

**Development of a 7-item Dietary Screener Questionnaire for Determining Intakes of
Eicosapentaenoic and Docosahexaenoic Acid in Canadians**

by

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Author's Declaration

This thesis consists of material all of which I authored or co-authored: see Statement of Contributions included in the thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Statement of Contributions

The experiment was designed by the author of this thesis, Ming Rong Liu with feedback from Dr. Ken Stark. Data regarding fatty content of seafood items were acquired from the Canadian Nutrient File which is publicly available: <https://www.canada.ca/en/health-canada/services/food-nutrition/healthy-eating/nutrient-data/canadian-nutrient-file-2015-download-files.html>. Data regarding food and nutritional intake of Canadians was acquired from the 2015 Canadian Community Health Survey which access was required and granted by Statistics Canada: <https://www.statcan.gc.ca/en/microdata/data-centres/access>.

The foundation for Canadian Screener was created by C.N Kuratko at Martek Biosciences Corp (now DSM-Firmenich). The data dictionary for the online version of the screener was provided by D.N Christifano, S.E Carlson, et al.

The fatty acid composition of eggs was determined as part of an undergraduate project under my supervision led by A. Olson with assistance from G. Smith. Fatty acid composition of liver and organic chicken was determined under my supervision with the help from L. Hogan-Schmidt and J. Hawkins respectively.

Abstract

The intakes of the omega-3 polyunsaturated acids, eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) are associated with various health benefits. The main dietary sources of EPA and DHA are seafood, but there are non-seafood sources that contain significant amounts of EPA and DHA. Determining the dietary intake of EPA and DHA can be challenging due to the sporadic nature of seafood intake. An online 7-item dietary screener has been used to estimate the DHA intake of pregnant women from both seafood and non-seafood sources in the United States. Using this screener as a template, a Canadian screener for estimating EPA and DHA intakes was developed. In Project 1, EPA values were added and DHA values revised to match those in the Canadian Nutrient File, the questions were reordered to focus on seafood commonly consumed, questions about eggs were expanded to include options for size and omega-3 enrichment, and a French version of the screener was developed. In Project 2, fatty acid quantitation analyses were completed on screener items when the Canadian Nutrient File EPA and DHA data was either missing or not consistent with literature. This included eggs (medium, large, extra-large, omega-3, and omega-3 plus), liver (beef, pork, and chicken), and chicken (regular and organic). For Project 3, the “Canadianized” screener was evaluated for errors using mock. To do this, intake information from the 24-hour dietary recalls in the 2015 Canadian Community Health Survey (CCHS) were extrapolated to bimonthly intakes and entered into the screener. The EPA and DHA intakes estimates from the screener agreed with those from the CCHS 2015 Nutrition, indicating that the Canadian screener is ready for a proper validation study in the future. Once validated, this online tool should be able to improve our ability to estimate the intakes of EPA and DHA by Canadians. In addition, the process that was used to “Canadianize” the screener can be used as a template to adapt the screener for different countries.

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List of Abbreviations

ALA	α -Linolenic Acid
ARA	γ -Arachidonic Acid
ASA24	Automated Self-Administered 24-hour Dietary Assessment
ANOVA	Analysis of Variance
CCHS	Canadian Community Health Survey
CHD	Coronary Heart Disease
CNF	Canadian Nutrient File
CV	Coefficient of Variation
DHA	Docosahexaenoic Acid
DHQ	Diet History Questionnaire
DRI	Dietary Reference Intake
EPA	Eicosapentaenoic Acid
FDC	Food Description Code
FFQ	Food Frequency Questionnaire
FID	Food Intake Data
FRL	Food Recipe Linkage
GC-FID	Gas Chromatograph-Flame Ionization Detector
HS	Household/Socio-demographic
HSD	Honestly Significant Different
IUPAC	International Union of Pure and Applied Chemistry
LCPUFA	Long Chain Polyunsaturated Fatty Acid
LOA	Limits of Agreement
NCI	National Cancer Institute
PUFA	Polyunsaturated Fatty Acid
R24	24-Hour Recall
RCT	Randomized Controlled Trial
REDCap	Research Electronic Data Capture
SPM	Specialized Pro-Resolving Lipid Mediator
TAG	Triacylglycerol
U.S.	United States of America
U.S.D.A	United States Department of Agriculture

Chapter 1

Introduction

The intakes of long chain polyunsaturated fatty acids (LCPUFA), particularly eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) are associated with a decreased risk in cardiovascular disease (W. Harris, 2007; Kris-Etherton et al., 2002; B. Lands, 2014; Mason et al., 2020), a decreased risk of preterm birth (Best et al., 2022; Olsen et al., 2018; Simmonds et al., 2020), and DHA has also been shown to support infant neurodevelopment (Carlson & Colombo, 2016; M. A. Crawford et al., 1976; McCann & Ames, 2005; Nevins et al., 2021). Recommendations for intakes of EPA and DHA vary across expert groups and international associations but usually fall between 250-500 mg/d for general health. However, the intake of EPA + DHA by Canadians is much lower, estimated to range from 98 ± 34 mg/d to 245 ± 189 mg/d (CCHS, 2015; Friesen & Innis, 2009; Denomme et al., 2005).

Estimating EPA and DHA intakes can be difficult, as most Canadians do not consume seafood regularly. Nutrients in foods that are consumed sporadically such as EPA and DHA in seafood can be easily misvalued when using tools that are based on short-term dietary assessments such as dietary records, food duplications, and 24-hour recalls (Bates et al., 2017; Hanson & Haub, 2010; Rahmawaty & Meyer, 2021; Spence, 2021). Assessments that can capture sporadic intakes are longer-term tools, such as food frequency questionnaires (FFQ), diet histories and multiple 24-hour recalls are often recommended for estimating EPA and/or DHA intakes (Liu et al., 2016), but these typically encompass hundreds of questions or repeated assessments which result in a high participant burden (Cui et al., 2023). Tailored dietary screeners can assess the intake of specific nutrients while greatly reducing participant and researcher burden (Thompson et al., 2015). Rapid determination of an individual's dietary EPA and DHA consumption through a targeted food frequency questionnaire,

also known as a screener, has been documented (Christifano et al., 2022; Kuratko, 2013; Lucas et al., 2009; Shen et al., 2019; Sullivan et al., 2006).

The foundation for our Canadian Screener comes from an online-screener hosted on the Research Electronic Data Capture (REDCap) platform by a research group from Kansas City, Kansas, USA. This version was focused on estimating DHA intake of pregnant women (Christifano et al., 2022). This online version was adapted from a previous 7-question paper-based screener used to estimate EPA and DHA intakes from seafood and select non-seafood food items that was developed by Martek Bioscience Corp (now DSM-Firmenich) in Columbia, Maryland. The first 3 questions focused on fish and shellfish intake, but it was then followed by 3 questions about liver, egg, and poultry, followed by a question about fish oil supplement intake.

This thesis involved the development of a seven-question online DHA screener to estimate EPA and DHA intakes of Canadians. To do so, food items in the screener were replaced with those based on national consumption data from the Canadian Community Health Survey (CCHS) 2015, followed up with the addition of EPA content for each of the existing food items. Omega-3 enriched eggs, which appear to have a greater market share in Canada compared with the US, were included in the Canadian screener as well, and a French version of the screener was included to allow nationwide use. The next stage involved the verification of EPA and DHA values as reports have suggested EPA and DHA content of the non-seafood animal sources (liver, eggs, and chicken) is higher than values in the Canadian Nutrient File (CNF) (Enser et al., 1998; Wang et al., 2024), which allowed us to change EPA and DHA values for liver, and add in omega-3 enriched eggs along with organic chicken. Preliminary testing of the Canadian Screener involved stratified random sampling. Twenty-five individuals representing a range of intakes were randomly selected from the CCHS. The two 24-hour food item intake information was combined and extrapolated to represent 100g servings over 2

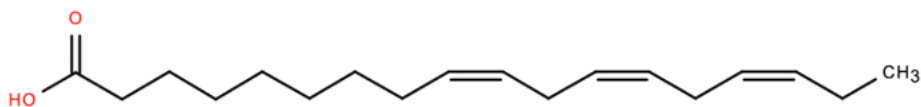
months to be entered into the screener. The EPA+DHA intakes from the CCHS were compared to those estimated values by Canadian Screener using t-tests, Pearson correlation, Bland-Altman analysis, and Cohen's weighted kappa. These tests suggest that the online screener can generate EPA+DHA estimates from the extrapolated food item data from individuals that reflects the EPA+DHA intake estimated by the two 24-hour recalls in CCHS. The Canadian online screener for estimating EPA and DHA intakes should be validated in a future study against an appropriated dietary assessment tool and blood biomarkers.

Chapter 2

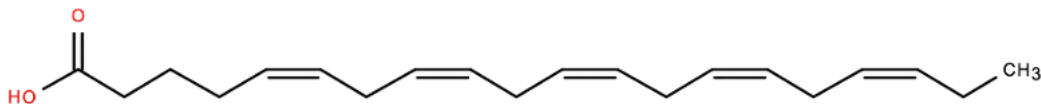
Scientific Background

2.1 Omega-3 Polyunsaturated Fatty Acids and Their Health Benefits

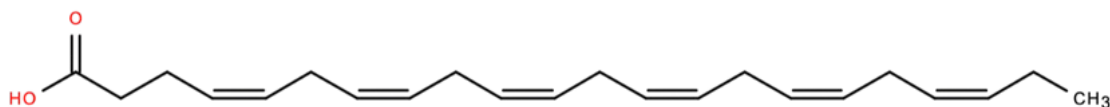
Omega-3 polyunsaturated fatty acids (n-3 PUFA) are fatty acids which contain a carbon-carbon double bond after the third carbon atom from the methyl or “omega” end of the fatty acid. This omega naming system is used as the omega end of the fatty acid stays metabolically inert and the omega location (ie, n-3) remains consistent with fatty acid elongation and shortening. In contrast, the International Union of Pure and Applied Chemistry (IUPAC) identifies double bonds in “delta” positions based on first carbon being the most oxidized or “alpha” carbon. In fatty acids, the “alpha” end is subject to beta-oxidation and/or elongation, which causes shifts in the “delta” numbering system. The omega-3 nomenclature therefore better reflects biochemical metabolism rather than purely chemical structure (Holman, 1964). The most abundant n-3 PUFA in the North American food supply is α -linolenic (ALA, 18:3n-3) (**Figure 2.1**) as it is found in soybean and canola oil (CCHS, 2015; NHANES Pre-Pandemic, 2022; Kris-Etherton et al., 2002). While ALA is an essential fatty acid and cannot be synthesized *de novo*, it can be elongated and desaturated to longer chain n-3 PUFA (Mohrhauer & Holman, 1963a, 1963c, 1963b). The conversion of the dietary ALA to EPA and DHA is limited in humans (Burdge, 2006; Burdge & Wootton, 2002; Gerster, 1998), as such, the consumption of preformed EPA and DHA (**Figure 2.1**) is often recommended to achieve tissue levels associated with health benefits (Hibbeln et al., 2006).



Linolenic Acid (ALA, 18:3n-3)



Eicosapentaenoic Acid (EPA, 22:5n-3)



Docosahexaenoic acid (DHA, 22:6n-3)

Figure 2.1. Line-structure diagrams of common omega-3 fatty acids in the North American food supply. From top to bottom, the image describes: α -linolenic acid (ALA, 18:3n-3), eicosapentaenoic acid (EPA, 20:5n-3), and docosahexaenoic acid (DHA, 22:6n-3)

2.2 Recommendations for Dietary Intakes of EPA & DHA

Recommendations for dietary intakes of EPA and DHA are focused on three tiers. For general health, various expert groups and organizations recommend between 250-500 mg/d of EPA+DHA, which can typically be achieved with two servings of fatty fish a week (Flock et al., 2013; P. M. Kris-Etherton et al., 2003). For pregnant and breastfeeding women, the recommendation changes to between 200-800 mg/d of DHA (Koletzko et al., 2014). Finally, for adults diagnosed with cardiovascular disease, a minimum of 1000 mg/d of EPA and DHA has been recommended (P. M. Kris-Etherton et al., 2002), with up to 4g/d for the treatment of hypertriglyceridemia (Skulas-Ray et al., 2019).

Despite the various recommendations for EPA and DHA intake, there are still no Dietary Reference Intakes (DRI). EPA and DHA are not essential nutrients as they can be biosynthesized from 18:3n-3 (Cholewski et al., 2018), making it difficult to fit into the traditional DRI framework. Most of the dietary recommendations for EPA and DHA are based on chronic disease endpoints which are beginning to be considered as a basis for establishing a DRI (Racey et al., 2021). While various observational studies demonstrate the benefits of dietary EPA and DHA (Del Gobbo et al., 2016; Albert et al., 2002; Olsen et al., 1993), there is also a lack of consistent results in clinical trials using fish oil supplements. a recent meta-analysis of randomized control trials (RCT) assessing the impact of EPA and DHA supplementation on CHD yielded mixed findings (Khan et al., 2021). However, many of the RCT without benefit did not assess dietary intakes of EPA + DHA at baseline. In some of these studies (Kalstad et al., 2021; Nicholls et al., 2020; The ASCEND Study Collaborative Group, 2018), the blood status at baseline suggest that participants in the treatment and control arms of the study were consuming 800-1800 mg/d of EPA and DHA. Therefore, participants in these trials may have already reached a plateau for benefits from the dietary intake of EPA+DHA on cardiovascular health.

2.3 Mechanisms of the Benefits of EPA & DHA

The high degree of unsaturation and long carbon structure of EPA and DHA can impact several molecular pathways including membrane fluidity, ion-channel activity, bioactive lipid synthesis, and gene expression. Collectively, these mechanisms influence a range of health outcomes, which include decreasing coronary heart disease risk (Yu et al., 2022), decreasing preterm birth incidence (Middleton et al., 2018), and supporting infant neurodevelopment (Lauritzen & Carlson, 2011).

One of the challenges of examining the benefits of EPA and DHA, is that they can impact various mechanisms through different pathways. EPA and DHA can have direct effects through interactions as unesterified fatty acids, by getting incorporated into phospholipids and changing membrane characteristics and/or by serving as substrates for various bioactive lipid signaling molecules (Mozaffarian & Wu, 2011).

2.4 Effect of Unesterified EPA & DHA

Within the bilayer, EPA and DHA alter the activity of membrane-bound ion pumps and channels through protein-lipid interactions. Elevating tissue and blood EPA and DHA levels has been shown to optimize endothelial-cell function through fluidizing actions on the membranes, an effect that can translate into anti-arrhythmic properties and lowers the risk of CHD (Leaf et al., 2005).

EPA and DHA can also act as ligands for nuclear receptors and transcription factors that are involved in lipid metabolism and energy homeostasis (Shearer et al., 2012). Hepatic synthesis of triglycerides is one mechanism that is downregulated by EPA and DHA, resulting in lowering of plasma triglycerides (W. S. Harris, 1989), which has been shown to improve insulin sensitivity and support glycaemic controls in individuals with diabetes (Chan et al., 2002).

2.5 Effect of EPA & DHA on Membranes

With increased intake, EPA and DHA replace other fatty acids in the phospholipid cell membrane via the Land's cycle (W. E. M. Lands, 1958, 1960). The increased membrane unsaturation can increase membrane fluidity and decrease membrane thickness (SanGiovanni & Chew, 2005). These membrane dynamics then influence embedded receptors and ion-channels which can impact their stability and functions such as signal transduction, and protein trafficking (Valentine & Valentine, 2004; Williams et al., 2012). Examples are evident in G-protein signalling and retinal photoreceptors, where DHA content in rod cells support visual acuity (Campoy et al., 2012). In the

brain, DHA intake is shown to lower the ARA/DHA ratio (McNamara et al., 2018), where a lower ratio supports key neurodevelopmental processes in infants including neurogenesis, synaptic pruning, and synaptic function (Shahidi & Ambigaipalan, 2018).

2.6 Signaling Molecules Derived from EPA & DHA

EPA and DHA can be used as substrates to form potent signalling molecules. This includes oxidation by cyclooxygenases, lipoxygenases and/or cytochrome p450 monooxygenases to form oxylipins such as specialized pro-resolving mediators (SPM) (Christie & Harwood, 2020; Gabbs et al., 2015) or N-acyl ethanolamines (Watson et al., 2019). These signalling molecules have important roles on neurodevelopment, cardiovascular function, and inflammation.

EPA and DHA can be made into classes of SPM such as protectins, resolvins, and maresins which act to suppress inflammation, resolve the immune response, and promote healing respectively. Together, these have shown benefits in chronic inflammation conditions including asthma, and rheumatoid arthritis (Fredman & Serhan, 2024; Serhan et al., 2015).

Prostanoids (prostaglandins and thromboxanes) produced by cyclooxygenases are involved in cardiovascular health (Braune et al., 2020). While ARA-derived prostanoids (TXA₂ and PGE₂) enhance platelet aggregation and vasoconstriction to aid clotting (Badimon et al., 2021), EPA and DHA derived prostanoids (TXA₃ and PGE₃) can reduce clotting through reduced platelet aggregation and suppresses vasoconstriction (Calder, 2013), lowering the risk of stroke and CHD compared with ARA-derived prostanoids (Badimon et al., 2021).

Finally, EPA and DHA can competitively inhibit the production of ARA-derived ethanolamines in the brain by serving as an alternative substrates for the same pathway (Kim & Spector, 2013). Unlike the ARA-derived anandamide and 2-acylglycerol, the EPA- and DHA-derived ethanolamines such as N-Docosahexaenoyl ethanolamine (synaptamide) tend to be weak agonists for

the cannabinoid receptors (Watson et al., 2019) which have been shown to support synapse formation and neurite growth (Kim & Spector, 2013; Park et al., 2016).

2.7 EPA & DHA in the North American Food Supply

Fish and seafood are the main source of preformed dietary EPA and DHA. In Canadians, the top five most common seafoods are salmon, tuna, crab, shrimp, and cod (Hu & Chan, 2020). Fatty fish such as salmon contain higher concentrations of EPA and DHA (~1500 mg/100g) compared with leaner fish such as cod (~200 mg/100g) and shellfish such as shrimp (~138 mg/100g).

Canadians on average consume low amounts of EPA and DHA ranging from 98 ± 34 mg/d to 245 ± 189 mg/d with higher intakes occurring on coastal areas (CCHS, 2015; Denomme et al., 2005; Dolecek & Grandits, 1991; Friesen & Innis, 2009). Using short-term dietary assessment methods such as the Automated Self-Administered 24-Hour Dietary Assessment Tool (ASA24) ((National Cancer Institute, 2025) (or 3d recall rarely accurately captures fish consumption in Canadians (Cade et al., 2002) due to the sporadic nature of fish consumption (Hanson & Haub, 2010), and the negatively skewed intake distribution. Fish oil supplementation can be an alternative source of EPA and DHA in those who do not consume seafood, however research has shown individuals who take fish oil supplements typically consume high amounts of seafood (Richter et al., 2017).

In people that do not consume seafood or fish oil supplements, the main dietary source of EPA and DHA are certain animal products that contribute lower, but significant amounts of EPA and DHA. Among retail meats, chicken contains the highest levels of EPA+DHA that range between 30 mg/100g to 112 mg/100g (*Canadian Nutrient File*, 2015; Wang et al., 2024) compared with other commonly consumed meats such as beef, pork, and turkey. Given that 58% of Canadians consume chicken on any given day (CCHS, 2015), chicken represents a significant source of dietary EPA and DHA by the population. In addition to chicken, organ meats, particularly liver is believed to be

another non-seafood source of EPA and DHA (Enser et al., 1998; Gladyshev et al., 2015). Other non-seafood sources of EPA and DHA include functional foods that have been enriched in EPA and DHA. In the past, omega-3 fortified foods included cow's milk, yogurt, cheese, margarine, eggs, peanut butter, bread, and juice (Patterson et al., 2012), but many of these products except omega-3 eggs have been removed from mainstream grocery retail stores.

In the past, omega-3 enriched eggs in the past were produced by feeding flaxseed, fish meal or fish oils to egg-laying hens (González-Esquerria & Leeson, 2001). Regular eggs contains about 50 mg of EPA and DHA per egg (*Canadian Nutrient File*, 2015). However, laying hens had a relatively high ability to convert ALA to EPA and DHA, producing up to 100 mg/egg of EPA and DHA from dietary flaxseed which is high in ALA (González-Esquerria & Leeson, 2001), and supplementing their feed with preformed EPA and DHA from fish meal or oil can increase the concentrations of EPA and DHA to over 200 mg per egg. (González-Esquerria & Leeson, 2001; Lawlor et al., 2010; Wei & Jacobson, 2011). Although eggs enriched with omega-3s via fish oil or meal omega-3 enriched eggs were once available, producers have largely switched to flaxseed feeding on biochemical analysis.

Finally, omega-3 supplements such as fish oil and algal oil can have high amounts of EPA and DHA. Commercial fish oils typically have EPA and DHA in three chemical forms, Natural triacylglycerols (TAG), ethyl-esters, and re-esterified TAG. Simple extraction of fish body oil results in natural triacylglycerols that are typically 30% EPA+DHA. Natural TAG can be further refined by removing the fatty acids from the glycerol and forming ethyl esters that can be chemically distilled to create commercial fish oils of approximately 70% EPA+DHA. When there are regulations requiring the fatty acids to be sold in a natural chemical form, the purified ethyl esters can be used to create a re-esterified TAG fish oil that is approximately 50% as EPA+DHA. Algal oils typically contain DHA without EPA and are natural TAG products marketed towards vegetarians and vegans and/or brain

health. There are also pharmaceutical grade fish oil products such as Vascepa[®] that contain over 96% EPA as an ethyl ester available as prescription drugs.

Chapter 3

Dietary Assessment

3.1 Types of Dietary Assessments

Dietary assessments involve the collection of consumed foods and/or drinks over a specified time to measure food consumption or estimate the intake of a nutrient (Labonté et al., 2016; Dao et al., 2019; Esquivel & Lozano, 2024). The application of dietary assessment tools can differ from estimating the dietary intake of a population versus estimating the dietary intake of an individual (Gibson, 2005). In population assessments, the goal is to identify challenges and develop public health policies and food guide policies. Single day assessments are often used across large sample sizes as statistical modelling can be used to correct errors while generating group averages. For individual assessments, the goal is to identify the specific intakes of nutrients to determine the risk of or manage malnutrition and disease. The assessment tool used for individual nutrients can vary between the need for determining precise estimates of recent intake versus understanding long-term dietary patterns, but the approach should try to account for day-to-day variation depending on the nutrients of primary interest. Intervention researchers can use dietary assessment to determine dietary intake of participants to control for variations in nutrient intakes that may impact outcomes. Individual dietary assessments can be categorized as prospective or retrospective. Prospective approaches involve the participant recording their current diet, whereas retrospective approaches allow a participant to recall their diet (Magkos & Yannakoulia, 2003)

Prospective Methods

- Diet Records – also known as food diaries are where the participant records all foods and beverages consumed over a period of time. Diet records can be highly accurate when

determining the recent intake of an individual; however, they often have a high participant burden (Bates et al., 2017).

- Food Duplications – involve quantifying the consumption of foods and beverages via a duplicated portion (Bates et al., 2017). These can be accurate and precise if the duplicated portions collected represent actual intakes, however the cost and participant and researcher burden are warranted only when there are concerns about the ability of a nutrient database to accurately reflect the nutrient content of the food items being consumed.

Retrospective

- 24-hour Recalls – involve the participant recalling foods and beverages they consumed in the past 24-hours. These can have a lower participant and researcher burden but may not be able to assess usual intakes unless multiple days are collected. However, extra days of assessment increase the burden. The day-to-day variation in seafood intake in Canadians requires assessments over multiple days (S. Kirkpatrick, 2025).
- Diet Histories – involve capturing an individual's food consumption pattern over months or years (Bates et al., 2017). These can capture infrequently consumed foods and habitual intake; however, they require a trained interviewer and therefore have a high researcher burden.
- Food Frequency Questionnaires (FFQ) – involve capturing habitual diet using a list of foods or drinks that the researcher is focused on (Bates et al., 2017). These can have a lower researcher burden; however, the participant burden can be high as the questionnaire can take over 30 minutes to complete. FFQ results can be imprecise as the estimates are typically semi-quantitative or qualitative, however, they have been used to estimate EPA and DHA intakes (Shiraishi et al., 2013).

- Dietary Screeners – are a shorter form FFQ aimed to capture habitual intake of a particular nutrient or food group. These have a lower participant and researcher burden as they typically take 15 minutes to complete. However, they need to be focused on specific nutrient and/or food items and therefore do not capture overall dietary patterns. When it comes to assessing dietary intakes of EPA and DHA, there is only a need to focus mainly on seafood and select non-seafood animal sources (Richter et al., 2017) and this approach is often recommended to capture the intakes of EPA and DHA (Serra et al., 2021).

3.2 Challenges to Dietary Assessments

Inaccuracies in dietary assessments can arise within the tool itself (systemic error) or from day-to-day variations from the participant (random). The most common systemic error is the underestimation of self-reported energy intake compared with actual energy intake (McKenzie et al., 2021). Energy underestimation can be due to under reported food intake such as forgotten items (i.e. dietary recalls and FFQ) and changing dietary habits during the assessment to obtain a desirable outcome (i.e. dietary records) (Hill & Davies, 2001; Burrows et al., 2019; Esquivel & Lozano, 2024). To capture variations in food intake, using longer-term and/or multiple dietary assessments has been shown to return a more accurate and precise estimated intake. (Amoutzopoulos et al., 2018; Cade et al., 2002; Dao et al., 2019). Estimating accurate and precise nutrient intakes in dietary assessments also requires the ability recall portion sizes by participants (Cade et al., 2002). Although there is no consensus on best practices for portion size estimations, there are approaches that add clarity which include specifying the quantity, asking the participant to describe their portion size, using reference images, and/or weighing the food (Salesse et al., 2025).

3.3 Determining EPA and DHA Intakes

Determining EPA and DHA in Canadians is challenging. Most short-term dietary assessments often misreport fish consumption as they do not capture the sporadic intake of fish in Western societies (Casey et al., 2024; Rahmawaty & Meyer, 2021; United States Environmental Protection Agency, 2016). Short-term assessments such as 3d diet records are can to generate random error in EPA and DHA intake estimates as seafood intake may be missed, or a seafood intake that is recorded overrepresents the frequency of consumption (Fratesi et al., 2009). While multiple 24-hour recalls have been used as a standard to validate EPA and DHA dietary assessments (Patterson et al., 2012; Sullivan et al., 2008), studies have shown that assessment over a minimum of 30 days is recommended to capture the variability of dietary fats (Basiotis et al., 1987; Tokudome et al., 2002). As an alternative to repeated dietary assessments spread over a longer period of time, dietary assessments such as an FFQ that ask about longer time periods can reliably estimate EPA and DHA intake (Patterson et al., 2012; Shiraishi et al., 2013; St-Jules et al., 2013; Wallingford et al., 2013). For the assessment of EPA and DHA specifically, we can reduce participant burden, by focusing on seafood and select non-seafood items with appreciable amounts of EPA and DHA (Richter et al., 2017). In the past, there were two in-person FFQ developed for estimating EPA and DHA intake by Canadians. The first determined EPA and DHA intakes from seafood only, while non-seafood sources of EPA and DHA, such as poultry and eggs, were not considered (Lucas et al., 2009). The second was more comprehensive and contained questions for non-seafood sources of EPA and DHA (Patterson et al., 2012), but included numerous omega-3 enriched functional foods (ex., orange juice, milk, margarine, etc.) that are no longer available in major supermarket chains, making these functional food questions an unnecessary burden. Portion size images are important for estimating EPA and DHA intake as there are different edible components of seafood depending on the species (e.g., finfish and shellfish).

Biomarkers of EPA and DHA are often used to assess disease risk such as cardiovascular disease (Djuricic & Calder, 2025; W. Harris, 2007), but they can also be used to estimate dietary intakes (W. E. M. Lands et al., 1992; Patterson et al., 2015). There is no consensus on which biomarker should be used, as tissue biopsies and various blood fractions (erythrocytes, plasma, and whole blood) have been examined (Brenna et al., 2018). While tissue biopsies such as adipose tissue reflect long-term intake of EPA and DHA due to its slow turnover rate of about 6 months (Katan et al., 1997), it is too invasive for routine use. Erythrocyte fatty acid composition is often preferred for determining EPA and DHA status, as it is not impacted as much as plasma by recent dietary intakes (Brenna et al., 2018), and represents intake over 2-3 months (W. Harris, 2007). Erythrocyte EPA and DHA content correlates with EPA and DHA content of various blood fractions and lipid classes (Stark et al., 2016), although plasma EPA and DHA can be more responsive to recent dietary intakes (Patterson 2014, Metherel PLEFA). Plasma levels of EPA and DHA usually plateau 6-8 weeks after consistent changes in dietary intake of EPA and DHA (Cao et al., 2006; Katan et al., 1997; Patterson et al., 2014, 2015).

The collection of dried blood spots for fatty acid analyses has practical advantages as it can be collected by fingertip prick, requires relatively little sample handling and chemical processing (Brenna et al., 2018). The associations between whole blood and erythrocyte EPA and DHA with dietary intakes of EPA and DHA in typical ranges of Canadians are similar (Patterson et al., 2015) and dried blood spots samples have been used to validate EPA and DHA dietary assessment tools in the past (Patterson et al., 2012). While there have been attempts to define the relationship between blood levels and dietary intakes of EPA and DHA (Patterson et al., 2015), the prediction of intake amounts from the various possible biomarkers remains relatively imprecise as various metabolic factors such as genetic polymorphisms and sex hormone levels appear to influence blood and tissue

levels along with intake (De Groot & Meyer, 2020). Biological sampling is also difficult for large-scale population use as it is invasive and requires advanced analytical equipment (Saravia et al., 2022). Dietary assessments can allow for a more cost-effective way of determining levels of EPA and DHA compared with biological sampling, while reducing both the participant and researcher burden (Subar et al., 2012).

3.4 Canadian Community Health Survey

The CCHS 2015 Nutrition is a cross-sectional survey conducted by Statistics Canada and Health Canada to determine the nutrient intakes of Canadians across 10 Canadian provinces between January 2015 to December 2015. The number of participants from a province and ethnicity of the participants were selected to represent the distribution within Canada. The survey had two questionnaires: (1) 24-hour dietary recall(s) and (2) a general health questionnaire to support the 24-hour dietary recall. In 2015, a total of 20,487 respondents completed the initial 24-hour dietary recall while 7608 completed a secondary dietary recall. The data from the CCHS 2015 Nutrition is organized using a database structure across eight interconnected data files, of which we used four (Table 3.1).

3.5 Canadian Nutrient File

The Canadian Nutrient File (CNF) is the official food composition database for Canada which is maintained by Health Canada (<https://food-nutrition.canada.ca/cnf-fce/>). It contains detailed nutrient profiles including energy, macronutrients, micronutrients, vitamins, minerals and individual fatty acids for over 5500 commonly consumed food items. While it is the official source of food composition in Canada, there are concerns about its accuracy and precision as the last update was in 2015 and approximately 75% of the current food sources are derived from the USDA. Informal discussions with Health Canada indicate that deployment of updates has been delayed due to software

issues. Using the current CNF database may or may not reflect the current nutrient composition of Canadian food items in 2025. Specifically, the database is missing omega-3 enriched eggs and organic chicken (*Canadian Nutrient File*, 2015) that contain considerable amounts of EPA and/or DHA (González-Esquerro & Leeson, 2001; Wang et al., 2024). These products are available in Canadian supermarkets.

Table 3.1 The CCHS Public Use Microdata Files Used for Preliminary Testing

File Name	File Description
Household/ Socio-demographic data hs.sas7bdat (HS)	Contains overview data for each participant, including number of total calories, total protein, total EPA, and total DHA intake.
24-hour recall data r24.sas7bdat (R24)	Contains the raw data from the dietary recall. Selecting different variables allows selection of day 1 or day 2 data, specific participants, specific food categories, etc.
Food description codes fdc.sas7bdat (FDC)	Contains food descriptions including amount of nutrient per gram of a specific food. This file can be linked to the 24-hour recalls.
Food intake data fid.sas7bdat (FID)	Contains the amounts consumed for each food item and can be linked back to the 24-hour recall data.

3.6 Development of a 7-Question Canadian Screener

Determining accurate and precise EPA and DHA intakes requires an assessment period of at least 30 days (Basiotis et al., 1987; Tokudome et al., 2002). A tailored FFQ or dietary screener for estimating EPA and DHA has the potential for the least amount of burden while generating relatively accurate and precise estimates (Serra et al., 2021). A paper-based EPA and DHA screener was developed by Martek Biosciences Corp (now DSM-Firmenich) in Columbia, Maryland, in 2013 to provide a rapid tool for estimating omega-3 fatty acid intake in clinical research (Kuratko, 2013). The screener had 7 questions with the first three gathering information about the dietary intake of seafood. These questions about fish were grouped so that question one asked about the monthly intake of fish

with high amounts of EPA+DHA (36mg/serving) while question two and three had medium (15mg/serving) and low (11mg/serving) amounts of EPA+DHA, respectively. The four additional questions about the monthly intake of liver (9mg/serving), weekly intake of eggs (3.25mg/egg yolk) and chicken (8mg/serving), and participant were asked to indicate the EPA and DHA content and frequency of n-3 supplements to estimate the participants intake of EPA+DHA (Kuratko, 2013). At first, the use of the 7-question screener was limited to their in-house clinical research group, enabling estimation of EPA and DHA intake without biological sampling.

In 2022, a research group from Kansas City, Kansas, adapted the 2013 dietary screener for pregnant women in the U.S. and focused exclusively on DHA (Christifano et al., 2022). They also transitioned the tool to an online format to reduce researcher and participant burden associated with paper-based or telephone-based dietary assessments (Carter et al., 2015; S. Kirkpatrick & Collins, 2016; Rollo et al., 2016). They used Research Electronic Data Capture (REDCap), a secure platform developed in 2004 by Vanderbilt University for designing and distributing research surveys and the online survey is hosted by the University of Kansas Medical Centre:

<https://redcap.kumc.edu/surveys/?s=XLP7DJDWF4>. This updated online screener was validated against red blood cell phospholipid biomarkers in 1400 women and showed strong correlations ($r = 0.88$) for estimating DHA intake (Christifano et al., 2022). For the Canadian screener, we will continue to use the REDCap platform as it allows for rapid survey deployment, and it is easy to share with other research groups. REDCap also has numerous logic and validation features through its back-end software that allow real-time tailoring of the survey based on the participants' responses, validation of data to ensure correct entry, and real-time calculations to provide immediate feedback to both the participants and researchers. REDCap surveys can also be updated easily if EPA and DHA content of food items changes. To develop the Canadian Screener, the U.S. DHA Screener was shared with us as a REDCap data dictionary (.csv file) through a collaboration with D.N. Christiano and S.E.

Carlson (Christifano et al., 2022). The Canadian screener will be hosted by the University of Waterloo, Faculty of Health.

Challenges that have been identified for this adaptation include checking the EPA and DHA content of food items against the CNF and making relevant changes. Regarding eggs, the U.S. based 2022 DHA screener, and the CNF do not distinguish between different types of eggs, but in Canada, omega-3-enriched eggs are available in most supermarkets. For poultry, there is a recent report suggesting EPA and DHA content has increased with modern husbandry practices (Wang et al., 2024), and there is evidence that organic chicken may have higher levels of EPA and DHA (Giampietro-Ganeco et al., 2020). In addition, the 2022 DHA screener had a question about liver intake due to significant DHA content, but this is not reflected in the CNF. Due to these discrepancies, we completed fatty acid analyses using the gas chromatograph with a flame ionization detector (GC-FID) on eggs, poultry and liver available in major supermarkets (see Chapter 6). A French language option was also added (Chapter 5).

Despite the challenges, the revised 7-question screener for EPA and DHA intake for Canadians will continue to use the basic structure of the 2022 DHA online screener. There will still be three questions focused on seafood, but the seafood groupings were altered. Instead of groupings by high (36 mg of EPA and DHA per 85 g serving), medium (15 mg of EPA and DHA per 85 g serving), and low (11 mg of EPA and DHA per 85 g serving) levels of EPA and DHA, the three seafood questions will then be rearranged to 1) commonly consumed seafood (salmon, crab, cod, etc.), 2) other finfish (sardine, mackerel, bass, etc.), and 3) shellfish/molluscs (lobster, shrimp, squid, etc.). Seafood consumption frequencies were based on data from the Canadian Community Health Survey (CCHS). This was done to allow most participants to complete their main seafood intake assessment in the first section. We will also take advantage of the online system and add distinct EPA and DHA values for each individual fish and shellfish species, and add visual aids (e.g., a deck of

cards beside a picture of 100 g of shrimp) (Nelson & Haraldsdóttir, 1998) to improve portion size estimation. The section on the intakes of eggs, and poultry were expanded to include omega-3 enriched eggs and organic chicken due to their higher EPA and/or DHA content than the regular options (Chapters 5 and 6). Questions about liver and dietary supplements that contain EPA and DHA were retained, although the EPA and DHA content of liver was increased based on our analyses (Chapter 6).

Chapter 4

Homogenizing to Capture EPA and DHA in Food

4.1 Addressing EPA & DHA Content Discrepancies

Since seafood consumption in Canada is generally low (Hu & Chan, 2020), quantification of dietary EPA and DHA intake of Canadians from both marine and non-marine sources is essential for developing a valid EPA and DHA screener. The CNF has the nutritional composition of over most commonly consumed foods in Canadians, but approximately three-quarters of the entries are drawn directly from the USDA Nutrient Database and may not reflect the Canadian food supply. In addition, the presently available CNF database has not been updated since 2015. Therefore, potential discrepancies will be exemplified to be addressed through biochemical determinations of fatty acids in popular food items in our laboratory.

4.2 Homogenization Techniques for the Quantification of EPA & DHA

Accurate quantification of EPA and DHA in food items requires proper sample preparation, which starts with thorough homogenization to obtain a representative sample. Mechanical blending and pulverization using a mortar and pestle are two common methods for homogenizing, however, novel milling tools for food homogenization are now available that can greatly reduce the burden of sample preparation (Burden, 2008). Bead milling is a technique that can efficiently and consistently disrupt solid samples using a relatively cost-efficient benchtop unit (Nitsos et al., 2020), but reliability of its use for fatty acid analysis is still limited.

4.3 Literature and Findings on Bead Milling for Lipid Analysis

We recently compared the homogenization of a blender, mortar and pestle, and a bead miller for the fatty acid analyses of five seafoods most commonly consumed by Canadians (presented at

the 16th ISSFAL Congress in Quebec City, July 2, 2025). Analytical variability can be represented using a coefficient of variation (CV), with CV under 5% representing consistent analyses (Brenna et al., 2018). For total fat concentration, the bead miller had the lowest variability with an CV of less than 5% for salmon, cod, tuna, crab, and shrimp individually. The average CV for the blender was 7% and for mortar and pestle was 8% with homogenization of salmon being particularly problematic. In salmon, bead milling and mortar and pestle grinding resulted in fully disrupted salmon samples, while unprocessed regions were observable using an optical microscope after blending as compared with raw salmon (**Figure 4.1**). The bead miller also had a 100% sample recovery compared with the 85% recovery by the blender and 75% by the mortar and pestle. Previous literature has shown that the bead mill has lower variability than both the blender and mortar and pestle for total fat determinations of lean turkey and fatty pork samples (Segura et al., 2015; Segura & Lopez-Bote, 2014). For the upcoming project, the bead miller will be used to determine the fatty acid content of solid samples that include organic and non-organic chicken cuts, and various liver samples. For liquid samples (regular and omega-3 eggs), complete homogenization can be completed using a blender.

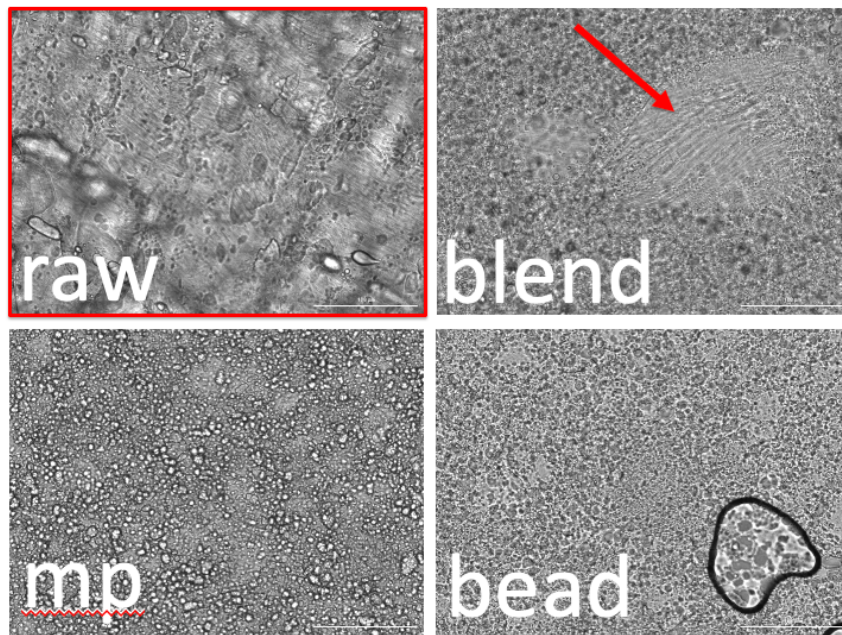


Figure 4.1. Optical Microscope Slides of Unprocessed and Processed Salmon. Four-panel brightfield images (raw, blender, mortar and pestle, bead miller; top left, top right, bottom left, bottom right) of raw Atlantic salmon homogenate; red highlights and arrows mark unprocessed regions; black spot in bead panel mark water droplets; acquired on BioTek Cytation 5 at 400 $\times$ magnification with 50 μ m scale bar.

Chapter 5

Project 1: Development of a Canadian EPA & DHA Intake Screener

5.1 Introduction

Determining the intakes of EPA and DHA is important for clinical studies examining cardiovascular disease (Djuricic & Calder, 2025) and maternal-infant health (Martinat et al., 2021). A non-invasive 7-question online dietary screener had been used in the U.S. to examine the DHA intakes of pregnant women and assess the risk of preterm birth (S. Crawford et al., 2022). The REDCap coding for this U.S. screener was shared with us and in Project 1, modifications were made to use CNF data and add foods consumed often in Canada but not the U.S., add the ability to determine EPA, replaced Spanish with French, and decrease user burden to tailor the screener for Canadians. The ability to accurately estimate EPA and DHA intakes in individuals is needed to support observational studies to link EPA and DHA intakes to disease risks and health outcomes. A quick dietary assessment tool such as the screener is also important for dietary intervention trials as it allows researchers to determine habitual intakes, or in the case of fish oil supplementation studies, it can assess background dietary intakes of EPA and DHA from food. As an online tool, the screener also has the potential to be used by dietitians or even the general public for assessing individual dietary habits regarding EPA and DHA intakes.

5.2 Methods and Results

The modifications to the screener included: refining the Canadian screener categories, adding EPA and DHA values to either align with those in the CNF or replace based on our biochemical analyses, adding egg types that commonly exist in Canadian supermarkets, switching the response prompts from open-ended to fixed frequencies, adding images to assist portion size determinations, and allow the screener to be accessed in the official languages of Canada (French and English). Due

to the iterative nature of these processes, the results are indicated along with the detailed descriptions of the methods in the sections below.

5.2.1 Refining Screener Seafood Categories

The seafood items and the categorization of the types of seafood of the U.S. screener were examined. Geographic location can influence the types of seafood consumed by a population (Sioen et al., 2009), so it was necessary to check if different seafood species are consumed in Canada. The U.S. screener had also categorized the seafood by the concentration of DHA as several species shared a single DHA content, which was a remnant from an effort to reduce investigator burden in the original paper survey (Kuratko, 2013). With the online version, each seafood species could be assigned its own EPA and DHA value, therefore the seafood was recategorized with the most commonly eaten seafood in the first category (ex. Salmon, tuna, shrimp, cod, crab, etc.) followed by other fish (finfish) in the next and then other shellfish in the third category (see **Appendix B**). The ranking of seafood by individuals that consume in the Canadian population were determined using data as reported in the CCHS data files. All seafood intakes were extracted from the CCHS FID and FDC files shown in Table 3.1. Individuals reporting extremely high seafood item intakes (above 10kg) in a single 24-hour recall were removed as outliers. The code to perform this calculation is included in **Appendix A**. The steps to extract and calculate percent consumption of a specific seafood from all 2015 CCHS participants to get a seafood consumption ranking in Canadians included:

- 1.) Foods and beverages reported on the first recall from the CCHS were filtered by food group code from the FID (individual food items, amount in grams, food codes) and FDC (food codes, food descriptions) files using CNF food group codes (e.g. 34A - Fish, Less than 6% total fat; 34B – Fish, Superior or equal to 6% total fat; 35A – Shellfish) for each individual.

2.) The food group code data was separated into the individual seafood items in the screener by using keywords from the food descriptions (e.g. “salmon” was used to collapse all forms of salmon into one category). The number of individuals consuming each species of seafood was summed.

3.) Steps 1 and 2 were repeated for the CCHS 2015 day 2 of the 24-hour recall.

4.) The total number of individuals consuming each individual seafood species were summed and ranked by the number of individuals that consumed each species.

Following the seafood ranking of the CCHS 2015 data, 43 seafood species were consumed. To reduce participant burden, seafood that did not contain any EPA and DHA were excluded from the screener. The final rankings of all fish in the CCHS 2015 Nutrition can be found in Appendix B.

Determining intake percentages for Canadians from the CCHS 2015 Nutrition was repeated for all other screener questions including question 4 (liver), question 5 (eggs), and question 6 (poultry). The results from the CCHS 2015 NUTRITION indicates that out of the 20,483 participants, only 91 (0.4%) had consumed liver, 11,788 (57.6%) had consumed eggs, and 11,032 (53.9%) had consumed poultry. For liver intake, beef, chicken, and pork accounted for 95% of all specified liver intake in the CCHS 2015 Nutrition. For poultry, chicken accounted for 92% of all poultry intake in the CCHS 2015 Nutrition. Based on these high percentages, and to avoid increased analytical burden, we did not consider adding additional species to the screener.

5.2.2 Introduction of EPA Values and Alignment with the Canadian Nutrient File

The source for the EPA and DHA values in the original paper-based screener was indicated to be the USDA, Nutrient Database for Standard Release 14, 2002. Details of how a single value for a food item in the screener were generated from groups of food items (ex. raw salmon, baked salmon,

cooked salmon, etc.) were not provided as the publication was a correspondence that summarized an abstract at a conference (Kuratko, 2013). To ensure that the screener is appropriate for the Canadian population, the values for the same food items in the CNF were substituted. However, if the CNF values for EPA and DHA were different from the values in the previous screeners (Christifano et al., 2022; Kuratko, 2013), additional checks were completed (See Chapter 6). Although the CNF may be outdated and values may have changed (Parpia et al., 2018), it is the most appropriate source in the absence of up to date values, therefore, EPA and DHA values for the Canadian screener should reflect the CNF unless we can demonstrate specific evidence to the contrary. To complete this objective, the full CNF database was exported with all its nutrient values standardized to a 100-gram serving size. Items to be included in the Canadian screener were identified in the CNF database as “raw” and then entered into the screener coding. The list of EPA and DHA values of seafood derived from the CNF can be found in Appendix B. For non-seafood items, inconsistencies were observed for liver, eggs, and chickens and these were investigated in more detail in Chapter 6. It was also observed that raw whole eggs in CNF were derived from a single nutrient profile and then scaled to the average egg size (ie. small, medium, large, and extra-large). These CNF options and nutrient profiles do not reflect common product choices available in Canadian supermarkets such as omega-3 enriched eggs but also ignore literature indicating different size eggs have distinct nutrient profiles (Réhault-Godbert et al., 2019).

5.2.3 Inclusion of Egg Types Commonly Consumed in Canada

Omega-3 enriched egg products which have been advertised to contain up to 125 mg of DHA per egg (Burnbrae Farms, 2025), are commonly found in major supermarket shelves in Canada allowing the general population to reach the recommended intake of EPA+DHA (250 mg/day) with just two eggs a day. The U.S. screener focused on egg yolks and had no option for the type of egg

consumed. The Canadian screener will allow for participants to choose between medium, large, extra-large, omega-3, and omega-3 plus eggs. The CNF has nutrient values including EPA and DHA for “egg, chicken, whole, fresh or frozen, raw” 100g of egg which can be scaled to different-sized eggs based on the average mass of the egg type and omega-3 enriched eggs are not an available option in CNF. Given that there is evidence that the nutritional value of eggs is not consistent across all egg sizes (Réhault-Godbert et al., 2019) and the lack of data for omega-3 enriched eggs, fatty acid analysis on all egg types was completed as part of an undergraduate project under my supervision (See Chapter 6).

5.2.4 Converting Frequency Response Options to a Fixed Interval

Open-ended questions such as “How many servings of x do you eat monthly” (where x = species or food) place a cognitive burden on participants which may cause misreporting (Herforth et al., 2019). Instead, it has been recommended in scientific literature to ask closed questions such as “How often do you eat a 100-gram serving of x” (Cade et al., 2002). To revise the Canadian screener, specific frequency intervals were derived from the NCI Diet History Questionnaire (DHQ) III (National Cancer Institute, 2025b). However, rather than using the full interval list, a subset was chosen to reflect the common sporadic consumption patterns of seafood (Spence, 2021). Participants can now select one of the eight ordered categories for each seafood item, which will have a conversion factor to normalize the concentration to concentration per day (**Table 5.1**).

Table 5.1 Frequency and Daily Consumption Conversion Factors for Screener

Frequency	REDCap Servings-per-Day Conversion Factor
Never	0
Once every 2 months (Bimonthly)	1/60
Monthly	1/30
Twice a month	1/14
Weekly	1/7
Twice a week	2/7
Every other day	1/2
Daily	1

For the calculation of EPA and DHA intake amounts, REDCap allows the creation of functions that total the EPA or DHA intake from the selected seafood questions per day by using the following logic:

1.) If no-intake is reported: $C_{total} = 0$

Where:

- C_{total} = Total intake of EPA or DHA from question

2.) else: $C_{total} = \Sigma (Concentration \times Frequency)$

Where:

- Σ = Summation of each seafood species
- Concentration = Amount of EPA or DHA in one serving size of the species
- Frequency = Conversion Factor to Normalize to per-day

This logic was used for all three fish questions, with slight alterations for calculating dietary EPA and DHA from liver, egg, and poultry intake. In the screener, preformed EPA and DHA intakes was kept as two separate functions, which allowed independent summation of each fatty acid.

5.2.5 Inclusion of Standardized Portion Size Images

Portion size information is necessary to estimate quantities of nutrient intakes from participants (Cade et al., 2002; Sharma & Chadha, 2017). The use of portion size images has been shown to improve estimation of amounts eaten (S. I. Kirkpatrick et al., 2016). Initially, portion size images were borrowed from the ASA24, but these images were of low resolution and lacked a reference object to provide scale. High-resolution images utilizing AI-based image creation tools were used, which included a 100-gram portion of the screener food item positioned on a plate beside

a standard deck of playing cards. Images for all portion sizes can be found in **Appendix C**, **Appendix D**, and **Appendix E**. Code to include portion size images in REDCap can be found in **Appendix F**.

5.2.6 Providing the Screener in the Official Languages of Canada

The screener was updated to allow the questions to be stated in the official languages of Canada (English and French). The initial language translation from English involved using DeepL (*DeepL Translator*, 2025) followed by verification from individuals fluent in French, and checks against websites of major supermarkets and fish markets in Quebec. The first round of review was done by Dr. Melanie Plourde from the University of Sherbrooke, which resulted in adjustments for frequency categories, and seafood translations and questions about fish species such as flounder, perch, catfish, bass, crayfish, and imitation crab. A second round of review was completed by two students at McGill University with a select number of seafood samples to verify the most known name of specific fish such as flounder (Plie vs Flet vs Limande) and imitation crab (Surimi vs Simili-Crabes vs Goberge). These names were then confirmed by checking Quebec-based supermarkets and fish markets.

5.3 Conclusion

The U.S. based DHA screener was tailored to capture the EPA and DHA intake in the Canadian population (Screenshots of the English version of revised Screener are available in **Appendix G**, but the screener is also available in French). The participant burden of the new screener was reduced as the open-ended questions in the screener were shifted to a closed, fixed-frequency interval format. By incorporating data from the CCHS 2015 Nutrition and CNF, we changed the types of seafood species and the EPA and DHA content of food items in the screener to reflect those consumed by Canadians. However, during the switch to CNF values, we discovered that omega-3 enriched eggs that we intended to add were not listed in the CNF and the U.S. Screener DHA content

of eggs did not match the CNF data. Further examination indicated that the nutrient composition of different size eggs in CNF was scaled for a single nutrient composition and may not reflect actual differences in the EPA and DHA content of different size eggs. It was also discovered that there were discrepancies between CNF values and the U.S. Screener for liver and chicken. Follow up research is necessary to resolve these differences, which is examined in Chapter 6.

Chapter 6

Project 2: Determining Fatty Acid Composition of Selected Food Items

6.1 Introduction

Reliable reference values for the food items in the screener are required to accurately estimate EPA and DHA intake in individuals. We observed inconsistencies between EPA and DHA content for the non-seafood animal items between the U.S. 7-question Screener, the CNF and the scientific literature (**Table 6.1**). The CNF EPA + DHA content of liver and chicken, was lower than the U.S. Screener and the scientific literature (Enser et al., 1998; Gladyshev et al., 2015; Wang et al., 2024). Egg EPA + DHA was higher than the U.S. Screener, but also lower than the literature (Wang et al., 2024). In addition, the U.S. Screener and CNF have no data on omega-3 enriched eggs, which are common in Canadian major grocery stores. The EPA+DHA values in the U.S. Screener were developed by Martek Biosciences Corp. in 2002, which became DSM Nutritional Products, and details about the determinations are limited as it was published as a correspondence referring to an abstract presented at a conference (Kuratko, 2013). The available CNF data values may also be outdated as the last available update was in 2015. Also, the scientific literature on the fatty acid composition of liver, eggs and chicken may not be representative of Canada as the publications that were available were either dated or from other countries, or potentially oversampled specialty products (Hennessy et al., 2021; Pikul et al., 1985; Wang et al., 2024).

Table 6.1. EPA + DHA of liver, egg, and chicken from the Martek Screener, CNF, and the Literature

Food	EPA+DHA Content		
	Martek Screener ¹	CNF	Literature ²
Liver (mg/100g)	10.5	0	234
Egg (mg/egg)	3.25	39	72
Chicken (mg/100g)	9.5	7	40

¹Martek Screener values from Kuratko, Nutrition. 2013, 29(5), 807–808. doi:

10.1016/j.nut.2012.10.013 ²Liver values were taken from Enser et al., Meat Sci. 1998, 49(3).321-327. doi:10.1016/S0309-1740(97)00143-5, and egg and chicken values were taken from Wang et al., J. Agric. Food Chem.2024, 72(27):15311-15320. doi: 10.1021/acs.jafc.4c03546. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; CNF, Canadian Nutrient File.

The fatty acid composition of liver (beef, pork and chicken), egg (medium, large, extra-large, omega-3, and omega-3 plus), and chicken meat (regular and organic) was examined by direct biochemical analyses in our laboratory. These products were all available in local supermarkets. For chicken meat, breasts, thighs, drumsticks, and wings in both regular and organic varieties were examined. Organic varieties were added due to a recent publication suggesting organic chicken has higher EPA and DHA content (Wang et al., 2024). Each type of cuts analysed made up at least 10% of all chicken consumption, with the combination of these four cuts covering more than 90% of total chicken consumption in the CCHS 2015 Nutrition when unspecified/mixed chicken meat was excluded. The code to determine the percent of chicken consumption from the CCHS 2015 Nutrition is listed in **Appendix H**.

Hypotheses:

- 1.) The EPA and DHA content of liver, egg, and chicken samples determined by our laboratory analyses will be higher than content in the CNF and show similar content to values in the literature.
- 2.) Omega-3 and omega-3 plus eggs will have higher content of EPA and DHA as compared with medium, large, and extra-large eggs.

6.2 Methods

6.2.1 Sample Collection and Preparation

Liver and eggs were purchased from major supermarkets in the Waterloo region based on what was consistently available. For liver, different species were offered at different stores catering to different ethnic populations, so the requirement for consistent offering was overlooked. Beef liver was purchased from Sobeys (Waterloo, ON) and pork and chicken liver were purchased from T&T Supermarket (Waterloo, ON). For eggs, the types of eggs commonly available were determined by examining the egg section of several local supermarkets. Those that were consistently available were purchased by the dozen (12) eggs and included medium, large, extra-large, omega-3, and omega-3 plus. Chicken cuts were purchased from the local Costco (Waterloo, ON).

Egg preparation for fatty acid analysis was completed as part of an undergraduate project led by Avah Olsen with assistance from Grady Smith under my in-lab supervision. As a liquid, eggs can be homogenized easily through blending. Individual eggs were homogenized using a heavy-duty blender (CB15BU, Waring, CT, USA) on the lowest setting for 30 seconds. Four technical replicates of 50 mg for each egg were then taken for fatty acid analysis. Therefore, each egg category was determined from 48 analyses of the 12 eggs.

Blending of solids can be problematic as discussed in Chapter 4. Therefore, for the liver and chicken samples, a bead milling protocol that was developed for seafood (unpublished) was used. For the liver types and chicken cuts, samples were chopped into small pieces and 50 mL of each liver type and chicken cut were transferred into stainless steel grinding jars with steel balls for bead milling. Prior to bead milling, the jars were fully submerged into liquid nitrogen for a minimum of two minutes to make samples brittle and then bead milled at 25 Hz for two minutes (TissueLyser II, Qiagen, Venlo, NL). The homogenates for each liver type and chicken cut were then combined.

6.2.2 Fatty Acid Composition Analysis of Selected Food Items

Lipids were extracted from the food homogenates using 2:1 (v:v) chloroform:methanol (Folch et al., 1957). Five technical replicates were completed on the chicken cut and liver type homogenates. The lipid extraction solvents contained ethyl nonadecanoic acid (C 19:0 ethyl ester, Nu-Check Prep Inc., Elysian, MN, USA) as the internal standard, and butylated hydroxytoluene as an antioxidant (Metherel et al., 2013). The samples were vortexed and held for 24 hours at 4°C. After 24 hours, the organic phase of a sample was isolated by adding sodium phosphate buffer (0.2 mol/L), mixing gently by inversion, followed by centrifugation at 1734 x g for 5 min. The lower organic phase containing the lipids was collected. The lipid extract was then dried under a stream of nitrogen, and fatty acid methyl esters were generated through a trans-esterification procedure by adding 14% boron trifluoride (BF₃) in methanol with hexane to the extract and heating at 95°C (Morrison & Smith, 1964).

Fatty acid methyl esters were isolated in heptane and then analyzed using a Scion 8300 gas chromatograph equipped with a DB-FFAP 15 m x 0.1 mm ID x 0.1 µm film thickness, nitroterephthalic acid-modified, polyethylene glycol capillary column (J&W Scientific from Agilent Technologies, Mississauga, ON, Canada) with hydrogen as the carrier gas (Masood et al., 2005). Samples were introduced into the 250°C injector at a 50:1 split ratio using a Scion 8400Pro autosampler. The initial oven temperature was 150°C with a 0.25 min hold, followed by a 35°C per minute ramp to 200°C, a 1°C per minute ramp until 211°C, and finally a rapid 80°C per minute ramp until 245°C for a 4-minute hold. The flame ionization detector was set to 300°C with flow rates at 300 ml/min for air and 30 ml/min for nitrogen makeup gases, with a sampling frequency of 50 Hz. Individual fatty acid methyl esters were identified by matching retention times to an external reference standard (GLC-462, Nu-Check Prep Inc., Elysian, MN, USA) which contains a mixture of 28 known fatty acid methyl esters and quantified based on the response of the internal standard.

6.2.3 Fatty Acid Handling and Statistical Analysis

All data processing and statistical tests were conducted using RStudio (Version 2025.05.0+496, 2025). Individual fatty acids were expressed both as a percentage of total fatty acids and as concentrations in their source sample (ug/mg). Values were expressed as mean $\pm$ standard deviation. The differences between each egg types were examined using one-way Analysis of Variance (ANOVA) with post-hoc testing by Tukey's Honestly Significant Difference (HSD) when appropriate. The differences between regular and organic chicken (breast, thighs, drumsticks, and wings) were determined using a two-way ANOVA followed by Tukey's HSD post-hoc test when appropriate. Statistical significance was inferred when $p < 0.05$.

6.2.4 Determining a Single Value of EPA and DHA for Selected Food Items

To derive EPA and DHA values that represented the intake of “liver” and the intake of “chicken” for the single screener question 4 for “liver” and the single screener question 6 for “chicken”, it was necessary to combine the liver type fatty acid data and then the chicken cut fatty acid data. This was completed by calculating a weighted average based on consumption from the CCHS 2015 Nutrition data using the following equation:

$$FA_{Weighted} = \Sigma (FA \text{ Concentration} \times Intake \text{ Percent})$$

Where:

- $FA_{Weighted}$ = The weighted concentration of EPA or DHA from chicken or liver
- Σ = Summation of weighted EPA or DHA from chicken cuts or liver types
- FA Concentration = Amount of EPA or DHA in portion of chicken cuts or liver types
- Intake Percent = Proportion of total chicken or liver intake from a certain cut or type

The intake percentages for chicken cuts and liver types were extracted from the CCHS 2015 Nutrition FID and FDC files. The code to perform this calculation is included in Appendix H and **Appendix I.**

Steps to extract and calculate percent consumption included:

- 1.) The CCHS 2015 Nutrition data was filtered by food group code from the FID (individual food items, amount (grams), food codes) and FDC (food codes, food descriptions) files using CNF food group codes (e.g. 27A – Chicken, Meat Only; 27B – Chicken, Meat + Skin, etc.) and keywords from the food descriptions (e.g. the keyword “chicken” was added as a filter across the food groups) for day 1 recalls.
- 2.) The extracted data was then grouped into different cuts using keywords from the food descriptions (e.g. “Thigh” or “Breast” or etc. was searched across the extracted data)
- 3.) When multiple items were identified for the same keyword, the gram intakes were summed up to generate a total intake for that cut/type. The percent intake for each cut/type was determined by calculating its contribution to overall consumption.

Because chicken breasts, thighs, wings, and drumsticks make up the majority of specified chicken intake in the CCHS 2015 Nutrition, we assumed that the four chicken cuts represent 100% of the chicken intake. In the CCHS 2015 Nutrition, chicken breasts contribute 68.5% to total chicken intake, chicken thighs contribute 11%, chicken drumsticks contribute 10.5% and chicken wings contribute 10%. For liver samples, beef liver intake contributed 82.6% of total liver intake, with 14% being chicken liver, while pork liver intake was only 2% (*CCHS*, 2015).

6.3 Results

6.3.1 Laboratory Determinations of EPA+DHA in Selected Food Items

Chicken liver has the highest EPA+DHA content with 113.5 ± 3.77 mg/100g as compared with pork liver with 25.2 ± 1.7 mg/100g and then finally beef liver with 14.55 ± 0.23 mg/100g (**Figure 6.1**). In the CNF, beef, pork and chicken liver are all listed as having 0 mg of EPA + DHA.

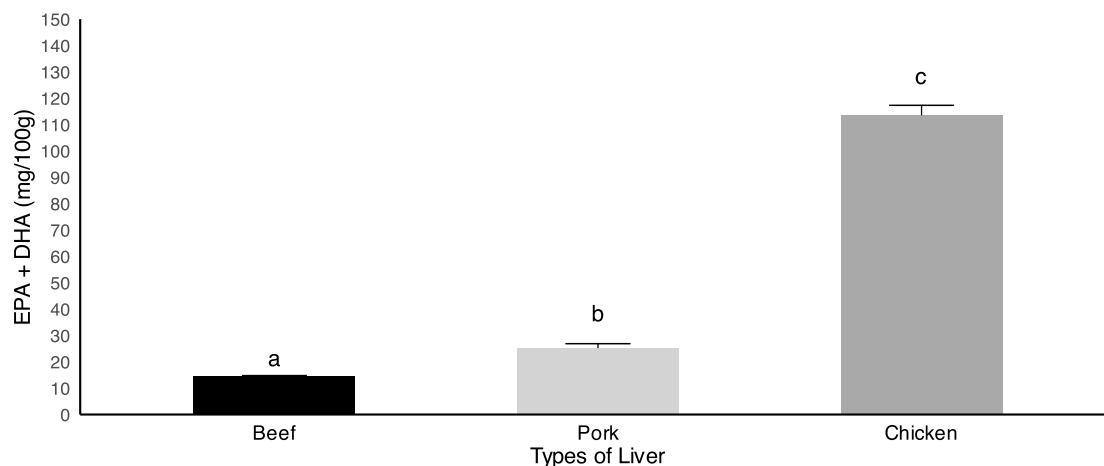


Figure 6.1. EPA+DHA concentrations in beef, pork, and chicken liver from laboratory fatty acid analysis. Values were expressed in mean $\pm$ SD. Significant differences were indicated when $p < 0.05$ using a one-way ANOVA followed with Tukey's post-hoc testing. Different letters are significantly different. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

For eggs, the omega-3 plus eggs have the highest EPA+DHA content with 74.4 ± 11.7 mg/egg, which is almost double compared with the other egg types, and almost triple compared with medium eggs (11.5 ± 2.8 mg/egg). Surprisingly, the other “regular” omega-3 eggs contained 42.9 ± 6.1 mg/egg, which was statistically higher than regular eggs but not dramatically so. The large and extra-large eggs did not differ from each other with EPA+DHA content around 33 mg/egg. Other than the omega-3 enriched eggs that are not available in CNF, these directly determined EPA+DHA

content of eggs were within the same range for the large and extra-large eggs but the medium eggs had much less EPA+DHA than what was reported in CNF (**Figure 6.2**).

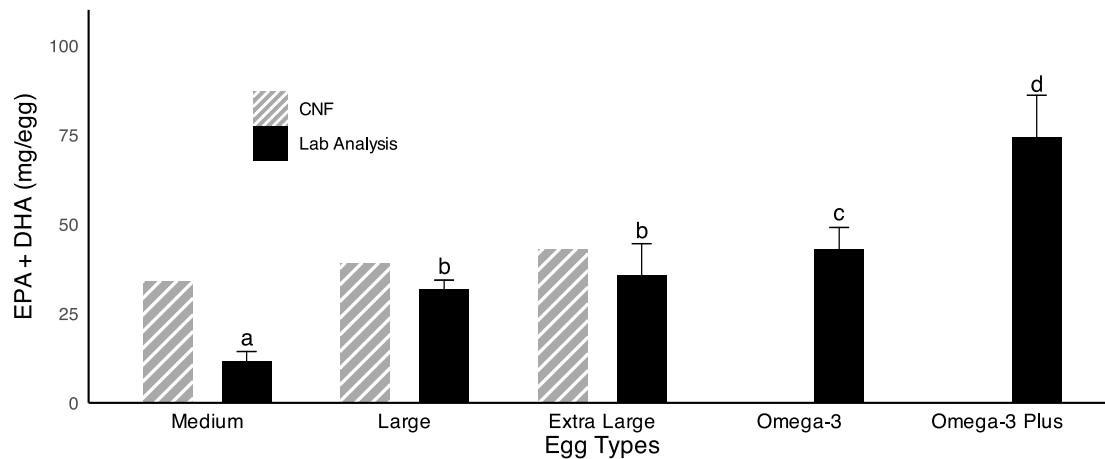


Figure 6.2. EPA+DHA concentrations in medium, large, extra-large, omega-3 and omega-3 plus eggs from laboratory fatty acid analysis and the CNF.

Values were expressed in mean $\pm$ SD for Significant differences were indicated when $p < 0.05$ using a one-way ANOVA followed with post-hoc testing. CNF did not have values for Omega-3 and Omega-3 Plus eggs. Different letters are significantly different. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; CNF, Canadian Nutrient File.

Analysis of 40 chicken samples (20 organic and 20 regular) showed that only organic chicken drumsticks and wings were statistically different compared with their regular counterparts, however, overall, the organic cuts still had about twice as much EPA+DHA compared with the regular chicken at 28.5 mg/200g and 14.8 mg/200g respectively (**Figure 6.3**). The CNF values for chicken breast, thigh, and wings fell within the regular chicken standard deviations with the CNF drumsticks being only 5 mg/200g more than the regular counterpart indicating that the CNF is representative of conventionally raised chicken in major Canadian supermarkets (**Figure 6.3**).

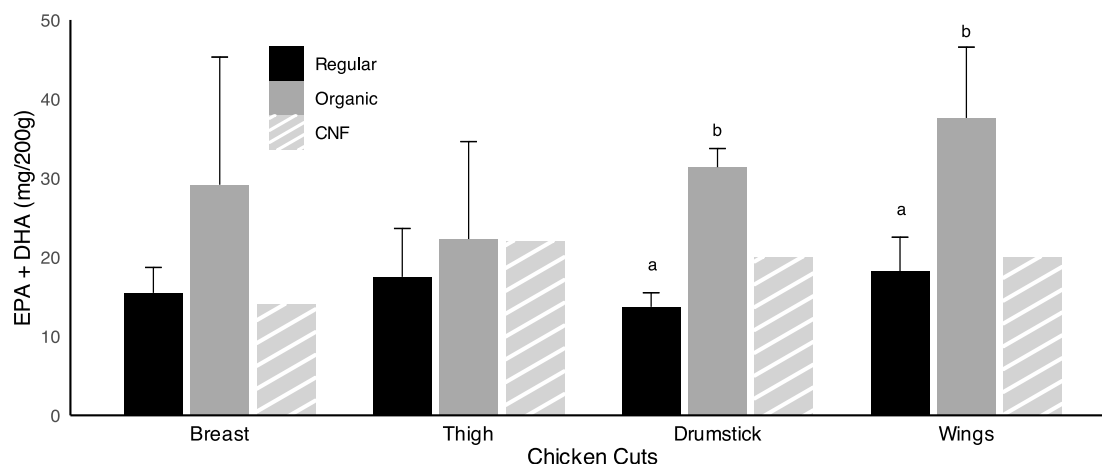


Figure 6.3. Laboratory fatty acid analysis of EPA+DHA concentrations in regular and organic chicken cuts and EPA+DHA values from the CNF. Values are expressed as mean $\pm$ SD. Significant differences were indicated when $p < 0.05$ using a two-way ANOVA followed with Turkey's post-hoc testing. Different letters are significantly different. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; CNF, Canadian Nutrient File.

6.3.2 EPA & DHA Concentrations Determined for the Screener

To determine a single value for EPA and DHA content for question 4 (liver intake) in the Canadian screener, a weighted average concentration was calculated using the equation described in the methods section. The final calculated liver values added to the screener are 10.9 mg/100g for EPA and 20.9 mg/100g for DHA (**Table 6.2**). The CNF did not have values of EPA or DHA for the liver types.

Table 6.2. Weighted Liver EPA and DHA Concentrations

Liver Type	Consumption Percent	EPA (mg/100g)	DHA (mg/100g)
Beef	82.6%	7.3 $\pm$ 0.2	7.2 $\pm$ 0.1
Chicken	14.4%	27.8 $\pm$ 0.6	85.7 $\pm$ 2.5
Pork	2.0%	6.7 $\pm$ 0.4	18.4 $\pm$ 1.8
Total	100%	10.9	20.9

Mean $\pm$ SD. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

EPA and DHA concentrations for question 5 (egg intake) in the Canadian screener were changed to ask about the intake of each distinct egg type (medium, large, extra-large, omega-3, and omega-3 plus). This assessment was based on reports in the literature suggested that the dated CNF data for eggs was not accurate (Wang paper, Avah egg size) and by our direct biochemical analyses.

The final values for the Canadian screener are 0.4 mg of EPA and 11.2 mg of DHA per medium egg, 1.0 mg of EPA and 30.7 mg of DHA per large egg, 1.1 mg of EPA and 34.7 mg of DHA per extra-large egg, 4.9 mg of EPA and 38.0 mg of DHA per omega-3 egg, and 11.0 mg of EPA and 63.4 mg of DHA per omega-3 plus egg (**Table 6.3**).

Table 6.3. EPA and DHA Concentrations for Common Egg Types in Canada

Chicken Egg Type	CNF (mg/egg)		Stark Lab Analysis (mg/egg)	
	EPA	DHA	EPA	DHA
Medium	1	33	0.4 ± 0.2	11.2 ± 2.8
Large	1	36	1.0 ± 0.3	30.7 ± 2.6
Extra-Large	1	40	1.1 ± 0.4	34.7 ± 8.6
Omega-3	n/a	n/a	4.9 ± 0.8	38.0 ± 5.9
Omega-3 Plus	n/a	n/a	11.0 ± 1.3	63.4 ± 11.0

Mean ± SD. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; CNF, Canadian Nutrient File.

Our direct biochemical analyses included regular and organic chicken, indicating that organic chicken had higher values of EPA and DHA, while our direct biochemical analyses agreed with existing CNF values. Chicken breasts, thighs, wings, and drumsticks make up the majority of specified chicken intake in the CCHS 2015 Nutrition, therefore, the EPA and DHA amounts for question 6 (chicken intake) are a single value derived from the weighted intake of the chicken cuts from our analyses. The percentages were multiplied by the EPA and DHA content from fatty acid analysis to derive values of 6.5 mg of EPA per 200g of regular chicken and 7.0 mg of DHA per 200g of regular chicken and for organic chicken, the values are 8.3 mg/200g for EPA and 20.4/200g mg for DHA (**Table 6.4**).

Table 6.4. Weighted Chicken EPA and DHA Concentrations

Chicken Cut	Consumption Frequency	Regular (mg/200g)		Organic (mg/200g)	
		EPA	DHA	EPA	DHA
Breast	68.5%	5.6 ± 0.2	7 ± 0.2	9.1 ± 5.1	20 ± 11.3
Thigh	11%	8 ± 2.1	8 ± 1.5	4.0 ± 1.5	20.2 ± 3.3
Drumstick	10.5%	8 ± 1.0	4 ± 1.2	4.1 ± 2.9	18.8 ± 8.5
Wings	10%	9.0 ± 2.0	9.3 ± 1.5	12.1 ± 3.5	25.5 ± 11.5
Total	100%	6.5	7.0	8.3	20.4

Mean ± SD. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

6.4 Discussion

EPA and DHA content values for non-seafood animal sources (livers, eggs, and chickens) were determined for the Canadian screener by direct biochemical analyses. Hypothesis 1, which stated that the EPA and DHA content of liver, egg, and chicken samples would be higher than both Screener and the CNF, was partially accepted as liver, and organic chicken had higher values. For eggs, medium eggs were considerably lower in EPA+DHA as compared with the CNF. Hypothesis 2 was accepted as the omega-3 and omega-3 plus eggs did have higher EPA+DHA concentrations compared with regular eggs, and they were therefore added as additional options to the egg choices in the Canadian screener.

Chicken liver contained greater EPA and DHA content compared with beef liver and pork liver. The EPA+DHA content of chicken liver was likely higher due to two reasons. Chickens are always in the process of developing yolks, which increases the conversion of essential fatty acids into EPA and DHA (Cherian & Sim, 1991) and microorganisms in the rumen of beef cattle can limit LCPUFA absorption and subsequent accumulation in tissues as they remove carbon-carbon double bonds and/or change their location (Bionaz et al., 2020).

Large and extra-large egg EPA+DHA values from laboratory analysis are comparable with EPA+DHA values from the CNF. Laboratory analyzed EPA+DHA content for medium eggs was

lower compared with their CNF counterparts, most likely due to the CNF standardizing the nutrient values across egg types by weight (Medium eggs = 46.4g, Large = 52.7g, and Extra-large = 58.1g), which does not account for biological variations that could affect the egg size and content, such as diet (Heo et al., 2023; Kowalska et al., 2021). Medium eggs are often produced by developing young hens, resulting in eggs with different nutrient quality as compared with established laying hens that tend to generate large and extra-large eggs (Réhault-Godbert et al., 2019). Omega-3 and omega-3 plus eggs contained statistically higher EPA+DHA values compared with regular eggs, however the omega-3 eggs are advertised to contain 75 mg of DHA per egg (Burnbrae Farms, 2025) and our fatty acid analysis indicated only 40 mg of DHA per egg. The same pattern was found in omega-3 plus eggs where they were advertised to contain 125 mg of DHA (Burnbrae Farms, 2025), but were shown to only contain 65 mg of DHA. Fish meal feeding can increase the content of DHA in eggs above what is obtainable by feeding ground flaxseed (Elkin & Harvatine, 2023; Gonzalez-Esquerria & Leeson, 2000; González-Esquerria & Leeson, 2001). The cost of fish meal, however, can vary depending on fishing quotas, so it is possible that it may be omitted from feeding when it becomes cost prohibitive. As such regular monitoring of omega-3 enriched eggs may be required to determine the range of production variation throughout and across years.

Chicken analysis revealed that EPA and DHA content for regular chicken was similar to the CNF data. The CNF data is originally derived from USDA data from 2013, suggesting that any changes to general animal husbandry practices for conventional chicken in North America have not affected EPA+DHA content in the last decade. This contrasts with a recent publication from the U.S. suggesting that the EPA+DHA content of chicken has increased due to changes in the advancement of animal nutrition and feeding being incorporated into the production industry (Wang et al., 2024). In this publication, equal amounts of regular and organic chicken were combined and only the EPA

and DHA values for this combined chicken were reported. Our analyses indicate that organic chicken has approximately twice as much EPA and DHA, but organic chicken consumption is much lower than regular chicken (Van Loo et al., 2010). As such, we have added an organic chicken option to the Canadian screener.

6.5 Limitations

This project had several limitations. Liver, chicken, and egg products analyzed were only purchased from the Waterloo region and therefore may not represent EPA and DHA values across Canada. Also, true biological variation may not be reflected as the number of biological replicates used was limited. For the assessment of liver, only one sample per species was used, for chicken only three per species, and egg at 12 per egg type. Furthermore, we collected food items within a relatively limited time frame, which may not account for EPA and DHA variations in food items throughout the year. To confirm the precision and accuracy of the nutritional content of these food items, biological replicates should be collected from a wider region at different time points, but this would require additional funding. Another limitation is the EPA and DHA values from the chicken samples were only derived from four popular chicken cuts (breast, wing, thigh, and drumsticks) as the determination of the fatty acid composition all available chicken products was not possible. However, given that the EPA+DHA content of chicken samples was not statistically different within chicken types, the inclusion of all chicken cuts may not have a large impact upon the final EPA and DHA value in the Canadian. Finally, changes in the EPA and DHA content due to food preparation and cooking were not assessed as all the food products were analyzed in the raw form..

6.6 Conclusion

This project examined the EPA and DHA values of various non-seafood items (livers, eggs, and chicken). From these direct analyses, new values for EPA and DHA were entered into the

Canadian screener for question 4 (liver), question 5 (egg), and question 6 (chicken). Our first hypothesis was partially supported as regular eggs and chicken had EPA and DHA values were similar with the CNF. The biochemical analysis however revealed that liver contained considerable amounts of EPA+DHA (15 mg – 120 mg per 100 g of tissue) which was lower than some reports in the scientific literature (150 mg – 500 mg per 100 g of tissue) (Enser et al., 1998, 2000; Shin et al., 2012), but much higher than EPA+DHA content for liver in the CNF database. Organic chicken was found to be twice as high in EPA+DHA as regular chicken and we accepted the second hypothesis EPA and DHA value for omega-3 and omeg-3 plus eggs were higher than regular egg types. These EPA and DHA values were comparable to those in the scientific literature values for omega-3 eggs (Gonzalez-Esquerria & Leeson, 2000) and organic chicken (Wang et al., 2024). Based on these findings, the CNF needs some updates for the fatty acid composition of some but not all foods. Also, adding additional items should be considered for food products that have emerged and persisted in the Canadian food supply such as omega-3 enriched eggs and organic chicken that differ in nutrient content from counterparts that are generated through regular production processes. Through discussions with Health Canada representatives, a new CNF update has been prepared. Once the update is launched, it will be interesting to check the EPA and DHA content of the food items we have listed in the screener.

Chapter 7

Project 3: Preliminary Testing of the Canadian Screener

7.1 Introduction

The objective of Project 3 was to complete preliminary testing of the Canadian 7-question EPA and DHA screener to determine if the online screener/software can return expected values to determine if it is ready for a validation study. To do this, we used anonymized intake data from the CCHS 2015 Nutrition dataset. The CCHS 2015 Nutrition data was collected using a 24-hour Dietary Recall component on two separate days, therefore an extrapolation of this daily intake data was made to match the duration of the screener questions. The intention was not to use the comparison as a validation as the CCHS 2015 is focused on population intake assessment, while the 7-question screener is designed to assess individual intakes. It was intended to quickly generate mock data that reflects ranges in the Canadian intakes of EPA and DHA to determine if and why there the screener and CCHS 2015 Nutrition values do not align before a validation study is attempted in the future. Bivariate statistics and concordance testing which are typically used for validation studies were used to compare the actual CCHS 2015 EPA+DHA intake estimates to those generated by the screener after entering the mock data to help formally assess the potential of the screener, but also as practice with these statistical approaches prior to a future validation study.

Stratified random sampling of participants from the CCHS 2015 Nutrition data was completed and extrapolated for entry into the screener by using the those with two 24-hour food item intake information. Participants were stratified by ranking participants by CCHS 2015 EPA+DHA intake estimates to generate of very low, low, medium, high, and very high intakes according to dietary recommendation in the literature. Five participants were randomly selected from each group then food items related to the Canadian screener were identified and intake quantities for days 1 and 2

were combined and then extrapolated to represent 100g servings over a 2-month period. This mock serving frequency was then entered into the Screener and provided additional estimates of EPA and DHA. Preliminary testing of the data included an examination of basic descriptive statistics, and simple correlations between the CCHS 2015 Nutrition and the 7-question screener intake estimates. Concordance testing using Bland-Altman plots and categorical agreements. Notably, Project 3 is a preliminary test and not a validation, as the assumption that a 2-day recall can be representative of a long-term intake is not typically valid as it can miss day-to-day variations in intake. This preliminary test is being used to identify potential biases or areas for improvement before committing to a full validation study.

Hypotheses:

- 1.) The mean estimated daily EPA + DHA intake estimated by the Screener will not be significantly different from the CCHS 2015 Nutrition estimated intake for the medium, high, and very high intake categories. However, the screener will generate higher estimates of EPA+DHA intakes than those derived from the CCHS 2015 Nutrition particularly when EPA + DHA intakes are in the lower range (individuals that do not eat seafood), as the screener is using higher values of EPA and DHA than the CCHS 2015 Nutrition for liver.
- 2.) The Canadian screener will show acceptable to strong linear agreement when compared with estimated EPA+DHA values from the CCHS 2015 Nutrition
- 3.) Concordance testing and categorical agreements will show a high degree of agreement between the Screener and the CCHS 2015 Nutrition estimated EPA+DHA intakes

7.2 Methods

A stratified random sampling approach was used to select 25 participants from the CCHS 2015 Nutrition, which resulted in 5 participants from five EPA+DHA intake categories. The

EPA+DHA categories were determined based on current dietary recommendations (Flock et al., 2013; Kris-Etherton et al., 2003). The data used for preliminary testing required EPA and DHA intake estimates, food intake patterns, and food descriptions and their coding. This data was extracted from four of the CCHS 2015 Nutrition files as described in Chapter 3 previously (Table 3.1).

7.2.1 Selecting Participants from the Canadian Community Health Survey – Nutrition

Using the Household/socio-demographic data (HS) file (N = 20,487), participants were excluded if they were pregnant or lactating, outside the age range of 18-65, and had a caloric intake outside the range of 400-8000 kcal. Participants who had two 24-hour recalls were selected as two recalls accounts for some day-to-day variation. From the remaining participants, those with an extreme value of EPA, DHA, or weight of over 9999 g for a single food item were excluded. The final sample set for the preliminary testing of the screener included 1130 participants (**Figure 7.1**).

The code of the exclusion criteria can be found in **Appendix J**.

Participants were categorized into five EPA+DHA groups using their averaged 2-day recalls based on daily recommendations for EPA+DHA intake (Flock et al., 2013; Koletzko et al., 2014; Kris-Etherton et al., 2003): very low (0-100 mg), low (101-250 mg), medium (251-500 mg), high (501-1000 mg), and very high (>1000 mg). To ensure that the testing of the screener was done across a wide range of possible intakes and not all from one group, five individuals were randomly selected from each group using a random number generator (Random.org, 2025), for a total of 25 participants. Because participants were not ordered consecutively within each group (e.g. 1,2,3,4, etc.), but rather ordered based on participant IDs (120, 144, 250, etc...), we were unable to use a random number generator to match to the participant ID's themselves. Rather, we ranked participants from lowest to highest participant ID within each group and assigned them a rank starting at 1. This allowed us to use the random number generator between 1 and the total number of participants in that group,

ensuring each participant had a fair chance at being selected. The code used for the ranking of individuals from lowest to highest EPA+DHA intake then separation into intake groups can be found in **Appendix K** and **Appendix L**. Random numbers generated along with their respective participant ID's can be found in **Appendix M**.

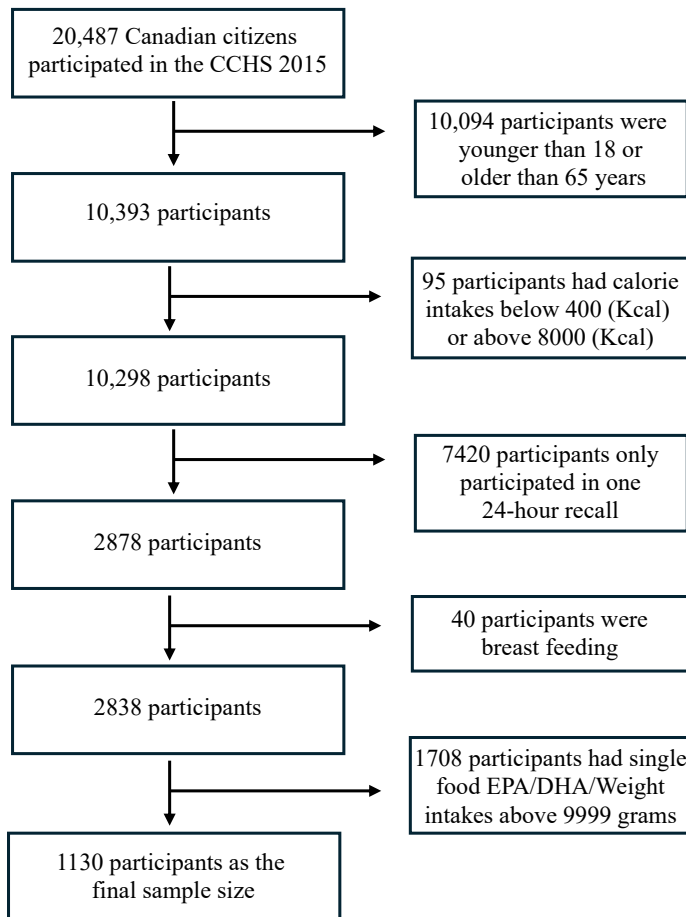


Figure 7.1. Flow chart for selection of participants in the CCHS 2015 Nutrition for comparison with the Canadian 7-Question EPA and DHA Screener

7.2.2 Data Extraction

To answer the Canadian Screener questions for each participant, the intake of foods that are in the Canadian 7-question EPA+DHA screener was extracted from the CCHS 2015 Nutrition data files. This required matching and combining the data of individuals using unique participant IDs from the CCHS 2015 Nutrition FID and FDC files. The steps to do this were:

- 1.) The CCHS 2015 Nutrition data was filtered by food group code from the FID (individual food items, amount in grams, food codes) and FDC (food codes, food descriptions) files using the CNF food group codes (e.g. 34A - Fish, Less than 6% total fat; 34B – Fish, Superior or equal to 6% total fat; 35A – Shellfish) for each individual.
- 2.) The food group code data was separated into individual screener items by using keywords from the food descriptions (e.g. “salmon” was searched across the seafood food group codes).
- 3.) Gram amounts of each item in the screener were summed using the keyword for a food item (e.g. “salmon”). When multiple items were identified for the same keyword, the gram intakes were combined to generate a total intake.
- 4.) The total intake (grams) for food items identified by each keyword over 2 days was extrapolated to in the intake over 2 months (60 days) and then divided by 100g to represent habitual intake of bimonthly servings of that food item. The calculated serving frequency was mapped to the nearest categorical option in the Canadian screener (i.e. “I eat a 100g serving of salmon once a week).
- 5.) This process was repeated for each food group code (Liver, Eggs, and Chicken)

The code used for extracting intake specific to the screener questions can be found in **Appendix N** (Seafood), **Appendix O** (Liver), **Appendix P** (Egg), and **Appendix Q** (Chicken). An

example of the result from data extraction process of an individual from the CCHS 2015 Nutrition (Participant ID 13813) and how it would be entered in the new screener is shown in **Appendix R**.

7.2.3 Statistical Tests

All data processing and statistical tests were conducted using RStudio (Version 2025.05.0+496, 2025). Statistical significance was inferred when $p < 0.05$. T-tests were used to determine if EPA+DHA estimates from the Canadian Screener are different from the CCHS 2015 Nutrition values within each group. The relationship between estimated intake values from Canadian Screener and those in the CCHS 2015 Nutrition was examined using Pearson correlation. Concordance was examined visually by plotting EPA + DHA intake estimates generated by the Canadian Screener and those estimated by the CCHS 2015 Nutrition dietary assessment using a Bland-Altman plot. Categorical concordance by Cohen's weighted kappa was also used to examine if the intake estimates of the participants were binned into EPA+DHA intake categories similarly.

7.3 Results

7.3.1 Participant Selection and Comparison of Estimated EPA+DHA

A total of 1130 participants that completed two 24-hour dietary recalls were selected from the CCHS 2015 Nutrition were categorized into five EPA+DHA groups (**Figure 7.2**). About 90% of the 1130 participants fell within the very low and low groups, therefore, to test the screener in a wide range of intakes, five random participants from each group (Appendix M) were selected, generating 25 participants and 50 EPA+DHA estimates (CCHS 2015 Nutrition and Screener). Using a two-way ANOVA with a Tukey's post-hoc test showed differences between the groups, however there were no statistical differences within the groups, showing that the Canadian Screener and the CCHS 2015 Nutrition estimated EPA+DHA were not different (**Figure 7.3**).

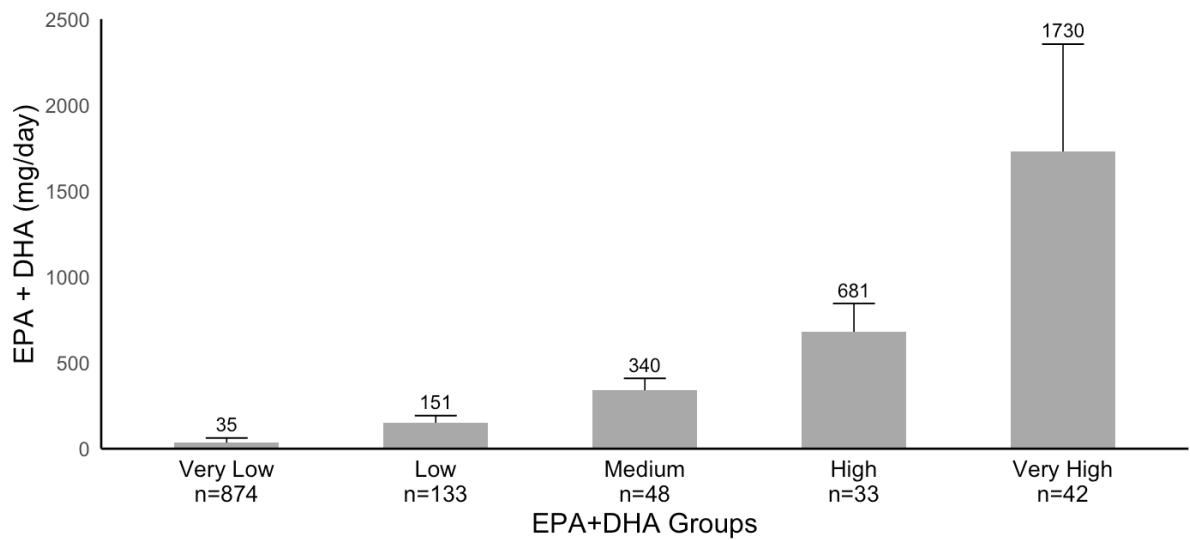


Figure 7.2. Average intakes and number of individuals consuming EPA+DHA according to intake recommendation rankings. Bars and error bars represent the mean $\pm$ SD for daily EPA+DHA estimate groups in selected participants from the CCHS 2015 Nutrition (total n = 1130). Very low = 0-100 mg/d, low = 101-250 mg/d, medium = 251-500 mg/d, high = 501-1000 mg/d, and very high >1000 mg/d. CCHS, Canadian Community Health Survey. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

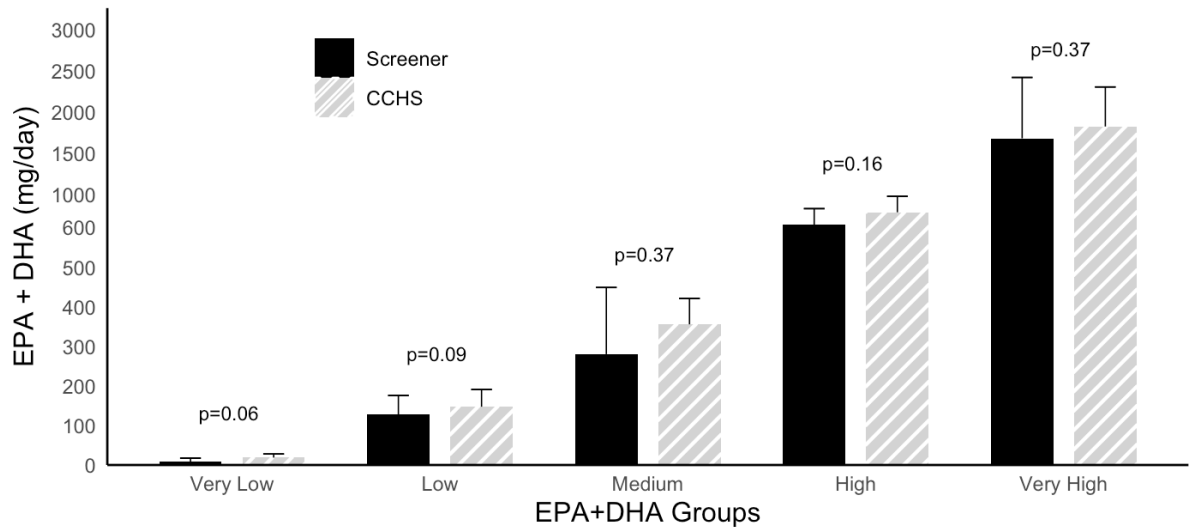


Figure 7.3. EPA+DHA intake estimates from the screener as compared with estimates from CCHS 2015 24-hour recalls according to intake recommendation rankings. Bars and error bars represent the mean $\pm$ SD. P-values were determined by t-tests within each ranking group. CCHS, Canadian Community Health Survey. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

7.3.2 Linear Association

The linear relationship of estimated EPA+DHA between the Canadian screener and the CCHS 2015 Nutrition was also assessed using a Pearson correlation, which showed a significant ($p < 0.0001$) and positive relationship ($R = 0.90$). The relationship was modelled by the equation $y = 0.89x - 16$, which is relatively close to the perfect $y = x$ line showing a strong relationship. The data points at the lower estimated (below 1000 mg/day) showed decreased variability compared with higher estimates as they were visually closer to the line of best fit (**Figure 7.4**).

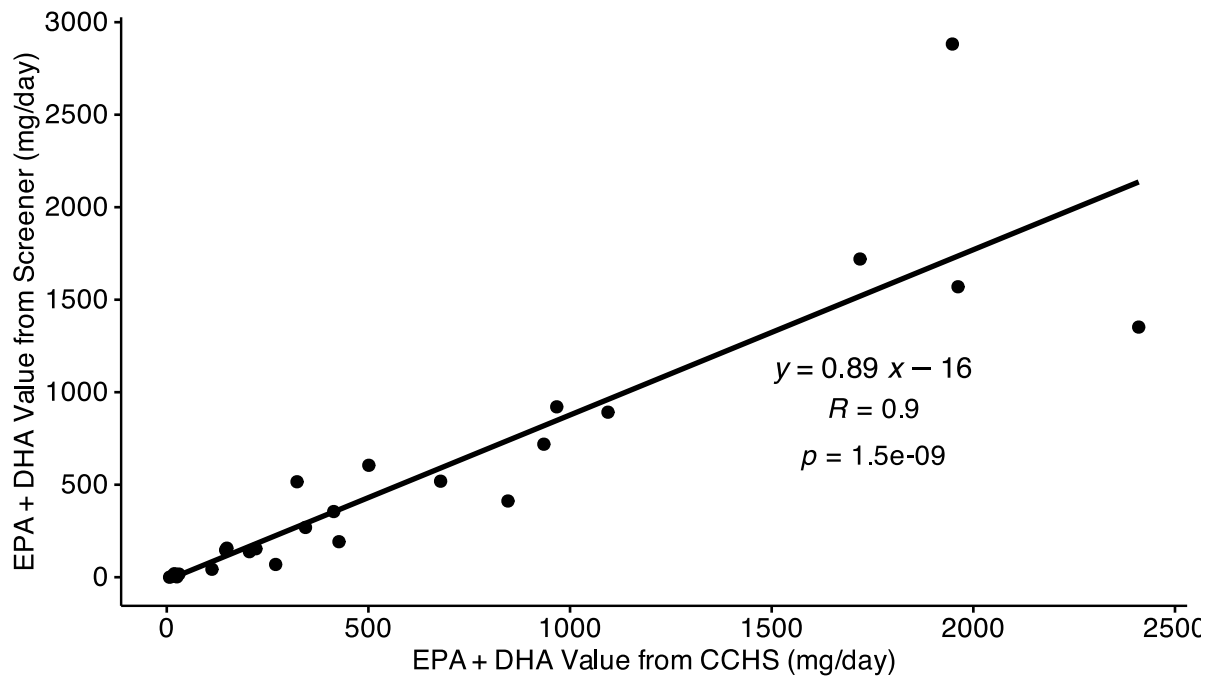


Figure 7.4. Scatterplot of estimated EPA+DHA intake between the Canadian Screener and the CCHS 2015 Dietary Recalls. Equation of the best fit line, Pearson correlation and p-values were determined, $n = 25$

7.3.3 Bias Assessment

Agreement between the Canadian Screener and the CCHS 2015 Nutrition was examined using the Bland-Altman plot (**Figure 7.5**) to assess for bias. A one-sample t-test on the differences between Screener and CCHS 2015 Nutrition estimated EPA+DHA intakes showed that there was no statistically significant bias ($p = 0.20$), indicating that the Canadian Screener and the CCHS 2015 Nutrition values were not different. The 95% upper and lower limits of agreement (LOA) were determined to be 541 mg/day and -708 mg/day, respectively. Visual assessment of the plot indicated that as the estimated intake values increased, the difference between the two values increased as well, shown by the two values outside the LOA (Figure 7.5).

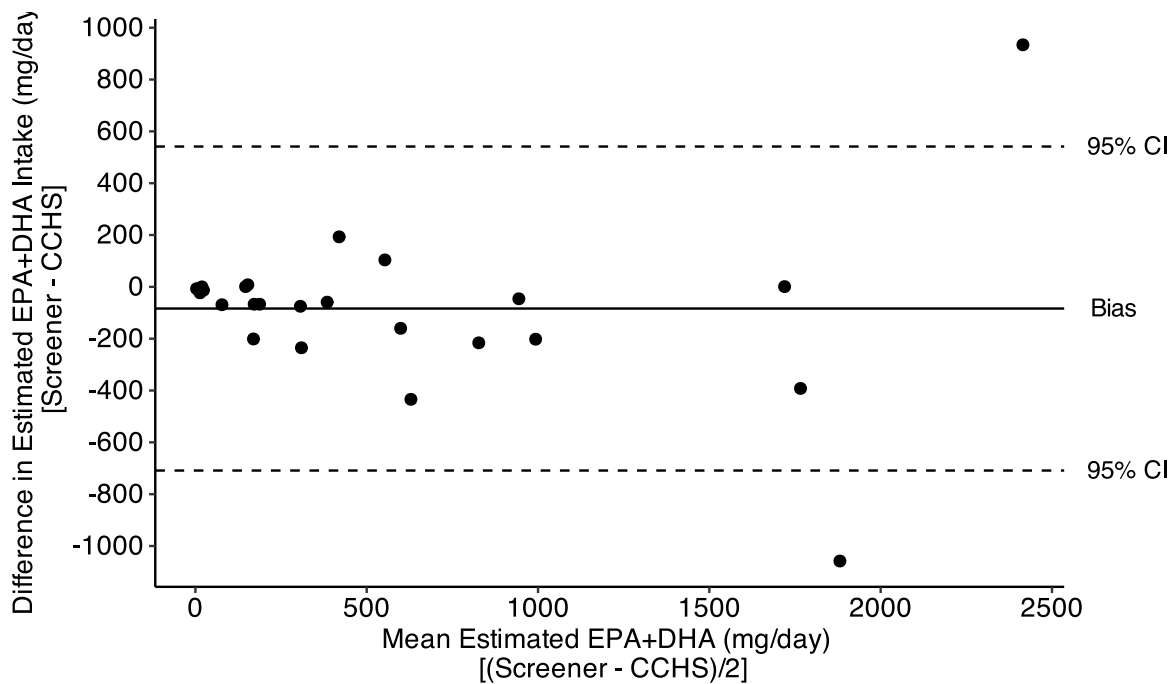


Figure 7.5 - Concordance testing of Canadian Screener and CCHS 2015 Dietary Recalls estimated EPA+DHA intake using a Bland-Altman plot. One sample t-test completed on difference. $p = 0.20$, $n = 25$

7.3.4 Categorical Agreement

Categorical agreement using Cohen's weighted kappa between the Canadian Screener and the CCHS 2015 Nutrition intake demonstrated almost perfect categorical agreement ($K = 0.91$, $p < 0.001$). The very low intake group exhibited perfect agreement while the low, high, and very high groups had 80% of their participants in the same categories. The medium group exhibited the lowest agreement with only 40% of its values being in the same group, however, 80% of its values were still at most one adjacent category away (**Table 7.1**).

Table 7.1 Categorical agreement between assignment into EPA+DHA intake groups between the Canadian Screener and the CCHS 2015 Nutrition

Group	Actual, n	Agreement, n (%)			
		Same	Adjacent Below	Adjacent Above	Other
Very Low	5	5 (100%)	-	-	-
Low	5	4 (80%)	1 (20%)	-	-
Medium	5	2 (40%)	1 (20%)	1 (20%)	1 (20%)
High	5	4 (80%)	1 (20%)	-	-
Very High	5	4 (80%)	1 (20%)	-	-
Total	25	19 (76%%)	4 (16%%)	1 (4%)	1 (4%)

^aCohen's weighted kappa for concordance between measurements, calculated using the quadratic (Fleiss-Cohen) weighting due to ordered categories, $K = 0.91$, $p < 0.001$

7.4 Discussion

The results indicate that the Canadian Screener is ready for a validation study as the Canadian screener-estimated EPA+DHA intakes were not statistically different compared with the mock data from across all the five groups of the CCHS 2015 Nutrition. This was generally expected as both the EPA and DHA values for the Canadian Screener and CCHS 2015 Nutrition are derived from the CNF.

Hypothesis 1, which projected that the screener would have higher estimates of EPA+DHA at lower levels of intake was rejected. This was based on the intake recommendation categories being statistical similar across all levels for the Screener and the CCHS 2015 estimates. Pearson correlations were also strong across all intakes and residuals at intakes below 250 mg/d were negligible. Both Bland-Altman and Cohen's Kappa concordance testing suggested very high agreement between the estimates of the two dietary assessment tools. Hypothesis 1 was based on the Canadian screener having higher amounts of EPA and DHA in liver (that we added) as compared with the CCHS 2015 dietary recalls which relied on the CNF underestimates of EPA and DHA in liver. Upon closer examination, this effect was not observed due to the 25 randomly selected participants having no liver intake. An *ad hoc* examination manually selecting for participants that consumed liver did result in

the Canadian screener generating higher EPA and DHA intake estimates as compared with the CCHS 2015 Nutrition estimates from the same participants (data not shown).

Hypothesis 2 was accepted as a strong linear agreement by Pearson's correlation ($R = 0.09$, $p < 0.0001$) was confirmed between the dietary assessment tools although higher correlation residuals were observable in the very high intake ranges ($>1500\text{mg/d}$). Hypothesis 3 was also accepted as concordance testing and categorical agreements showed a high degree of agreement between the Screener and the CCHS 2015 Nutrition estimated EPA+DHA intakes. The Bland-Altman plot demonstrated no significant bias between the Screener and the CCHS 2015 – Nutrition estimated EPA and DHA intakes. In addition, categorical concordance was a near perfect agreement with the values generated from the CCHS 2015 Nutrition for each individual with 96% of the participants being categorized within 1 group adjacent to their expected group. There was, however, slightly lower agreement in the medium intake groups that was not expected. The use of a single raw seafood EPA and DHA value for a species in the Canadian Screener may be the source of this discretion. The CCHS 2015 Nutrition data had food reported in various cooked states such as baked, broiled, steamed, and canned. An *ad hoc* informal comparison of the average EPA+DHA values from cooked seafood in the CNF versus raw seafood in the CNF suggests that cooked seafood has approximately 135% higher concentrations of EPA+DHA when compared with their raw counterparts (data not shown). This was unexpected as literature in this area has shown that cooking seafood typically results in the losses of EPA and DHA due to fat rendering out with heating (Gladyshev et al., 2014; Kitson et al., 2009; Sioen et al., 2006). It is not clear why the CNF cooked seafood has a gain in EPA and DHA with cooking and appears to warrant additional research to determine the impact of cooking on seafood EPA and DHA.

7.5 Limitations

The current assessment uses validation techniques, but it should not be considered a validation of the Canadian Screener. The current study utilizes the CCHS 2015 Nutrition results which have a 2-day 24-hour recall, while the Screener requires recall from the past 2 months. The assumption that the 2-day intake is representative of everyday intake is not typically true, but it was used to quickly generate mock data to test the logistics of the new screener and examine and use the data generated from the REDCap system. In addition, we have a small sample size of 25, with only 5 participants in each intake group. The Bland-Altman analysis suggests an increased variability at higher EPA+DHA intake amounts. This phenomenon has been observed previously with higher estimates of EPA+DHA intake (Patterson et al., 2012) and with other nutrient intake assessments (Filippi et al., 2014), however, there are only two points outside the 95% confidence interval. A larger sample size would be required to verify this pattern.

7.6 Conclusion

Preliminary testing of the Canadian Screener demonstrated a high agreement between EPA+DHA intake estimates by the Screener and by the CCHS 2015 24-hour dietary recalls. Estimations from the screener show that it can consistently estimate EPA+DHA intake and place participants into the correct EPA+DHA intake groups. While this was expected as most of the EPA + DHA since both the Canadian Screener and the CCHS 2015 Nutrition derive their nutrient data from the CNF, it is important to note that the information in the screener was captured by seven questions, while CCHS 2015 Nutrition used for comparison was derived from two detailed 24-hour recalls. The Screener appears to be ready for a proper validation study.

Chapter 8

General Discussion

8.1 Overview

The purpose of this thesis was to establish a current dietary assessment tool that can be used to determine the habitual intakes of dietary EPA and DHA in individual Canadians. Previous screeners used in Canada were either missing non-seafood sources (Lucas et al., 2009) of EPA and DHA, or were outdated with food items that no longer exist in the Canadian food supply (Patterson et al., 2012). The REDCap coding of an online U.S. screener was used as a template and updated to reflect the EPA and DHA containing food items in the Canadian food supply. Layout modifications were also made to reduce participant and researcher burden, and portion size images were added to improve the ability to estimate intake quantities. When we noticed the EPA and DHA values from the non-seafood items in the screeners did not align with other sources (i.e. CNF and scientific literature), direct biochemical analyses to determine fatty acid compositions were completed. Finally, mock food item intake data was generated from the CCHS 2015 Nutrition dataset to allow preliminary comparisons between EPA+DHA estimates by the new Canadian screener with EPA+DHA intake estimates from the 24-hour recalls of CCHS 2015. This testing indicated a very high agreement and correlation between assessments; therefore, the Canadian screener is ready for a proper validation study against another dietary assessment tool along with comparisons to blood biomarkers of EPA and DHA.

The REDCap online screener we used as a template (Christifano et al., 2022) was based on a 7-item paper screener (Kuratko, 2013). This version of the online screener did not take full advantage of the processing abilities of the online environment. In the paper and original online screener, fish with similar amounts of EPA and DHA were grouped into three categories and assigned a single

value. In the new Canadian screener, each seafood species has been assigned unique EPA and DHA. This allowed us to group the seafood by consumption patterns of Canadians to make the flow of questions more intuitive to reduce participant burden. In addition, we recognized that omega-3 enriched eggs and organic chicken have become common in the Canadian food supply, therefore we added them to the Canadian screener. We also realized that the CNF values for liver, did not accurately reflect true values and did determinations of Canadian food products to generate our own EPA and DHA values for the screener. Previous screeners in Canada have been paper based and relied on the 2005 CNF (Lucas et al., 2009) and 2007b CNF along with direct fatty acid determinations of EPA and DHA containing products not listed in the CNF at that time (Patterson et al., 2012). The former screener did not include non-seafood items, while the latter included various n-3 enriched products available at the time including eggs, along with poultry but not liver or organic chicken. The omission of liver was likely a result of the CNF database indicating liver did not contain EPA and DHA, which appears to be an error that persist in the CNF 2015 file, while the emergence and growing popularity of organic is a more recent phenomenon.

Before the Canadian screener can be used, it needs to be validated. In the future, we will compare the EPA and DHA intake estimates of the new screener to estimated derived from multiple 24-hour recalls completed over two months. In addition, EPA and DHA blood biomarkers will be compared as they have been demonstrated to predict EPA and DHA intakes (Patterson et al., 2015). The online screener we used as a template was validate using Pearson's and Spearman's correlation testing (S. Crawford et al., 2022). Reliance on Pearson's and/or Spearman's correlation testing for validated EPA and DHA based dietary assessment tools appears to be a common practice (Lucas et al., 2009; Sullivan et al., 2008). However, correlation testing only examines the strength and direction of a linear relationship. Proper validation requires of dietary assessment tools requires the use of

concordance testing to determine the degree of agreement between methods (Martin Bland & Altman, 1986; Willett & Lenart, 2012). Therefore, our future validation study will include a Bland-Altman plot between the dietary assessment tools and a Cohen's Kappa examination of the screener intakes against the EPA+DHA blood biomarker status.

8.2 Conclusion

Following validation of the Canadian screener, it can be shared with other Canadian researchers interested in the assessment of habitual EPA and DHA intake using the existing platform hosted by the University of Waterloo. If participant data needs to remain within a host institute, the REDCap platform allows the code to be exported easily so other institutions can host the screener on their local REDCap server. For international collaborations, we recommend that researcher consider the process we underwent in this thesis to adapt the screener to the nutrient database that best reflects that country and to include checks for the EPA and DHA content of local food products to be included in the final screener. Adaptations for different countries should also consider examining seafood consumption patterns within that country, to allow for listings/question that are intuitive for the population of that country/region, similar to what we did. The screener also has the potential to be used by healthcare professionals for quick screening of EPA and DHA intakes of those at risk for cardiovascular disease as well as pregnant mothers. Follow-up blood biomarker assessments may then be necessary to confirm findings. If low cost, large volume, secure server architecture is available, the screener could potentially be used by the general public to self-monitor their dietary intake of EPA and DHA. Overall, this screener once validated should become a quick and useful tool to improve the ability to determine intake estimates of EPA and DHA in Canadians.

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Appendix A

R Functions to Acquire Seafood Consumption in Grams

```
CNF_fish <- fdc %>%
  filter(FID_FGR %in% c("34A", "34B", "35A"))

fish_fid <- fid %>%
  inner_join(CNF_fish, by = "FID_CDE")

fish_fid_v2 <- fish_fid %>%
  select(
    ADM_RNO,
    SUPPID,
    FID_CDE,
    FDC_DEN,
    FID_WTG,
    FDC_DHA,
    FDC_EPA,)

fish_fid_v3 <- fish_fid_v2 %>%
  mutate(
    fish_type = case_when(
      str_detect(FDC_DEN, regex("salmon", ignore_case = TRUE)) ~ "Salmon",
      str_detect(FDC_DEN, regex("cod", ignore_case = TRUE)) ~ "Cod",
      str_detect(FDC_DEN, regex("tuna", ignore_case = TRUE)) ~ "Tuna",
      str_detect(FDC_DEN, regex("haddock", ignore_case = TRUE)) ~ "Haddock",
      str_detect(FDC_DEN, regex("crab", ignore_case = TRUE)) ~ "Crab",
      str_detect(FDC_DEN, regex("shrimp", ignore_case = TRUE)) ~ "Shrimp",
      str_detect(FDC_DEN, regex("eel", ignore_case = TRUE)) ~ "Eel",
      str_detect(FDC_DEN, regex("squid", ignore_case = TRUE)) ~ "Squid",
      str_detect(FDC_DEN, regex("herring", ignore_case = TRUE)) ~ "Herring",
      str_detect(FDC_DEN, regex("trout", ignore_case = TRUE)) ~ "Trout",
      str_detect(FDC_DEN, regex("perch", ignore_case = TRUE)) ~ "perch",
      str_detect(FDC_DEN, regex("snapper", ignore_case = TRUE)) ~ "Snapper",
      str_detect(FDC_DEN, regex("catfish", ignore_case = TRUE)) ~ "Catfish",
      str_detect(FDC_DEN, regex("tilapia", ignore_case = TRUE)) ~ "Tilapia",
      str_detect(FDC_DEN, regex("flatfish", ignore_case = TRUE)) ~ "Flatfish",
      str_detect(FDC_DEN, regex("pollock", ignore_case = TRUE)) ~ "Pollock",
      str_detect(FDC_DEN, regex("lobster", ignore_case = TRUE)) ~ "Lobster",
      str_detect(FDC_DEN, regex("sardine", ignore_case = TRUE)) ~ "Sardine",
      str_detect(FDC_DEN, regex("oyster", ignore_case = TRUE)) ~ "Oyster",
      str_detect(FDC_DEN, regex("mackerel", ignore_case = TRUE)) ~ "Mackerel",
      str_detect(FDC_DEN, regex("mussel", ignore_case = TRUE)) ~ "Mussel",
      str_detect(FDC_DEN, regex("milkfish", ignore_case = TRUE)) ~ "Milkfish",
      str_detect(FDC_DEN, regex("croaker", ignore_case = TRUE)) ~ "Croaker",
      str_detect(FDC_DEN, regex("butterfish", ignore_case = TRUE)) ~ "Butterfish",
      str_detect(FDC_DEN, regex("pompano", ignore_case = TRUE)) ~ "Pompano",
      str_detect(FDC_DEN, regex("smelt", ignore_case = TRUE)) ~ "Smelt",
```

```

str_detect(FDC_DEN, regex("clam", ignore_case = TRUE))~ "Clam",
str_detect(FDC_DEN, regex("scallop", ignore_case = TRUE))~ "Scallop",
str_detect(FDC_DEN, regex("halibut", ignore_case = TRUE))~ "Halibut",
str_detect(FDC_DEN, regex("turbot", ignore_case = TRUE))~ "Turbot",
str_detect(FDC_DEN, regex("bass", ignore_case = TRUE))~ "Bass",
str_detect(FDC_DEN, regex("caviar", ignore_case = TRUE))~ "Roe",
str_detect(FDC_DEN, regex("whitefish", ignore_case = TRUE))~ "Whitefish",
str_detect(FDC_DEN, regex("anchovy", ignore_case = TRUE))~ "Anchovy",
str_detect(FDC_DEN, regex("arctic", ignore_case = TRUE))~ "Arctic Char",
str_detect(FDC_DEN, regex("pike", ignore_case = TRUE))~ "Pike",
str_detect(FDC_DEN, regex("snail", ignore_case = TRUE))~ "Snail",
str_detect(FDC_DEN, regex("Octopus", ignore_case = TRUE))~ "Octopus",
str_detect(FDC_DEN, regex("Pickerel", ignore_case = TRUE))~ "Pickerel",
str_detect(FDC_DEN, regex("hake", ignore_case = TRUE))~ "Hake",
str_detect(FDC_DEN, regex("swordfish", ignore_case = TRUE))~ "Swordfish",
str_detect(FDC_DEN, regex("carp", ignore_case = TRUE))~ "Carp",
str_detect(FDC_DEN, regex("sunfish", ignore_case = TRUE))~ "Sunfish",
str_detect(FDC_DEN, regex("grouper", ignore_case = TRUE))~ "Grouper",
str_detect(FDC_DEN, regex("dolphinfish", ignore_case = TRUE))~ "Dolphinfish",
str_detect(FDC_DEN, regex("roe", ignore_case = TRUE))~ "Roe",
str_detect(FDC_DEN, regex("gefiltefish", ignore_case = TRUE))~ "Gefiltefish",
str_detect(FDC_DEN, regex("crayfish", ignore_case = TRUE))~ "Crayfish",
str_detect(FDC_DEN, regex("sea cucumber", ignore_case = TRUE))~ "Sea Cucumber",
str_detect(FDC_DEN, regex("shark", ignore_case = TRUE))~ "Shark",
str_detect(FDC_DEN, regex("scup", ignore_case = TRUE))~ "Scup",
str_detect(FDC_DEN, regex("abalone", ignore_case = TRUE))~ "Abalone",
str_detect(FDC_DEN, regex("kingfish", ignore_case = TRUE))~ "Kingfish"
))

recallday1_fish <- fish_fid_v3 %>%
  filter(SUPPID == 1)
recallday2_fish <- fish_fid_v3 %>%
  filter(SUPPID == 2)

fish_consumption_day1 <- recallday1_fish %>%
  group_by(fish_type) %>%
  summarize(
    total_grams = sum(FID_WTG, na.rm = TRUE),
    .groups = "drop"
  ) %>%
  arrange(desc(total_grams))

fish_consumption_day2 <- recallday2_fish %>%
  group_by(fish_type) %>%
  summarize(
    total_grams = sum(FID_WTG, na.rm = TRUE),
    .groups = "drop"
  )

```

```

) %>%
arrange(desc(total_grams))

avg_fish_consumption <- fish_consumption_day1 %>%
  rename(total_grams_day1 = total_grams) %>%
  full_join(
    fish_consumption_day2 %>%
      rename(total_grams_day2 = total_grams),
    by = "fish_type"
  )

avg_fish_consumption_v2 <- avg_fish_consumption %>%
  mutate(
    total_grams_day1 = coalesce(total_grams_day1, 0),
    total_grams_day2 = coalesce(total_grams_day2, 0),
    average_grams = (total_grams_day1 + total_grams_day2) / 2
  )

```

* R code to estimate total grams consumed and number of individuals who consumed the individual seafood species for both day 1 and day 2 in the Canadian Community Health Survey

Appendix B

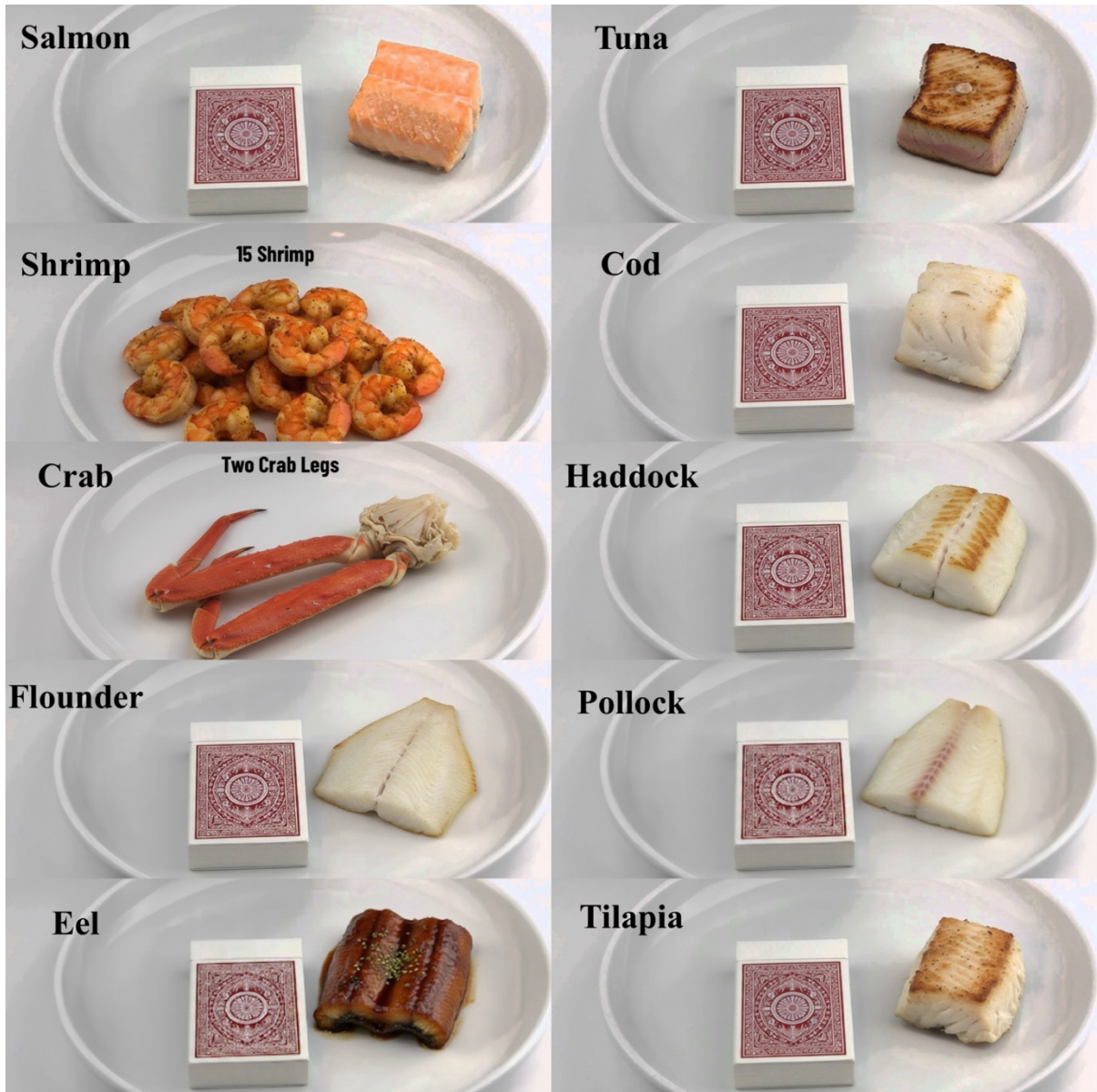
Distinct Number of Seafood Consumers from the CCHS 2015 with CNF EPA and DHA Values

Species of Fish	Number of Consumers (CCHS 2015 Day 1)	EPA (mg/100g)	DHA (mg/100g)
Salmon	934	321	1115
Tuna	647	71	185
Shrimp	632	68	70
Cod	459	64	120
Crab	229	113	259
Haddock	205	42	89
Flatfish	154	137	108
Pollock	137	71	350
Tilapia	99	5	86
Eel	78	84	63
Trout	75	202	528
Scallop	74	42	61
Lobster	69	76	46
Squid	65	146	342
Perch	56	79	174
Clam	53	43	64
Halibut	42	66	128
Catfish	36	130	234
Mussel	35	188	253
Herring	34	709	862
Sardine	34	473	509
Bass	30	161	434
Mackerel	29	898	1401
Anchovy	25	538	911
Oyster	21	438	250
Pike	16	73	200
Smelt	15	123	97
Whitefish	14	200	300
Croaker	9	275	418
Snapper	9	51	260
Pompano	8	175	393
Roe	7	983	1363
Snail	6	119	0
Scup	4	0	0
Carp	3	238	114
Hake	3	0	0
Dolphinfish	2	88	20
Grouper	2	27	220
Octopus	2	76	81
Swordfish	2	107	647
Abalone	1	49	0
Crayfish	1	104	38
Shark	1	316	527

* Grey Bar Corresponds to Excluded from the Canadian Screener
 CNF = Canadian Nutrient File, CCHS = Canadian Community Health Survey 2015

Appendix C

Question 1 Portion Size (100 grams) Images for Canadian Screener



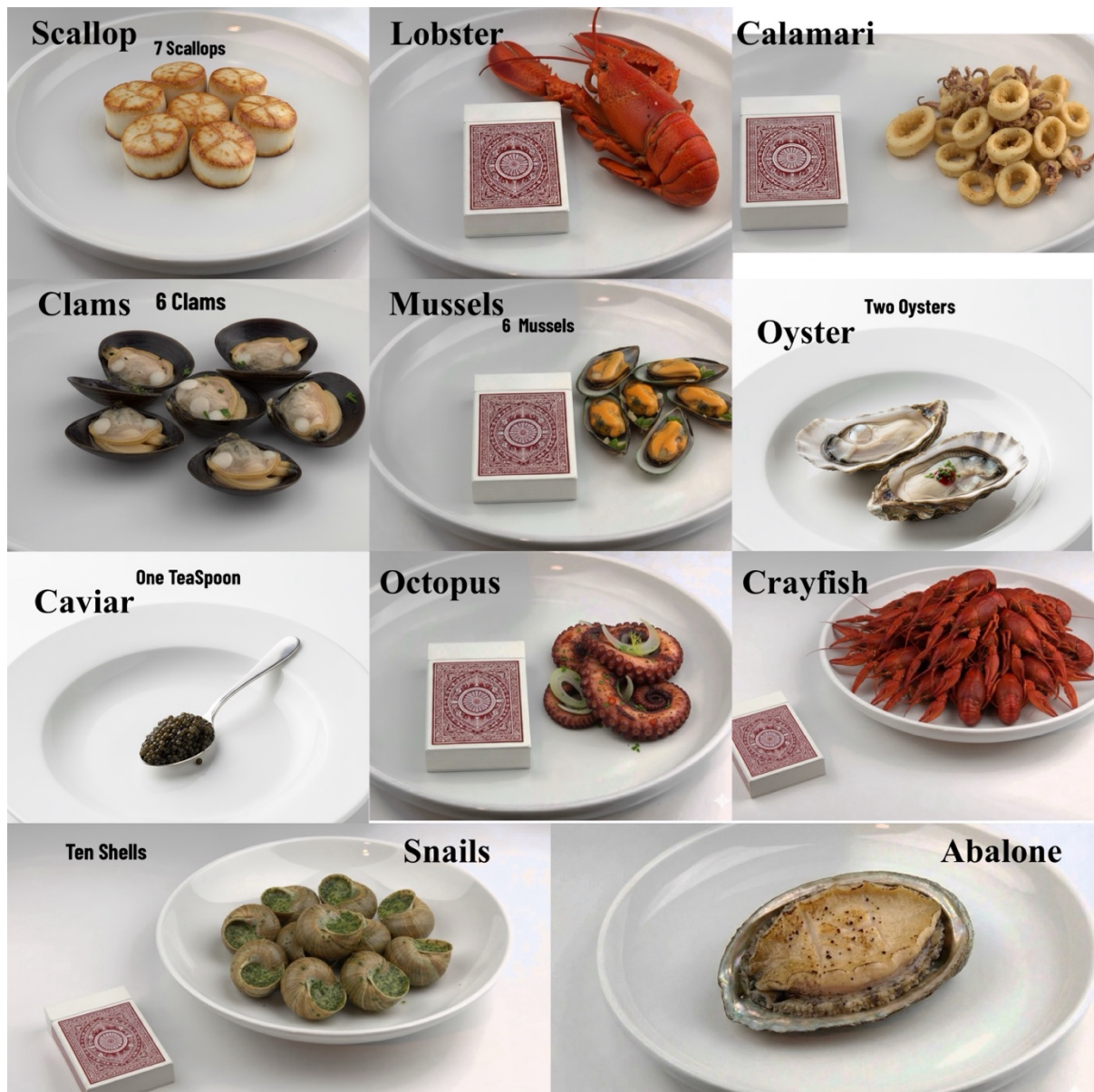
Appendix D

Question 2 Portion Size (100 grams) Images for Canadian Screener



Appendix E

Question 3 Portion Size (100 grams) Images for Canadian Screener



Appendix F

Code to Allow a Clickable Pop-up to see Portion Size Image of Abalone in the Screener

```
Abalone <a href="#abalone-modal" class="btn btn-outline-success btn-xs" style="margin-left:15px;">Serving Size<i class="fas fa-image"></i> <div id="abalone-modal" class="modal-window"> <div> <a href="#close" title="Close" class="modal-close">X</a>  </div> </div>
```

* Javascript code implementations of a portion size image that allows the screener user to launch a clickable pop-up window labelled “Serving Size” containing the reference food image.

Appendix G

Screenshots of Questions from the Canadian EPA and DHA Screener

What is your preferred language? Quelle est votre langue préférée ? * must provide value		☒ English ☐ Français	reset
First Name:		Last Name:	
Race * must provide value	☐ White ☐ Black or African American ☐ South Asian ☐ East Asian ☐ Indigenous ☐ Hispanic or Latino ☐ Other: reset		
Email Address * must provide value			
Phone Number			

1.) For each type of seafood listed below, please indicate how often you typically eat a 100-gram serving

		Never	Once in 2 Months	Monthly	Twice a month	Weekly	Twice a week	Every Other Day	Daily
Salmon	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									reset
Tuna	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									reset
Shrimp	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									reset
Cod	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									reset
Crab	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									reset
Haddock	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									reset
Flounder	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									reset
Pollock	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									reset
Eel	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									reset
Tilapia	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									reset

2.) For each type of fish listed below, please indicate how often you typically eat a 100-gram serving

		Never	Once in 2 Months	Monthly	Twice a month	Weekly	Twice a week	Every Other Day	Daily
Perch	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									
									reset
Halibut	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									
									reset
Catfish	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									
									reset
Herring	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									
									reset
Sardine	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									
									reset
Bass	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									
									reset
Mackerel	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									
									reset
Anchovy	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									
									reset
Imitation Crab or Whitefish	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									
									reset
Trout	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									
									reset
Croaker	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									
									reset
Snapper	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									
									reset
Pompano	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									
									reset
Carp	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									
									reset
Mahimahi	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									
									reset
Grouper	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									
									reset
Swordfish	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									
									reset
Shark	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									
									reset

3.) For each type of shellfish listed below, please indicate how often you typically eat a 100 gram serving									
		Never	Once in 2 Months	Monthly	Twice a month	Weekly	Twice a week	Every Other Day	Daily
Scallop	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									reset
Lobster	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									reset
Squid/Calamari	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									reset
Clam	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									reset
Mussel	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									reset
Oyster	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									reset
Caviar (Teaspoon)	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									reset
Octopus	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									reset
Crayfish	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									reset
Snail	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									reset
Abalone	Serving Size	☐	☐	☐	☐	☐	☐	☐	☐
* must provide value									reset

4.) How often do you typically eat a 100-gram serving of liver?

(Note: only include chicken, beef, or pork liver)

* must provide value

- ☐ Never
- ☐ Once in 2 months
- ☐ Monthly
- ☐ Twice a month
- ☐ Weekly
- ☐ Twice a week
- ☐ Every other day
- ☐ Daily

[Serving Size](#) 

[reset](#)

5.) In the **past week, how many eggs did you consume?
Specify the type of egg**

(Include egg yolks used in cooking)

* must provide value

Egg Type:

- ☐ Medium
- ☐ Large
- ☐ Extra-Large
- ☐ Omega-3
- ☐ Omega-3 Plus

[reset](#)

6.) In the **past week, how often did you typically eat a 200-gram serving of chicken?**

(A 200-gram serving is about 1 chicken breast, 1 chicken thigh, or 2 chicken drumsticks.)

* must provide value

Chicken Type:

- ☐ Regular
- ☐ Organic

[reset](#)

7.) Are you taking a fish oil supplement or a prenatal vitamin with EPA and/or DHA in it?

* must provide value

- ☐ Yes
- ☐ No

[reset](#)

Appendix H

R Function to Acquire Chicken Consumption Frequency from CCHS 2015 Nutrition

```
CNF_poultry <- fdc %>%
  filter(FID_FGR %in% c("25C", "27A", "27B", "27C", "27D", "27E", "211A", "211B", "211C"))
chicken_consumption_day1 <- CNF_poultry %>%
  filter(bird_species == "Chicken")
meat_cuts <- function(data) {
  data %>%
    mutate(
      cut = case_when(
        str_detect(FDC_DEN, regex("Tender", ignore_case = TRUE)) ~ "Tenders",
        str_detect(FDC_DEN, regex("Breast", ignore_case = TRUE)) ~ "Breast",
        str_detect(FDC_DEN, regex("Thigh", ignore_case = TRUE)) ~ "Thigh",
        str_detect(FDC_DEN, regex("Drumstick", ignore_case = TRUE)) ~ "Drumstick",
        str_detect(FDC_DEN, regex("Wing", ignore_case = TRUE)) ~ "Wing",
        str_detect(FDC_DEN, regex("Leg", ignore_case = TRUE)) ~ "Leg",
        str_detect(FDC_DEN, regex("Light", ignore_case = TRUE)) ~ "Light",
        str_detect(FDC_DEN, regex("Dark", ignore_case = TRUE)) ~ "Dark",
        str_detect(FDC_DEN, regex("Bacon", ignore_case = TRUE)) ~ "Bacon"
      )
    )
}
```

* R program designed to categorize chicken consumption into specific meat cuts such as tenders, breast, thighs, drumsticks, etc.

Appendix I

R Function to Acquire Liver Consumption Frequency

```
CNF_liver <- fdc %>%  
  filter(FID_FGR %in% c("28A", "28B"))  
liver_fid <- fid %>%  
  inner_join(CNF_liver, by = "FID_CDE") %>%  
  select(  
    ADM_RNO,  
    SUPPID,  
    FID_CDE,  
    FDC_DEN,  
    FID_WTG)
```

* R Function to isolate liver consumption by filtering the CCHS 2015 Nutrition database for liver-specific food groups then using those food groups to isolate consumption data in the 24-hour recall. The participants ID, recall day, food code, food description, and food weight for returned

Appendix J

R Function for Participant Exclusion Criteria in Project 3

```
hs_2day_recalls <- read_sas("HS.sas7bdat") %>%
  filter(is.na(DHH_AGE) | (DHH_AGE >= 18 & DHH_AGE <= 65)) %>%
  filter(is.na(FSDDEKC) | (FSDDEKC >= 400 & FSDDEKC <= 8000)) %>%
  filter(is.na(ADMFSID) | ADMFSID == 1) %>%
  filter(is.na(WHC_05) | WHC_05 != 1) %>%
  filter(is.na(R24FLOW) | R24FLOW != 1) %>%
  filter(is.na(R24DCNT) | R24DCNT > 0) %>%
  select(ADM_RNO)

outlier_participants <- fid %>%
  inner_join(fdc, by = "FID_CDE") %>%
  filter(FDC_DHA > 10000 | FDC_EPA > 10000 | FID_WTG > 10000) %>%
  select(ADM_RNO) %>%
  distinct()
```

* R program for the inclusion criteria and outliers for participants. Adults had to be within the age of 18-65, with a daily intake of 400-8000 kcal. Participants had to be non-pregnant, had to have food intake in the recall, and any single food intake above 10kg of EPA, DHA, or weight were removed

Appendix K

R Function for Calculating Average Daily EPA+DHA Intake per Participant in Project 3

```
participant_epadha_totals <- fid %>%
  inner_join(fdc, by = "FID_CDE") %>%
  filter(ADM_RNO %in% hs_2day_recalls$ADM_RNO) %>%
  filter(!ADM_RNO %in% outlier_participants$ADM_RNO) %>%
  mutate(
    actual_dha_intake_g = (FDC_DHA * FID_WTG),
    actual_epa_intake_g = (FDC_EPA * FID_WTG)
  ) %>%
  group_by(ADM_RNO, SUPPID) %>%
  summarise(
    total_dha_g = sum(actual_dha_intake_g, na.rm = TRUE),
    total_epa_g = sum(actual_epa_intake_g, na.rm = TRUE),
    .groups = "drop"
  ) %>%
  mutate(
    total_epadha_g = total_dha_g + total_epa_g,
    total_epadha_mg = total_epadha_g * 1000
  ) %>%
  group_by(ADM_RNO) %>%
  summarise(
    avg_epadha_mg = mean(total_epadha_mg, na.rm = TRUE),
    .groups = "drop"
  )
```

* R program for the calculation of mean intake of EPA + DHA values for each participant specified in both day 1 and day 2 recalls. Participants are chosen using the inclusion criteria described in Appendix J. The code merges dietary intake records with nutrient compositions from the CNF, which the is summed and converted to milligrams.

Appendix L

R Function for Categorizing Participants into EPA+DHA Intake Groups

```
CCHS_verylow <- participant_epadha_totals %>%  
  filter(avg_epadha_mg <= 100)  
CCHS_low <- participant_epadha_totals %>%  
  filter(between(avg_epadha_mg, 101, 250))  
CCHS_medium <- participant_epadha_totals %>%  
  filter(between(avg_epadha_mg, 251, 500))  
CCHS_high <- participant_epadha_totals %>%  
  filter(between(avg_epadha_mg, 501, 1000))  
CCHS_veryhigh <- participant_epadha_totals %>%  
  filter(avg_epadha_mg > 1000)
```

* R program for grouping participants into EPA+DHA groups for random selection. Participants are chosen using the inclusion criteria described in Appendix J.

Appendix M

Stratified Random Participant Selection for the Preliminary Testing of the Canadian EPA+DHA Screener

EPA+DHA Intake Group	Random Number Generated to Identify Participant to Use *	Participant ID Selected with Assigned Rankings *
Very Low (0-100 mg/day) n = 874	58	1134
	247	5992
	317	7571
	664	15196
	819	19204
Low (101-250 mg/day) n = 133	16	2217
	55	8104
	92	14360
	99	15164
	105	16226
Medium (251-500 mg/day) n = 48	1	146
	5	3177
	42	16915
	44	18374
	45	19208
High (501-1000 mg/day) n = 33	8	3686
	12	5674
	25	12061
	28	13813
	29	15707
Very High (1001+ mg/day) n = 42	3	1909
	15	4079
	18	5807
	19	7670
	32	14952

* The total study population for project 3 was divided into five groups based on EPA+DHA consumption (very low, low, medium, high, and very high). Within each group, participants were sorted into ascending order based on participant ID, then a ranking variable was introduced assigning 1 to the first participant, and up to the total participant count for the group for the last participant. To perform the selection, five random integers was generated between 1 and the total participant count for that group, and the participants with the corresponding integers were selected.

Appendix N

R Function to Calculate Total Consumption (grams) of Specific Seafood Intake per Participant

```
individual_fish_intake <- function(adm_rno_get) {  
  fid %>%  
    filter(ADM_RNO %in% adm_rno_get) %>%  
    filter(FID_FGR %in% c("34A", "34B", "35A")) %>%  
    inner_join(fdc, by = "FID_CDE") %>%  
    rename(FID_WTG_grams = FID_WTG) %>%  
    mutate(  
      Fish_Type = case_when(  
        str_detect(FDC_DEN, regex("salmon", ignore_case = TRUE)) ~ "Salmon",  
        str_detect(FDC_DEN, regex("cod", ignore_case = TRUE)) ~ "Cod",  
        str_detect(FDC_DEN, regex("tuna", ignore_case = TRUE)) ~ "Tuna",  
        str_detect(FDC_DEN, regex("haddock", ignore_case = TRUE)) ~ "Haddock",  
        str_detect(FDC_DEN, regex("crab", ignore_case = TRUE)) ~ "Crab",  
        str_detect(FDC_DEN, regex("shrimp", ignore_case = TRUE)) ~ "Shrimp",  
        str_detect(FDC_DEN, regex("eel", ignore_case = TRUE)) ~ "Eel",  
        str_detect(FDC_DEN, regex("squid", ignore_case = TRUE)) ~ "Squid",  
        str_detect(FDC_DEN, regex("herring", ignore_case = TRUE)) ~ "Herring",  
        str_detect(FDC_DEN, regex("trout", ignore_case = TRUE)) ~ "Trout",  
        str_detect(FDC_DEN, regex("perch", ignore_case = TRUE)) ~ "perch",  
        str_detect(FDC_DEN, regex("snapper", ignore_case = TRUE)) ~ "Snapper",  
        str_detect(FDC_DEN, regex("catfish", ignore_case = TRUE)) ~ "Catfish",  
        str_detect(FDC_DEN, regex("tilapia", ignore_case = TRUE)) ~ "Tilapia",  
        str_detect(FDC_DEN, regex("flatfish", ignore_case = TRUE)) ~ "Flatfish",  
        str_detect(FDC_DEN, regex("pollock", ignore_case = TRUE)) ~ "Pollock",  
        str_detect(FDC_DEN, regex("lobster", ignore_case = TRUE)) ~ "Lobster",  
        str_detect(FDC_DEN, regex("sardine", ignore_case = TRUE)) ~ "Sardine",  
        str_detect(FDC_DEN, regex("oyster", ignore_case = TRUE)) ~ "Oyster",  
        str_detect(FDC_DEN, regex("mackerel", ignore_case = TRUE)) ~ "Mackerel",  
        str_detect(FDC_DEN, regex("mussel", ignore_case = TRUE)) ~ "Mussel",  
        str_detect(FDC_DEN, regex("milkfish", ignore_case = TRUE)) ~ "Milkfish",  
        str_detect(FDC_DEN, regex("croaker", ignore_case = TRUE)) ~ "Croaker",  
        str_detect(FDC_DEN, regex("butterfish", ignore_case = TRUE)) ~ "Butterfish",  
        str_detect(FDC_DEN, regex("pompano", ignore_case = TRUE)) ~ "Pompano",  
        str_detect(FDC_DEN, regex("smelt", ignore_case = TRUE)) ~ "Smelt",  
        str_detect(FDC_DEN, regex("clam", ignore_case = TRUE)) ~ "Clam",  
        str_detect(FDC_DEN, regex("scallop", ignore_case = TRUE)) ~ "Scallop",  
        str_detect(FDC_DEN, regex("halibut", ignore_case = TRUE)) ~ "Halibut",  
        str_detect(FDC_DEN, regex("turbot", ignore_case = TRUE)) ~ "Turbot",  
        str_detect(FDC_DEN, regex("bass", ignore_case = TRUE)) ~ "Bass",  
        str_detect(FDC_DEN, regex("caviar", ignore_case = TRUE)) ~ "Roe",  
        str_detect(FDC_DEN, regex("whitefish", ignore_case = TRUE)) ~ "Whitefish",  
        str_detect(FDC_DEN, regex("anchovy", ignore_case = TRUE)) ~ "Anchovy",  
        str_detect(FDC_DEN, regex("arctic", ignore_case = TRUE)) ~ "Arctic Char",  
      )  
    )  
  }
```

```

str_detect(FDC_DEN, regex("pike", ignore_case = TRUE))~ "Pike",
str_detect(FDC_DEN, regex("snail", ignore_case = TRUE))~ "Snail",
str_detect(FDC_DEN, regex("Octopus", ignore_case = TRUE))~ "Octopus",
str_detect(FDC_DEN, regex("Pickerel", ignore_case = TRUE))~ "Pickerel",
str_detect(FDC_DEN, regex("hake", ignore_case = TRUE))~ "Hake",
str_detect(FDC_DEN, regex("swordfish", ignore_case = TRUE))~ "Swordfish",
str_detect(FDC_DEN, regex("carp", ignore_case = TRUE))~ "Carp",
str_detect(FDC_DEN, regex("sunfish", ignore_case = TRUE))~ "Sunfish",
str_detect(FDC_DEN, regex("grouper", ignore_case = TRUE))~ "Grouper",
str_detect(FDC_DEN, regex("dolphinfish", ignore_case = TRUE))~ "Dolphinfish",
str_detect(FDC_DEN, regex("roe", ignore_case = TRUE))~ "Roe",
str_detect(FDC_DEN, regex("gefiltefish", ignore_case = TRUE))~ "Gefiltefish",
str_detect(FDC_DEN, regex("crayfish", ignore_case = TRUE))~ "Crayfish",
str_detect(FDC_DEN, regex("sea cucumber", ignore_case = TRUE))~ "Sea Cucumber",
str_detect(FDC_DEN, regex("shark", ignore_case = TRUE))~ "Shark",
str_detect(FDC_DEN, regex("scup", ignore_case = TRUE))~ "Scup",
str_detect(FDC_DEN, regex("abalone", ignore_case = TRUE))~ "Abalone",
str_detect(FDC_DEN, regex("kingfish", ignore_case = TRUE))~ "Kingfish"
)) %>%
filter(!is.na(Fish_Type)) %>% # ensure fish type exists
group_by(ADM_RNO, Fish_Type) %>% # group by participant and fish type
summarise(
  total_consumption_g = sum(FID_WTG_grams, na.rm = TRUE),
  .groups = "drop"
)
}

```

* R program to categorize and quantify species-specific seafood consumption in the entire CCHS 24-hour recall. The program uses food codes to only include seafood then uses keywords to group species. The final intake weight in grams and the frequency of intake specific to the species is calculated as the output.

Appendix O

R Function to Calculate Total Consumption (grams) of Specific Liver Intake per Participant

```
individual_liver_intake <- function(adm_rno_get) {  
  fid %>%  
    filter(ADM_RNO %in% adm_rno_get) %>%  
    filter(FID_FGR %in% c("28A", "28B")) %>%  
    inner_join(fdc, by = "FID_CDE") %>%  
    rename(FID_WTG_grams = FID_WTG) %>%  
    mutate(  
      Liver_Type = case_when(  
        str_detect(FDC_DEN, regex("beef", ignore_case = TRUE)) ~ "Beef",  
        str_detect(FDC_DEN, regex("chicken", ignore_case = TRUE)) ~ "Chicken",  
        str_detect(FDC_DEN, regex("fish", ignore_case = TRUE)) ~ "Fish",  
        str_detect(FDC_DEN, regex("seal", ignore_case = TRUE)) ~ "Seal",  
        str_detect(FDC_DEN, regex("goose", ignore_case = TRUE)) ~ "Goose",  
        str_detect(FDC_DEN, regex("lamb", ignore_case = TRUE)) ~ "Lamb",  
        str_detect(FDC_DEN, regex("Pâté", ignore_case = TRUE)) ~ "Pate",  
        str_detect(FDC_DEN, regex("pork", ignore_case = TRUE)) ~ "Pork",  
        str_detect(FDC_DEN, regex("turkey", ignore_case = TRUE)) ~ "Turkey",  
        str_detect(FDC_DEN, regex("veal", ignore_case = TRUE)) ~ "Veal"  
      )) %>%  
    filter(!is.na(Liver_Type)) %>%      # ensure fish type exists  
    group_by(ADM_RNO, Liver_Type) %>%  # group by participant and fish type  
    summarise(  
      total_consumption_g = sum(FID_WTG_grams, na.rm = TRUE),  
      .groups = "drop"  
    )  
  }
```

* R program to categorize and quantify liver type consumption in the entire CCHS 24-hour recall. The program uses food codes to only include liver then uses keywords to group types. The final intake weight in grams and the frequency of intake per type is calculated as the output.

Appendix P

R Function to Calculate Total Consumption (grams) of Specific Chicken Egg Intake per Participant

```
individual_egg_intake <- function(adm_rno_get) {  
  fid %>%  
    filter(ADM_RNO %in% adm_rno_get) %>%  
    inner_join(fdc, by = "FID_CDE") %>%  
    filter(  
      FID_FGR.x %in% c("16A") &  
      str_detect(FDC_DEN, regex("chicken", ignore_case = TRUE)) &  
      !str_detect(FDC_DEN, regex("white", ignore_case = TRUE))  
    ) %>%  
    rename(FID_WTG_grams = FID_WTG) %>%  
    group_by(ADM_RNO) %>%  
    summarise(  
      total_consumption_g = sum(FID_WTG_grams, na.rm = TRUE),  
      .groups = "drop"  
    )  
}
```

* R program to categorize and quantify egg consumption in the entire CCHS 24-hour recall. The program uses food codes to only include eggs then uses keywords to group egg types. The final intake weight in grams and the frequency of intake per type is calculated as the output.

Appendix Q

R Function to Calculate Total Consumption (grams) of Specific Chicken Intake per Participant

```
individual_chicken_intake <- function(adm_rno_get) {
  fid %>%
    filter(ADM_RNO %in% adm_rno_get) %>%
    filter(FID_FGR %in% c("25C", "27A", "27B", "27C", "27D", "27E", "211A", "211B", "211C"))
  %>%
    inner_join(fdc, by = "FID_CDE") %>%
    filter(str_detect(FDC_DEN, regex("chicken", ignore_case = TRUE))) %>%
    rename(FID_WTG_grams = FID_WTG) %>%
    mutate(
      Chicken_Cut = case_when(
        str_detect(FDC_DEN, regex("Leg", ignore_case = TRUE)) ~ "Tenders",
        str_detect(FDC_DEN, regex("Breast", ignore_case = TRUE)) ~ "Breast",
        str_detect(FDC_DEN, regex("Thigh", ignore_case = TRUE)) ~ "Thigh",
        str_detect(FDC_DEN, regex("Drumstick", ignore_case = TRUE)) ~ "Drumstick",
        str_detect(FDC_DEN, regex("Wing", ignore_case = TRUE)) ~ "Wing",
        str_detect(FDC_DEN, regex("Tender", ignore_case = TRUE)) ~ "Leg",
        str_detect(FDC_DEN, regex("Light Meat", ignore_case = TRUE)) ~ "Light",
        str_detect(FDC_DEN, regex("Dark Meat", ignore_case = TRUE)) ~ "Dark",
        str_detect(FDC_DEN, regex("Bacon", ignore_case = TRUE)) ~ "Bacon",
        TRUE ~ "Other"
      )
    ) %>%
    filter(!is.na(Chicken_Cut)) %>%           # ensure chicken cut exists
    group_by(ADM_RNO, Chicken_Cut) %>%       # group by participant and chicken type
    summarise(
      total_consumption_g = sum(FID_WTG_grams, na.rm = TRUE),
      .groups = "drop"
    )
}
```

* R program to categorize and quantify chicken consumption in the entire CCHS 2015 Nutrition 24-hour recall. The program uses food codes to only include chickens then uses keywords to group chicken cuts. The final intake weight in grams and the frequency of intake per cut is calculated as the output.

Appendix R

Extrapolation of CCHS 2015 Nutrition intake data from participant ID 13813 for
input into the 7-item Canadian Screener

Question #	Fish Species	Intake (g/2 days)	# of servings (100g)
Question 1	Salmon	0	Never
	Tuna	52	Twice a week
	Shrimp	50	Twice a week
	Cod	0	Daily
	Crab	26	Weekly
	Haddock	0	Never
	Flatfish (Flounder/Sole)	0	Never
	Pollock	0	Never
	Eel	26	Weekly
	Trout	0	Never
Question 2	Perch	0	Never
	Halibut	0	Never
	Catfish	0	Never
	Herring	0	Never
	Sardine	0	Never
	Bass	0	Never
	Mackerel	0	Never
	Anchovy	0	Never
	Whitefish	0	Never
Question 3	Scallop	0	Never
	Lobster	0	Never
	Squid	0	Never
	Clam	0	Never
	Mussel	0	Never
	Oyster	0	Never
	Roe	0	Never
	Octopus	0	Never
	Crayfish	0	Never
	Snail	0	Never
Question 4	Liver	0	Never
Question 5	Eggs	14	1 (egg/week)
Question 6	Chicken	92	2 (200 grams/week)