Whole Food, Functional Food, and Supplement Sources of Omega-3 Fatty Acids and Omega-3 HUFA Scores among U.S. Soldiers

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Abstract

A questionnaire to measure consumption of contemporary sources of omega-3 fatty acids including i) seafood, ii) poultry and eggs, iii) omega-3 functional foods, and iv) dietary supplements was completed by U.S. soldiers (N = 191). Omega-3 highly unsaturated fatty acid (HUFA) scores were higher ($p < 0.01$) among those who i) took an omega-3 supplement, ii) consumed sushi, iii) consumed omega-3 functional foods, iv) consumed beef less than twice per week, and v) ate seafood at least twice a week. Omega-3 HUFA was positively correlated with EPA + DHA intake ($\beta = 0.39$; $p < 0.01$) and age ($\beta = 0.14$; $p = 0.03$) and negatively correlated with beef intake ($\beta = -0.13$; $p = 0.05$) and smoking ($\beta = -0.12$; $p = 0.08$). Intake of contemporary foods rich in omega-3s were associated with higher biomarker values while smoking and beef intake were associated with lower values.

Keywords: omega-3 fatty acids; functional food, food frequency questionnaire; docosahexaenoic acid; eicosapentaenoic acid; military personnel

Chemical compounds studied in this article:

docosahexaenoic acid (PubChem CID:445580); and eicosapentaenoic acid (PubChem CID:446284)

1. Introduction

Omega-3 fatty acids are naturally occurring lipids 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 U.S., the estimated mean dietary intake of the long-chain omega-3 fatty acids (U.S. Department of Agriculture, 2012) is below optimal levels (Vannice & Rasmussen, 2014). However, most large government nutrition studies (e.g. NHANES) are not inclusive of the military, and in a large study of dietary supplement use in the military (Lieberman et al., 2010) fish oil was reported as “other” thus preventing the establishment of any firm conclusions about omega-3 supplement use in this population. Applying the Land’s Equation to food intake estimates based on sample garrison menus and historical consumption patterns, investigators predicted omega-3 highly unsaturated fatty acids (HUFA; fatty acids with 20 or more carbons and three or more double bonds) would comprise 23.6 % of the total HUFA of the plasma phospholipids of a servicemember consuming the garrison diet (Marriott, Yu, Majchrzak-Hong, Johnson, & Hibbeln, 2014). Yet, U.S. military service members consume foods from a variety of military-affiliated (e.g., dining facilities, commissary, feeding rations) and non-military-affiliated (e.g., grocery stores, restaurants, convenience stores) sources. In addition, the omega-3 food environment is constantly 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 provide relatively few foods rich in omega-3 fatty acids (Davis & Prall, 2014), rations comprise only part of the dietary

intake picture (Hanson, Johnston, Dretsch, Lin, & Haub, 2015), and it would be incorrect to make assumptions regarding intake or omega-3 status based solely on ratios.

The relationship between omega-3 fatty acids and health outcomes has been the topic of numerous population-based studies (Huang, 2010; Vannice & Rasmussen, 2014). 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 status have been utilized in research (Fekete, Marosvölgyi, Jakobik, & Decsi, 2009), and many including whole blood docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (Farzaneh-Far et al., 2010), plasma phosphatidylcholine DHA (Schaefer et al., 2006), and erythrocyte membrane omega-3 fatty acids (Thorseng et al., 2009) 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 (Beydoun et al., 2013; Naqvi, Buettner, Phillips, Davis, & Mukamal, 2010), diet history (van Gelder, Tijhuis, Kalmijn, & Kromhout, 2007), and food frequency questionnaire (FFQ) (de Goede, Geleijnse, Boer, Kromhout, & Verschuren, 2010; Golding, Steer, Emmett, Davis, & Hibbeln, 2009; Gopinath, Flood, Rochtchina, McMahon, & Mitchell, 2010) 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 (Lepsch et al., 2015; Øverby, Serra-Majem, & Andersen, 2009). Individual responses to changes in omega-3 intake have been shown to vary dramatically (Köhler, Bittner, Löw, & von Schacky, 2010), and factors such as gender (Childs, Romeu-Nadal, Burdge, & Calder, 2008), age (Block, Harris, & Pottala, 2008; Sands, Reid, Windsor, & Harris,

2005; Wagner et al., 2015), body mass index (BMI) (Sands et al., 2005; Lepsch et al., 2015), and smoking status (Block et al., 2008; Wagner et al., 2015) are associated with differences in various omega-3 biomarkers. Variants of the fatty acid desaturase (FADs) genes as well as variants of the elongase of very long fatty acids 2 (ELOVL2) gene are associated with differences in omega-3 fatty acids (Tanaka et al., 2009). Plourde et al. (2009) detected differences in plasma omega-3 fatty acids based on allele variant of the apolipoprotein E (APOE) gene, and recently, Chouinard-Watkins et al. (2015) found an interaction between APOE genotype and BMI that was associated with variations in plasma omega-3 biomarker response to a fish-oil supplement. 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.

In this study designed to investigate the omega-3 status of 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 food 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 U.S. Omega-3 tissue status was determined by measuring the percent of omega-3 HUFA in total HUFA of whole blood, herein referred to as the omega-3 HUFA score (Stark, 2008). Compared to other whole blood biomarkers of omega-3 fatty acids, the omega-3 HUFA score was shown to have the highest correlations with dietary intake of EPA

+ DHA as determined by both food record (0.45, $p < 0.001$) and FFQ (0.49, $p < 0.001$) (Patterson, Hogg, Kishi, & Stark, 2012). In addition, the omega-3 HUFA score 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 characterize the relationship between the percent of omega-3 highly unsaturated fatty acids in the total HUFA of whole blood (omega-3 HUFA score) and dietary intake in a sample of deploying soldiers.

2. Subjects and Methods

2.1 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 and occurred during scheduled duty hours. It was made clear to all potential subjects that participation was strictly voluntary and was not part of their required military duty. Only those soldiers whose military duties were not hindered by study participation were eligible; however, the exact number of those eligible is unknown given the dynamic nature of the setting. The soldiers did not receive compensation for their participation. This protocol was conducted in accordance with the Declaration of Helsinki and approved by the Kansas State University Institutional Review Board. After being provided a

complete description of the study, the soldiers recruited for this study were required to provide written informed consent prior to participation.

2.2 EPA + DHA Intake

Based on the concept of generic memory (Willet, 1998), 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. The FFQ was developed by modifying an existing FFQ that had been validated in an Australian population (Mina, Fritsch, & Knuiman, 2007), and based upon the initial assessment of content validity and reliability appears to be a comprehensive and reliable ($n = 54$, $\rho = 0.86$, $p < 0.001$) instrument for measuring EPA + DHA intakes in young adults (Hanson, Rosenkranz, Holcomb, & Haub, 2012). Consumer research (Mintel, 2008) 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 the initial assessment study. 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. In addition to the eleven items used to measure EPA + DHA, participants were asked to indicate their usual serving size and frequency of consumption of beef and pork.

The omega-3 HUFA content of each food item was determined using the U.S. Department of Agriculture National Nutrient Database for Standard Reference, Release 22 (U.S. Department of Agriculture, 2009). Because docosapentaenoic acid (DPA) values were generally only available for the seafood items and dark chicken meat, EPA and DHA became the primary dietary focus. Nonetheless, in an effort to better understand the contribution of all dietary HUFA, we measured a secondary intake variable (i.e., omega-3 HUFA intake), which captured intake of EPA + DHA from all sources plus the intake of DPA from seafood and dark chicken meat. For supplements and items not listed in the U.S. 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.

2.3 Demographic and Health-Related Behaviors

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

2.4 Omega-3 HUFA Score

Finger-pricked blood sampling, combined with microwave accelerated fatty acid assay, fast gas chromatography data acquisition, and automated data processing were used to determine omega-

3 HUFA score. The development of this methodology has have been described previously in detail (Lin et al., 2014). In brief, non-fasting fingertip prick blood samples were collected on strips of chromatography paper treated with BHT and acetone as outlined by Ichihar et al. (2002). The samples were air-dried for 3 hours at room temperature and stored in a closed sample container overnight during transport before being stored at -80°C until analysis. A one-step, direct transesterification fatty acid analysis accelerated by microwave irradiation was developed from Lepage and Roy (1986) 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, USA). Afterwards, the samples were chilled in ice, neutralized by an addition of 5 mL of 6% Na₂CO₃ 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, USA) 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 (Masood, Stark, & Salem, 2005). Data were expressed as the percentage of each fatty acid in total weight of the identified fatty acids in each sample (wt %). The omega-3 HUFA score was calculated using the following formula: (EPA + DHA + DPA) / (HUFA total) X 100.

2.5 Statistical Analysis.

Data analyses were performed using SPSS version 22 (IBM Corporation, Armonk, NY, USA).

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. Seafood consumption was categorized as twice per week or less. Similar categorizations were made for frequency of beef and pork consumption. Descriptive analyses were based on the number of complete responses. Pairwise exclusion was used to exclude cases only if they were missing data for a specific analysis. Neither the EPA + DHA intakes, nor the omega-3 HUFA scores were normally distributed. The EPA + DHA intakes skewness value was 2.289 (SE 0.176) while the kurtosis value was 6.385 (SE 0.350). The omega-3 HUFA scores skewness value was 1.859 (SE 0.176) with the kurtosis value at 5.879 (SE 0.350). As such, nonparametric tests were used for all analysis with the exception of the regression analysis in which z-score transformations were used. Spearman's correlation coefficients were conducted to measure bi-variant correlations, and Mann-Whitney U 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. Linear regression analysis using backwards deletion was conducted using the z-score transformed omega-3 HUFA score as the dependent variable and z-score transformed FFQ-based intake of EPA + DHA, age, smoking status, and z-scores transformed intake of beef as the independent variables. Except for removal of variables from the regression analysis which was based on a significance of $p < 0.10$, two-tailed tests and a significance level of $p < 0.05$ were used for all analyses.

3. Results

3.1 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%; $n = 113$) 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 body BMI, and were less likely to report regular daily alcohol consumption. Additional demographic and health related characteristics by service component are summarized in Table 1.

3.2 FFQ-Based EPA + DHA Intakes

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, 42.9 % ($n = 82$) 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) 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 2) and among those 29 years old and older (Table 2).

3.3 Omega-3 HUFA Scores

The omega-3 HUFA scores ranged from 11.8 to 34.0% with a mean of 18.2 (SD = 3.1) and a median of 17.7%. The omega-3 HUFA score did not differ significantly based on military service category (Table 2). Omega-3 HUFA score was positively correlated with DHA, EPA, and DPA (Table 3). The correlation between omega-3 HUFA score and time to last meal was not significant (Table 3). The omega-3 HUFA scores were lower among current smokers and those 28 years old and younger (Table 2).

3.4 FFQ-Based Dietary Intake and Omega-3 HUFA Scores

The FFQ-based intake of EPA + DHA was positively correlated with omega-3 HUFA score ($r = 0.40$), as well as with DHA, and omega-3 polyunsaturated fatty acids (PUFA) (Table 3).

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 4). Omega-3 HUFA scores were lower among those who reported consuming beef more often than twice per week (Table 4).

The FFQ-based omega-3 HUFA intake (i.e., EPA + DHA plus DPA from seafood and dark chicken) was positively correlated with the omega-3 HUFA score (Table 3) and nearly perfectly correlated with the FFQ-based intake of EPA + DHA (0.999; $P < 0.001$).

The results of the regression analysis with z-score transformed omega-3 HUFA score as the predictive variable and z-score transformed FFQ-based intake of EPA + DHA, smoking status, age, and z-score transformed FFQ-based intake of beef as predictive variables are presented in Table 5. All of the predictive variables were retained with reported intake of z-score transformed EPA + DHA ($\beta = 0.39$) making 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 6. Sixty-six percent of the participants were assigned to either the same or an adjacent quintile.

4. 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 (Øverby et al, 2009), 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, Williams, and Meyer (2006) attained a correlation of 0.54 between FFQ estimates of omega-3 long-chain PUFA and plasma fatty acids, while Lucas, Asselin, Mérette, Poulin, and Dodin (2009) obtained a correlation of 0.42 between FFQ estimates of EPA + DHA and red blood cell membrane measurements of the corresponding fatty acids.

Patterson et al. (2012) reported a correlation of 0.49 between FFQ estimates of DHA + EPA and n-3 HUFA score among Canadian adults. The study by Patterson is similar to the current study in that the FFQ used to measure dietary intake of EPA + DHA was developed to measure contemporary source of EPA + DHA and included non-marine whole foods, functional foods, and nutraceutical sources. The mean omega-3 HUFA score (18.2%) and FFQ-based intake of EPA + DHA (301mg/day; SD = 325) in this present study of soldiers were lower than the 24.4% omega-3 HUFA score and the 340 mg/day (SD = 340) FFQ estimate for EPA + DHA reported by Patterson et al. (2012).

The finding that omega-3 HUFA scores were higher among soldiers reporting functional food use is the first of its kind to identify an association between functional food use and biomarker status among U.S. soldiers while the finding that omega-3 HUFA scores were lower among smokers and younger participants is consistent with that which has been reported previously (Block, Harris, Pottala, 2008; Sands, Reid, Windsor, & Harris, 2005; Wagner, et al., 2015). Using bivariate analysis, Li, Zhang, Hsu-Hage, Wahlqvist, & Sinclair (2001) found that meat intake was positively correlated with DPA and negatively correlated with n-6 DHA, EPA, and DHA. However, unlike our study, in the study by Li et al. (2001) the relationship between meat intake and fatty acid markers disappeared when examined using multiple linear regression. Furthermore, unlike our study, total meat intake rather than intake of each of the individual meat types was analyzed. Although additional research is needed to confirm these relationship, the association between diet and omega-3 HUFA score has potential implication for the military foodservice program. The growing concerns about the psychological health of servicemembers, 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 (Cornum, 2014). As such, the role of diet is of particular importance to military health.

The participants in the current study also had lower omega-3 HUFA scores (18.2%) compared to the 20.3% calculated from reported fingertip blood values for young men and women from Ontario who were consuming 0.22% of energy as omega-3 polyunsaturated fat (Metherel, Armstrong, Patterson, & Stark, 2009). In addition, the omega-3 HUFA score and the FFQ-based intake of EPA + DHA in the present study were 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. (2009) for older adults. Noteworthy in the study by Fratesi et al. 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 (Sublette et al. 2011). In that study, which utilized an FFQ and did not include supplement users or collect intake from functional foods, eggs, or poultry, mean EPA + DHA intake was 76 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. Also worth noting, is the observation that the strength of the relationship between the omega-3 HUFA score and the FFQ-based omega-3

HUFA intake was essentially the same as the strength of the relationship between the omega-3 HUFA score and the FFQ-based EPA + DHA intake.

Several limitations need to be considered with regard to the interpretation of the current results. Given the 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 (Burdge and Calder, 2005), this individual who was taking flaxseed oil had an estimated EPA + DHA intake of 480 mg/day and an omega-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, we have characterized the relationship between omega-3 HUFA score and the dietary intake of US soldiers. To the best of our knowledge this is the first study of its kind to report on the results obtained using an FFQ designed to include contemporary sources of omega-3 fatty acids in a US population. Our finding that contemporary foods, including functional foods, rich in omega-3 fatty acids were associated with higher biomarker values suggests that these food items should not be overlooked when assessing omega-3 status. Lastly, the finding of an inverse relationship between beef intake and omega-3 HUFA score is an important area for future research.

5. Conflict of Interest

The authors of this paper do not have any conflicts of interest or potential conflicts of interest to report.

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Table 1. Characteristics by military service category

Characteristic	<u>National Guard</u>			<u>Active Duty</u>					
	n	%		n	%		χ^2	p	
Gender									
Male	116	93.5		60	89.6		0.96	0.327	
Female	8	6.5		7	10.4				
Marital Status									
Married	70	56.5		43	64.2		1.08	0.300	
Single, divorced, separated	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	64.2		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	0.413	
Current smoker	39	31.5		25	37.3				
	n	mean	SD	n	mean	SD	df	t	p
Age*	123	30.5	8.7	67	27.2	6.1	176.5	3.02	0.003
BMI*	123	27.9	4.2	67	26.7	3.4	163.5	2.12	0.036

Table 2. Comparison of dietary intake of EPA + DHA and omega-3 HUFA score by sample characteristics

Characteristic	n	Median EPA + DHA mg/day	Mean Rank	U	p	Median n-3 HUFA score	Mean Rank	U	p
Gender									
Male	176	207	96.2	1283.0	0.857	17.68	95.5	1237.0	0.686
Female	15	130	93.5			18.16	101.5		
Age*									
28 or younger	104	171	88.1	3701.0	0.041	17.25	84.0	3275.0	0.002
29 or older	86	241	104.5			18.36	109.4		
Marital Status									
Married	113	202	96.2	4388.0	0.960	17.98	101.5	3784.0	0.097
Single, divorced, separated	78	207	95.8			17.17	88.0		
Education									
Less than college degree	147	196	93.2	2826.0	0.205	17.52	93.6	2885.0	0.278
College degree	44	228	105.3			18.03	103.9		
Ethnic Category									
Non-Hispanic Caucasian	124	210	97.0	4026.0	0.726	17.54	92.4	3712.0	0.225
All other	67	184	94.1			17.96	102.6		
Alcohol Use									
One or more drinks/day	132	208	94.3	3671.0	0.653	17.80	96.5	3670.0	0.697
Less than one drink/day	58	178	98.2			17.55	93.2		
Smoking Status*									
Never or former smoker	127	208	99.2	3659.0	0.261	18.17	103.8	3074.0	0.006
Current smoker	64	195	89.7			17.06	80.5		
Military Service Category*									
National Guard	124	214	102.9	3294.0	0.018	17.62	95.6	4099.0	0.880
Active Duty	67	117	83.2			17.82	96.8		

Table 3. Fatty acids and correlations with omega-3 HUFA score, intake, and time to last food

				<u>Correlations</u>			
				omega-3 HUFA Score	Intake EPA + DHA ^a	Intake omega-3 HUFA ^b	Time to Last Food ^c
				(n = 191)	(n = 191)	(n = 191)	(n = 149)
	<u>Whole Blood Fatty Acid^c</u>						
	Median	Minimum	Maximum				
14:0	0.48	0.00	7.02	.04	.01	.01	.15
16:0	20.03	8.60	32.81	.04	-.10	-.11	.09
18:0	10.47	6.21	22.62	-.05	-.12	-.12	.15
20:0	0.62	0.33	1.99	-.17*	-.09	-.09	.03
22:0	1.71	0.88	4.15	-.19**	-.09	-.09	.05
24:0	2.73	0.66	5.28	-.12	-.01	-.01	.04
16:1 n7	1.14	0.42	9.10	.01	-.07	-.08	-.01
18:1 n9	18.15	11.01	31.59	.08	.14	.14	-.18*
18:1n7	1.84	1.11	2.63	-.05	.17*	.17*	-.01
20:1n9	0.17	0.06	0.84	-.02	.12	.13	-.08
24:1n9	1.84	0.86	3.21	-.10	.04	.04	.08
18:2 n6	22.33	12.16	28.94	-.06	.10	.11	-.24**
18:3 n6	0.35	0.08	1.08	.14*	-.11	-.11	.07
20:2 n6	0.22	0.11	5.39	-.08	-.02	-.02	.01
20:3 n6	1.43	0.46	2.68	-.19*	-.04	-.05	-.05
20:4 n6	9.27	4.78	14.38	-.06	-.07	-.07	.07
22:2 n6	0.36	0.12	4.97	-.15*	-.03	-.02	-.06
22:4 n6	1.44	0.75	2.22	-.21**	-.16*	-.17*	.08
22:5 n6	0.60	0.04	5.04	-.29**	-.05	-.05	.06
18:3 n3	0.49	0.21	1.15	.09	.06	.06	-.21*
20:5 n3	0.36	0.09	3.83	.49**	.11	.10	.07
22:5 n3	0.96	0.45	1.51	.21**	.01	.01	.05
22:6 n3	1.47	0.84	3.41	.55**	.35**	.35**	.05
SFA	36.05	25.91	56.14	.00	-.19**	-.19**	.19*
MUFA	23.40	14.88	35.46	.05	.13	.14	-.16
n-6 PUFA	36.47	26.09	46.59	-.15*	-.01	-.01	-.12
n-3 PUFA	3.40	2.31	6.38	.71**	.27**	.27**	.01
n-6 HUFA	13.38	7.54	19.37	-.21**	-.10	-.11	.10
n-3 HUFA	2.86	1.79	6.05	.64**	.25**	.25**	.06
Total HUFA	16.25	9.97	23.62	.00	-.02	-.03	.12
n-3 HUFA Score	17.69	11.84	34.00		.40**	.41**	.01

^a Intake of EPA + DHA as determined by food frequency questionnaire (FFQ).^b Intake of EPA + DHA plus DPA from seafood and dark chicken meat as determined by FFQ.^c Time to last food as self-reported in hours^d Weight % of total fatty acids

Spearman Rank Order Correlation used to determine significance

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

Table 4. Comparison of omega-3 HUFA score by dietary practices

Dietary Practice	n	Median Omega-3 HUFA Score	Mean Rank	U	<i>p</i>
Omega-3 Supplement Use*					
Yes	25	19.39	122.0	1274.0	0.004
No	160	17.52	88.5		
Functional Food Use*					
Yes	35	18.45	97.5	1486.0	0.006
No	122	17.43	73.7		
Sushi Consumption*					
Yes	68	18.32	114.0	2959.0	0.001
No	123	17.31	86.1		
Consumed Seafood at Least Twice per Week*					
Yes	82	18.23	110.9	3250.0	0.001
No	109	17.19	84.8		
Consumed Beef More than Twice per Week*					
Yes	122	17.29	86.6	3067.0	0.002
No	69	18.38	112.6		
Consumed Pork More than Twice per Week					
Yes	37	17.54	92.0	2702.0	0.626
No	154	17.81	97.0		

* Groups differ, $p < 0.01$

Table 5. Results of linear regression analysis with omega-3 HUFA score^a as dependent variable

Independent variables	<i>B</i>	SE <i>B</i>	β	t	F	p
Intake EPA + DHA ^b	0.39	0.07	0.39	5.92		<0.001
Age	0.02	0.01	0.14	2.16		0.032
Intake Beef ^c	-0.13	0.07	-0.13	-1.97		0.051
Smoking	-0.24	0.14	-0.12	-1.78		0.077
Model					15.43	<0.001

^a Z-score transformed % omega-3 highly unsaturated fatty acids (HUFA) in total HUF of whole blood

^b Z-score transformed values obtained using the food frequency questionnaire

^c Z-score transformed values as determined by frequency and portion size reported on questionnaire

Model R = 0.50

Table 6. Agreement of quintile assignment between EPA + DHA intakes^a and omega-3 HUFA score^b

Intake EPA + DHA Quintile	Intake EPA + DHA ^a vs. Omega-3 HUFA Score ^b		
	Same	Adjacent	Misclassified
1 (n = 39)	15	7	17
2 (n = 38)	8	19	11
3 (n = 37)	4	20	13
4 (n = 39)	11	18	10
5 (n = 38)	15	9	14
Total n (%)	53 (28%)	73 (38%)	65 (34%)

^a Value obtained using the FFQ

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