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# Levels of nutrients in relation to fish consumption among older male anglers in Wisconsin



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## ABSTRACT

Fish is an important source of nutrients including omega-3 fatty acids, which may reduce risk of adverse health outcomes such as cardiovascular disease; however, fish may also contain significant amounts of environmental pollutants. The Wisconsin Departments of Health Services and Natural Resources developed a survey instrument, along with a strategy to collect human biological samples to assess the risks and benefits associated with long-term fish consumption among older male anglers in Wisconsin. The target population was men aged 50 years and older, who fish Wisconsin waters and live in the state of Wisconsin. Participants provided blood and hair samples and completed a detailed (paper) questionnaire, which included questions on basic demographics, health status, location of catch and species of fish caught/eaten, consumption of locally caught and commercially purchased fish, and awareness and source of information for local and statewide consumption guidelines. Biological samples were used to assess levels of docosahexaenoic acid (DHA), docosapentaenoic acid (DPA), eicosapentaenoic acid (EPA); vitamin D; and selenium in blood. Quantile regression analysis was used to investigate the associations between biomarker levels and self-reported consumption of fish from the Great Lakes and other areas of concern, other locally caught fish, and commercially purchased fish (meals per year). Respondents were largely non-Hispanic white men in their 60's with at least some college education, and about half were retired. Fish consumption was high (median of 54.5 meals per year), with most fish meals coming from locally-caught fish. Multivariate regression models showed that the effect of supplement use was much greater than that of fish consumption, on nutrient levels, although consumption of fish from the Great Lakes and areas of concern was significantly associated with higher levels of vitamin D even after controlling for supplement usage.

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## 1. Introduction

Fish represents a dietary source of lean protein and important nutrients, including omega-3 fatty acids, selenium, and vitamin D (NIH, 2015a, 2015b, 2015c). Increased consumption of fatty acids may reduce the risk of adverse health outcomes including diabetes (Zhang et al., 2013), cerebrovascular disease (Chowdhury et al., 2012) and cardiovascular disease (Joensen et al., 2010; Levitan et al., 2010; Wilk et al., 2012). Selenium has also been hypothesized as beneficial to health due to its antioxidant activity

(Rayman, 2000, 2012; Tinggi, 2008; Rees et al., 2013; Roman et al., 2014); although the evidence is mixed for cardiovascular health (Rees et al., 2013), studies have found that inadequate selenium is associated with poorer cognition and immune function, as well as overall mortality (Rayman, 2012). Vitamin D has been shown to reduce risk of cardiovascular disease (Wang et al., 2008; Vacek et al., 2012), and there is some evidence for decreased risk of overall mortality (Bjelakovic et al., 2014b) and certain types of cancer (Bjelakovic et al., 2014a). Although vitamin D is produced endogenously during exposure to sunlight (NIH 2015a, 2015b, 2015c), individuals living in northern climates with a long, cold season may be at particular risk for vitamin D deficiency. Few foods are naturally rich in vitamin D, but fish is a natural source to combat the seasonal decline in vitamin D levels. Consequently, fish consumption is thus particularly encouraged for populations at

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high risk of these adverse health outcomes, including older men. However, certain types of fish may also contain high levels of contaminants such as mercury. Consequently, advice regarding fish consumption may be unclear or contradictory depending upon the source of the advice (e.g., state agencies, healthcare providers, popular media), goal of the advice (focus on health benefits or avoidance of contaminants), target population, and other factors.

To balance the risks and benefits of consuming fish, fish consumption guidelines issued by Great Lakes states are designed to encourage consumption of fish that are high in nutrients yet low in contaminants. In Wisconsin, such guidelines were first issued in 1976 by the Departments of Health Services (DHS) and Natural Resources (DNR) and provide more restrictive advice for women of childbearing age (under age 50) and children under age 15. While older men are not specifically targeted by Wisconsin's consumption advisories, they may particularly benefit from consumption of fish due to increased risk of stroke and heart disease (Salonen et al., 1995; He et al., 2002, de Goede et al., 2012). Older men also may have higher body burden of contaminants perhaps due in part to lifelong bioaccumulation and consumption patterns (Knobeloch et al., 2006, 2009; Turyk et al., 2012).

In order to assess the risks and benefits associated with long-term fish consumption among this subpopulation, the Wisconsin DHS' WI Fish Consumption Advisory Program (WFCAP) developed a survey instrument, along with a strategy to collect human biological samples to assess the risks and benefits associated with long-term fish consumption among older male anglers in Wisconsin. While there have been some studies of nutrient content in Great Lakes basin fish (as noted by Turyk et al. (2012) and Neff et al. (2014)), there are few which have been able to directly link reported fish consumption habits with biomarkers. Herein, we focus on the association between fish consumption and biomarker levels of nutrients in this angler cohort. Findings from this analysis will aid DHS and DNR in crafting and communicating appropriate fish consumption guidelines to this potentially vulnerable population of older male anglers.

## 2. Materials and methods

Study participants were recruited from those who previously participated in an online survey administered by the DHS, and had indicated when completing this survey, that they would be interested in future studies (Imm et al., 2013). An additional 43 persons (who had not participated in the online survey) were recruited via flyers and other methods (Fig. 1).

As with the original online survey, the target population was men aged 50 years and older, who fish Wisconsin waters and live in the state of Wisconsin. Participants provided blood and hair samples and completed a detailed (paper) questionnaire, which

included questions on basic demographics, health status, location of catch and species of fish caught/eaten, consumption of locally caught and commercially purchased fish, and awareness and source of information for local and statewide consumption guidelines. Hair samples were used to evaluate mercury body burden (not reported here). The Survey of the Health of Wisconsin program conducted follow-up phone calls and coordinated bio-sample collection in the homes of study participants (Nieto et al., 2010). The study was reviewed by the University of Wisconsin Human Subjects Review Board and determined to be exempt, as it was conducted for the purpose of public health research.

### 2.1. Biological samples

Biological samples were used to assess levels of lipids (cholesterol and triglycerides) and nutrients (omega-3 fatty acids including Docosahexaenoic acid (DHA), Docosapentaenoic acid (DPA), Eicosapentaenoic acid (EPA); vitamin D as the sum of 25-hydroxyvitamin D<sub>2</sub> and 25-hydroxyvitamin D<sub>3</sub>; and selenium, in blood. Each participant provided 47 mLs of whole blood; all blood collection vials were frozen immediately at  $-20^{\circ}$ , with the exception of the vial for fatty acids analysis (frozen at  $-80^{\circ}$  in vertical position) and the vial for lipids analysis (refrigerated in vertical position for no longer than one week). The Wisconsin State Laboratory of Hygiene (WSLH) analyzed the serum collected from whole blood samples for selenium and fatty acids. WSLH determined selenium in plasma following the WSHL Environmental Health Division's CLIN TOX Method 12 CT/Clinical Blood Mercury by Inductively Coupled Plasma-Mass Spectrometry. For fatty acids analysis, WSLH validated and analyzed human serum using NuChek's 455 fatty acids standards mixture (NuChek, 2015). Technical assistance from the Minnesota Health Department was requested to successfully transfer this methodology from their laboratory. Frozen plasma samples were thawed, extracted, and derivatized with acetyl chloride and butylated hydroxytoluene to form fatty acid methyl esters (FAMES) and were reconstituted in hexane. FAME fatty acid derivatives were injected onto a capillary gas chromatographic (GC) column with flame ionization detection (FID; Agilent 6890; Santa Clara, CA). Sixteen fatty acids (4 saturated, 1 monounsaturated, and 11 polyunsaturated) were quantified by FID detector. Reporting limits for 16 *n*-3, *n*-6 and *n*-9 fatty acids ranged from 0.85 to 3.1 mg/L. NuChek's fatty acid calibration standard mixture and extraction blanks are used for each batch of 10 samples (NuChek, 2015). A five-point calibration curve was performed at the start of the sample set. The quantification standard included all the fatty acids reported. The calibration curves were used to quantify the corresponding compound with the sample matrix. The quantification standards were spiked in the plasma, extracted and then derivatized prior to injecting on the GC/FID. Check standards, blanks and duplicates were run with each sample set. Check standards were run to validate the calibration curve and on regular intervals to monitor the stability of the instrument and reproducibility of the method. The check standard was passed if the individual compound was within 50–125%. Duplicate samples were run at least every 10 samples analyzed and or if enough sample volume was available. All compounds within the calibration curve achieved 0.990 correlation coefficient or greater. All blanks associated with this project were below reporting limit concentrations.

Marshfield Laboratory analyzed serum samples for triglycerides and cholesterol. For both tests, an enzymatic/timed endpoint method was performed on a Beckman DXC analyzer. Serum lipids were used as an adjustment factor in multivariate models.

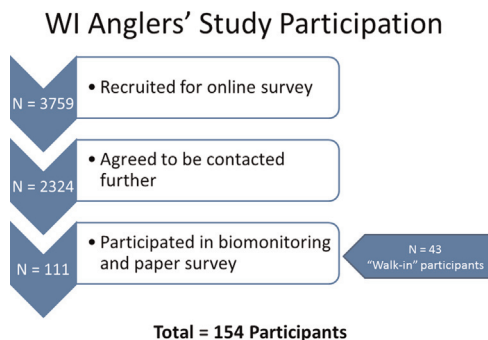


Fig. 1. Flowchart of participation in the biomarker study.

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