

Relations between n-3 fatty acid status and cardiovascular disease risk factors among Quebecers¹⁻³

Éric Dewailly, Carole Blanchet, Suzanne Gingras, Simone Lemieux, Louise Sauvé, Jean Bergeron, and Bruce John Holub

ABSTRACT

Background: Epidemiologic evidence shows an inverse relation between fish consumption and death from ischemic heart disease. This beneficial effect is attributed to n-3 fatty acids.

Objectives: The purpose of this study was to examine the association between plasma phospholipid concentrations of the n-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and various cardiovascular disease risk factors among Quebecers.

Design: The study population consisted of 1460 subjects aged 18-74 y who participated in the 1990 Quebec Heart Health and Nutrition Survey. Data were obtained through home interviews and clinic visits.

Results: Expressed as the percentage of total fatty acids in plasma phospholipids, the geometric means of EPA, DHA, and their combination were 0.47%, 1.19%, and 1.70%, respectively. Concentrations of n-3 fatty acids were positively associated with fish intake. We found positive associations between EPA and total cholesterol, LDL cholesterol, HDL cholesterol, plasma glucose, and systolic and diastolic blood pressure. We found positive associations between DHA and total cholesterol, the ratio of total to HDL cholesterol, triacylglycerols, systolic blood pressure, and plasma glucose and insulin. We also found positive associations between the ratio of EPA to arachidonic acid and total cholesterol, HDL cholesterol, and systolic blood pressure and a negative association with the ratio of total to HDL cholesterol.

Conclusions: Our results indicate that concentrations of EPA and DHA in plasma phospholipids reflected Quebecer fish consumption. Results also show that EPA and the ratio of EPA to arachidonic acid can positively influence HDL-cholesterol concentrations. *Am J Clin Nutr* 2001;74:603-11.

KEY WORDS n-3 Fatty acids, eicosapentaenoic acid, docosahexaenoic acid, fish intake, cardiovascular disease risk factor, cholesterol, LDL, HDL, triacylglycerol, blood pressure, glucose, insulin, Quebec

INTRODUCTION

Cardiovascular disease is the leading cause of death and hospitalization in the province of Quebec. In 1993-1995, the age-standardized mortality rates (per 100,000 person-years) for ischemic heart diseases for men and women were 199.9 and 100.1, respectively (1). Research in cardiovascular health has

shown that high blood pressure, abnormal blood lipid concentrations, and diabetes are major risk factors for cardiovascular disease (CVD) (2, 3). In turn, these risk factors are strongly linked to abdominal obesity, inadequate levels of physical activity, and diets high in saturated fat (3-5). Numerous studies reported that a diet rich in fish and marine mammals protects against CVD (6-11). In fact, populations who consume large amounts of marine foods have a low prevalence of arterial thrombosis and CVD (8, 12-19). In addition, epidemiologic evidence shows an inverse relation between fish consumption and death from coronary heart disease (12, 16, 20-22). These beneficial effects are attributed to the n-3 fatty acids eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), for which the main dietary sources are fish, shellfish, and marine mammals. n-3 Fatty acids favorably act on risk factors implicated in the pathogenesis of atherosclerotic and thrombotic diseases. Observed positive effects of n-3 fatty acids include the lowering of blood lipid concentrations, especially of triacylglycerols and VLDL cholesterol (8). The reported effects on total cholesterol, LDL cholesterol, and HDL cholesterol are less consistent (8). The ingestion of fish oils containing EPA and DHA and of marine fatty fish (eg, mackerel) is associated with reduced blood pressure (7, 23-26). n-3 Fatty acids may also prevent the occurrence of diabetes and improve insulin action; however, data on the effects of n-3 fatty acids on plasma glucose and insulin are scarce and surrounded by controversy (8, 9, 27).

Many studies reporting beneficial effects of fish consumption were conducted among populations who consumed large quantities of fish (6, 28, 29). However, no epidemiologic studies have examined the profiles of plasma fatty acids, in particular the n-3 fatty acids of marine origin, and their association with selected

¹From the Public Health Research Unit and the Lipid Research Center, CHUL Research Center, Centre Hospitalier Universitaire de Québec, Ste-Foy, Canada; the Departments of Social and Preventive Medicine and of Food Sciences and Nutrition, Laval University, Ste-Foy, Canada; and the Department of Human Biology and Nutritional Sciences, University of Guelph, Guelph, Canada.

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³Address reprint requests to É Dewailly, Public Health Research Unit, Laval University Medical Research Center, 2400 d'Estimauville, Beauport G1E 7G9, Quebec, Canada. E-mail: eric.dewailly@crchul.ulaval.ca.

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CVD risk factors in a large population with low fish intake. Quebecers generally consume small quantities of fish; the average daily consumption is ≈ 15 g (30). In the present study, *n*-3 fatty acids were measured in plasma phospholipids, with particular attention given to the proportion of EPA and DHA, in a representative sample of Quebecers. The first objective was to examine the association between concentrations of EPA and DHA and fish consumption. The second objective was to verify the relations between concentrations of *n*-3 fatty acids and various CVD risk factors.

SUBJECTS AND METHODS

Study population

As part of the federal-provincial Canadian Heart Health Initiative, Santé Québec, an organization of the Quebec Health and Social Services Ministry, conducted the Heart Health and Nutrition Survey among Quebecers. The primary objective of the survey was to determine the prevalence of CVD risk factors and participants' knowledge and awareness of the causes and consequences of these factors (31, 32). The target population of the survey was composed of noninstitutionalized men and women aged 18–74 y. The participants constituted a stratified probability sample of the population and were selected according to a statistical sampling design. The sampling design, developed by the Quebec Bureau of Statistics, is described elsewhere (31, 32). Briefly, 2354 subjects were recruited for home interviews. Of these, 2118 subjects participated in the dietary survey and 2056 participated in the clinical sessions. Written informed consent was obtained from all subjects after approval of the protocol by the ethics committee of Maisonneuve-Rosemont Hospital, Montreal. A random sample of subjects was drawn from subjects attending both the home interviews and the clinical sessions. Thus, 1460 blood samples were used to analyze concentrations of *n*-3 fatty acids.

Plasma lipids, glucose, and insulin

Participants in the clinical sessions were asked to fast for 12 h before giving blood samples. Concentrations of plasma total cholesterol, triacylglycerols, LDL cholesterol, and HDL cholesterol were analyzed according to the methods of the Lipid Research Clinics (33). Cholesterol and triacylglycerol concentrations were measured in plasma and in lipoprotein fractions by use of an Auto-Analyzer II (Technicon, Tarrytown, NY). The HDL-cholesterol fraction was obtained after precipitation of LDL cholesterol in the infranant fluid with heparin and manganese chloride. Plasma glucose was measured enzymatically, and fasting insulin concentrations were measured with a commercial double-antibody radioimmunoassay (LINCO Research, St Louis) that showed little cross-reactivity ($<0.2\%$) with human proinsulin; CVs were $\leq 5.5\%$ (34).

Plasma phospholipid fatty acids

Plasma samples used for fatty acid analyses were stored at -80°C until analyzed in 1996. To measure the fatty acid composition in plasma phospholipids, 200- μL aliquots of plasma were extracted after the addition of chloroform:methanol (2:1, by vol), in the presence of a known amount of internal standard (diheptadecanoyl phospholipid) (35). The total phospholipid was isolated from the lipid extract by thin-layer chro-

matography with heptane:isopropyl ether:acetic acid (60:40:3, by vol) as the developing solvent. After transmethylation with boron trifluoride:methanol, the fatty acid profile was determined by capillary gas-liquid chromatography. The fatty acid composition of plasma phospholipids was expressed as a percentage of the total area of all fatty acid peaks from 14:0 to 24:1. In this study, plasma phospholipid concentrations of fatty acids correspond to relative percentages of total fatty acids by weight.

Blood pressure

Four blood pressure measurements were taken by a trained survey nurse according to the recommendations of the consensus conference on the management of mild hypertension in Canada (36). Standard mercury sphygmomanometers, 38.1-cm (15-inch) stethoscopes, and appropriately sized cuffs were used. Pressure readings were taken at the beginning and at the end of both the home interview and the clinical visit. These values are reported as the arithmetic mean of the 4 readings.

Lifestyle assessment and anthropometry

Using a questionnaire covering diet, smoking, alcohol intake, previous medical history of CVD, and socioeconomic characteristics, nurses conducted face-to-face interviews with the participants in their homes. These same participants also attended a clinical session in which anthropometric measurements such as height, weight, and waist and hip girth were recorded. The mean (\pm SD) body mass index (in kg/m^2) of the subjects was 24.9 ± 4.3 , and their mean waist girth was 83.2 ± 12.9 cm. In this study, the accumulation of adipose tissue in the abdominal area, measured by the waist girth, was used to measure abdominal obesity (4, 37). Recent studies showed that the amount of visceral adipose tissue is associated with many CVD risk factors such as insulin resistance, type 2 diabetes, hypertension, and changes in the concentrations of plasma lipids and lipoproteins (38). Waist circumference was measured by positioning the measuring tape horizontally at the level of noticeable waist narrowing. The measurement was made at the end of a normal expiration and was recorded to the nearest centimeter. Waist girth ≥ 100 cm for subjects <40 y of age and ≥ 90 cm for subjects ≥ 40 y of age was defined as abdominal obesity (39).

Dietary assessment

Fish intake data were obtained with use of a food-frequency questionnaire administered by a nutritionist during a face-to-face at-home interview. The questionnaire was used to measure the consumption of 56 food items and beverages during the month before the survey. The food-frequency questionnaire included only 2 fish items: fish and fried fish. Each subject was asked to report the usual frequency of fish consumption and the usual portion size.

Data analysis

All statistics in this paper were obtained from weighted data to reestablish the equiprobability of an individual being selected for the sample and to take into account nonresponse by age, sex, and geographic strata. For this, each respondent was given a value (weight) corresponding to the number of subjects he or she represented in the Quebec population (31). The population sample covered by the survey extended to 5 000 371 adults (18–74 y). Thus, all data in the present report were weighted and are representative of the entire Quebec adult population. Crude *n* values are presented for information only.

TABLE 1

Relative concentrations of n-3 fatty acids in plasma phospholipids according to category of fish intake per week¹

Fatty acid	Category of fish intake per week				P ²	All subjects (n = 1460)	
	0 g (n = 316)	1-55 g (n = 419)	56-120 g (n = 369)	≥121 g (n = 356)		Geometric \bar{x} (95% CI)	Arithmetic \bar{x} (range)
	<i>% by wt of total fatty acids</i>					<i>% by wt of total fatty acids</i>	
EPA	0.44 ± 0.17 ^a	0.45 ± 0.22 ^{ab}	0.48 ± 0.22 ^b	0.53 ± 0.28 ^c	0.0001	0.47 ± 0.23 (0.46, 0.48)	0.52 (0.09-3.69)
DHA	1.04 ± 0.38 ^a	1.13 ± 0.44 ^b	1.25 ± 0.47 ^c	1.36 ± 0.63 ^d	0.0001	1.19 ± 0.51 (1.17, 1.21)	1.28 (0.35-4.36)
EPA+DHA	1.51 ± 0.46 ^a	1.60 ± 0.58 ^a	1.76 ± 0.60 ^b	1.92 ± 0.81 ^c	0.0001	1.70 ± 0.65 (1.67, 1.72)	1.79 (0.63-8.05)
AA	6.38 ± 1.36	6.17 ± 1.36	6.24 ± 1.49	6.21 ± 1.51	0.22	6.24 ± 1.43 (6.17, 6.31)	6.40 (2.50-12.15)
EPA:AA	0.068 ± 0.03 ^a	0.072 ± 0.03 ^{ab}	0.077 ± 0.04 ^b	0.086 ± 0.04 ^c	0.0001	0.076 ± 0.04 (0.074, 0.077)	0.083 (0.010-0.407)
PUFA, n-3 series ³	2.31 ± 0.62 ^a	2.36 ± 0.68 ^a	2.53 ± 0.71 ^b	2.70 ± 0.92 ^c	0.0001	2.47 ± 0.76 (2.44, 2.51)	2.57 (1.11-8.87)
PUFA, n-6 series ⁴	28.90 ± 3.09 ^a	28.35 ± 2.52 ^{ab}	28.15 ± 2.59 ^b	28.25 ± 2.69 ^b	0.002	28.39 ± 2.73 (28.25, 28.53)	28.52 (18.43-39.03)
n-3:n-6	0.080 ± 0.02 ^a	0.083 ± 0.02 ^a	0.090 ± 0.03 ^b	0.095 ± 0.03 ^c	0.0001	0.087 ± 0.03 (0.086, 0.088)	0.090 (0.038-0.298)

¹ \bar{x} ± SD. Values in the same row with different superscript letters are significantly different (Scheffe's procedure), $P < 0.05$. EPA, eicosapentaenoic acid (20:5n-3); DHA, docosahexaenoic acid (22:6n-3); AA, arachidonic acid (20:4n-6); PUFA, polyunsaturated fatty acids.

²Obtained by analysis of variance.

³18:3 + 18:4 + 20:3 + 20:4 + 20:5 + 22:5 + 22:6.

⁴18:2 + 18:3 + 20:2 + 20:3 + 20:4 + 22:2 + 22:4 + 22:5.

Our goal in the statistical analysis was to describe plasma phospholipid concentrations of n-3 fatty acids. Subjects were grouped according to categories of fish intake (g/wk) and according to biological and lifestyle factors. The statistical distribution of plasma fatty acid concentrations was checked and was found to be skewed. Therefore, geometric means were used to describe fatty acid concentrations. Arithmetic means are also presented to facilitate comparisons with other surveys. Analysis of variance on the logarithm of plasma fatty acids and Scheffe's procedure were used to compare geometric means between groups. The SDs of the arithmetic means and 95% CIs of the geometric means are also presented. The chi-square test was used to compare the prevalence of CVD risk factors according to sex and age.

The association between plasma phospholipid concentrations of n-3 fatty acids, in particular of EPA and DHA, and values of CVD risk factors was assessed by multiple linear regression analysis. Variables with a skewed distribution were log transformed. The regression analyses were conducted for subjects who were not taking prescribed drugs for hypercholesterolemia, high blood pressure, or diabetes. Adjustments were made for the potential confounding effects of age, sex, waist girth, smoking, alcohol intake, and personal history of CVD (pectoris angina, myocardial infarction, and cardiac arrhythmia). The potentially modifying effect of sex was checked by using an interaction term between sex and CVD risk factors.

We also conducted additional analyses in which we calculated conditional odds ratios to examine the association between the prevalence of CVD risk factors and quintiles of plasma n-3 fatty acid concentrations. Logistic regression was performed to control for the same confounding variables as described above and excluded the same subjects. All statistical analyses were performed with the SAS software package [version 6.12; SAS Institute, Inc, Cary, NC (40)], and statistical significance was set at $P = 0.05$.

RESULTS

The study population consisted of 722 men (\bar{x} ± SD age: 40.6 ± 17.0 y) and 738 women (\bar{x} ± SD age: 39.6 ± 16.4 y) aged 18-74 y. The subjects' average fish intake was 95 g/wk (geometric \bar{x} : 83 g/wk) during

the month before the survey. Fifty percent of all subjects had consumed ≤55 g fish weekly, and 43.3% of these subjects reported that they had not consumed fish at all during the month before the survey. Approximately 25% of subjects reported having consumed between 56 and 120 g fish weekly, and 24% of subjects reported having consumed >120 g fish weekly.

The fatty acid composition of plasma phospholipids found among the study population is shown in **Table 1** according to category of fish intake (g/wk). For the entire population, concentrations of EPA, DHA, and their combination (EPA+DHA) were 0.47%, 1.19%, and 1.70%, respectively. Concentrations of EPA, DHA, and their combination were associated with higher fish intake. For arachidonic acid (AA), concentrations did not vary according to fish consumption. The ratio of EPA to AA and the total amount of n-3 polyunsaturated fatty acids (PUFAs) were positively associated with fish intake. Concentrations of n-6 PUFAs were significantly lower in subjects who consumed ≥56 g fish/wk than in subjects in the lowest fish intake categories. In contrast, higher ratios of n-3 to n-6 PUFAs were found among subjects with higher fish intake.

The relations between fish intake, plasma phospholipid n-3 fatty acids, and potentially confounding variables are summarized in **Table 2**. Fish intake was significantly higher in men than in women. Although differences were not marked, DHA and EPA+DHA concentrations were significantly higher in women than in men, whereas no significant sex differences were observed for EPA, the ratio of EPA to AA, and the ratio of n-3 to n-6 PUFAs. Older persons had higher n-3 fatty acid concentrations, but differences were most significant between the 18-34- and 35-49-y age groups and between the 18-34- and 50-74-y age groups. Fish intake was also positively associated with age, especially when the youngest age group was compared with the oldest age group. Subjects with elevated waist girth values had higher concentrations of EPA and EPA+DHA than did subjects with normal waist girth, but there was no significant difference in fish intake between these groups. Nonsmokers had higher concentrations of DHA and EPA+DHA and a higher ratio of n-3 to n-6 PUFAs than did smokers, even though fish intake was not significantly different between these groups. Subjects who consumed ≥20 alcoholic drinks/wk had higher concentrations of EPA and

TABLE 2

Fish intake and relative concentrations of n-3 fatty acids in plasma phospholipids according to potential confounding variables¹

	Fish intake g/wk	Relative concentrations of n-3 fatty acids			EPA:AA	n-3:n-6
		EPA	DHA	EPA+DHA		
		% by wt of total fatty acids				
Sex						
Male (n = 722)	87.80 ± 132.6 ²	0.48 ± 0.23	1.15 ± 0.53	1.66 ± 0.66	0.075 ± 0.03	0.087 ± 0.03
Female (n = 738)	77.93 ± 96.2	0.46 ± 0.24	1.23 ± 0.49	1.73 ± 0.64	0.076 ± 0.03	0.087 ± 0.03
P ³	0.02	0.07	0.0005	0.04	0.93	0.85
Age (y)						
18-34 (n = 784)	75.30 ± 85.8 ^a	0.44 ± 0.17 ^a	1.09 ± 0.34 ^a	1.56 ± 0.43 ^a	0.071 ± 0.03 ^a	0.081 ± 0.02 ^a
35-49 (n = 432)	83.51 ± 172.8 ^{a,b}	0.49 ± 0.33 ^b	1.23 ± 0.75 ^b	1.75 ± 0.95 ^b	0.077 ± 0.05 ^b	0.089 ± 0.04 ^b
50-74 (n = 244)	90.74 ± 117.3 ^b	0.50 ± 0.25 ^b	1.30 ± 0.54 ^b	1.83 ± 0.70 ^b	0.080 ± 0.04 ^b	0.093 ± 0.03 ^b
P ³	0.01	0.0001	0.0001	0.0001	0.0002	0.0001
Waist girth						
Normal (n = 1296)	81.86 ± 116.4	0.47 ± 0.23	1.19 ± 0.51	1.69 ± 0.65	0.075 ± 0.04	0.087 ± 0.03
Elevated (n = 151)	87.81 ± 112.6	0.52 ± 0.22	1.25 ± 0.51	1.79 ± 0.66	0.079 ± 0.03	0.090 ± 0.03
P ³	0.41	0.01	0.09	0.03	0.21	0.09
Smoking status						
Smoker (n = 424)	78.04 ± 109.9	0.46 ± 0.18	1.10 ± 0.47	1.59 ± 0.56	0.074 ± 0.03	0.084 ± 0.02
Nonsmoker (n = 1034)	84.34 ± 117.9	0.48 ± 0.25	1.23 ± 0.52	1.74 ± 0.68	0.076 ± 0.04	0.089 ± 0.03
P ³	0.19	0.07	0.0001	0.0001	0.45	0.0002
Alcohol intake						
None (n = 177)	85.57 ± 124.6	0.47 ± 0.28 ^a	1.25 ± 0.54 ^a	1.75 ± 0.74 ^{a,b}	0.076 ± 0.04 ^a	0.090 ± 0.03 ^a
1-19 drinks/wk (n = 1235)	81.59 ± 113.0	0.47 ± 0.21 ^a	1.18 ± 0.49 ^a	1.68 ± 0.61 ^a	0.075 ± 0.04 ^a	0.086 ± 0.02 ^a
≥20 drinks/wk (n = 47)	96.42 ± 149.8	0.62 ± 0.36 ^b	1.31 ± 0.71 ^a	1.97 ± 0.99 ^b	0.089 ± 0.04 ^b	0.102 ± 0.04 ^b
P ³	0.43	0.0001	0.02	0.001	0.04	0.0001
Previous history of CVD						
Yes (n = 136)	77.61 ± 144.9	0.51 ± 0.24	1.25 ± 0.56	1.80 ± 0.71	0.079 ± 0.04	0.091 ± 0.03
No (n = 1324)	83.03 ± 112.4	0.47 ± 0.23	1.18 ± 0.50	1.69 ± 0.64	0.075 ± 0.04	0.087 ± 0.03
P ³	0.46	0.03	0.09	0.03	0.18	0.06

¹ Values in the same row with different superscript letters are significantly different (Scheffe's procedure), $P < 0.05$. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; AA, arachidonic acid; CVD, cardiovascular disease.

² Geometric $\bar{x} \pm SD$.

³ Obtained by analysis of variance.

EPA+DHA and higher ratios of EPA to AA and of n-3 to n-6 PUFAs than did other alcohol intake groups. However, fish intake did not vary significantly according to alcohol intake. Concentrations of EPA and EPA+DHA were higher among subjects who had a previous medical history of CVD than in those without such a medical history, but fish intake did not vary significantly according to a previous medical history of CVD. Subjects using medication for hypercholesterolemia had higher concentrations of DHA and EPA+DHA than did nonusers (data not shown). Concentrations of EPA, DHA, and EPA+DHA and ratios of n-3 to n-6 PUFAs were also higher among subjects receiving treatment for high blood pressure (data not shown). The ratio of EPA to AA was significantly lower in subjects being treated for diabetes than in subjects not being treated (data not shown). Fish intake was higher among subjects using medication for high blood pressure than among nonusers, whereas no significant difference was found between groups using or not using medication for hypercholesterolemia and diabetes (data not shown).

For subsequent analyses, 290 of the 1460 subjects were excluded because they reported using medication for hypercholesterolemia, high blood pressure, or diabetes. Among the remaining subjects ($n = 1170$), the prevalence of high-risk concentrations of total and LDL cholesterol was 13.6% and 12.6%, respectively, and was positively associated with age but was not significantly different between men and women (Table 3). In contrast, the prevalence of low HDL-cholesterol concentrations

was 9.1% in men and only 1.9% in women and did not vary significantly according to age. A greater proportion of men than of women were in the high-risk range for the ratio of total to HDL cholesterol (≥ 6) and for triacylglycerol concentrations (≥ 2.3 mmol/L), and the prevalence of both was positively associated with age. High blood pressure was also more prevalent in men than in women and was positively associated with age in both sexes. The prevalence of impaired fasting glucose did not vary according to sex but was positively associated with age in both sexes. Finally, the prevalence of insulin ≥ 90 pmol/L was not significantly different between men and women and did not vary significantly by age.

Shown in Table 4 are the regression coefficients (β values) from the multiple linear regression analysis with CVD risk factor values as dependant variables and plasma phospholipid concentrations of n-3 fatty acids as predictor variables. We found positive associations between EPA and total cholesterol, LDL cholesterol, HDL cholesterol, systolic and diastolic blood pressure, and plasma glucose. In parallel, we found positive associations between DHA and total cholesterol, the ratio of total to HDL cholesterol, triacylglycerols, systolic blood pressure, and plasma glucose and insulin. We found a negative association between DHA and HDL cholesterol. EPA+DHA was also negatively associated with HDL cholesterol and positively associated with the other CVD risk factors. The ratio of EPA to AA had positive associations with total cholesterol, HDL cholesterol, and

TABLE 3

Prevalence (weighted) of cardiovascular disease (CVD) risk factors according to sex and age¹

CVD risk factors	Men					Women					All subjects	
	18-34 y	35-49 y	50-74 y	All	P ²	18-34 y	35-49 y	50-74 y	All	P ²	(n = 1170)	P ³
	(n = 368)	(n = 93)	(n = 124)	(n = 585)		(n = 361)	(n = 127)	(n = 97)	(n = 585)			
	%					%					%	
TC ≥ 6.2 mmol/L	6.6	18.6	29.8	15.1	0.001	3.5	11.8	30.7	12.1	0.001	13.6	0.14
LDL ≥ 4.1 mmol/L	8.1	14.3	21.7	12.8	0.001	3.0	10.4	35.7	12.4	0.001	12.6	0.87
HDL ≤ 0.9 mmol/L	7.2	8.5	14.3	9.1	0.08	2.3	1.8	1.1	1.9	0.73	5.4	0.001
TC:HDL ≥ 6	6.9	12.3	13.9	10.0	0.05	1.5	4.9	9.9	4.4	0.001	7.2	0.001
Triacylglycerols ≥ 2.3 mmol/L	11.0	17.1	28.6	16.5	0.001	4.9	8.8	14.0	8.2	0.01	12.2	0.001
SBP/DBP ≥ 140/90 mm Hg	8.0	8.9	37.8	14.5	0.001	1.4	6.5	25.8	8.3	0.001	11.3	0.001
Glucose ≥ 6.1 mmol/L	0.5	5.5	9.0	3.8	0.001	0.7	0.7	8.2	2.3	0.001	3.0	0.12
Insulin ≥ 90 pmol/L	21.8	20.9	26.3	22.5	0.52	19.1	14.3	23.2	18.2	0.12	20.3	0.08

¹TC, total cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure.²Chi-square test by age.³Chi-square test by sex.

systolic blood pressure and a negative association with the ratio of total to HDL cholesterol. We found positive associations between the ratio of n-3 to n-6 PUFAs and CVD risk factors, except HDL cholesterol and insulin.

To reduce the possibility of a residual modifying effect of sex on the observed associations, separate regression analyses were conducted in men and women for HDL cholesterol, the only CVD risk factor that showed a modification effect with sex (data not shown). No modification effect was found for total cholesterol, LDL cholesterol, the ratio of total to HDL cholesterol, triacylglycerols, systolic and diastolic blood pressure, and plasma glucose and insulin. A positive association between the ratio of EPA to AA and HDL cholesterol was still found in men and women, but the association was stronger in women ($\beta = 0.30$, $P = 0.0001$) than in men ($\beta = 0.18$, $P = 0.008$). In addition, a significant positive association between EPA and HDL cholesterol was found in women ($\beta = 0.21$, $P = 0.002$) but not in men ($\beta = 0.06$, $P = 0.41$). In women, there were no longer inverse associations between HDL cholesterol and DHA, EPA+DHA, and the ratio of n-3 to n-6 PUFAs. We observed only slight differences in the estimates for men when compared with those for the total group.

Additional analyses were performed to examine the association between the prevalence of low HDL-cholesterol concentrations and quintiles of the ratio of EPA to AA by using conditional

odds ratios and comparing subjects in quintiles 2-5 with those in quintile 1. When modeling the probability of HDL-cholesterol concentrations ≤ 0.9 mmol/L, the odds ratio for the group with the highest ratio of EPA to AA was 0.18 (95% CI: 0.05, 0.57) (Figure 1). The threshold value of the ratio of EPA to AA (in the highest quintile) that afforded this protective effect was 0.11.

DISCUSSION

One of the purposes of this investigation was to determine plasma phospholipid concentrations of n-3 fatty acids (EPA and DHA) in a representative sample of the Quebec population. This study was the first to examine the n-3 fatty acid profile of a large population in whom fish intake is relatively low. Fish consumption is not a significant part of the cultural food habits of Quebecers (41). Our results indicate that Quebecers consume, on average, smaller quantities of fish and have substantially lower concentrations of EPA and DHA than do the Japanese and Inuit, but concentrations of EPA and DHA in Quebecers are similar to those of Americans (19, 42-47).

Many published reports showed that n-3 fatty acids measured in phospholipids reflect fish intake (8, 9, 48, 49). In the present study, the dietary questionnaire used to quantify fish intake was not detailed: only 2 questions were used to measure

TABLE 4

Regression coefficients (β values) from multiple linear regression analysis with cardiovascular disease (CVD) risk factor values as dependent variables and relative concentrations of n-3 fatty acids in plasma phospholipids as predictor variables¹

CVD risk factors	n-3 Fatty acids (n = 1170)				
	Log EPA	Log DHA	Log EPA+DHA	Log EPA:AA	Log n-3:n-6
TC	0.52 (0.0003)	0.51 (0.004)	0.71 (0.0003)	0.43 (0.002)	1.17 (0.0001)
LDL	0.28 (0.03)	0.27 (0.09)	0.37 (0.04)	0.21 (0.09)	0.60 (0.006)
HDL	0.14 (0.004)	-0.27 (0.0001)	-0.16 (0.02)	0.24 (0.0001)	-0.11 (0.16)
Log TC:HDL	-0.002 (0.92)	0.13 (0.0001)	0.11 (0.0001)	-0.04 (0.02)	0.13 (0.0001)
Log triacylglycerols	0.06 (0.07)	0.32 (0.0001)	0.31 (0.0001)	-0.04 (0.17)	0.38 (0.0001)
Log SBP	0.02 (0.007)	0.02 (0.01)	0.03 (0.0008)	0.01 (0.03)	0.05 (0.0001)
Log DBP	0.02 (0.01)	0.01 (0.17)	0.02 (0.03)	0.01 (0.06)	0.03 (0.006)
Log glucose	0.02 (0.005)	0.03 (0.004)	0.04 (0.002)	0.01 (0.13)	0.04 (0.002)
Log insulin	0.01 (0.67)	0.13 (0.0001)	0.12 (0.002)	-0.04 (0.19)	0.09 (0.06)

¹One model for each combination of CVD risk factor and n-3 fatty acid. Each model included age, sex, waist girth, smoking, alcohol intake, and personal history of CVD. P values in parentheses. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; AA, arachidonic acid; TC, total cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure.



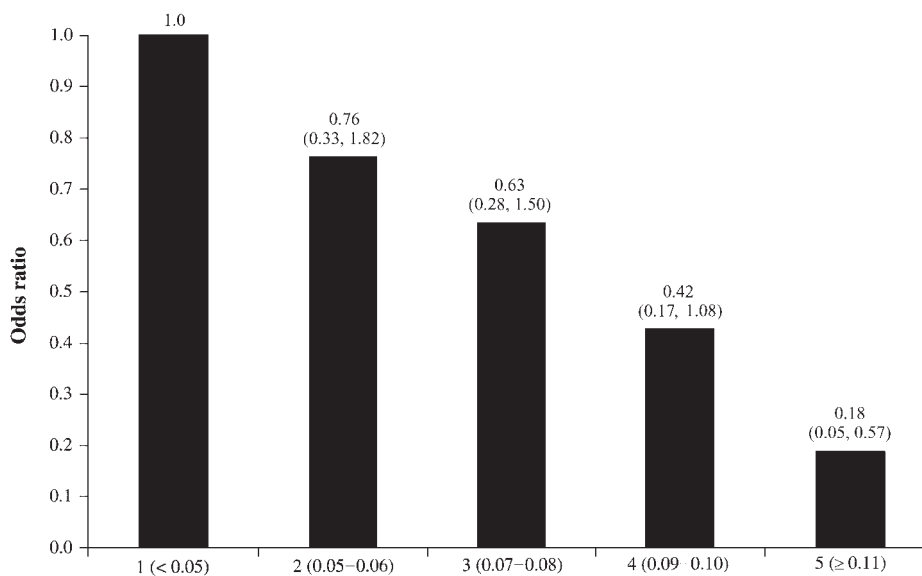


FIGURE 1. Odds ratios (95% CIs) of a prevalent high-risk concentration of plasma HDL cholesterol by quintiles of the plasma phospholipid ratio of eicosapentaenoic acid to arachidonic acid.

fish intake. However, we believe that the food-frequency questionnaire was a fair measure of Quebecer fish intake because plasma phospholipid EPA and DHA concentrations correlated with fish intake. Differences between the geometric means of EPA and DHA were not pronounced between different fish intake groups even though the differences were significant. When values for individuals in the 90th percentile of EPA+DHA ($n = 154$) were examined, the geometric mean EPA+DHA was 2.03% (data not shown), and fish intake for this group was 328 g/wk (geometric \bar{x}). Higher values of EPA+DHA had been expected for this group. Possible explanations for this finding may involve the type of fish eaten and its preparation, as well as the quantity of fish consumed (45, 50, 51).

The EPA and DHA content of fish varies according to species and can depend on habitat, stage of maturity, size, diet, season, and so forth (45, 50, 52, 53). Aquaculture fish contain less $n-3$ fatty acids than do wild fish because of their different feeding patterns and differences in the lipid content of food items (45, 54). Quebecers consume mostly commercial fish as well as some popular species, such as salmon and trout, which originate from aquaculture (41). Cod and plaice are also popular among consumers of fish in Quebec. These species are classified as lean-flesh fish and contain only 0.23% and 0.33% by wt, respectively, of EPA+DHA (55).

We also determined whether fish consumed by Quebecers was fried. For 22% of the sampled population, all fish consumed the month before the survey was fried. When comparing the plasma concentrations of EPA+DHA of fried-fish consumers with those of non-fried-fish consumers, we found that the fried-fish consumers had lower concentrations of EPA+DHA (1.61%; 95% CI: 1.56%, 1.67%) than did the non-fried-fish consumers (1.75%; 95% CI: 1.70%, 1.80%). These results may indicate the importance of dietary EPA and DHA sources and of the preparation methods of fish consumed. Moreover, frying fish often involves the addition of other fats such as saturated fats (50).

No significant differences were found in concentrations of $n-3$ fatty acids (with the exception of DHA) between the group

with no weekly fish intake and the group with fish intake between 1 and 55 g weekly. This may reflect the possibility that subjects who ate little (<55 g/wk) or no fish compensated for this with other dietary sources of EPA and DHA such as poultry and eggs, although these are not normally major sources of dietary EPA and DHA. This discrepancy may also suggest a minimum threshold below which fish intake is not reflected in plasma concentrations of EPA and DHA.

Although it is well known that $n-3$ fatty acids reduce triacylglycerol concentrations in humans, our study did not reveal this beneficial effect. Moreover, EPA and DHA had different relations with triacylglycerol concentrations. Such results may reflect different mechanisms whereby EPA and DHA modify plasma lipids (29, 56–60). In the present study, fish intake was only 15 g fish/d, equivalent to ≈ 170 mg EPA+DHA. It has been estimated that a minimum of 1 $n-3$ fatty acid consumed (EPA+DHA combined)/d can be expected to significantly lower triacylglycerols (57). Recent studies indicate that supplementary intakes of EPA+DHA of $\approx 0.9-1.5$ g/d both retard the progression of CVD in patients (61) and reduce sudden cardiac death rates in patients having previously experienced a myocardial infarction (62). It seems likely that the range of fish intake of Quebecers was not high enough to see a beneficial effect of EPA+DHA on plasma triacylglycerols.


Our results showed a protective effect of EPA and the ratio of EPA to AA on plasma HDL cholesterol. Lower ratios of total to HDL cholesterol were also associated with higher ratios of EPA to AA. In our study, a threshold value of 0.11 for the ratio of EPA to AA, corresponding to an average fish intake of 110 g/wk (median = 82.8 g/wk), afforded this protective effect. In contrast, DHA was negatively associated with HDL cholesterol and the ratio of total to HDL cholesterol. The explanation of the observed relation between the ratio of EPA to AA and the ratio of total to HDL cholesterol is unclear because the association between EPA and the ratio of total to HDL cholesterol was not significant. When EPA is consumed as fish or fish oil in the diet, its concentrations increase in platelet membrane phospholipids,

thereby reducing the availability of AA in tissue phospholipids (49). In contrast, DHA may not efficiently inhibit AA metabolism (29). Bonna et al (60) indicated that the increase of HDL cholesterol after n-3 fatty acid intake is positively related with changes of plasma EPA concentrations and negatively related with the increase of DHA, but the underlying mechanisms remain unexplained. However, Simon et al (63) showed that the concentrations of DHA in serum phospholipids were inversely correlated with the risk of ischemic heart disease. Hence, it seems that, when plasma concentrations of AA are taken into account, the beneficial effect of EPA on the ratio of total to HDL cholesterol is more easily detected.

Most studies that examined the effects of n-3 fatty acids on blood pressure were done using fish oil supplements. In their meta-analysis of 31 controlled trials, Morris et al (64) concluded that the hypotensive effect of fish oil at high doses might be strongest in hypertensive subjects and in those with clinical atherosclerotic disease or hypercholesterolemia. According to Knapp (65), both the amount and type of fat consumed can exert different effects on blood pressure. Thus, our results suggest that EPA and DHA have no beneficial effect on blood pressure when only small quantities of fish are consumed.

We found no protective effects of n-3 fatty acids on plasma glucose and insulin. There are few reports concerning the effect of n-3 fatty acids on plasma glucose and insulin in general populations. Most studies were conducted among patients with type 1 or 2 diabetes (66). It has been suggested that n-3 fatty acids impair glycemic control in patients with type 2 diabetes (67-69). In other studies, glucose control was improved or remained unchanged (67, 70, 71). According to Puhakainen et al (72), differences in the dosage of n-3 fatty acids may provide a potential explanation for the differences in effects of fish oil on glycemia. Storlien et al (27) indicated that a low ratio of n-3 to n-6 PUFAs may be a critical factor in insulin resistance as well as atherosclerosis. Hence, because the fish intake of Quebecers was relatively low, it was probably not high enough to show a protective effect of n-3 fatty acids on plasma glucose and insulin.

In this study we determined the plasma phospholipid concentrations of n-3 fatty acids in a representative sample of Quebecers. Fish intake among the Quebec population was reflected in plasma concentrations of EPA and DHA. Results of our study showed a protective effect of EPA on plasma HDL cholesterol. Results also suggest that when the ratio of EPA to AA is >0.11 in plasma phospholipids, corresponding to an average fish intake of ≥ 110 g/wk, n-3 fatty acids of marine origin may favorably influence some CVD risk factors such as concentrations of HDL cholesterol and ratios of total to HDL cholesterol. However, our data also indicate that Quebecers, in general, have to further increase their daily intake of n-3 fatty acids to obtain recognizable beneficial effects on CVD risk factors. The Government of Canada recommends a total n-3 fatty acid intake of 1.1-1.8 g/d, but it does not distinguish between linolenic acid and n-3 fatty acids of marine origin (73). In the general population, the minimal recommended EPA+DHA intake for adults is 650 mg/d for optimal health and CVD prevention (74). This recommendation corresponds to $\approx 20-62$ g fish/d, depending on the n-3 fatty acid content and amount of fat in the fish consumed (45). These recommendations are subsequent to the numerous studies showing that fish is the main source of EPA and DHA and that a fish-based diet is associated with a low prevalence of CVD. This

knowledge must now be applied to nutrition education programs promoting fish consumption. 

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