Influence of n-3 fatty acids on cardiac autonomic activity among Nunavik Inuit adults

Beatriz Valera¹, Eric Dewailly¹,², Elhadji Anassour-Laouan-Sidi¹, Paul Poirier³,⁴

¹ Axe Santé des Populations et Environnement, Centre de Recherche du CHUQ, Quebec, Canada
² Department of social and preventive medicine, Laval University, Quebec, Canada
³ Quebec Heart and Lung Institute, Laval Hospital Research Centre, Quebec, Canada
⁴ Faculty of Pharmacy, Laval University, Quebec, Canada

ABSTRACT

Objectives. Inuit from Nunavik (northern Quebec) consume large amounts of fish and marine mammals, which are important sources of n-3 polyunsaturated fatty acids (n-3 PUFAs). These substances have a beneficial impact on heart rate (HR) and heart rate variability (HRV). However, it is unknown if this beneficial impact remains significant in populations with high mercury exposure. The study assessed the impact of n-3 PUFAs (Docosahexaenoic [DHA] and Eicosapentaenoic acid [EPA]) on resting HR and HRV among Nunavik Inuit adults considering mercury and other potential confounders.

Study design. Cross-sectional study employing clinical measurements.

Methods. Complete data were collected among 181 adults ≥40 years old (109 women and 72 men) living in the 14 coastal villages of Nunavik. Several indices of HRV were derived from a 2-hour Holter monitoring assessment. n-3 PUFAs levels were measured in membrane erythrocytes. Simple linear regression was used to analyse the relationship between n-3 PUFAs levels and resting HR and HRV parameters while multiple linear regressions were carried out to control for confounders.

Results. In the overall analyses, EPA was associated with SDANN (β=0.07, p=0.04) and LF norm (β=-1.84, p=0.03) after adjusting for confounders. Among women, DHA was associated with resting HR (β=-1.40, p=0.03) while EPA was associated with SDNN (β=0.08, p=0.03), SDANN (β=0.09, p=0.02) and resting HR (β=-2.61, p=0.002). No significant association was observed in men.

Conclusions. These results suggest a beneficial impact of n-3 PUFAs on resting HR and HRV among Nunavik Inuit women.

(Int J Circumpolar Health 2011; 70(1):6-18)

Keywords: n-3 fatty acids, Docosahexaenoic acid (DHA), Eicosapentaenoic acid (EPA), resting heart rate, heart rate variability
INTRODUCTION

n-3 polyunsaturated fatty acids (PUFAs) are essential fatty acids that are found in high concentrations in marine fish and oils. The main n-3 PUFAs of marine origin are Docosahexaenoic (DHA) and Eicosapentaenoic acid (EPA). Levels of these substances are high in Arctic populations since their traditional diet is mainly based on the consumption of fish and marine mammals. In northern Quebec (Canada), high plasma phospholipids concentrations of EPA (mean: 1.99% of fatty acids) and DHA (mean: 4.52% of fatty acids) were detected among Inuit from Nunavik during a health survey conducted in 1992 (1). These levels were higher than those detected in southern Quebec (mean EPA: 0.47% of fatty acids and mean DHA: 1.19% of fatty acids) (2) and in other populations whose diets are not primarily based on seafood consumption (3).

n-3 PUFAs have been associated with decreased risk of ventricular arrhythmias (4,5) and sudden cardiac death (SCD) (6). Some authors have suggested that the beneficial impact on SCD could be mediated by enhancing the heart rate variability (HRV), which represents the sympathetic and parasympathetic activities of the autonomic nervous system (ANS). A positive association between n-3 PUFAs and HRV has been observed in patients with type 1 diabetes mellitus (DM) (7), in those with coronary heart disease (8,9) as well as in healthy subjects (10–13). In contrast, no changes in HRV parameters were observed in some studies conducted among healthy subjects (14–16) and haemodialysis patients (17). Regarding HR, a beneficial impact of n-3 PUFAs has been observed among coronary artery disease patients (18) and among healthy subjects (3,19,20). In addition, the results of a meta-analysis conducted by Mozaffarian et al. showed that fish oil decreased HR by 1.6 bpm compared to placebo (21).

Despite the potential beneficial effects attributed to n-3 PUFAs, fish consumption could also involve some cardiovascular risk. Fish and marine mammals accumulate high quantities of mercury that has been associated with decreased HRV in adults (22–24). Since it is important for Inuit to know the risks and benefits of the traditional diet, we aimed to assess the impact of n-3 PUFAs on HRV and resting HR taking into account potential confounding factors. Also, we tested the potential interaction between mercury and n-3 PUFAs in order to know if mercury modified the impact of n-3 PUFAs on HRV and resting HR.

MATERIAL AND METHODS

Study population and sampling

Data used in this study were collected in the 14 coastal communities of Nunavik in 2004. The target population included all permanent residents, except for non-Inuit households and individuals living full time in public institutions. The survey plan was a complex 2-stage stratified random sampling. The first stage consisted of selecting a stratified random sample of private Inuit households with proportional allocation. The community was the only stratification variable used. This stratification allowed the representation of the target population to be up to standard. Since home addresses (civic numbers) in some municipalities are consecutive, the survey frame was sorted first by home addresses, followed by a systematic draw of a predetermined number of households to avoid selection of 2 immediate neighbours. Since many Inuit regularly move from one house to another, it was decided to sample households instead of individuals. The assumption was that recruiting a
member of a household rather than a specific individual, would increase coverage of the target population. To obtain a good representation of each community, a proportional allocation of sample units corresponding to the size of each village was chosen. It was important to choose households from all 14 communities since the distances separating villages could be associated with significant differences in lifestyle. In the second stage, all eligible people were asked to participate according to the survey steps or instruments. Among the 670 eligible households, 521 agreed to participate which corresponds to a response rate of 77.8%. HRV measurements were restricted to adults ≥40 years. Among 472 individuals eligible to this section, 211 accepted to participate. Forty-seven percent (47%) of exclusions (n=122) were due to refusal to sign a consent form. Moreover, 69 individuals had non-valid Holter recordings (26%) and 70 (27%) were excluded due to having a pacemaker, having no time, an unspecified reason, device not available or being handicapped. Furthermore, we excluded 1 individual with no information on blood mercury concentration, 5 individuals who were not Inuit, and 24 individuals with anti-arrhythmic or beta-blocker medication at the moment of data collection. Thus, 181 individuals (109 women and 72 men) were included in the statistical analyses.

**Data collection**

All the information used in this study was gathered on board the research icebreaker *Amundsen*. For this purpose, each participant was invited to fill out questionnaires and to attend a clinical evaluation. Each individual who agreed to participate in the survey signed a consent form. The study protocol was approved by the ethics committee of Laval University.

Questionnaires were used to gather information on age, sex, smoking habits, alcohol consumption, total income, menopausal status as well as leisure time physical activity. Details on the method used to estimate the physical activity are published elsewhere (25). Information regarding cardiovascular disease (CVD) and medication was obtained from medical files.

During the clinical session, blood samples were drawn and anthropometric and physiological measurements were taken. Waist circumference (WC) was obtained using a graduated inelastic tape when subjects were in a standing position (26). Body mass index (BMI) was calculated by dividing the weight by the squared height.

Physiological measurements included HRV and resting HR. HRV indices were derived from an ambulatory Holter monitoring system (GE MARQUETTE SERIES 8500) using 7 leads (derivations V5, V1, and AVF). The HRV recording was carried out on board the icebreaker *Amundsen* and started around 8:00 a.m. or 1:00 p.m. The HRV was registered during a 2-hour period and participants were allowed to do different activities such as walking, fill out questionnaires and undergo anthropometric measurements. Interpretation and extraction of HRV parameters were performed automatically by using the software provided by General Electric (MARS PC Ambulatory ECG Analysis System) while the complete signal was carefully edited using visual checks and manual corrections of individual R-R intervals and QRS complex classifications. For the calculation of HRV parameters, only R-R intervals between QRS complexes of sinus origin were used. Intervals whose duration was <80% or >120% of that of the running R-R average were excluded. Time domain parameters included the standard deviation of R-R intervals (SDNN), the standard deviation of the average R-R intervals calculated over 5-minute periods (SDANN), the square root of the mean squared differences of successive R-R inter-
vals (rMSSD) and the proportion of interval differences of successive R-R intervals >50 ms (pNN50). Both rMSSD and pNN50 are indices of cardiac parasympathetic modulation, while SDNN represents the overall variability and SDANN represents the long-term variability. Fast Fourier transformation was used to obtain frequency domain indices such as low frequency (LF=0.04–0.15 Hz), which represents both sympathetic and parasympathetic activity, and high frequency (HF=0.15–0.40 Hz), which is an index of parasympathetic activity. The LF/HF ratio represents the sympatho-vagal balance (27). LF and HF were also expressed in normalized units that represent the relative value of each power component in proportion to the total power minus the VLF component (28). Resting HR was measured by taking the pulse in the right wrist for 30 seconds or 60 seconds if the pulse was irregular. Before the pulse measurement was taken, subjects were required to stay quiet for at least 5 minutes and to have not eaten or smoked for at least 30 minutes prior to the measurement.

**Laboratory analyses**

For the determination of the fatty acid composition of the membranes of erythrocytes, a quantity of 600 µL of red blood cells were thawed at room temperature, centrifuged at 3000g for 5 minutes and washed 3 times with 0.9% saline solution. Lipids were extracted with chloroform/methanol (2:1, by volume). Then the extracted lipids were methylated with methanol/benzene 4:1 (v/v) and 200 µL acetyl chloride. The fatty acid profile was determined by gas chromatography (HP 5890 gas chromatograph equipped with an automated injector 7673A) and a flame ionization detector (Hewlett Packard, Toronto, Canada). Concentrations of plasma total cholesterol (total-cholesterol), triglycerides, low-density lipoprotein (LDL-cholesterol) and high-density lipoprotein (HDL-cholesterol) were analysed according to methods of the Lipid Research Clinics (U.S. Department of Health). Cholesterol and triglycerides concentrations were determined in plasma and in the lipoprotein fractions using an Auto-Analyzer II (Technicon Instruments Corporation, Tarrytown, New York). The HDL fraction was obtained after precipitation of LDL in the infranatant with heparin and manganese chloride. LDL-cholesterol was calculated using Friedewald's formula (29). Insulin determination was performed with an Auto-analyzer Roche Modular analytics E170 (Elecsys module) using a commercial double-antibody radioimmunoassay. Plasma glucose was measured enzymatically through a reaction with hexokinase. Insulin sensitivity was estimated from the HOMA model (Homeostasis model assessment) as fasting insulin times fasting glucose/22.5 (30). Blood mercury concentrations were measured by inductively coupled plasma mass spectrometry (ICP-MS). Blood samples were diluted 20 fold in a solution containing ammonium hydroxide before analysis. The detection limit was 0.5 nmol/L.

**Statistical analyses**

In the first step, all the variables were described. Continuous variables with skewed distribution were log-transformed and the geometric means (95% confidence interval) were presented. For continuous variables with normal distribution, the arithmetic means (95% confidence interval) were presented. Descriptive statistics were presented by sex, and characteristics were compared using student t-test for continuous variables and chi-square for categorical variables. Simple regressions were applied to study the relationship between n-3 PUFAs and the outcomes (HRV and resting HR), while multivariable linear regressions were used to control for confounders. In selecting the confounding factors, all variables associated with
RESULTS

The characteristics of the participants are presented in Table I. Mean age was 51.1 years (95% CI: 49.8–52.5 years) and the sample was composed of 109 women (60.2%) and 72 men (39.8%). Concentrations of DHA and EPA increased significantly with age (r=0.40, p<0.0001 and r=0.49, p<0.0001, respectively). Levels of DHA tended to be higher in women than in men (6.94 vs. 6.46% of fatty acids, p=0.06) while no significant differences were observed for EPA levels (2.21 vs. 2.01% of fatty acids, p=0.26). Moreover, women had higher HDL levels than men (1.96 vs. 1.54 mmol/L, p<0.0001) and lower SBP (120 vs. 125 mmHg, p=0.004) and DBP levels (73 vs. 77 mmHg, p=0.009). Fifty-three participants suffered from CVD (hypertension, ischemic heart disease and/or other CVD). Also, 26 participants were taking anti-hypertensive medication other than beta-blockers at the moment of data collection, and 4 participants were taking nitroglycerin for angina pectoris treatment. A description of the dependent variables is presented in Table II. Women had higher HF (160 vs. 119 ms², p=0.02) and higher resting HR than men (76 vs. 71 bpm, p=0.0008). In addition, LF/HF ratio (2.4 vs. 3.7, p<0.0001) was lower among women. DHA and EPA were correlated with blood mercury concentrations (r=0.53, p<0.0001 and r=0.63, p<0.0001, respectively).

In the overall analyses (Table III), the association between EPA and LF norm remained significant (β=-1.84, p=0.02), while the association with SDANN became significant (β=0.07, p=0.04) after adjusting for confounders. The interaction terms between DHA, EPA and mercury were not statistically significant (p>0.05 in all models). The interaction term between DHA and sex was statistically significant for SDNN (p=0.04) and SDANN (p=0.04), and it was near the significance level.
for HR \((p=0.05)\). The interaction term between EPA and sex was significant for SDNN \((p=0.02)\), SDANN \((p=0.03)\) and resting HR \((p=0.03)\). When analyses were stratified by sex, DHA and EPA were negatively correlated with resting HR among women \((r=-0.24; p=0.005\) and \(r=-0.29, p=0.0007\), respectively). The correlation between EPA+DHA and resting HR is presented in Figure 1. In addition, a positive correlation was observed between EPA and SDANN \((r=0.19, p=0.048)\). In multivariable analyses (Table IV), the association between DHA and resting HR \((\beta=-1.40, p=0.03)\) remained significant. Furthermore, we observed significant associations between EPA and SDNN \((\beta=0.08, p=0.03)\), SDANN \((\beta=0.09, p=0.02)\) and resting HR \((\beta=-2.61, p=0.002)\). No significant association was observed between n-3 PUFAs and resting HR or HRV in men.

### Table 1. Characteristics of the participants.

| Clinic and sociodemographic variables | n | All (n=181) Mean (95% CI) Range | Women (n=109) Mean (95% CI) | Men (n=72) Mean (95% CI) | p-value^b |
|---------------------------------------|---|---------------------------------|-------------------------------|---------------------------|-----------|
| Age (years)                           | 181 | 51.1 (49.8-52.5) 40-71 | 50.2 (48.5-51.8) 42.5-55.3 | 52.2 (50.0-54.4) 50.0-56.5 | 0.13 |
| EPA (% fatty acids)*                  | 181 | 2.1 (1.9-2.3) 0.5-7.3 | 2.2 (2.0-2.4) 1.5-3.5 | 2.0 (1.7-2.3) 1.8-3.2 | 0.26 |
| DHA (% fatty acids)                   | 181 | 6.7 (6.5-6.9) 1.6-10.1 | 6.9 (6.7-7.2) 5.8-8.5 | 6.5 (6.0-6.9) 5.4-7.9 | 0.062 |
| Fasting insulin (pmol/L)^a            | 155 | 46 (43-49) 15-243 | 51 (46-57) 47-216 | 46 (41-51) 38-191 | 0.79 |
| Fasting glucose (mmol/L)^a            | 153 | 4.7 (4.6-4.8) 2.8-8.3 | 4.7 (4.5-4.8) 2.6-8.0 | 4.7 (4.5-4.8) 3.5-9.1 | 0.97 |
| Homa-IR                               | 148 | 9.1 (8.4-9.9) 1.9-51.2 | 9.3 (8.4-10.3) 2.9-26.0 | 9.0 (7.9-10.3) 1.9-27.0 | 0.77 |
| Total-Cholesterol (mmol/L)            | 153 | 5.5 (5.4-5.7) 3.5-8.2 | 5.6 (5.4-5.8) 3.1-8.0 | 5.5 (5.2-5.7) 3.4-7.6 | 0.27 |
| LDL-Cholesterol (mmol/L)              | 153 | 3.2 (3.1-3.4) 1.3-6.1 | 3.1 (2.9-3.2) 1.4-6.3 | 3.4 (3.1-3.6) 2.2-7.4 | 0.098 |
| HDL-Cholesterol (mmol/L)              | 153 | 1.76 (1.67-1.84) 0.86-3.11 | 1.96 (1.86-2.06) 0.86-3.11 | 1.5 (1.4-1.6) 0.86-3.11 | <0.0001 |
| Triglycerides (mmol/L)^a              | 153 | 1.1 (1.0-1.2) 0.3-5.5 | 1.1 (1.0-1.2) 0.4-5.5 | 1.1 (1.0-1.2) 0.4-5.5 | 0.82 |
| BMI (Kg/m^2)                          | 178 | 28.2 (27.0-28.4) 18.4-45.3 | 28.1 (27.0-29.1) 18.4-45.3 | 28.3 (27.3-29.4) 18.4-45.3 | 0.72 |
| Waist circumference (cm)              | 180 | 94 (93-96) 71-131 | 93 (90-95) 71-131 | 96 (93-98) 71-131 | 0.17 |
| Mercury (nmol/L)^a                    | 181 | 96.5 (86.1-108.2) 2.4-760.0 | 99.8 (87.2-114.2) 2.4-760.0 | 93.3 (77.5-112.4) 2.4-760.0 | 0.55 |
| SBP (mm Hg)                           | 181 | 122 (120-125) 85-187 | 120 (122-129) 85-187 | 125 (117-122) 85-187 | 0.004 |
| DBP (mm Hg)                           | 181 | 75 (74-76) 55-111 | 73 (72-75) 55-111 | 77 (75-79) 55-111 | 0.009 |
| Smoking habits (n)                    | 176 | Daily smoker 109 69 40 | 107 66 39 | 122 73 43 | 0.23 |
|                                      |    | Occasionally 6 5 1 | 6 5 1 | 6 5 1 | 0.93 |
|                                      |    | Not at all 61 33 28 | 61 33 28 | 61 33 28 | 0.93 |
| Physical activity (n)                 | 176 | Active 23 14 23 | 23 14 23 | 23 14 23 | 0.93 |
|                                      |    | Moderate active 14 9 14 | 14 9 14 | 14 9 14 | 0.93 |
|                                      |    | Somewhat Active 18 10 18 | 18 10 18 | 18 10 18 | 0.93 |
|                                      |    | Sedentary 121 75 121 | 121 75 121 | 121 75 121 | 0.93 |
| No menses in the last 12 months (n)   | 41  | Menopause 22 | 22 | 22 | 0.09 |
|                                      |    | Hysterectomy 19 | 19 | 19 | 0.09 |
| Total income (n)                      | 151 | <$20,000 73 42 31 | 73 42 31 | 50 22 15 | 0.09 |
|                                      |    | $20,000-$40,000 41 29 12 | 41 29 12 | 29 12 7 | 0.09 |
|                                      |    | $40,000-$60,000 26 12 14 | 26 12 14 | 26 12 14 | 0.09 |
|                                      |    | >$60,000 11 4 7 | 11 4 7 | 11 4 7 | 0.09 |
| Alcohol consumption (n)               | 158 | Yes 130 73 57 | 130 73 57 | 73 40 23 | 0.001 |
|                                      |    | No 28 25 5 | 28 25 5 | 28 25 5 | 0.001 |

'a For log-transformed variables, geometric means are presented.

'b Results of the student t-test (continuous variables) and the chi-square test (categorical variables).

EPA: Eicosapentaenoic acid; DHA: Docosahexaenoic acid; LDL: low density lipoprotein; HDL: high density lipoprotein; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure.
n-3 fatty acids and heart rate variability

Table II. Resting HR and HRV parameters.

| Resting HR and HRV parameters | All (n=181) Mean (95% CI) | Women (n=109) Mean (95% CI) | Men (n=72) Mean (95% CI) | p-value |
|-------------------------------|---------------------------|-------------------------------|--------------------------|---------|
| LF (ms²)ᵃ | 410 (362-464) / 6.0ᵇ | 27-6596 | 385 (326-456) / 5.9ᵇ | 437 (365-523) / 6.1ᵇ | 0.32 |
| LF norm (nu) | 73 (71-75) | 44-93 | 70 (68-72) | 78 (76-79) | <0.0001 |
| HF (ms²)ᵇ | 138 (122-156) / 4.9ᵇ | 19-2329 | 160 (135-189) / 5.1ᵇ | 119 (100-143) / 4.8ᵇ | 0.02 |
| HF norm (nu) | 25 (7-56) | 7-55 | 30 (28-32) | 22 (21-24) | <0.0001 |
| LF/HF | 3.0 (2.8-3.2) | 0.8-13.3 | 2.4 (2.2-2.6) | 3.7 (3.3-4.1) | <0.0001 |
| SDNN (ms)ᵃ | 77 (73-80) | 29-226 | 75 (71-77) | 78 (73-85) | 0.40 |
| SDANN (ms)ᵃ | 47 (45-50) | 12-189 | 47 (44-50) | 48 (44-53) | 0.76 |
| rMSSD (ms)ᵃ | 28 (27-29) | 12-93 | 28 (26-30) | 28 (26-30) | 0.76 |
| pNN50 (%)ᵇ | 5.3 (4.4-6.3) | 0-57.4 | 5.3 (4.2-6.7) | 5.5 (4.1-7.0) | 0.84 |
| Resting HR (bpm) | 73 (72-74) | 53-100 | 76 (74-77) | 71 (68-72) | 0.0008 |

ᵃ For variables not normally distributed, the geometric means are shown. ᵇ Ln mean. ᶜ Student’s t-test.

LF: low frequency; HF: high frequency; SDNN: standard deviation of R-R intervals; SDANN: standard deviation of the average R-R intervals calculated over 5-minute periods; rMSSD: square root of the mean squared differences of successive R-R intervals; pNN50: proportion of interval differences of successive R-R intervals >50 ms.

Table III. Results of the simple and multivariable regression (overall analysis).

| Dependent variables | DHA (% of fatty acids) |  |  |  | EPA (% of fatty acids) |  |  |  |
|---------------------|------------------------|------------------------|----------------|------------------------|------------------------|------------------------|----------------|----------------|
|                     | Crude ğ (p-value)       | Adjusted ğ (p-value)   |  |  | Crude ğ (p-value)       | Adjusted ğ (p-value)   |  |  |  |
| LF (ms²)ᵃ | -0.11 (0.004) | -0.0001 (0.99) |  |  | -0.18 (0.0004) | -0.03 (0.70) |  |  |  |
| LF norm (nu) | -1.02 (0.01) | -0.85 (0.08) | -1.47 (0.01) | -1.84 (0.03) |  |  |  |  |
| HF (ms²)ᵇ | -0.06 (0.12) | 0.02 (0.76) | -0.11 (0.02) | 0.05 (0.56) |  |  |  |  |
| HF norm (nu) | 1.02 (0.01) | 0.04 (0.94) | 1.47 (0.01) | 1.16 (0.18) |  |  |  |  |
| LF/HF | -0.05 (0.01) | -0.01 (0.76) | -0.07 (0.01) | -0.04 (0.28) |  |  |  |  |
| SDNN (ms)ᵃ | -0.01 (0.38) | 0.01 (0.65) | -0.02 (0.27) | 0.03 (0.23) |  |  |  |  |
| SDANN (ms)ᵃ | -0.01 (0.76) | 0.01 (0.57) | 0.002 (0.92) | 0.07 (0.04) |  |  |  |  |
| rMSSD (ms)ᵃ | -0.02 (0.13) | -0.004 (0.87) | -0.03 (0.08) | 0.03 (0.38) |  |  |  |  |
| pNN50 (%)ᵇ | -0.05 (0.44) | 0.001 (0.99) | -0.07 (0.25) | 0.11 (0.32) |  |  |  |  |
| Resting HR (bpm) | -0.23 (0.60) | -0.06 (0.92) | -0.67 (0.16) | -1.08 (0.17) |  |  |  |  |

ᵃ Log-transformed variables.

LF: low frequency; HF: high frequency; SDNN: standard deviation of R-R intervals; SDANN: standard deviation of the average R-R intervals calculated over 5-minute periods; rMSSD: square root of the mean squared differences of successive R-R intervals; pNN50: proportion of interval differences of successive R-R intervals >50 ms.

For the selection of confounders, we included all variables associated with the outcomes at p<0.20 in the initial models. Afterwards, the change-in-estimate method was used to select those variables that modulated the regression coefficient 10% or more.

LF was adjusted for age, HOMA-IR, waist circumference, smoking, alcohol and mercury; LF norm was adjusted for age, HOMA-IR, waist circumference, smoking, alcohol and mercury; HF was adjusted for age, HOMA-IR, waist circumference, triglycerides, HDL-cholesterol, smoking, alcohol, sex and mercury; HF norm was adjusted for age, HOMA-IR, waist circumference, triglycerides, HDL-cholesterol, smoking, alcohol, sex and mercury; LF/HF was adjusted for age, LDL-cholesterol, HDL-cholesterol, sex and mercury; SDNN was adjusted for HOMA-IR, triglycerides, waist circumference, smoking and mercury; SDANN was adjusted for HOMA-IR, triglycerides, waist circumference, smoking and mercury; rMSSD was adjusted for HOMA-IR, triglycerides, age, waist circumference, smoking, alcohol and mercury; pNN50 was adjusted for HOMA-IR, triglycerides, age, waist circumference, smoking, alcohol and mercury; resting HR was adjusted for age, sex, waist circumference, HOMA-IR, smoking, alcohol and mercury.
n-3 fatty acids and heart rate variability

Figure 1. Pearson correlation between DHA+EPA and resting HR among women.

Table IV. Adjusted regression coefficients (p-value) between n-3 PUFAs and HRV stratified by sex.

| Dependent variables | Women          | Men            |
|---------------------|----------------|----------------|
| DHA (% of fatty acids) | 0.06 (0.58) | -0.01 (0.83) |
| EPA (% of fatty acids) | -0.10 (0.25) | -0.21 (0.82) |
| LF (ms²)            | -0.03 (0.38) | 0.03 (0.30)   |
| LF norm (nu)        | 0.06 (0.06)  | -0.01 (0.56)  |
| HF (ms²)            | 0.06 (0.13)  | -0.04 (0.65)  |
| HF norm (nu)        | 0.03 (0.40)  | -0.02 (0.42)  |
| rMSSD (ms)          | 0.06 (0.58)  | -0.14 (0.87)  |
| pNN50 (%)           | 0.06 (0.02)  | -0.14 (0.87)  |
| Resting HR (bpm)    | -1.40 (0.03) | -1.00 (0.31)  |

*Log-transformed variables.

LF: low frequency; HF: high frequency; SDNN: standard deviation of R-R intervals; SDANN: standard deviation of the average R-R intervals calculated over 5-minute periods; rMSSD: square root of the mean squared differences of successive R-R intervals; pNN50: proportion of interval differences of successive R-R intervals >50 ms.

Confounding factors among women:
- LF was adjusted for age, waist circumference, smoking, alcohol, menopause and mercury; LF norm was adjusted for age, waist circumference, smoking, alcohol, menopause and mercury; HF was adjusted for age, waist circumference, HOMA-IR, smoking, alcohol, menopause and mercury; HF norm was adjusted for age, waist circumference, HOMA-IR, smoking, alcohol, menopause and mercury; LF/HF was adjusted for age, smoking, menopause and mercury; SDNN was adjusted for age, waist circumference, HOMA-IR and mercury; SDANN was adjusted for HOMA-IR, menopause and mercury; rMSSD was adjusted for age, waist circumference, HOMA-IR, menopause and mercury; pNN50 was adjusted for age, waist circumference, HOMA-IR and mercury; resting HR was adjusted for age, smoking and mercury

Confounding factors among men:
- LF was adjusted for age, waist circumference, HOMA-IR and mercury; LF norm was adjusted for age, waist circumference, HOMA-IR and mercury; HF was adjusted for age, waist circumference, HOMA-IR and mercury; HF norm was adjusted for age, waist circumference and mercury; LF/HF norm was adjusted for age and mercury; SDNN was adjusted for waist circumference, HOMA-IR and mercury; SDANN was adjusted for waist circumference, HOMA-IR and mercury; rMSSD was adjusted for waist circumference, HOMA-IR, alcohol and mercury; pNN50 was adjusted for waist circumference, alcohol and mercury; resting HR was adjusted for waist circumference, HOMA-IR, alcohol and mercury.
DISCUSSION

In the present study, EPA was associated with a decreasing LF norm and an increasing SDANN. However, when the analyses were stratified by sex, EPA and DHA were associated with decreasing resting HR only among women. In addition, EPA was associated with increasing SDNN and SDANN while the association between DHA and SDNN was near the significance level. These results remained significant after adjusting for mercury and other confounders, which is a relevant information considering the high mercury levels present in this population. To our knowledge, this is the first population-based study to show an association between n-3 PUFAs and resting HR and HRV while controlling for mercury exposure.

The results of the present study suggest a beneficial impact of n-3 PUFAs on resting HR and HRV. The latter represents the sympathetic and parasympathetic activities of the ANS. Since resting HR is mainly regulated by the parasympathetic activity, we could hypothesize that EPA and DHA have a beneficial impact on this branch of the ANS. However, the HRV analysis showed only significant associations with parameters reflecting the overall HRV (SDNN and SDANN), namely, the variations due to both sympathetic and parasympathetic activity.

A beneficial impact of n-3 PUFAs on resting HR and HRV has been reported in clinical trials involving patients with various illnesses (7–9,32) as well as healthy subjects (10,11,13,19,20). In contrast, few population-based studies have assessed the influence of n-3 PUFAs consumption on these outcomes (3,12,33). Mozaffarian et al. have observed a significant increase in normalized HF and a decrease in normalized LF among American adults when they increase their tuna or other fish consumption (12). Our results agree with the previous study, since EPA was associated with decreasing normalized LF in the overall analyses. However, when the analyses were stratified by sex, we observed significant associations between DHA, EPA and resting HR and SDNN and SDANN only in women. The results regarding HR are in line with a clinical trial that assessed the effects of supplementation with DHA (free of EPA) on selected cardiovascular disease risk factors, including HR in postmenopausal women (20). Also, our results are in line with those observed by Mozaffarian et al. among 5096 American men and women (33). In that study, 1 g/day higher EPA + DHA intake was associated with 2.3 beats/min lower HR. Our results also agree with those reported by Dallongeville et al. (3), who studied 9,758 men without coronary heart disease (CHD) and who were recruited in Lille, Strasbourg, and Toulouse, France and Belfast, Ireland. Among these men, the resting HR remained lower among fish consumers after adjusting for confounders. Our results that show a 1% increment of DHA and EPA as being associated with a decrease of 1.4 bpm and 2.6 bpm, respectively, among women are in line with a pooled estimate obtained in a meta-analysis that assessed the effect of using fish oil supplementation on HR (21). Differences in the impact of n-3 PUFAs between women and men could be due to varying levels of estrogen, since these hormones have been suggested to have a cardio-protective effect in young women as well as in postmenopausal women who are undergoing hormone replacement therapy (34–36). In this sample, 41 women (30%) were classified as being menopausal if they had not had a period in the last 12 months or if they had had a hysterectomy. Adjustment for menopausal status did not change the associations between n-3 PUFAs and resting HR.
or HRV. However, it would be more appropriate to adjust for estrogen levels since 70% of women might still have high estrogen levels. No adjustment for this variable could lead to an overestimation of the n-3 PUFAs effects.

The potential anti-arrhythmic effect of n-3 PUFAs is supported by results of animals and in vitro studies. Some studies have observed a beneficial impact of n-3 PUFAs on the prevention of ventricular arrhythmias in dogs (37), rats (38,39) and nonhuman primates (40). Regarding in vitro studies, Kang and Leaf observed that addition of low micromolar concentrations of EPA or DHA slowed the beating rate of the myocytes (41). The anti-arrhythmic effects of n-3 PUFAs may be mediated by increased parasympathetic activity but also by modulation of ion channels (Na⁺, K⁺, Ca²⁺) (42). To our knowledge, no experimental evidence exists regarding the impact of n-3 PUFAs on the parasympathetic activity. However, Nishimura et al. observed that the administration of EPA to diabetic rats decreased cardiac noradrenalin concentration, which suggests a decrease in cardiac sympathetic activity (43).

A limitation of our study could be the relatively low participation rate for ambulatory electrocardiograms (34.2%), which could lead to a selection bias. However, we compared the excluded subjects due to invalid Holters to those included in the analyses, and both groups were similar regarding levels of DHA and EPA. Since the exclusion of these individuals was not linked to the exposure, the risk of selection bias was unlikely. In addition, we compared excluded individuals versus those with valid HRV data regarding age, sex, cholesterol (LDL and HDL), triglycerides, insulin resistance, alcohol consumption, smoking and waist circumference. The results did not reveal significant differences, which suggest that the individuals included in the analyses represented those with no valid HRV data. Furthermore, the weighting method used permits to minimize the bias due to non-response, which consequently minimized the risk of selection bias. Another possible limitation could be related to the relatively small sample size among men which could have reduced the statistical power and thus, affect the detection of significant associations. Of note, most of the studies reporting a significant association between n-3 PUFAs and HR and HRV in men involved a larger sample size (3,19). Finally, our study dealt with the limitation of cross-sectional designs where it was not possible to establish a cause–effect relationship. However, the associations obtained among Inuit women will be assessed during the follow-up of participants that will be implemented in this population in the future.

Strengths of our study involved the use of biological measures of n-3 PUFAs. Some population-based studies reporting an impact of n-3 PUFAs on resting HR or HRV have assessed the intake of n-3 PUFAs using food frequency questionnaires (12,33,44). Even if those questionnaires are validated, the use of biological measures provides a more accurate measurement of the n-3 PUFAs intake and consequently, minimizes the information bias. Furthermore, the method used for recording HRV is placebo-free since it permits to measure HRV during routine activities and minimise the stress of being in a doctor’s office. The relatively low sampling frequency used in this study may have affected the spectrum (45). However, the method used in the spectral analysis (Fourier transformation) performed an interpolation of R-R intervals, which refined the R-wave and resulted in satisfactory results (28). Also, in addition to the HRV
traditional risk factors (age, sex, obesity, insulin sensitivity [measured as HOMA-IR], cholesterol [HDL and LDL-cholesterol] and triglycerides levels, smoking habits, alcohol consumption, physical activity, menopausal status and socioeconomic status), we considered the impact of mercury exposure. These results are particularly important since mercury has been associated with decreased HRV in populations with a high mercury intake, including the Inuit from Nunavik (22–24). However, no impact of mercury on resting HR was observed in this population (unpublished observations) and in other studies (22,24), which suggests that fish consumption may offer some protection regarding resting HR even in highly mercury-exposed populations. Consequently, the consumption of fish that are rich in n-3 PUFAs and low in mercury is encouraged in order to maximize the protective effect of n-3 PUFAs on resting HR and HRV.

Among Nunavik Inuit adults, EPA was associated with enhanced HRV. Moreover, both DHA and EPA were associated with slower resting HR and increased HRV among women while considering the impact of mercury among several confounders. These results support the hypothesis of a potential anti-arrhythmic effect of marine n-3 PUFAs on mercury-exposed adults.

Acknowledgements
We would like to thank the Quebec Ministry of Health, Nunavik Board of Health, Canadian Institute for Health Research, the Northern Contaminant Program (Indian and Northern Development), and the Fonds de recherche en santé du Québec (FRSQ), as well as the ArcticNet network for their support. We also thank the participants and Claudette Fortin from Laval Hospital who analysed the electrocardiograms, and Belkacem Abdous for his useful statistical comments. Beatriz Valera is a doctoral scholar from FRSQ, Paul Poirier is a clinician-research scholar from the FRSQ.

Conflict of interest statement
None to declare.

REFERENCES
1. Dewailly E, Blanchet C, Lemieux S, Sauvé L, Gingras S, Ayotte P et al. n-3 Fatty acids and cardiovascular disease risk factors among the Inuit of Nunavik. Am J Clin Nutr 2001;74(4):464–473.
2. Dewailly E, Blanchet C, Gingras S, Lemieux S, Sauvé L, Bergeron J et al. Relations between n-3 fatty acid status and cardiovascular disease risk factors among Quebecers. Am J Clin Nutr 2001;74(5):603–611.
3. Dallongeville J, Yarnell J, Ducimetiere P, Arveiller D, Ferrieres J, Montaye M et al. Fish consumption is associated with lower heart rates. Circulation 2003;108(7):820–825.
4. Christensen JH, Riahi S, Schmidt EB, Melgaard H, Kirstein Pedersen A, Heath F et al. n-3 Fatty acids and ventricular arrhythmias in patients with ischaemic heart disease and implantable cardioverter defibrillators. Eurpace 2005;7(4):338–344.
5. Aarsetoy H, Ponitz V, Nilsen OB, Grunth H, Harris WS, Nilsen DW. Low levels of cellular omega-3 increase the risk of ventricular fibrillation during the acute ischaemic phase of a myocardial infarction. Resuscitation 2008;78(3):258–264.
6. Marchioli R, Barzi F, Bomba E, Chieffo C, Di Gregorio D, Di Mascio R et al. Early protection against sudden death by n-3 polysaturated fatty acids after myocardial infarction: time-course analysis of the results of the Gruppo Italiano per lo Studio della Sopravvivenza nell’Infarto Miocardico (GISSI)-Prevenzione. Circulation 2002;105(16):1897–1903.
7. Christensen JH, Skou HA, Madsen T, Tørring I, Schmidt EB. Heart rate variability and n-3 polysaturated fatty acids in patients with diabetes mellitus. J Intern Med 2001;249(6):545–552.
8. Christensen JH, Gustenhoff P, Korup E, Aarøe J, Tøft E, Møller J et al. Effect of fish oil on heart rate variability in survivors of myocardial infarction: a double blind randomised controlled trial. BMJ 1996;312(7032):677–678.
9. Villa B, Calabresi L, Chiesa G, Rised P, Galli C, Sirtori CR. Omega-3 fatty acid ethyl esters increase heart rate variability in patients with coronary disease. Pharmacol Res 2002;45(6):475.
10. Holguin F, Tellez-Rojo MM, Lazo M et al. Cardiac autonomic changes associated with fish oil vs soy oil supplementation in the elderly. Chest 2005; 127 (4): 1102–1107.
11. Romieu I, Tellez-Rojo MM, Lazo M, Manzano-Patiño A, Cortez-Lugo M, Julien P et al. Omega-3 fatty acid prevents heart rate variability reductions associated with particulate matter. Am J Respir Crit Care Med 2005; 172(12):1534–1540.
12. Mozaffarian D, Stein PK, Prineas RJ, Siscovick DS. Dietary fish and omega-3 fatty acid consumption and heart rate variability in US adults. Circulation 2008;117 (9):1130–1137.
13. Christensen JH, Christensen MS, Dyerberg J, Schmidt EB. Heart rate variability and fatty acid content of blood cell membranes: a dose-response study with n-3 fatty acids. Am J Clin Nutr 1999;70(3):331–337.
14. Geelen A, Zock PL, Swenne CA, Brouwer IA, Schouten EG, Katan MB. Effect of n-3 fatty acids on heart rate variability and baroreflex sensitivity in middle-aged subjects. Am Heart J 2003;146(2):E4.

15. Dyerberg J, Eskesen DC, Andersen PW, Astrup A, Buemann B, Christensen JH, et al. Effects of trans- and n-3 unsaturated fatty acids on cardiovascular risk markers in healthy males. An 8 weeks dietary intervention study. Eur J Clin Nutr 2004;58(7):1062–1070.

16. Christensen JH. n-3 fatty acids and the risk of sudden cardiac death. Emphasis on heart rate variability. Dan Med Bull 2003;50(4):347–367.

17. Fiedler R, Mall M, Wand C, Osten B. Short-term administration of omega-3 fatty acids in hemodialysis patients with balanced lipid metabolism. J Ren Nutr 2005;15(2):253–256.

18. Bairati I, Roy L, Meyer F. Effects of a fish oil supplement on blood pressure and serum lipids in patients receiving coronary artery disease. Can J Cardiol 1992;8(1):41–46.

19. Grimsgaard S, Banaa KH, Hansen JB, Myhre ES. Effects of highly purified eicosapentaenoic acid and docosahexaenoic acid on hemodynamics in humans. Am J Clin Nutr 1998;68(1):52–59.

20. Stark KD, Holub BJ. Differential eicosapentaenoic acid elevations and altered cardiovascular disease risk factor responses after supplementation with docosahexaenoic acid in postmenopausal women receiving and not receiving hormone replacement therapy. Am J Clin Nutr 2004;79(5):765–773.

21. Mozaffarian D, Geelen A, Brouwer IA, Geleijnse JM, Zock PL, Katan MB. Effect of fish oil on heart rate in humans: a meta-analysis of randomized controlled trials. Circulation 2005;112(13):1945–1952.

22. Yaginuma-Sakurai K, Murata K, Shimada M, Nakai K, Kurokawa N, Kameo S, et al. Intervention study on cardiac autonomic nervous effects of methylmercury exposure to seafood. Neurotoxicol Teratol 2009;32(2):240–245.

23. Valera B, Dewally E, Poirier P. Cardiac autonomic activity and blood pressure among Nunavik Inuit adults exposed to environmental mercury: a cross-sectional study. Environ Health 2008;7:29.

24. Lim S, Chung HU, Paek D. Low dose mercury and heart rate variability among community residents nearby to an industrial complex in Korea. Neurotoxicology 2010;31(1):10–16.

25. Nolan B, Lamontagne P, Tremblay A. Qanuippitaa. How are we? Physical activity, anthropometry and perception of body weight [Internet]. Quebec: Institut national de santé publique du Québec and Nunavik Regional Board of Health and Social Services; 2007 [revised 2008 nov 24; cited 2010 may 10]. Available from: http://www.inspq.qc.ca/pdf/publications/nunavik.asp

26. Pouliot M, Despres J, Lemieux E, Moorjani S, Bouchard C, Tremblay A, et al. Waist circumference and abdominal sagittal diameter: best simple anthropometric indexes of abdominal visceral adipose tissue accumulation and related cardiovascular risk in men and women. Am J Cardiol 1994;73(7):460–468.

27. Poirier P, Hernandez TL, Weil KM, Shepard TJ, Eckel RH. Impact of diet-induced weight loss on the cardiac autonomic nervous system in severe obesity. Obes Res 2003;11(9):1040–1047.

28. Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Eur Heart J 1996;17(3):354–381.

29. Friedewald WT, Levy RJ, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18(6):499–502.

30. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28(7):412–419.

31. Rochelette L, Blanchet C. Methodological Report. Nunavik Health Survey 2004 [Internet]. Quebec: Institut national de santé publique du Québec and Nunavik Regional Board of Health and Social Services; 2007 [revised 2008 may 1; cited 2008 Dec 11]. Available from: http://www.inspq.qc.ca/pdf/publications/nunavik.asp

32. Christensen JH, Schmidt EB, Molenberg D, Toft E. Alpha-linolenic acid and heart rate variability in women examined for coronary artery disease. Nutr Metab Cardiovasc Dis 2005;15(5):345–351.

33. Mozaffarian D, Prineas RJ, Stein PK, Siscovick DS. Dietary fish and n-3 fatty acid intake and cardiac electrophysiologic parameters in humans. J Am Coll Cardiol 2006;48(3):478–484.

34. Mercuro G, Podda A, Pitzalis L, Zoncu S, Mascia M, Melis GB, et al. Evidence of a role of endogenous estrogen in the modulation of autonomic nervous system. Am J Cardiol 2000;85(6):787–789, A789.

35. Ribeiro TF, Azevedo GD, Crescencio JC, Marâes VR, Paiva V, Caiati AM, et al. Heart rate variability under resting conditions in postmenopausal and young women. Braz J Med Biol Res 2003;36(7):871–877.

36. Liu CC, Kuo TB, Yang CC. Effects of estrogen on gender-related autonomic differences in humans. Am J Physiol Heart Circ Physiol 2003;285(5):H2188–2193.

37. Billman GE, Kang JX, Leaf A. Prevention of sudden cardiac death by dietary pure omega-3 polyunsaturated fatty acids in dogs. Circulation 1999;99(20):2452–2457.

38. Dhein S, Michaelis B, Mohr FW. Antiarrhythmic and electrophysiological effects of long-chain omega-3 polyunsaturated fatty acids. Naunyn Schmiedebergs Arch Pharmacol 2005;371(3):202–211.

39. McLennan PL. Relative effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on cardiac arrhythmias in rats. Am J Clin Nutr 1993;57(2):207–212.

40. McLennan PL, Bridle TM, Abeywardena MY, Charnock JS. Dietary lipid modulation of ventricular fibrillation threshold in the marmoset monkey. Am Heart J 1992;123(6):1555–1561.
n-3 fatty acids and heart rate variability

41. Kang JX, Leaf A. Effects of long-chain polyunsaturated fatty acids on the contraction of neonatal rat cardiac myocytes. Proc Natl Acad Sci USA 1994;91(21):9886–9890.

42. Xiao YF, Sigg DC, Leaf A. The antiarrhythmic effect of n-3 polyunsaturated fatty acids: modulation of cardiac ion channels as a potential mechanism. J Membr Biol 2005;206(2):141–154.

43. Nishimura M, Nanbu A, Komori T, Ohtsuka K, Takahashi H, Yoshimura M. Eicosapentaenoic acid stimulates nitric oxide production and decreases cardiac noradrenaline in diabetic rats. Clin Exp Pharmacol Physiol 2000;27(8):618–624.

44. Chrysohoou C, Panagiotakos DB, Pitsavos C, Skoumas J, Krinos X, Chloptsios Y. Long-term fish consumption is associated with protection against arrhythmia in healthy persons in a Mediterranean region--the ATTI-CA study. Am J Clin Nutr 2007;85(5):1385–1391.

45. Ziemssen T, Gasch J, Ruediger H. Influence of ECG sampling frequency on spectral analysis of RR intervals and baroreflex sensitivity using the EUROBAVAR data set. J Clin Monit Comput 2008;22(2):159–168.

Eric Dewailly, MD, Ph.D.
Axe Santé des Populations et Environnement, Centre de Recherche du CHUQ
2875 Boulevard Laurie, Édifice Delta 2, Bureau 600
Québec (QC) G1V 2M2
CANADA
Email: eric.dewailly@crchul.ulaval.ca