional	108	-0.23*	-0.25**	-0.15
Mood				
Vigor	110	-0.22*	-0.25**	-0.05
Happiness	110	-0.34**	-0.27**	-0.24*
Depression	110	0.41**	0.28**	0.31**
Anger	110	0.28**	0.17	0.25**
Fatigue	110	0.31**	0.25**	0.19*
Anxiety	110	0.35**	0.24*	0.26**
Restlessness	110	0.43**	0.21*	0.41**
PCL-17				
Re-experiencing	62	0.11	-0.03	0.22
Avoidance	63	0.37**	0.18	0.34**
Hyperarousal	63	0.25*	0.12	0.21
Total	62	0.33**	0.16	0.30*

Spearman Rank Order Correlation was used to determine significance

*$p \leq 0.05$; **$p \leq 0.01$

Table 4.5 ASRS screening category and PCL-M scores

	Negative ASRS		**Positive ASRS**			
PCL-M Scores	N	Mean Rank	N	Mean Rank	U	p^a
Re-experiencing	53	30.4	9	38.3	177.5	0.192
Avoidance	54	30.0	9	43.9	135.5	0.031*
Hyperarousal	54	30.8	9	39.3	177.0	0.191
Total	53	29.1	9	45.7	111.0	0.011*

[a]Mann-Whitney U tests were used to determine significance

*$p \leq 0.05$

Table 4.6 Deployment injury and measures of ADHD, cognition, mood, and frequency of PTSD symptoms

	No Injury		Injury			
	N	Mean Rank	N	Mean Rank	U	p[a]
Cognitive						
HVLT	25	26.1	25	24.9	296.5	0.756
COWA	23	26.2	21	18.5	157.0	0.047*
Target Tracking	26	27.3	23	22.4	240.0	0.237
Simple Reaction	26	29.0	23	20.5	195.5	0.038*
Code Substitution	26	30.9	23	18.4	146.5	0.002**
Procedural Reaction	26	27.9	23	21.7	224.0	0.132
Go-No-Go	26	27.4	23	22.3	237.5	0.218
Match-to-Sample	26	27.4	23	22.4	238.0	0.221
Code Substitution Recall	26	28.7	23	20.8	203.0	0.054
Stroop Congruent	26	31.0	23	18.2	142.5	0.002**
Stroop Incongruent	26	29.6	23	19.9	180.5	0.018*
Stroop Neutral	26	29.4	23	20.0	184.0	0.021*
Stroop Emotional	26	29.2	23	20.2	189.5	0.028*
Mood						
Vigor	26	24.8	23	25.2	294.5	0.928
Happiness	26	25.8	23	24.2	279.5	0.695
Depression	26	24.0	23	26.1	273.0	0.599
Anger	26	25.5	23	24.4	285.5	0.785
Fatigue	26	24.2	23	25.9	278.0	0.674
Anxiety	26	24.7	23	25.4	291.0	0.872
Restlessness	26	26.3	23	23.5	265.0	0.495
PCL-M						
Re-experiencing	31	24.8	30	37.4	273.5	0.003**
Avoidance	32	31.6	30	31.4	477.0	0.966
Hyperarousal	32	28.3	30	35.0	376.0	0.140
Total	31	27.6	30	34.5	360.5	0.131
ADHD						
Inattention	37	39.0	34	32.7	517.0	0.194
Hyperactivity/Impulsiveness	37	32.2	34	40.1	496.0	0.105
Total	37	36.8	34	35.2	601.0	0.746
Sleep Scale	26	24.7	23	25.4	290.0	0.841

[a]Mann-Whitney U tests were used to determine significance
$^{*}p \leq 0.05$; $^{**}p \leq 0.01$;

Chapter 5 - Omega-3 Status and Psychological Outcomes among Soldiers Deploying to Combat

Abstract

Psychological problems and human error are two leading causes of death and disability among military service members. Strategies to improve the psychological health and cognitive performance of those in the military are much needed. Recent advances suggest omega-3 fatty acids may play an important role in the psychological well being of those in the military. The purpose of this study was to explore the relationship between omega-3 status and psychological outcome variables among soldiers deploying to combat. Measures of omega-3 status included: a) eicosapentaenoic aid (EPA) + docosahexaenoic acid (DHA) intake, b) the percent of omega-3 highly unsaturated fatty acids (HUFA) in total HUFA of whole blood (13), and c) the percent of DHA in total fatty acids of whole blood. Corrected p values were utilized to account for multiple tests. Intake of EPA + DHA was not significantly correlated with any of the measures of mood. Correlations between the omega-3 exposure variables and cognitive performance variables were deemed not significant following comparison with the corrected p value (≤ 0.001). Analyses of participants with EPA + DHA intakes at or below the median, revealed omega-3 HUFA scores were related ($p \leq 0.002$) to happiness ($\beta = -0.46$), depression ($\beta = 0.44$), and fatigue ($\beta = 0.43$). Although exploratory in nature, the results of this study suggest that among soldiers a relationship between omega-3 fatty acids and mood may exist. Given the current concerns regarding the psychological health of those in the military, additional research is warranted to better understand the potential diet-mood relationship.

Introduction

Depression, posttraumatic stress disorder (PTSD) and traumatic brain injury (TBI) are serious health problems common among soldiers returning from deployments in Afghanistan and Iraq (1, 2). Rising suicide rates among soldiers and veterans have become a somber concern (3). At the same time, and despite remarkable technological advances, human cognitive factors remain a major limiting factor in current military operations (4). The operational limitations imposed by human factors are well evidenced by the fact that human error accounts for approximately 80-85% of all accidents in the military (5).

Strategies to improve the psychological health and cognitive performance of those in the military are much needed and depend on advances in neuroscience. An increased understanding of the molecular basis of neurological functioning offers hope of a better understanding of the human mind and its ability to function in adverse conditions. One set of biological molecules that has recently received considerable attention with regard to neurological functioning is the family of omega-3 fatty acids. Two omega-3 fatty acids in particular, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been linked to positive psychological effects in an overwhelming number of studies.

DHA is a 22-carbon fatty acid containing six double bonds with the omega-3 configuration. It is the most prominent omega-3 fatty acid in the brain and retina (6), and it plays a critical role in cognition and vision (7). EPA is a 20-carbon fatty acid containing five double bonds with the omega-3 configuration. Both DHA and EPA can be can be synthesized from alpha linoleic acid (ALA) an 18-carbon omega-3 fatty acid. However, in general, the conversation of ALA to DHA is quite low (8).

Dietary intakes of omega-3 fatty acids vary among populations. Dietary sources of ALA include walnuts, canola oil, soybeans, and flaxseed oil. The major dietary sources of EPA and DHA are fish and shellfish, and although they contain lower levels, eggs and poultry are considered important sources due to the fact that these foods are consumed in much greater quantities. In addition, numerous omega-3 enriched products, such as margarine and juice are now being made available to consumers. However, the estimated median intake of EPA and DHA in the US (9) is below the suggested optimal level (10).

The relationship between omega-3 fatty acids and cognitive outcomes has been identified in numerous epidemiologic, molecular, and clinical studies. In animal models, chronic dietary restriction of omega-3 fatty acids during development reduces brain DHA, increases brain omega-6 fatty acids, and results in problems related to cognition (6, 11). In humans, omega-3 fatty acids are associated with favorable cognitive and neurological measurements (12-16). In general, the effects of omega-3 fatty acids are believed to be due to the roles they play in: a) neurological cell functioning, b) the neurological system's development and ability to adapt (neuroplasticity), and c) the neurological system's ability to successfully overcome injury (neuroprotection). A discussion of each of these roles follows.

Neuron Function

Two lines of thought have developed to explain the roles omega-3 fatty acids play in neurological cell function (6). The first is based on DHA's role as a component of the cell membrane. The second is based on the notion that DHA and its metabolic products serve specific functions in signaling pathways (6).

Diet is known to modulate neurological cell membrane composition. Deficiencies in DHA's dietary precursors lead to compensatory increases in docosapentaenoic acid (DPA) as well as other omega-6 fatty acids in brain tissue cells (6, 11). Although the differences may be subtle, each fatty acid has unique physical properties. These properties may affect fluidity and interactions with membrane proteins. In turn, signal transduction and neurotransmission may be affected.

In addition to functioning as a component of the cell membrane, lipids serve as messengers relaying information from one cell to another or from one subcellular compartment to another. Plasma membrane lipids cleaved by phospholipids A2 form extracellular metabolites known as eicosanoids (17). Other phospholipases cleave cell membrane lipids and release intracellular products that play an important role in signal transduction. DHA appears to be involved in ion channel activities (6), gene expression (6), cell protection (18), and has been shown to stimulate neurite growth (19, 20) and synaptic function (21).

Neuroplasticity

Research on DHA and cognitive function has focused heavily on infants, the elderly, and individuals with conditions such as attention deficit hyperactivity disorder (ADHD). In infants, a positive association between DHA status and neurodevelopmental outcome has been well-documented (7). Among preschool-aged children, maternal supplementation with omega-3 fatty acids during pregnancy improved hand and eye coordination (13), and in a case-control study of young adults, plasma levels of DHA and EPA were significantly lower in those with ADHD compared to those without the diagnosis (22).

Given the neurological changes that occur during infancy and childhood it is understandable that research has concentrated on these periods of the lifespan. However, new insight into the molecular functioning of DHA suggests that it may play a greater role in mid-life neurological functioning than was once believed; this may be particularly so in scenarios involving active learning, or extreme environmental conditions.

The network of connections between neurons forms the basis of memory and learning, and the adult brain appears to have the capacity to alter neural conductivity by either modifying the strength of synapses or forming new axon terminals or dendrite processes (23). In rat embryonic cell cultures, DHA has been shown to promote neurite growth. Cells cultured in a DHA containing medium developed a significantly greater number of neurons with longer neurites compared to those cells cultured in mediums containing oleic acid, arachidonic acid, or DPA (19). In addition, the DHA-containing medium significantly increased the number of branches per neuron (19). Feeding pregnant rats a diet void of omega-3 fatty acids significantly decreased DHA content of the embryonic hippocampus, and resulted in significantly shorter neurite length (19).

Neurite growth necessitates an increase in cell surface area, and plasma membrane expansion occurs as transport organelles fuse with the plasma membrane. This fusion is thought to be SNARE (soluble N-ethylmaleimide-sensitive-factor attachment protein receptor) protein dependent. The release of several neurotransmitters including acetylcholine and dopamine are also believed to be SNARE dependent, and dietary deficiencies of omega-3s have been shown to modify neurotransmitters release (24). In the process of neurotransmitter release, a vesicle associated membrane protein (VAMP) pairs with a target membrane SNARE. Once the contents of the intercellular vesicle have been released, disassembly of the SNARE complex occurs, thereby allowing continuation of the transport cycle. In the case of neurotransmitter release, regulation of the cycle occurs via reversible S-nitrosylation of the SNARE which inhibits its disassemble and decreases neurotransmitter release. Rats fed a diet deficient in omega-3 fatty acids had reduced S-nitrosylation levels suggesting omega-3 deficiencies may interfere with the vesicle transport system of neurotransmitter release (25).

It is plausible to consider the possibility that reduced levels of DHA may also interfere with vesicle transport systems that supports plasma membrane expansion and neurite growth. Should stronger and more numerous neurological synapses be one effect of increased DHA intake, improved cognitive performance may result. In a recent study of 35-54 year olds, an association was observed between higher serum phospholipid DHA and better performance on tests of working memory, vocabulary, and nonverbal learning and mental flexibility (26), lending support to the notion that fatty acids play a role in cognition in middle adulthood.

Neuroprotection

In addition to its role in cognitive functioning, evidence suggests DHA plays an important role in protecting neurons. As many as 1 in 10 soldiers deployed to combat experience symptoms indicative of mild brain trauma (2), and many who suffer a TBI experience long-term symptoms. Following the initial mechanical damage caused by a TBI, a chain reaction of biochemical events occurs leading to delayed neuron damage (27). The cellular changes that occur after a TBI are in many ways like an accelerated form of brain aging (28).

Reducing the damaging effects of TBI has become an important area of research and one in which the neuroprotective effects of EPA and DHA may play a role (29). Both plasma omega-3 fatty acids (15, 30) and increased fish consumption (14, 31) were associated with a decreased risk of cognitive decline in older adults. In a randomized double-blind trial, omega-3 fatty acids reduced cognitive decline among a subgroup of participants categorized as having very mild cognitive decline (32).

Neuroprotectin D1 (NPD1), a recently identified neuroprotective molecule that is a biosynthetic product of DHA, has increased interest in the protective effects of omega-3 fatty acids. NPD1 appears to be involved in steps to counter inflammation and neuronal cell damage. DHA derived neuroprotectins seem to redirect cellular fate away from cell death and toward survival (18). In rodent models, dietary supplementation of omega-3 fatty acids counters the decrease in hippocampus levels of AMP-activated protein kinase (AMPK), phosphorylated AMP activated protein kinase (pAMPK), and expression of silent information regulator 2 (SIR 2) that accompanies mild TBI (33). Similarly, in rodent models of spinal cord injury, treatment with DHA reduced oxidative stress, decrease inflammation, increased neuronal survival, and improved functional outcomes (34). This neuroprotection is not limited to TBI and spinal injuries, as research suggests DHA may be protective in brain ischemia (18) and may guard against macular degeneration (35), and hearing loss (12).

Military personnel in combat areas must rapidly adapt to new experiences and make split second decisions. They may also face extreme environmental conditions capable of causing neurological damage (e.g. intense ultraviolet light, excessive noise, and sudden air pressure changes caused by blast waves from mortars and improvised explosive devises), and are often exposed to psychologically traumatizing experiences. Given the role EPA and DHA play in neuron function, neuroplasticity and neroprotection, the potential consequences of low dietary intakes in soldiers is an important concern. Omega-3 dietary intake levels among military personnel have not been established (29). For the most part, government nutrition studies (e.g. NHANES) are not inclusive of the military, and in a recent study of dietary supplement use among soldiers (36), fish oil was categorized as "other," thereby preventing the establishment of any firm conclusions about omega-3 supplement use in this population.

Purpose

The purpose of this study was to explore the relationship between omega-3 status and psychological outcome variables among soldiers deploying to combat. Measures of omega-3 status included: a) EPA + DHA intake, b) the percent of omega-3 highly unsaturated fatty acids (HUFA) in total HUFA of whole blood (37), and c) the percentage of DHA in total fatty acids of whole blood. Highly unsaturated fatty acids are defined as those fatty acids with 20 or more carbons and three or more double bonds. The percent of omega-3 HUFA in total HUFA of whole blood will herein be referred to as the omega-3 HUFA score.

Methods

Subjects

A volunteer sample of soldiers (n = 272) scheduled for deployment to Iraq completed one or more of the omega-3 fatty acid assessment measurements. Due to the operational constraints of the military, not all participants were able to complete all components of the study. As a result, subsamples were used to explore the relationship between omega-3 exposure variables and outcome variables. Participants were from one of two combat brigades which were a brigade combat team (BCT) from the Texas National Guard and an active duty brigade from Kansas. Data were collected between October 2009 and February 2010. The participants completed this study approximately one month prior to deployment. It was made clear to all potential subjects that participation was strictly voluntary and was not part of their required military duty. The soldiers were not awarded compensation for their participation. This protocol was approved by, and was conducted in accordance with the ethical standards of, the Kansas State University Institutional Review Board. After being provided a complete description of the study, all of the soldiers recruited for this study were required to provide written informed consent prior to participation.

Instruments

Intake of EPA + DHA

A self-administered 11-item FFQ was used to measure consumption of EPA+ DHA during the preceding six-month period. This FFQ included items to capture intake of EPA and DHA from: 1) seafood, 2) poultry and eggs, 3), omega-3 functional foods, and 4) dietary supplements. Development and assessment of the FFQ is described in detail in Chapter 2 and Chapter 3.

Omega-3 HUFA Score and DHA

Non-fasting fingertip prick blood samples were collected on strips of chromatography paper treated with BHT and acetone as described previously (38). The samples were placed in cryogenic containers and allowed to dry overnight before being stored at -80°C until analysis. A microwave-facilitated, one-step transesterification fatty acid analysis was used. The percent of each fatty acid by weight was then determined using gas chromatography. Additional details regarding these procedures are provided in Chapter 3.

Cognitive performance

Cognitive performance was assessed using the Combat Stress Assessment (CSA) (39), the Controlled Oral Word Association Test (COWT) (40), and the Hopkins Verbal Learning Test-Revised (HVLT-R) (41). The CSA includes a battery of 11 validated subtests of memory, attention, and executive functions administered on a handheld computer. The COWAT is a test of semantic fluency and the HVLT-R is a test of memory of semantic categorical words. Trained research personnel administered both the COWT and the HVLT-R. Additional details regarding the cognitive performance measures can be found in Chapter 4 (submission pending).

Mood

Mood was assessed using the mood subtest of the CSA. This subtest includes seven mood categories including vigor, happiness, depression, anger, fatigue, anxiety, and restlessness. A total of 42 adjectives describing feelings (six for each scale) appear on the screen and participants use a zero-to-six visual analog scale to indicate how well each of these words represents their feelings. The lower the scale scores the less agreement indicated.

Sleep scale score

Participants degree of alertness was also measured using a subtest of the CSA. This subtest includes a seven-step scale in which participants indicate their degree of alertness with the value of one being assigned to the most rested and alert state.

Demographic and health-related behaviors

A self-administered questionnaire was used to measure demographic characteristics, height, weight, alcohol use, and smoking status.

Statistical Analysis

Data analyses were performed using Predictive Analytics Software (version 18, 2009, SPSS, Inc, Chicago, IL). Blank responses on the FFQ were quantified as zero. Frequencies and percentages were calculated for categorical data. Measures of central tendency, dispersion, and distribution were calculated for continuous variables. Chi-square and t-tests were used to compare participant characteristics based on type of military service. Because EPA + DHA intakes, omega-3 HUFA scores, and many of the cognitive test and mood scale scores were not normally distributed, non-parametric statistical analyses were utilized in analyses which included these variables. Mann-Whitney U tests were conducted to explore the relationship between categorical variable responses and the omega-3 exposure variables. Spearman rank order correlation coefficients were used to measure the correlation between the omega-3 exposure variables and the cognitive test and mood scales scores. Multiple regression analyses using backwards deletion were conducted with mood scale scores as dependent variables and omega-3 exposure variables, sleep scale scores, and age as the independent variables. For the purposes of the regression analysis mood scale and omega-3 HUFA scores were rank transformed. Pairwise exclusion was used to exclude cases only if they were missing data for a specific analysis, however; only cases with the full set of variables were included in the multiple regression analyses. Except for removal of variables from the regression analysis which was based on a significance of $p \leq 0.10$, two-tailed tests and a significance level of $p \leq 0.05$ were used for all analyses. Bonferroni corrected p values were calculated to account for multiple analyses.

Results

Participant Characteristics

The majority (65%; n = 177) of the participants were from the National Guard and 90.7% (n = 244) were male. Age in years ranged from 18 to 56 with a mean age of 29.0 (SD = 7.9). More than half (55.9%; n = 146) were married. All but one of the participants had completed high school or an equivalent, and 22% (n = 57) had a college degree. Mean body mass index (BMI) was 27.5 kg/m^2 (SD = 4.1). Fifty-nine percent (n = 155) identified themselves as non-Hispanic Caucasian. Compared to the National Guard, the Active Duty soldiers were younger, had a lower BMI, and were less likely to report regular daily alcohol consumption during the previous week. Additional demographic and health related characteristics by military service category are summarized in Tables 5.1 and 5.2.

Omega-3 Exposure Variables

Measures of central tendency, dispersion, and distribution for the omega-3 exposure variables are presented in Table 5.3. The EPA + DHA intakes were positively skewed with values clustering at the low end. The median EPA + DHA intake was 197 mg/day. Intake of EPA + DHA did not differ significantly among any of the groups based on participant characteristics (Table 5.4). The omega-3 HUFA scores were also positively skewed. The median omega-3 HUFA score was 16.5. The omega-3 HUFA scores were lower among smokers, and those 28 years old and younger (Table 5.5).

Mood and Omega-3 Exposure Variables

Scores for anger, depression, and anxiety were positively skewed with 27% of the participants (n = 30) scoring a zero on the anger scale, 23% (n = 25) scoring a zero on the depression scale, and 22% (n = 24) scoring a zero on the anxiety scale. Each of the mood variables was significantly correlated ($p \leq 0.05$) with the other mood variables measured. The strength of these correlations ranged from $\rho = -0.22$ for the correlation between anxiety and vigor to $\rho = 0.72$ for the correlation between anxiety and depression. Age and sleep scale scores were both correlated with numerous mood scales scores (Table 5.6).

Correlations between the omega-3 exposure variables and mood scale scores among participants at all intake levels of EPA + DHA are presented in Table 5.7. The FFQ-based intake of EPA + DHA was not significantly correlated with any of the mood scale scores among participants at all intake levels of EPA + DHA. The omega-3 HUFA scores were correlated with depression ($\rho = 0.23$, $p < 0.018$), happiness ($\rho = -0.23$, $p < 0.021$), anger ($\rho = 0.21$, $p = 0.030$) and fatigue ($\rho = 0.21$, $p < 0.034$), and DHA was significantly correlated with depression ($\rho = 0.21$, $p = 0.030$). The correlations between the omega-3 exposure variables and mood scale scores did not remain significant following comparison with the Bonferroni corrected p value (≤ 0.011) calculated using 21 tests.

Correlations between the omega-3 exposure variables and mood scale scores among participants at or below the median intake of EPA + DHA are presented in Table 5.8. As was the case among participants at all intake levels, FFQ-based intake of EPA + DHA was not significantly correlated with any of the mood scale scores among those at or below the median. In addition, DHA was not significantly correlated with any of the mood scale scores among those at or below the median. However, the omega-3 HUFA scores were significantly correlated with all of the mood scale scores. The correlations remained significant for omega-3 HUFA and depression, fatigue, happiness, and vigor following comparison with the Bonferroni corrected p value (≤ 0.002) calculated using 21 tests. Correlations between the omega-3 exposure variables and mood scale scores among participants above the median intake of EPA + DHA were not significant (data not shown).

The results of the regression analyses among participants with EPA + DHA intakes at or below the median are presented in Table 5.9. The relationship between omega-3 HUFA scores remained significant ($p \leq 0.05$) for all mood variable with the strength of the relationship ranging from $\beta = -0.46$ for happiness to $\beta = 0.31$ for anxiety.

Cognitive Performance and Omega-3 Exposure Variables

Among participants at all levels of EPA + DHA intake, HVLT scores were correlated with EPA + DHA intake ($\rho = -0.183$, $p = 0.037$). Among participants at or below the median intake of EPA + DHA, Go-No-Go scores were correlated with EPA + DHA intake ($\rho = -0.33$, $p = 0.022$), and among those with intakes above the median Simple Reaction time scores were correlated with EPA + DHA intake ($\rho = 0.25$, $p = 0.050$). All correlations between the omega-3 exposure variables and cognitive performance variables were deemed not significant following comparison with the Bonferroni corrected p value (≤ 0.001) calculated using 39 tests.

Discussion

This study is among the first to explore the relationship between omega-3 status and psychological outcome variables among soldiers. Although the observed relationship between omega-3 status and mood was not entirely consistent with those that have been reported earlier, the results clearly reinforce the postulation that the relationship between omega-3 status and mood is not a simple linear correlation. The finding that omega-3 HUFA score was positively correlated with depression scale scores appears to be contrary to the results obtained by Sublette et al (42) in which lower plasma DHA levels were observed in participants with major depressive disorder. The positive correlation between the omega-3 HUFA score and depression also appears to be in contradiction of the results from the clinical trial (43), which found that symptoms of depression decreased with omega-3 supplementation. However, one must keep in mind the fact that there are important differences between the subjects in this present study and those enrolled in the aforementioned studies. Namely, the depression scale scores obtained from the participants in this study were on average very low. In addition, due to the fact that the participants were soldiers scheduled for deployment, individuals with existing psychiatric conditions would have likely been identified and diverted from deployment prior to being given an opportunity to enroll in our study. Given the participant differences, as well as the methodological difference that exist between studies, the results we obtained regarding the relationship between the omega-3 HUFA score and depression are not necessarily startling.

Also noteworthy, was the lack of relationship between omega-3 status and cognitive outcomes. This finding regarding cognitive outcomes was particularly notable given the extensive number of cognitive tests (both computerized and research administered) utilized. However, the use of Bonferroni corrected p values is somewhat controversial, particularly in instances where correlations exist among the independent and as well as the dependent variables (as was the case in the present study).

This study is one of the first to utilize the omega-3 HUFA score to measure the relationship between psychological outcomes, and the present findings provide support for the use of this biomarker in predicting psychological outcomes.

Limitations

There are a number of limitations to consider in interpreting the results of the current study. In particular, the results were based on the self-reported behaviors and feelings of a volunteer sample. Participants' responses and cognitive performance may have differed from those not participating. In addition, the stigma associated with psychological health problem (44) may have resulted in reluctance to report negative behaviors and feeling. Although the potential differences between participants and non-participants are always a concern with volunteer samples, the demographic characteristics of the participants in the present study were similar to those typically noted in studies involving soldiers. For example in a study of 88,235 soldiers from the Active Army, the Reserves, and the National Guard, Milliken et al (1), reported that 90.8% of the subjects were male, 58.2% were married and the average age was 30.4 years. In the present study 90.7 % of the subjects were male, 55.9% were married, and the average age was 29.0 years. In general, it appears that the sample of soldiers in the present study was representative of the larger population of soldiers.

In addition, some concerns may be raised due to the fact that this is one of the first studies to utilize the omega-3 HUFA score to measure the relationship between omega-3 status and psychological outcomes. However, this method has been validated (37), and the present findings indicate that the omega-3 HUFA score predicts psychological outcomes. Additional limitations of this study have been address in Chapter 3 and Chapter 4.

Conclusion

Overall, the results of this study indicate the presence of a relationship between omega-3 fatty acids and mood. In this sample of soldiers, omega-3 status was not associated with cognitive performances. In addition, higher omega-3 HUFA scores were associated with higher unfavorable mood scores while whole blood DHA was associated with higher depression scores. Although additional research is necessary to further elucidate the results presented here, such work is imperative given the psychological health concerns currently being faced by the military.

References

1. Milliken CS, Auchterlonie JL, Hoge CW. Longitudinal assessment of mental health problems among active and reserve component soldiers returning from the Iraq war. JAMA. 2007;298(18):2141-8.

2. Schneiderman AI, Braver ER, Kang HK. Understanding sequelae of injury mechanisms and mild traumatic brain injury incurred during the conflicts in Iraq and Afghanistan: persistent postconcussive symptoms and posttraumatic stress disorder. Am J Epidemiol. 2008;167(12):1446-52.

3. Kuehn BM. Military probes epidemic of suicide: mental health issues remain prevalent. JAMA. 2010;304(13):1427, 9-30.

4. Wesensten N, Belenky, G, Balkin, T Cognitive readiness in network-centric operations. Parameters. 2005;Spring:94-105.

5. Warfighter Judgment and Decision Making. Military Operational Medicine Research Program 2008; Available from: https://www.momrp.org/cpa1.html.

6. Innis SM. Dietary (n-3) fatty acids and brain development. J Nutr. 2007;137(4):855-9.

7. Uauy R, Hoffman DR, Peirano P, Birch DG, Birch EE. Essential fatty acids in visual and brain development. Lipids. 2001;36(9):885-95.

8. Burdge GC, Calder PC. Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. Reprod Nutr Dev. 2005;45(5):581-97.

9. Institute of Medicine. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids. Washington, DC: National Academies Press; 2005.

10. Kris-Etherton PM, Innis S, American Dietetic Association, Dietitians of Canada. Position of the American Dietetic Association and Dietitians of Canada: dietary fatty acids. J Am Diet Assoc. 2007;107(9):1599-611.

11. Moriguchi T, Salem N, Jr. Recovery of brain docosahexaenoate leads to recovery of spatial task performance. J Neurochem. 2003;87(2):297-309.

12. Gopinath B, Flood VM, Rochtchina E, McMahon CM, Mitchell P. Consumption of omega-3 fatty acids and fish and risk of age-related hearing loss. Am J Clin Nutr. 2010;92(2):416-21.

13. Dunstan JA, Simmer K, Dixon G, Prescott SL. Cognitive assessment of children at age 2(1/2) years after maternal fish oil supplementation in pregnancy: a randomised controlled trial. Arch Dis Child Fetal Neonatal Ed. 2008;93(1):F45-50.

14. van Gelder BM, Tijhuis M, Kalmijn S, Kromhout D. Fish consumption, n-3 fatty acids, and subsequent 5-y cognitive decline in elderly men: the Zutphen Elderly Study. Am J Clin Nutr. 2007;85(4):1142-7.

15. Schaefer EJ, Bongard V, Beiser AS, Lamon-Fava S, Robins SJ, Au R, et al. Plasma phosphatidylcholine docosahexaenoic acid content and risk of dementia and Alzheimer disease: the Framingham Heart Study. Arch Neurol. 2006;63(11):1545-50.

16. Whalley LJ, Deary IJ, Starr JM, Wahle KW, Rance KA, Bourne VJ, et al. n-3 Fatty acid erythrocyte membrane content, APOE varepsilon4, and cognitive variation: an observational follow-up study in late adulthood. Am J Clin Nutr. 2008;87(2):449-54.

17. Voet D, Voet JG, Pratt W. Fundamentals of Biochemisty: 2nd Edition: Wiley; 2006.

18. Mukherjee PK, Chawla A, Loayza MS, Bazan NG. Docosanoids are multifunctional regulators of neural cell integrity and fate: significance in aging and disease. Prostaglandins Leukot Essent Fatty Acids. 2007;77(5-6):233-8.

19. Calderon F, Kim HY. Docosahexaenoic acid promotes neurite growth in hippocampal neurons. J Neurochem. 2004;90(4):979-88.

20. (ISSFAL) ISSFAL Policy Statement 3: Recommendations for intake of polyunsaturated fatty acids in healthy adults. 2004.

21. Cao D, Kevala K, Kim J, Moon HS, Jun SB, Lovinger D, et al. Docosahexaenoic acid promotes hippocampal neuronal development and synaptic function. J Neurochem. 2009;111(2):510-21.

22. Antalis CJ, Stevens LJ, Campbell M, Pazdro R, Ericson K, Burgess JR. Omega-3 fatty acid status in attention-deficit/hyperactivity disorder. Prostaglandins Leukot Essent Fatty Acids. 2006;75(4-5):299-308.

23. Purves D, Augustine GJ, Fitzpatrick D, Katz LC, LaMantia A-S, McNamara JO, et al., editors. Neuroscience: 2nd Edition [on-line]. Sunderlund, MA: Sinauer; 2001.

24. Aid S, Vancassel S, Poumes-Ballihaut C, Chalon S, Guesnet P, Lavialle M. Effect of a diet-induced n-3 PUFA depletion on cholinergic parameters in the rat hippocampus. J Lipid Res. 2003;44(8):1545-51.

25. Pongrac JL, Slack PJ, Innis SM. Dietary polyunsaturated fat that is low in (n-3) and high in (n-6) fatty acids alters the SNARE protein complex and nitrosylation in rat hippocampus. J Nutr. 2007;137(8):1852-6.

26. Muldoon MF, Ryan CM, Sheu L, Yao JK, Conklin SM, Manuck SB. Serum phospholipid docosahexaenonic acid is associated with cognitive functioning during middle adulthood. J Nutr. 2010;140(4):848-53.

27. Cernak I. Recent advances in neuroprotection for treating traumatic brain injury. Expert Opin Investig Drugs. 2006;15(11):1371-81.

28. Bhattacharjee Y. Neuroscience. Shell shock revisited: solving the puzzle of blast trauma. Science. 2008;319(5862):406-8.

29. Erdman J, Oria M, Pillsbury L, Editors;, Committee on Nutrition T, and the Brain; Institute of Medicine. Nutrition and Traumatic Brain Injury: Improving Acute and Subacute Health Outcomes in Military Personnel: The National Academies Press; 2011.

30. Beydoun MA, Kaufman JS, Satia JA, Rosamond W, Folsom AR. Plasma n-3 fatty acids and the risk of cognitive decline in older adults: the Atherosclerosis Risk in Communities Study. Am J Clin Nutr. 2007;85(4):1103-11.

31. Morris MC, Evans DA, Tangney CC, Bienias JL, Wilson RS. Fish consumption and cognitive decline with age in a large community study. Arch Neurol. 2005;62(12):1849-53.

32. Freund-Levi Y, Eriksdotter-Jonhagen M, Cederholm T, Basun H, Faxen-Irving G, Garlind A, et al. Omega-3 fatty acid treatment in 174 patients with mild to moderate Alzheimer disease: OmegAD study: a randomized double-blind trial. Arch Neurol. 2006;63(10):1402-8.

33. Wu A, Ying Z, Gomez-Pinilla F. Omega-3 fatty acids supplementation restores mechanisms that maintain brain homeostasis in traumatic brain injury. J Neurotrauma. 2007;24(10):1587-95.

34. Huang WL, King VR, Curran OE, Dyall SC, Ward RE, Lal N, et al. A combination of intravenous and dietary docosahexaenoic acid significantly improves outcome after spinal cord injury. Brain. 2007;130(Pt 11):3004-19.

35. Chua B, Flood V, Rochtchina E, Wang JJ, Smith W, Mitchell P. Dietary fatty acids and the 5-year incidence of age-related maculopathy. Arch Ophthalmol. 2006; 124(7):981-6.

36. Lieberman HR, Stavinoha TB, McGraw SM, White A, Hadden LS, Marriott BP. Use of dietary supplements among active-duty US Army soldiers. Am J Clin Nutr. 2010;92(4):985-95.

37. Stark KD. The percentage of n-3 highly unsaturated fatty acids in total HUFA as a biomarker for omega-3 fatty acid status in tissues. Lipids. 2008;43(1):45-53.

38. Ichihar K, Waku K, Yamaguchi C, Saito K, Shibahara A, Miyatani S, et al. A convenient method for determination of the C20-22 PUFA composition of glycerolipids in blood and breast milk. Lipids. 2002;37(5):523-6.

39. Elsmore TF, Reeves DL, Reeves AN. The ARES test system for palm OS handheld computers. Arch Clin Neuropsychol. 2007;22 Suppl 1:S135-44.

40. desRosiers G, Kavanagh D. Cognitive assessment in closed head injury: Stability, validity and parallel forms for two neuropsychological measures of recovery. International Journal of Clinical Neuropsychology. 1987;9(4):162-73.

41. Brandt J. The Hopkins Verbal Learning Test-Revised. Odessa, Fla: Psychological Assessment Resources (PAR); 1991.

42. Sublette ME, Segal-Isaacson CJ, Cooper TB, Fekri S, Vanegas N, Galfalvy HC, et al. Validation of a food frequency questionnaire to assess intake of n-3 polyunsaturated fatty acids in subjects with and without major depressive disorder. J Am Diet Assoc. 2011;111(1):117-23.

43. Hallahan B, Hibbeln JR, Davis JM, Garland MR. Omega-3 fatty acid supplementation in patients with recurrent self-harm. Single-centre double-blind randomised controlled trial. Br J Psychiatry. 2007;190:118-22.

44. Kim PY, Thomas JL, Wilk JE, Castro CA, Hoge CW. Stigma, barriers to care, and use of mental health services among active duty and National Guard soldiers after combat. Psychiatr Serv. 2010;61(6):582-8.

Tables

Tables begin on the following page.

Table 5.1 Characteristics of participants by service component

Characteristic	National Gaurd		Active Duty			
	n	%	n	%	χ^2	p
Gender						
Male	160	92.0	84	88.4	0.91	0.340
Female	14	8.0	11	11.6		
Marital Status						
Married	88	52.4	58	62.4	2.42	0.120
Single	74	47.6	41	37.6		
Education						
Less than college degree	131	78.0	73	78.5	0.01	0.923
College degree	37	22.0	20	21.5		
Race/Ethnic Category*						
Non-Hispanic Caucasian	92	54.8	63	67.7	4.18	0.041
All other	76	45.2	30	28.3		
Alcohol Use*						
Less often than daily	105	62.9	75	80.6	8.86	0.003
Daily	62	37.1	18	19.4		
Smoking Status						
Non or ex-smoker	109	64.9	59	63.4	0.05	0.816
Current smoker	59	35.1	25	36.6		

*Groups differ, $p \leq 0.05$

Table 5.2 Additional characteristics of participants by service component

Characteristic	National Gaurd			Active Duty			T-test		
	n	mean	SD	n	mean	SD	df[a]	t	p[a]
Age*	168	30.0	8.7	93	27.1	6.0	259.0	3.21	0.013
BMI*	167	27.9	4.4	93	26.7	3.4	229.6	2.50	0.002

[a]Equal variances not assumed, *Groups differ, $p \leq 0.05$

Table 5.3 Central tendency, dispersion, and distribution of omega-3 exposure variables

	N	Mean	Median	Range	Inter-Quartile Range	Skewness	Kurtosis
Intake EPA + DHA	254	298 mg/day	197 mg/day	0 - 2320 mg/day	82 – 400 mg/day	2.60	9.06
Omega-3 HUFA Score[a]	210	16.9	16.5	11.4 - 33.0	14.8 - 18.2	1.66	5.16
DHA[b]	210	1.4	1.3	0.7 – 3.2	1.1-1.6	1.19	2.12

[a]Percent omega-3 highly unsaturated fatty acids (HUFA) in total HUFA of whole blood

[b]Expressed as percentage by weight of total fatty acids

Table 5.4 Comparison of EPA + DHA intake[a] by sample characteristics[b]

Characteristics	n	Median mg/day	Mean Rank	*U*	*p*
Gender					
Male	229	203.8	129.5	2415.0	0.200
Female	25	125.2	109.6		
Military Service Category					
National Gaurd	159	207.8	133.4	6613.5	0.097
Active Army	95	169.0	117.6		
Age					
27 or younger	131	183.6	121.7	7294.5	0.381
28 or older	119	202.3	129.7		
Marital Status					
Married	138	198.9	128.7	7418.5	0.508
Single, divorced, separated	113	194.9	122.7		
Education					
Less than college degree	195	187.7	123.7	5012.5	0.350
College degree	56	206.1	134.0		
Ethnic Category					
Non-Hispanic Caucasian	155	195.6	124.4	7189.0	0.651
All other	96	196.5	128.6		
Alcohol Use					
Less than one drink/day	171	181.9	119.9	5795.0	0.071
One or more drinks/day	79	218.7	137.7		
Smoking Status					
Never or former smoker	164	196.9	128.22	6770.0	0.506
Current smoker	87	183.6	121.8		

[a] Value obtained using food frequency questionnaire

[b] Mann-Whitney U tests were used to determine significance

Table 5.5 Comparison of omega-3 HUFA score[a] by sample characteristics[b]

Characteristics	n	Median Omega-3 HUFA Score	Mean Rank	*U*	*p*
Gender					
Male	192	16.5	103.8	1408.0	0.886
Female	15	16.5	106.1		
Military Service Category					
National Gaurd	143	16.2	101.4	4207.0	0.155
Active Army	67	16.8	114.2		
Age*					
27 or younger	100	15.8	85.7	3515.0	0.000
28 or older	101	17.3	116.2		
Marital Status					
Married	121	16.9	107.5	4059.0	0.053
Single, divorced, separated	80	16.1	91.2		
Education					
Less than college degree	156	16.4	98.0	3042.0	0.173
College degree	45	17.0	111.4		
Ethnic Category					
Non-Hispanic Caucasian	124	16.5	98.5	4400.0	0.433
All other	77	16.5	105.1		
Alcohol Use					
Less often than daily	141	16.5	102.1	3940.0	0.557
Daily	59	16.5	96.8		
Smoking Status*					
Never or former smoker	131	16.9	110.8	3307.0	0.001
Current smoker	70	15.7	82.7		

[a]Percent omega-3 highly unsaturated fatty acids (HUFA) in total HUFA

[b]Mann-Whitney U tests were used to determine significance

*Groups differ, $p \leq 0.01$

Table 5.6 Correlations[a] between mood, age, and sleep scale scores

	n	Anger	Anxiety	Depression	Fatigue	Happiness	Restlessness	Vigor
Sleep Scale Scores	110	0.18	0.15	-0.27**	0.54**	-0.58**	0.25**	-0.65**
Age	111	-0.21*	-0.22*	-0.21*	0.06	0.17	-0.27*	0.08

[a]Spearman rank ordered correlation coefficients

*$p \leq 0.05$; ** $p \leq 0.01$

Table 5.7 Correlations[a] between omega-3 exposure variables and mood scale scores among participants at all EPA + DHA intakes

	n	Anger	Anxiety	Depression	Fatigue	Happiness	Restlessness	Vigor
Omega-3 HUFA Scores[b]	103	0.21*	0.11	0.23*	0.21*	-0.23	0.09	-0.13
EPA + DHA Intake	110	0.16	0.05	0.06	0.02	-0.02	0.10	0.08
DHA	103	0.16	0.07	0.21*	-0.07	0.07	0.12	0.03

[a]Spearman rank ordered correlation coefficients

[b]Percent omega-3 highly unsaturated fatty acids (HUFA) in total HUFA of whole blood

*$p \leq 0.05$, but not significant at Bonferroni corrected p value (≤ 0.002) calculated using 21 tests

Table 5.8 Correlations[a] between omega-3 exposure variables and mood among participants with intakes at or below median EPA + DHA intake

	n	Anger	Anxiety	Depression	Fatigue	Happiness	Restlessness	Vigor
Omega-3 HUFA Scores[b]	43	0.37*	0.31*	**0.48****	**0.56****	**-0.50****	0.26	**-0.49****
EPA + DHA Intake	48	-0.01	0.04	0.01	0.12	0.03	-0.06	0.02
DHA	43	0.12	0.08	0.26	0.18	-0.19	0.08	-0.13

[a]Spearman rank ordered correlation coefficients

[b]Percent omega-3 highly unsaturated fatty acids (HUFA) in total HUFA of whole blood

*$p \leq 0.05$, **$p \leq 0.01$

Bold significant at Bonferroni corrected p value (≤ 0.002) calculated using 21 tests

Table 5.9 Standardized β from regression models with mood[a] as the dependent variable

	Omega-3 HUFA Score[b]	
	β	p
Mood[a]		
Anger[c]	0.38	0.011
Anxiety[c]	0.31	0.042
Depression[d]	0.44	**0.004**
Fatigue[e]	0.43	**0.001**
Happiness[f]	-0.46	**0.002**
Restlessness[c]	0.32	0.030
Vigor[e]	-0.36	0.008

[a]Rank Transformed

[b]Percent omega-3 highly unsaturated fatty acids (HUFA) in total HUFA of whole blood

[c]Final Model included age

[d]Final Model did not include additional variables

[e]Final Model included sleep

[f]Final Model included sleep & age

Bold significant at Bonferroni corrected p value ($p \leq 0.007$) calculated using 7 tests

Chapter 6 - Summary

Introduction

The overall purpose of this dissertation was to explore the relationship between omega-3 status and psychological outcome variables among soldiers deploying to combat. The results presented in Chapter 5, do not support the hypothesis that omega-3 fatty acids are associated with favorable cognitive and mood outcomes. In this sample of deploying soldiers, omega-3 status was not associated with cognitive performances. In addition, higher omega-3 HUFA (highly unsaturated fatty acids) scores were associated with higher unfavorable mood scores and whole blood docosahexaenoic acid (DHA) was associated higher depression scores. These findings contradict the general hypothesis that omega-3 fatty acids are associated with favorable psychological outcomes. Although paradoxical in nature, the results of this study may also be viewed as an indication that more information is need on this research topic. According to Lands (1), "Paradoxes come easily from oversimplified stories that are missing key information. The apparent contradiction of two facts is often resolved by adding a third fact we need to know."

In light of the above statement, it should be kept in mind that research on the topic of omega-3 fatty acids among military service members is at a very early stage. Soldiers experience unusual circumstances and extraordinary challenges. They may respond to stress very differently than other populations. The primary results obtained in this dissertation may be an outcome of the unique characteristics of the military population. As such, a comprehensive understanding of the relationship between omega-3 fatty acids and psychological outcomes in this population warrants additional investigation.

Future Direction

The future research topics that have been identified as a result of this dissertation are addressed in the following sections.

Develop an improved omega-3 dietary assessment instrument.

Omega-3 supplement use and omega-3 functional food use have increased in recent years and the difficulty of measuring "usual" EPA + DHA intake has been addressed in Chapters 2 and 3. Although the correlation between dietary EPA + DHA was significant and the magnitude of this correlation was in line with what has generally been reported for similar studies (2), a stronger correlation between biomarker and dietary intake would be a welcome improvement. Strandjord (3) was able to achieve a correlation coefficient of 0.78 between blood fraction HUFA scores and the HUFA scores predicted by the Lands Equation using reported dietary intake of linoleic acid and alpha-linolenic acid as well omega-6 and omega-3 HUFA intake. Based on this report, when conducting future studies it appears to be worth the effort to include linoleic acid and alpha-linolenic acid in dietary assessment instruments designed to measure omega-3 HUFA status.

Ascertain whether military and civilian populations differ with regard to the relationship between omega-3 fatty acids and psychological outcomes.

As mentioned in the previous section, research is needed to determine whether the results regarding omega-3 and mood are unique to the military. Among the participants in this study, mean scores for depression, anger, and anxiety were low with roughly one- fourth of the participants who completed each scale achieving a score of zero. One would intuitively assume that higher scores for depression, anger, and anxiety increase psychological risk. However, given the circumstances under which the data was collected, these higher scores may actually be the more adaptive response. Additional research using validated instruments with established cut points would help identify differences between military and non-military populations.

Explore the genetic variances that mediate the relationship between diet, biomarker status, and psychometric outcomes.

Biomarker responses to increased omega-3 fatty acid intakes vary dramatically among individuals (4) and plasma omega-3 fatty acids have been shown to differ based on the presence of the epsilon four allele variant of the apolipoprotein E gene (E4). Carriers of E4 are known to be at an increased risk for Alzheimer's disease (5), and they have also been shown to have higher plasma DHA and EPA levels at baseline (i.e. at low intake levels) compared to non-carriers (6). Future research in which the participants' E4 status is known will likely clarify the relationship between omega-3 status and psychometric outcomes.

Determine whether the relationship between omega-3 fatty acids and mood varies over the course of time, particularly following a deployment.

While it was not presented as a component of my dissertation, post-deployment data were collected from the returning soldiers. My most immediate research plans are to use this data to explore the relationship between omega-3 status and psychological outcomes following military deployment. The post deployment data will also allow for the measurement of any changes in omega-3 status and outcomes variables that may have occurred.

Explore the possibility that soldiers may be using omega-3 supplements as a "self-treatment" for mood and/ or cognitive concerns.

Although cognitive and mood scores did not differ based on prior deployment status (data not shown), omega-3 supplement use did. Twenty-five percent of the soldiers who had been on a prior deployment reported taking an omega-3 supplement which was significantly greater than the 4% of those who had not deployed and reported taking omega-3 supplements ($n = 150$, $\chi^2 = 14.43$, $p < 0.001$). Although the relationship between omega-3 supplement use and prior deployments cannot be elucidated from this study, these results bring to light an important consideration for future studies.

Conclusions

Prior to this dissertation, very little was known about the omega-3 status of those in the military. Although limitations have been identified, the results suggest that the omega-3 HUFA score among soldiers is low in comparison to other populations and this score is also well below the desirable range (1). In addition, although the results of this study are not consistent with the general hypothesis that omega-3 fatty acids are associated with favorable psychological outcomes, they do indicate the presence of a relationship between omega-3 fatty acids and mood. Based on these findings and the psychological health concerns facing the military, additional research on the topic of omega-3 fatty acids among those in the military is well warranted.

References

1. Lands B. A critique of paradoxes in current advice on dietary lipids. Progress in Lipid Research, 2008. 47(2): p. 77-106.

2. Overby NC, Serra-Majem L, Andersen, LF. Dietary assessment methods on n-3 fatty acid intake: a systematic review. Br J Nutr. 2009; 102 Suppl 1: S56-63.

3. Strandjord SE, Lands WEB, Hibbeln JR. Validation of an empirical equation: Dietary intakes of omega-3 and omega-6 fatty acids predict human blood compositions of highly unsaturated omega-3 and omega-6 fatty acids. (Completed Manuscript).

4. Kohler A, Bittner D, Low A, von Schacky C. Effects of a convenience drink fortified with n-3 fatty acids on the n-3 index. Br J Nutr. 2010;104(5):729-36.

5. Laws SM, Hone E, Gandy S, Martins RN. Expanding the association between the APOE gene and the risk of Alzheimer's disease: possible roles for APOE promoter polymorphisms and alterations in APOE transcription. J Neurochem. 2003;84(6):1215-36.

6. Plourde M, Vohl MC, Vandal M, Couture P, Lemieux S, Cunnane SC. Plasma n-3 fatty acid response to an n-3 fatty acid supplement is modulated by apoE epsilon4 but not by the common PPAR-alpha L162V polymorphism in men. Br J Nutr. 2009;102(8):1121-4.

Appendix A - IRB Approval Documents

K-STATE
Kansas State University

University Research
Compliance Office
203 Fairchild Hall
Lower Mezzanine
Manhattan, KS 66506-1103
785-532-3224
Fax: 785-532-3278
http://urco.ksu.edu

TO: Mark Haub
Human Nutrition
127 Justin

Proposal Number: 5070

FROM: Rick Scheidt, Chair
Committee on Research Involving Human Subjects

DATE: April 23, 2009

RE: Proposal Entitled, "Development of a questionnaire designed to measure dietary intake of long-chain omega-3 fatty acids"

The Committee on Research Involving Human Subjects / Institutional Review Board (IRB) for Kansas State University has reviewed the proposal identified above and has determined that it is EXEMPT from further IRB review. This exemption applies only to the proposal - as written – and currently on file with the IRB. Any change potentially affecting human subjects must be approved by the IRB prior to implementation and may disqualify the proposal from exemption.

Based upon information provided to the IRB, this activity is exempt under the criteria set forth in the Federal Policy for the Protection of Human Subjects, **45 CFR §46.101, paragraph b, category: 2, subsection: i.**

Certain research is exempt from the requirements of HHS/OHRP regulations. A determination that research is exempt does not imply that investigators have no ethical responsibilities to subjects in such research; it means only that the regulatory requirements related to IRB review, informed consent, and assurance of compliance do not apply to the research.

Any unanticipated problems involving risk to subjects or to others must be reported immediately to the Chair of the Committee on Research Involving Human Subjects, the University Research Compliance Office, and if the subjects are KSU students, to the Director of the Student Health Center.

K-STATE
Kansas State University

University Research
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TO: Mark Haub
Human Nutrition
127 Justin

Proposal Number: 4926

FROM: Rick Scheidt, Chair
Committee on Research Involving Human Subjects

DATE: February 4, 2009

RE: Approval of Proposal Entitled, "Omega-3 fatty acids and cognitive outcomes in soldiers deployed to combat areas."

The Committee on Research Involving Human Subjects has reviewed your proposal and has granted full approval. This proposal is **approved for <u>one year</u> from the date of this correspondence, pending "continuing review."**

APPROVAL DATE: February 4, 2009

EXPIRATION DATE: February 4, 2010

Several months prior to the expiration date listed, the IRB will solicit information from you for federally mandated **"continuing review" of the research.** Based on the review, the IRB may approve the activity for another year. **If continuing IRB approval is <u>not</u> granted, or the IRB fails to perform the continuing review before the expiration date noted above, the project will expire and the activity involving human subjects must be terminated on that date. Consequently, it is critical that you are responsive to the IRB request for information for continuing review if you want your project to continue.**

In giving its approval, the Committee has determined that:

- ☒ There is no more than minimal risk to the subjects.
- ☐ There is greater than minimal risk to the subjects.

This approval applies only to the proposal currently on file as written. Any change or modification affecting human subjects must be approved by the IRB prior to implementation. All approved proposals are subject to continuing review at least annually, which may include the examination of records connected with the project. Announced post-approval monitoring may be performed during the course of this approval period by URCO staff. Injuries, unanticipated problems or adverse events involving risk to subjects or to others must be reported immediately to the Chair of the IRB and / or the URCO.

KSTATE
Kansas State University

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TO: Mark Haub
Human Nutrition
Justin Hall

Protocol Number: 4926.2

FROM: Rick Scheidt, Chair
Committee on Research Involving Human Subjects

DATE: February 1, 2010

RE: Approval of Your Proposal Entitled, "Omega-3 fatty acids and cognitive outcomes in soldiers deployed to combat areas."

Federal regulations stipulate that human subjects protocols can be approved by IRB's for only one year, and require "continuing review" and approval to continue past the expiration date.

On the basis of the IRB "continuing review," your project is classified as follows:

> **Active.** The activity is pending or in progress, and there have been no changes that have occurred or are contemplated that would affect the status of human subjects.

EXPIRATION DATE: 2/4/2011

If the activity persists, it will be eligible for continuing review several months prior to the new expiration date.

Appendix B - Food Frequency Questionnaire

Long-Chain Omega-3 Questionnaire

Department of Human Nutrition
Kansas State University

ID # _______________

If you have any concerns or questions about completing this survey, please contact Jennifer Hanson at 785-532-0175 or at jhanson2@ksu.edu

Instructions:

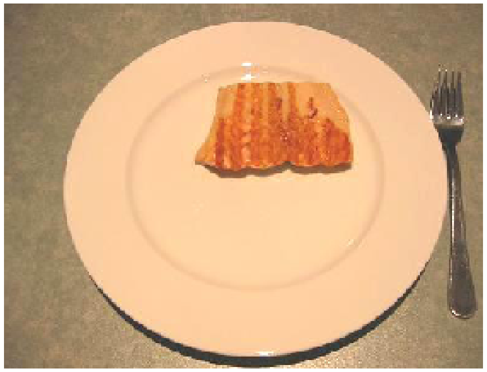
This questionnaire is about how much and how often you eat foods containing high levels of the long-chain omega-3 fatty acids. When answering, think about what you usually ate during the last six months. Please remember to include fish you ate in restaurants, as takeout food, and fish you or someone you know caught. Complete Parts 1-3.

Step 1: Mark the column with a cross to show how often; on average you ate the food.

Step 2: Mark your usual serving size with a cross, as small (S), medium (M), or large (L).

Please note:

- A small serving is about one-half (1/2) the medium serving size, or less.
- A large serving is about one-and-a half (1 ½) times the medium serving size or more
- If you never ate a food, mark "never" and omit the serving size.
- Please do not skip any foods or leave blanks
- When applicable, please write using BLOCK LETTERS
- 4 ounces of cooked fish looks like the picture on the right :



Example: This person never ate sushi, but ate a medium serving of baked or broiled white fish sardines once a week.

	NEVER or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2 + per day	Medium Serving size	S	M	L
Sushi	X									1 roll			
Baked or broiled white fish (such as snapper, cod, halibut, sole)				X						4 ounces			

PART 1
How often did you eat these foods during the last 6 months?

	NEVER or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2 + per day	Medium Serving size	S	M	L
Canned tuna, tuna salad, tuna sandwich, tuna casserole										1 can or 1 cup casserole			
Fried fish, fish sandwich, fish sticks										4 ounces or 1 sand-witch			
Fried Shellfish (shrimp and oysters)										4 ounces			
Sardines										1 can			
Baked or broiled white fish (such as snapper, cod, halibut, sole)										4 ounces			
Baked or broiled dark or oily fish such as salmon, mackerel, and bluefish										4 ounces			
Sushi Please write type										1 roll			
Beef										4 ounces			
Pork										4 ounces			
Chicken, dark meat										2 pieces			

How often did you eat these foods during the last 6 months?

	NEVER or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2 + per day	Medium Serving size	S	M	L
Eggs with yolks										2 egg			
"Omega-3" enriched eggs										2 egg			
"Omega-3" enriched margarine Please write brand name __________________										2 tea-spoons			
"Omega-3" enriched milk										1 cup			
"Omega -3" enriched yogurt										4 ounces			
"Omega-3" enriched juice										1 cup			

PART 2

Did you take any nutritional supplements during the last 6 months? (Circle) **Yes** or **No**
If NO skip to Part 3. If yes, how much and how often?

Supplement	How much did you normally take daily?
Cod liver oil, fish oil, or omega -3 supplements Please Specify 1.______________________ 2.______________________	
Multivitamin Please Specify 1.______________________ 2.______________________	
Other vitamin, mineral or nutritional supplements Please Specify 1.______________________ 2.______________________	

PART 3

Circle one answer for each numbered question. For each "yes" response, please explain below that item.

1. Does the amount of fish you eat change through-out the year for religious or cultural reasons (e.g. Lent)? **YES** or **NO**

   If yes, explain ____

2. Does the amount of fish you eat vary based on seasonal availability? **YES** or **NO**

   If yes, explain ____

3. Does the amount of fish you eat during the school year differ from the amount you eat when you are not at school? **YES** or **NO**

   If yes, explain ____

4. Do you eat any omega-3 rich foods that are not included on this survey? **YES** or **NO**

   If yes, explain ____

5. Are any of the questions on this questionnaire difficult to answer or understand? **YES** or **NO**

   If yes, explain ____

6. Is there anything else about your diet or supplement use that you think we might find interesting? **YES** or **NO**

   If yes, explain ____

Appendix C - Demographic and Health Behaviors Questionnaire

DEMOGRAPHIC & HEALTH BEHAVIORS

Please answer the questions below by circling the best answer. ID #______________

1. Which of the following best describes your ethnicity?
   A. African American
   B. Asian
   C. Caucasian
   D. Hispanic
   E. Native American
   F. Pacific Islander
   G. Other

2. Which of the following best describes your education level?
   A. Less than High School
   B. Completed High School or GED
   C. Some College
   D. College Degree or Higher

3. Which of the following best describes your usual alcohol consumption during the last week?
   A. None
   B. Less than one drink per day
   C. 1-2 drinks per day
   D. 3-4 drinks per day
   E. More than 4 drinks per day

4. Which of the following best describes your smoking habits?
   A. Never smoked
   B. Current smoker
   C. Ex-smoker

5. What is your gender?
   A. Male
   B. Female

6. What is your current marital status?
   A. Single
   B. Married
   C. Divorced
   D. Separated
   E. Widowed

7. During the last 6 months how many days per week have you done moderate or intense physical activities (including PT) for at least 10 minutes at a time.
A. 0
B. 1
C. 2-3
D. 4 or more

8. Which of the following best describes what you have done at work (not including PT) during the last 6 months?
A. Mostly sitting or standing
B. Mostly walking
C. Mostly physically demanding work (including walking with body armor)

9. Is English your first language?
A. Yes
B. No

10. How often do you have trouble wrapping up the final details of a project, once the challenging parts have been done?
A. Never
B. Rarely
C. Sometimes
D. Often
E. Very Often

11. How often do you have difficulty getting things in order when you have to do a task that requires organization?
A. Never
B. Rarely
C. Sometimes
D. Often
E. Very Often

12. How often do you have problems remembering appointments or obligations?
A. Never
B. Rarely
C. Sometimes
D. Often
E. Very Often

13. When you have a task that requires a lot of thought, how often do you avoid or delay getting started?
A. Never
B. Rarely
C. Sometimes
D. Often
E. Very Often

14. How often do you fidget or squirm with your hands or feet when you have to sit down for a long time?
A. Never
B. Rarely
C. Sometimes
D. Often
E. Very Often

15. How often do you feel overly active and compelled to do things, like you were driven by a motor?
A. Never
B. Rarely
C. Sometimes
D. Often
E. Very Often

Please answer the questions below by filling in the blank.

16. What is your height? _______feet ______inches

17. What is your current weight? __________pounds

18. What is your age? _________ years

END

Appendix D - Correspondence with Psychological Assessment Resources (PAR)

K-State Webmail jhanson2@k-state.edu

+ Font Size -

RE: Request: HVLT-R and COWA

From : Vicki McFadden <vmark@parinc.com> Wed, May 25, 2011 01:30 PM
Subject : RE: Request: HVLT-R and COWA
To : Jennifer Hanson <jhanson2@k-state.edu>

Hi Jennifer,

Written permission is not required for use of purchased tests from PAR. If you are modifying a test or including items in a publication/dissertation, then an Agreement with PAR is required. If you need a letter of permission for tests purchased, then please send me a copy of the Receipt/Order and I will be happy to prepare such a letter. This letter is not a requirement to use the materials.

I look forward to hearing from you.

Best Regards,

Vicki McFadden
Permissions Specialist

Psychological Assessment Resources, Inc., 16204 N. Florida Avenue, Lutz, FL 33549, www.parinc.com
Telephone: (888) 799-6082; Fax: (800) 727-9329; Intl Fax: (813) 449-4109; e-mail: vmark@parinc.com

Please note: I will be out of the office from May 26, 2011 until June 1, 2011.

-----Original Message-----
From: Jennifer Hanson [mailto:jhanson2@k-state.edu]
Sent: Wednesday, May 25, 2011 2:26 PM
To: Vicki McFadden
Subject: Re: Request: HVLT-R and COWA

Hi Vicki,
Thanks for the quick response. I was under the impression that we need to obtain a copyright release in addition to purchasing the forms. The forms were actually purchased thru the Army. Our graduate school requires a copyright release. The tests will be referred to, but items from the tests will not appear in my dissertation. Also I plan to submit manuscripts which will refer to tests and results.

I have to admit I am a little confused by the copyright rules and just want to be sure I doing things right.

----- Original Message -----
From: "Vicki McFadden" <vmark@parinc.com>
To: jhanson2@k-state.edu
Sent: Wednesday, May 25, 2011 1:10:12 PM
Subject: Request: HVLT-R and COWA

Dear Jennifer,

Thank you for completing the request forms. You state that you have already purchased the forms for use in your research, therefore, what permission are you looking for from PAR?

I look forward to hearing from you.

Thank you,

Vicki McFadden
Permissions Specialist

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Please note: I will be out of the office from May 26, 2011 until June 1, 2011.

-----Original Message-----
From: Jennifer Hanson [mailto:jhanson2@k-state.edu]
Sent: Wednesday, May 25, 2011 1:26 PM
To: Chad Sigmon
Subject: Permission Request Forms

Hi Chad,
I am attaching 2 copyright release requests. One is for the HVLT-R, & the other is for the Controlled Oral Word Association. I am a graduate student at KSU. The tests have already been purchased. Please let me know if you have any questions.
Thanks,

Jennifer

--
Jennifer A. Hanson, M.S., R.D., L.D.
Department of Human Nutrition
Kansas State University
Manhattan, KS 66506
Office: 785-532-0175
c: 913-306-3378

--
Jennifer A. Hanson, M.S., R.D., L.D.
Department of Human Nutrition
Kansas State University
Manhattan, KS 66506
Office: 785-532-0175
c: 913-306-3378

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Appendix E - Correspondence with Journal of Human Nutrition and Dietetics

K-State Webmail jhanson2@k-state.edu

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RE: One more question.. Manuscript JHND-11-03-0076-OA

From : Lisa - Oxford Walton <liwalton@wiley.com> Fri, Jul 08, 2011 08:16 AM
Subject : RE: One more question.. Manuscript JHND-11-03-0076-OA
To : Jennifer Hanson <jhanson2@k-state.edu>

Hi Jennifer,

Its fine to be published in the repository as well as this is common practice with dissertations. If you have any further questions please don't hesitate to contact me.

Kind Regards,
Lisa Walton

-----Original Message-----
From: Jennifer Hanson [mailto:jhanson2@k-state.edu]
Sent: Friday, July 08, 2011 12:00 AM
To: Walton, Lisa - Oxford
Subject: One more question.. Manuscript JHND-11-03-0076-OA

Hi Ms Walton,
Sorry again, but one more question. My question is, if I resubmit the manuscript can it also be "published" as part of my dissertation. Our graduate school requires that we publish our dissertations on K-Rex an electronic repository (see link below). I think this is common for graduate schools. I defended in June and will need to provide a copy of my dissertation in August to the Graduate School. If I submit to you and it is not allowed to be published in the schools repository I will submit a summary instead. Thanks.
http://krex.k-state.edu/dspace/handle/2097/4

Sincerely,
Jennifer

----- Original Message -----
From: "Ailsa M Brotherton" <ambrotherton@uclan.ac.uk>
To: "Jennifer Hanson" edu>
Cc: jhanson2@ksu.edu, liwalton@wiley.com
Sent: Thursday, July 7, 2011 4:47:53 PM
Subject: Re: Journal of Human Nutrition and Dietetics - Decision on Manuscript JHND-11-03-0076-OA

Jennifer

Apologies for the lack of clarity - if you are able to amend your manuscript in light of the reviewers comments then I would be happy to reconsider it

Thanks
Ailsa

>>> Jennifer Hanson edu> 07/07/2011 20:09 >>>
Dr. Brotherton,
Sorry to bother you, but I am unclear. Has the manuscript been "rejected" outright or is it possible that it might be accepted with revisions? I am uncertain. Could you please clarify.

Thanks,
Jennifer

----- Original Message -----
From: ambrotherton@uclan.ac.uk
To: jhanson2@ksu.edu
Cc: liwalton@wiley.com

K-State Webmail jhanson2@k-state.edu

+ Font Size -

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Jennifer

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Jennifer

----- Original Message -----
From: ambrotherton@uclan.ac.uk
To: jhanson2@ksu.edu
Cc: liwalton@wiley.com

Sent: Wednesday, July 6, 2011 6:23:57 PM
Subject: Journal of Human Nutrition and Dietetics - Decision on Manuscript JHND-11-03-0076-OA

06-Jul-2011

Dear Ms Hanson

I write with regard to manuscript JHND-11-03-0076-OA entitled "Clarity, Completeness and Reliability of a Food Frequency Questionnaire to Measure Eicosapentaenoic Acid and Docosahexaenoic Acid Intakes in Young Adults", which you submitted to the Journal of Human Nutrition and Dietetics.

Unfortunately I am unable to accept your manuscript for publication in the Journal of Human Nutrition and Dietetics in its current format.

Yours sincerely,

Dr Ailsa Brotherton
Editor, Journal of Human Nutrition and Dietetics
ambrotherton@uclan.ac.uk

Reviewer(s)' Comments to Author:

This paper, as the title indicates, deals with the clarity, completeness and reliability of a FFQ measuring ing EPA and DHA intake in young adults.

I am however, not clear about whether the validity of the FFQ has been established. You mentioned that this FFQ is a modified version of a Australian FFQ, but you do not give an indication of how much it has been change and whether that "validity" is still of relevance for you FFQ. This is after all, the main question that should be addressed. Without showing to be a valid method of determining EPA and DHA intake, the points such as clarity, completeness and reliability is not that relevant. You should address this issue in the methodology and discussion.

I suggest that you address the following points:

1. Give a detailed discussion of how much this Australian FFQ was changed?
2. Do you consider your FFQ to be valid tool for assessing EPA and DHA intake?
3. In addition to no.2, why did you not use another dietary method to validate your FFQ?
4. Why would seasonal changes affect fish intake? I thought it is more important in terms of fruit and vegetable intake?
5. Please provide a copy of your FFQ.
6. Please give very clear information of whether the FFQ, and which particular questions, you consider to be a valid measure of EPA and DHA intake.
7. Do you think your FFQ would be a valid tool to be used amongst different population groups?

--
Jennifer A. Hanson, M.S., R.D., L.D.
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Kansas State University
Manhattan, KS 66506
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