Lipid Analyses of Four Types of Fish from Manitoba Lakes

Mohammed H Moghadasian¹*, Paymahn Moghadasian¹, Khuong Le¹, Arnold Hydamaka² and Peter Zahradka¹

¹Department of Human Nutritional Sciences, University of Manitoba, and Canadian Centre for Agri-Food Research in Health and Medicine, St. Boniface Hospital Research Centre, Canada

²Department of Food Science, University of Manitoba, Canada

*Corresponding Author: Mohammed H Moghadasian, Department of Human Nutritional Sciences, University of Manitoba, 351 Tache Avenue, Winnipeg, Manitoba, Canada.

Abstract

Purpose: In this study, we compared the lipid profile of fillet samples from 4 types of fish including Arctic char (*Salvelinusalpinus*), pickerel (*Esoxreticulatus*), suckerfish (*Catostomuscommersoni*) and whitefish (*Coregonusclupeaformis*) obtained from Manitoba lakes with those from Atlantic and farmed salmon samples (*Salmosalar*).

Methods: Conventional lipid extraction was performed on all fish samples. Aliquots of extracted lipids were prepared for fatty acid profiling and sterol concentrations, using standard gas chromatography procedures.

Results: Fillet from Manitoba fish contained significantly less amounts of fat as compared to those from Salmon samples (5% vs 20% of fillet weight, p < 0.05). However the Manitoba fish samples had higher percentage of DHA (C22:6n-3) and EPA (C20:5n-3), as compared to the salmon samples. Both salmon samples had significantly higher amounts of MUFA, as compared to those in samples from the Manitoba lake fish. Similarly, the ratio of total n-6:n-3 fatty acids was at 4-8 times greater in either salmon samples than that in any of the samples from Manitoba fish. Furthermore, the fish samples from Manitoba lakes contained many times less cholesterol as compared to salmon samples (2 mg/100 g vs 80 mg/100 g, P < 0.05).

Conclusion: Lipid contents of fillets from Manitoba fish are different from those of salmon fillets.

Keywords: *Lipids; Fatty acids; DHA; EPA; Manitoba; Fish; Salmon; Whitefish; Sucker; Pickerel; Arctic Char*

Abbreviations: ALA: Alpha-linolenic acid; DHA: Docosahexaenoic acid; EPA: Eicosapentaenoic acid; MUFA: Mono-unsaturated fatty acids

Introduction

Cardiovascular disorders still remain one of the leading causes of morbidity and mortality in both developing and developed countries, including Canada [1]. Among several strategies for the prevention and/or treatment of such disorders, modifications in lifestyle and dietary habits have been very promising [2]. One aspect of changing dietary habits is regular consumption of fish and fish products. Several health promoting authorities, including the American Heart Association and Health Canada, recommend inclusion of 2 meals of fish per week for adults [3,4]. Fish and fish products may deliver health benefits through several mechanisms. However, the focus has been placed on a very long chain polyunsaturated fatty acid named Docosahexaenoic acid (DHA). DHA is synthesized by lower level organisms such as phytoplankton in the aquatic environment. Fish and other sea animals accumulate DHA in their tissues through consumption of such sources of DHA. Humans and animals may receive DHA through dietary intake of fish and other seafood products. Although this

dietary route is the main method of acquiring DHA, we and others have shown that humans and animals are able to synthesize very small amounts of DHA [5,6]. It appears that synthesis of DHA in higher level organisms is species and tissue dependent [7].

Not all fish and other sea animals contain the same amounts of DHA. Our current knowledge indicates that some types of fish including sardines, herring, salmon and trout are rich sources of DHA [8]. This variation in the amounts of DHA among various types of fish could be related to geographic location, diet and/or genetic background. These factors were the basis for our hypothesis that fish varieties living in cold environment including Manitoba lakes contain higher amounts of DHA. Thus, the purpose of this study was to compare lipid contents of 4 common types of Manitoba fish with those from commonly consumed salmon fish.

Materials and Methods

Fish samples

All fish samples were purchased at the same time from Gimli Fish in Winnipeg, Manitoba. Manitoba lake fish samples included Arctic char (*Salvelinusalpinus*), pickerel (*Esoxreticulatus*), suckerfish (*Catostomuscommersoni)* and whitefish (*Coregonusclupeaformis*). Samples from wild and farmed Atlantic salmon (*Salmosalar*) were used as controls. Attempts were made to have the fish samples taken from similar anatomical locations. All samples were frozen before analysis. Six specimens of 1 gram from each frozen fish sample were taken at the same time, and immediately used for lipid extraction and analysis (n = 6). No scientific attempts were made to confirm the scientific identity of the fish tested.

Fatty acid and sterol analyses

Specimens from the fish samples were used to extract total lipids based on the Folch method as previously used in our laboratory [9]. The lipid levels were calculated and expressed as percent of the tissue weight for each sample. Aliquots of lipid extracts were prepared for gas chromatography analysis to determine the levels of fatty acids as per our standard procedures [10]. Peaks representing major fatty acids were identified and the percentage of these fatty acids was calculated. Similarly, gas chromatography procedures were used to identify and estimate the amounts of various sterols including cholesterol and plant sterols (β-sitosterol, campesterol, sitostanol, campestanol, stigmasterol) in lipid samples per previously described methods [10]. For estimation of fatty acid concentrations by gas chromatography techniques, methylated samples were separated on a Varian WCOT Fused Silica CP-SELECT FAME column (100 m × 0.25 mm diameter and 0.25 μm film thickness; Varian Canada Inc., Mississauga, Ontario) using a Varian 450 GC with FID (Flame ionization Detector). The column was operated at 130°C for 2 min, followed by raising the temperature to 175°C at 25°C/min. After 25 min, the temperature rose again to 240°C at 3°C/min, and held for 10 min. Total run time was 60.47 min, and samples were run with a 20:1 split ratio and column flow of 0.8 ml/min, injector temperature 270°C and detector temperature 290°C. Hydrogen was used as the carrier gas. Percentage of major fatty acids from total fatty acids were calculated from the area under the curve of relevant peaks and reported as previously published [11,12].

For the estimation of the concentrations of cholesterol and plant sterols the following procedure was used. Sterol analysis was carried out using a Bruker 450GC/FID (flame ionization detector) gas chromatography instrument manufactured in the Netherlands. The column used was an Agilent DB-5MS capillary column (30 m x 250 um ID). Carrier gas used was hydrogen with a split ratio of 100:1 and a flow rate of 1 ml/min. The injector and detector temperatures were set at 295°C and 320°C, respectively. The temperature profile included: 180°C initial temperature held for 2 min. ramping 30°C/min to 300°C held for 15 min. ramping 20°C/min. to 320°C and held for 5 min. The following standards were used: 5α-Cholestane - (Sigma Canada cat#C8003), cholesterol (Sigma Canada cat#C8667), campesterol (Sigma Canada cat#C5157), stigmasterol (Sigma Canada cat#S2424), β-sitosterol (Sigma Canada cat#S1270), desmosterol (Sigma Canada cat# D6513), and lathosterol (Avanti Polar Lipids cat #700069).

Total lipid extraction was performed on the same day of sampling, while fatty acid analysis was performed within 3 days of lipid extraction. All lipid analyses were performed on six samples (n = 6).

Statistical analysis

Data are presented as mean±standard deviation. One-way ANOVA analysis followed by the Turkey test was used to perform statistical analysis on the data. P < 0.05 was set for the significant differences among the samples.

Results
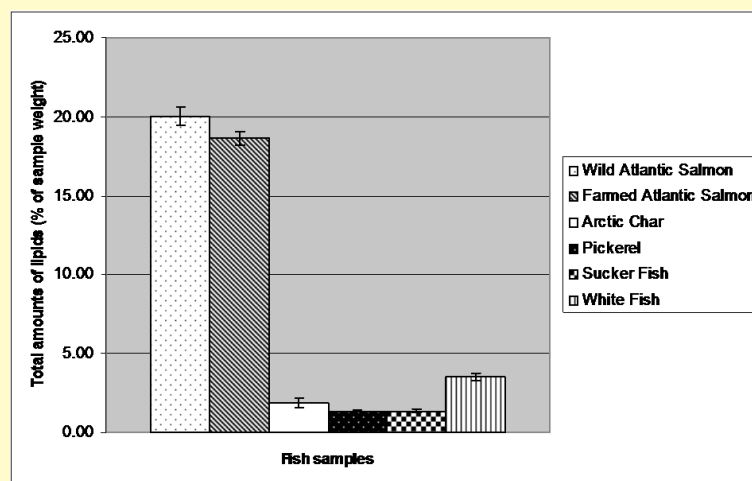
Figure 1: *Comparison of the amounts of lipids in fish samples from Manitoba lakes and farmed or wild salmon. Data are expressed as percentage of weight samples as mean and standard variation calculated from measurements of six samples per fish variety (n = 6). *, P < 0.05 compare to values from wild or farmed salmon samples.*

Figure 1 presents the quantity of total lipids in the freshly-thawed fish samples. Samples from wild and farmed salmon contain the highest amounts of total lipids, accounting for approximately 18%-20% of tissue weight. On the other hand, the amounts of lipids in the other 4 fish samples are less than 5% of the tissue weight.

Table 1 includes the levels of individual fatty acids detected at more than 1% in lipid extracts from the fish samples. The levels of myristic acid (C14:0) were significantly lower in Arctic char, pickerel and sucker, as compared with either types of salmon fish. Palmitic (C16:0) acid had the highest concentrations in Pickerel and the lowest concentrations in wild salmon. The levels of stearic acid (C18:0) were approximately 6% in pickerel followed by sucker (5.5%), farmed salmon (4.4%), Arctic char (4.1%), wild salmon (3.3%) and whitefish (3%). A major variation exists among these fish types in regard to the concentrations of oleic acid (C18:1), where pickerel had the lowest level at 6.5% and farmed Atlantic salmon contained the highest level at 32% among all fish samples examined. The levels of linoleic acid (C18:2) were significantly lower in all of Manitoba lake fish samples as compared to those of either salmon samples; compared to farmed salmon, wild salmon contains significantly lower levels of linoleic acid. It is interesting to note that the concentrations of the essential fatty acid ALA (C18:3n3) were relatively low in all of the 6 fish samples tested; the highest amount was found in wild salmon at 3.3%, and the lowest amount in the whitefish sample at < 1%.

Fatty Acid	Farmed Salmon	Wild Salmon	Arctic Char	Pickerel	Sucker	Whitefish
C14:0 (%)	2.76 ± 0.02	3.54 ± 0.22*	1.46 ± 0.165**	0.92 ± 0.12**	1.16 ± 0.25**	3.29 ± 0.26
C16:0 (%)	16.57 ± 0.18	13.61 ± 0.23*	19.97 ± 0.914	22.88 ± 1.31**	18.97 ± 1.02	15.88 ± 0.71
C16:1(%)	5.06 ± 0.05	5.57 ± 0.23	2.91 ± 0.18**	2.77 ± 0.26**	9.14 ± 1.12**	18.01 ± 1.4**
C18:0 (%)	4.38 ± 0.07	3.30 ± 0.07	4.08 ± 0.60	5.97 ± 0.91	5.51 ± 0.72	2.95 ± 0.21
C18:1 (%)	31.88 ± 0.49	25.77 ± 1.25*	14.20 ± 0.91**	6.51 ± 1.48**	9.95 ± 1.7**	18.33 ± 1.3**
C18:1n7 (%)	3.09 ± 0.01	3.64 ± 0.08	2.18 ± 0.06	2.27 ± 0.05	5.76 ± 0.2	5.05 ± 0.36
C18:2n6 (%)	10.93 ± 0.09	7.68 ± 0.28*	5.76 ± 0.25**	1.32 ± 0.21**	4.34 ± 0.33**	1.41 ± 0.2**
C18:3n3 (%)	1.56 ± 0.02	3.35 ± 0.069*	1.02 ± 0.055	1.74 ± 0.085	2.53 ± 0.24	0.97 ± 0.11
C20:1n12 (%)	0.89 ± 0.01	1.74 ± .38	0.475 ± 0.09	0.50 ± 0.04	0.24 ± 0.14	0.42 ± 0.02
C20:1n9 (%)	1.80 ± 0.14	1.66 ± 0.52	0.79 ± 0.04	0.26 ± 0.02	0.60 ± 0.05	0.50 ± 0.35
C20:4n6 (%)	0.69 ± 0.10	0.69 ± 0.047	2.27 ± 0.07**	3.67 ± 0.3**	4.49 ± 0.45**	1.17 ± 0.08**
C20:5n3 (%)	4.64 ± 0.15	6.521 ± 0..161	8.99 ± 0.49**	9.52 ± 0.82**	6.07 ± 0.62	9.85 ± 0.42**
C22:5n3 (%)	2.40 ± 0.06	3.396 ± 0.07*	1.86 ± 0.15**	2.31 ± 0.21	2.67 ± 0.21	2.76 ± 0.22
C22:6n3 (%)	5.10 ± 0.101	9.10 ± 1.13*	25.04 ± 1.8**	27.4 ± 2.0**	17.17 ± 1.97**	10.75 ± 1.2

Table 1: Fatty acid profile (% of total fatty acids) in various fish meat samples.
*Data are mean ± SD, n = 6; *, P < 0.05 as compared to farmed salmon; **, P < 0.05 as compared to either salmon samples*

The levels of arachidenoic acid (C20:4n6) were significantly lower in both salmon types as compared to those in samples from Manitoba lakes; the sucker samples showed the highest amount at 4.5% followed by 3.7% in pickerel, 2.3% in Arctic char and 1.2% in whitefish. Similarly, the levels of Eicosapentaenoic acid (EPA) were significantly higher in all of Manitoba lake fish samples as compared to those in either type of salmon. Except for the sucker samples, the other fish samples from Manitoba lakes contained approximately 9-10% Eicosapentaenoic acid. Furthermore, these fish samples also showed a great deal of variation in the content of DHA (C22:6n-3). All of the samples from Manitoba lake fish, except the whitefish sample, contained significantly higher amounts of DHA as compared with either type of salmon. Pickerel contained more than 5 times the DHA as compared to farmed Atlantic salmon. When comparing Manitoba fish with each other, the levels of DHA content from highest to the lowest were pickerel (27%), Arctic char (25%), suckerfish (17%) and whitefish (11%).

In addition to the above-mentioned contents of individual fatty acids, the 6 fish samples in this study contained various amounts of certain fatty acid groups. The data are summarized in Table 2. The percentage of total detected saturated fatty acids among all six fish samples varied from 21% to 31%. Wild salmon and Manitoba lake whitefish are at the lowest end at 21% and 23%, respectively, and Manitoba pickerel showed the highest amount at 31%. Total detected monounsaturated fatty acids (MUFA) showed a great deal of variation, representing 14% to 45% of total detected fatty acids among the 6 fish samples tested. In this regard, the order from the highest amounts of MUFA to the lowest was farmed Atlantic salmon (45%), whitefish (44%), wild Atlantic salmon (40%), suckerfish (28%), Arctic char (22%), and pickerel (14%). Similarly, all of these fish samples showed a large variation in regard to the contents of total detected polyunsaturated fatty acids (PUFA). This class of fatty acids accounted for 48% of total fatty acids in pickerel and Arctic char, 40% in sucker, 32% in wild Atlantic salmon, 28% in whitefish and 27% in farmed Atlantic salmon. Variations in the contents of PUFA reflect variations in the amounts of n-6 and n-3 fatty acids. Calculated total n-3 fatty acids ranged from 13% to 40% in fish samples tested. Overall, all of the Manitoba fish samples had several times more n-3 fatty acids than farmed salmon samples had. On the other hand, whitefish and pickerel samples contained 2-3 times less total n-6 fatty acids than farmed salmon had. The levels of total n-6 fatty

acids were comparable among wild salmon, Arctic char and sucker samples. Variations in the amounts and types of PUFA resulted in major variations in calculated total n6:n3 fatty acid ratios among these fish samples. This ratio was the lowest in whitefish and pickerel at 0.12, and 0.13, respectively and the highest in farmed salmon at 0.88.

Class of Fatty Acid	Farmed Salmon	Wild Salmon	Arctic Char	Pickerel	Sucker	Whitefish
Total saturated	24.63 ± 0.25	21.43 ± 0.35	26.84 ± 1.72	31.33 ± 2.22	27.15 ± 1.75	23.16 ± 0.94
Total MUFA	44.81 ± 1.38	40.17 ± 1.08	21.89 ± 1.12	14.53 ± 1.78	27.96 ± 2.95	43.76 ± 2.54
Total PUFA	27.15 ± 0.39	32.36 ± 1.46	48.0 ± 3.19	48.3 ± 3.45	40.37 ± 3.15	28.16 ± 1.7
Total n-3 FA (%)	13.85 ± 0.28	22.70 ± 1.34	37.55 ± 2.63	40.72 ± 3.13	28.83 ± 2.62	24.79 ± 1.57
Total n-6 FA (%)	12.12 ± 0.01	8.72 ± 0.25	9.12 ± 0.37	5.19 ± 0.20	9.86 ± 0.29	2.88 ± 0.15
Total n6:n3 ratio	0.88 ± 0.01	0.39 ± 0.03	0.24 ± 0.01	0.13 ± 0.01	0.34 ± 0.02	0.12 ± 0.01

Table 2: *Classes of fatty acids in six fish samples tested. Data are sum of the amounts of individual fatty acids identified in each class.*

The 6 fish samples tested form 2 clusters based on high and low cholesterol contents; the salmon samples had the highest amounts of cholesterol, approximately 80 mg/100 grams of fish meat samples, while all Manitoba lake fish had significantly lower levels of cholesterol at approximately 2 mg/100 grams of fish meat samples, except for the whitefish, which had an intermediate level of 12 mg/100 grams of meat sample. It is interesting that we were also able to detect measurable amounts of stigmasterol in all 6 fish samples. The levels of meat stigmasterol followed the patterns of cholesterol, but in lower amounts. Data on the sterol contents of fish meat samples are depicted in Figure 2.

Discussion

The health benefits of very long-chain n-3 polyunsaturated fatty acids have been well documented through clinical, observational, epidemiological and experimental studies [13,14]. Such strong evidence is behind current recommendations for regular consumption of fish and other seafood. Although alpha-linolenic acid (ALA) is the essential fatty acid that can be obtained through both plant and animal source foods, DHA can only be obtained through foods from animal origin, primarily fish and fish products.

Canadian and American authorities recommend consumption of two fish-meals per week [15]. Such recommendations endorse a number of health benefits including improvements in cardiovascular, immune, endocrine, nervous and reproductive systems [6]. Furthermore, studies have reported significant benefits of DHA in normal growth and development both during pregnancy and infancy [16]. Thus, intakes of appropriate amounts of DHA can enhance quality of life during various life stages. Despite such documented benefits of fish and fish product intakes, it seems that a significant portion of the population may not consume adequate amounts of these healthy food products [17].

Several factors may contribute to inadequate consumption of fish and fish products. Among them may be individuals' lifestyle factors, as vegan people will not consume animal products. Other factors could be availability, cost, preparation method, taste, odor, and others. This study provides information on the lipid composition of several types of fish caught from Manitoba lakes. Manitoba is known as "thousand lake" territory with cold temperatures. The Manitoba lakes are frozen for approximately 6 months of the year. Such long, severe winters have a significant impact on environment and living creatures. Among those, fish living in such extreme cold environment should have some protective mechanisms. Thus, Manitoba fish may have totally different lipid profile in comparison with ocean fish. Thus, we hypothesized that the lipid profile of fish fillets from Manitoba lakes may be different from that of commonly consumed salmon fish.
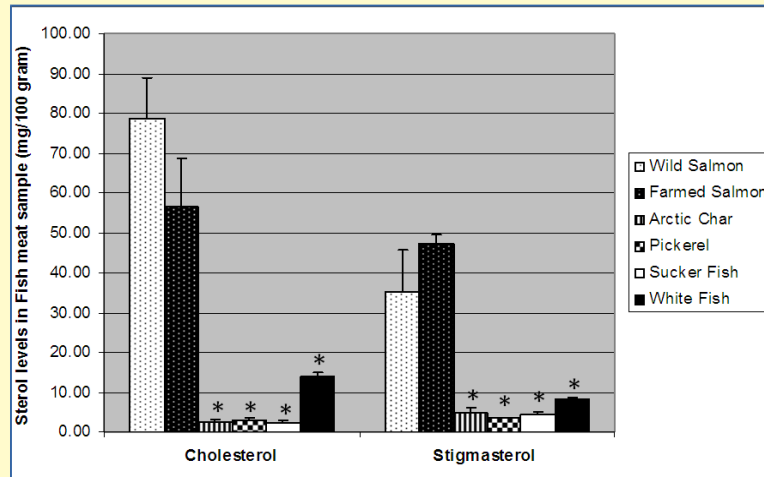
Figure 2: *Cholesterol and stigmasterol concentrations in four fish samples from Manitobav lakes in comparison with farmed or wild salmon. Data are expressed as mg/100 g meat samples as mean and standard variation calculated from measurements of six samples per fish variety (n = 6). *, p < 0.05 compare to values from wild or farmed salmon samples.*

We report that the fish from Manitoba lakes used in this study have different lipid content in both quality and quantity as compared with those from salmon. Salmon was used as the reference fish because it is a rich source of marine n-3 fatty acids and is commonly consumed worldwide. Among Manitoba lake fish examined, the Arctic char and pickerel contained a significant proportion of n-3 fatty acids in their tissue lipid extract. As summarized in Table 2, the proportion of total n-3 fatty acids in these two types of fish are 2-3 times higher than that in either farmed or wild Atlantic salmon. It is understood that both farmed and wild Atlantic salmon are 'fattier" than the types of Manitoba fish tested (Figure 1), however, the concentrations of DHA and EPA are greater in fish samples from Manitoba lakes. It is a general perception of the population that fat may not be good for you. Lower concentrations of total lipids in tissues of such varieties of fish will provide less DHA and EPA intake per serving size. Thus, one must consume such low-fat fish meals either at higher frequency or larger serving size to receive a dose of approximate 500 mg of DHA and EPA. Our calculation from the present study indicates that consumption of 50 grams of farmed salmon fillet can deliver approximately 500 mg of DHA, while that amount of DHA can be obtained from an intake of 100 grams of Arctic char fillet. It should be noted that Canadian information (http://www.hc-sc.gc.ca/fn-an/pubs/nutrition/omega3-eng.php) provides similar fatty acid information for these fish varieties [18]. Thus, it is possible that such high concentrations of DHA and low total fat contents are characteristics of the varieties, but not related to geographic locations. Another interesting observation from this study is that the ratio of n6:n-3 fatty acids is several time less in fish samples from Manitoba lakes as compared with salmon samples. For example, this ratio was calculated to be 0.88 in farmed salmon samples and 0.12 in Manitoba whitefish or 0.13 in Manitoba pickerel. Current recommendations suggest the dietary ratio of n-6: n-3 fatty acid be reduced to approximately 2-4:1 [19]. With this regard, consumption of fish with a low ratio of n-6: n-3 fatty acids such as the Manitoba fish seem very attractive.

In addition to their attractive fatty acid profile, fish from Manitoba lakes contained lower amounts of dietary cholesterol as compared with that in salmon fish. This could be an added attractive feature for both the general population and in particular for cardiovascular patients. Increased intakes of dietary cholesterol may be associated with increased levels of LDL cholesterol [20]. Thus, health authorities suggest that cardiovascular patients consume 200 mg or less of dietary cholesterol per day [21]. Animal-origin food is the main source of dietary cholesterol. However, this report shows that fish from Manitoba lakes contains small amounts of dietary cholesterol. Assuming that one individual consumes 250 grams of Manitoba fish per serving, the total amount of cholesterol intake will not exceed 10% of the recommended intake. Furthermore, we detected relatively high amounts of stigmasterol in all fish samples. Stigmasterol is recognized as a sterol synthesized primarily by plants; animals and humans do not absorb plant sterols to a large degree under normal conditions [22]. At this time, the source of stigmasterol in fish meat is unknown. It is possible that ocean plants and other botanical organisms produce significant amounts of stigmasterol. Regardless of origin, adequate amounts of plant sterols are known to reduce cholesterol absorption in both animals and humans [22]. Therefore, the presence of stigmasterol in Manitoba fish along with low levels of cholesterol makes Manitoba fish more attractive particularly to cardiovascular patients [23].

Conclusions

This study reports that fish from Manitoba lakes contain less percentage of total lipids but higher proportion of DHA in their tissue lipids as well as lower levels of tissue cholesterol as compared with those from salmon samples. Additional studies will be required to compare and contrast other food-related aspects of these fish varieties with other commonly consumed fish types. Similarly, it would be interesting to investigate whether the fatty acid profile observed in the tested fish samples also occurs in other types of fish from fresh water Northern lakes.

Acknowledgments

Bibliography

1. World Health Organization (2013) Global health observatory (GHO)
2. Health Canada. "Canadian Nutrient File", version 2010. Nutrient Data
3. American Heart Association. Fats 101 (2010).
4. Health Canada. "Make wise choices". (2007).
5. Burdge GC and Calder PC. "Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults". *Reproductive Nutritional Development* 45.5 (2005): 581-597.
6. Nicholson T., *et al*. "The role of marine n-3 fatty acids in improving cardiovascular health: A review". *Food & Function* 4.3 (2013): t 357-365.
7. Bezard J., *et al*. "The metabolism and availability of essential fatty acids in animal and human tissues". *Reproductive Nutritional Development* 34.6 (1994): 539-568.
8. Health Canada. "Prenatal nutrition guidelines for health professionals: Fish and omega-3 fatty acids". (2012).
9. Folch J., *et al.* "A simple method for the isolation and purification of total lipids from animal tissues". *Journal of Biological Chemistry* 226.1 (1957): 497-509.
10. Brooks CJ., *et al*. "Characterization of sterols by gas chromatography-mass spectrometry of the trimethylsilyl ethers". *Lipids* 3.5 (1968): 391-402.

11. Riediger ND., *et al*. (2009) "'Designer oils' low in n-6: n-3 fatty acid ratio beneficially alters cardiovascular risk in mice". *European Journal of Nutrition* 48.5 (2009): 307-314.

12. Riediger ND., *et al*. "Low dietary n-6: n-3 fatty acid ratio, with fish- or flaxseed oil, improves plasma lipids and alters tissue fatty acid composition in mice". *European Journal of Nutrition* 47.3 (2008): 153-160.

13. Rogers S., *et al*. "Effects of a Fish oil supplement on serum lipids, blood pressure, bleeding time, haemostatic and rheological variables. A double blind randomized controlled trial in healthy volunteers". *Atherosclerosis* 63.2-3 (1987): 137-143.

14. Singer P., *et al*. "Blood pressure lowering effect of eicosapentaenoic acid-rich diet in normotensive, hypertensive and hyper-lipemic subjects". *Experientia* 41.4 (1985): 462-464.

15. Smith KM and Sahyoun NR. "Fish consumption: recommendations versus advisories, can they be reconciled?" *Nutrition Reviews* 63.2 (2005): 39-46.

16. Health Canada. "Heart disease-heart health". (2009).

17. Kris-Etherton PM., *et al*. "Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease". *Arteriosclerosis Thrombosis Vascular Biolology* 23.2 (2003): e20-e30.

18. Health Canada. "Prenatal Nutrition Guidelines for Health Professionals-Fish and Omega-3 Fatty Acids (2013)".

19. Riediger N., *et al*. "A systemic review of the roles of n-3 fatty acids in health and disease". *Journal of the Academy of Nutrition and Dietetics* 109.4 (2009): 668-679.

20. Howell WH., *et al*. "Plasma lipid and lipoprotein responses to dietary fat and cholesterol: a meta-analysis". *American Journal of Clinical Nutrition* 65.6 (1997): 1747-1764.

21. Heart and Stroke Foundation. "Cholesterol control through food and activity". (2012).

22. Moghadasian MH and Frohlich JJ. "Effects of dietary phytosterols on cholesterol metabolism and atherosclerosis: Clinical and experimental evidence". *American Journal of Medicine* 107.6 (1999): 588-594.

23. Othman R and Moghadasian MH. "Beyond cholesterol-lowering effects of plant sterols: Clinical and experimental evidence on anti-inflammatory properties". *Nutrition Review* 69.7 (2011): 371-382.