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# Long-chain, n-3 fatty acids and physical activity — Independent and interactive associations with cardiac autonomic control ☆

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ABSTRACT

*Background/objectives:* Intake of the marine-based, n-3 fatty acids and engagement in physical activity are inversely related to cardiac morbidity and mortality. Among putative mechanisms, both n-3 fatty acids and physical activity may act through modulation of autonomic control of the cardiovascular system. This investigation examined the independent and interactive associations of n-3 fatty acids (eicosapentaenoic and docosahexanenoic acid; EPA, DHA) and physical activity with heart rate variability (HRV).

*Methods:* Subjects were 259 healthy 30–54 year-old adults. Serum phospholipid fatty acid composition was employed as a biomarker of dietary n-3 fatty acid exposure. Physical activity based on the Paffenbarger questionnaire was coded as $<$ or $\geq$2000 kcal/week. Standard time-domain (standard deviation of normal-to-normal intervals and root-mean squared of successive differences; SDNN, RMSSD) and frequency domain (high frequency and low frequency power) measures of HRV were derived from resting electrocardiographic recordings.

*Results:* In linear regression models with covariate adjustment for age, gender and race, greater n-3 fatty acid exposure was associated with greater SDNN and RMSSD, and high physical activity was associated with greater RMSSD. n-3 fatty acid exposure also predicted variation in SDNN, RMSSD, and high-frequency power in interaction with physical activity. Specifically, n-3 fatty acid exposure covaried positively with these three HRV indices only among participants expending 2000 kcal per week or more in physical activity. These latter findings were noted for DHA but not EPA.

*Conclusions:* These results suggest that the cardiovascular benefits of n-3 fatty acid consumption may be mediated, in part, by effects on cardiac autonomic control and may be dependent upon concomitant habitual exercise.


## 1. Introduction

Intake of n-3 long-chain, polyunsaturated fatty acids, naturally found in cold water fish, have documented utility in secondary prevention of cardiac death [1] and other coronary events [2]. The large, prospective US Physician's Health Study [3] found that consumption of long-chain n-3 fatty acids was associated with reduced incidence of sudden cardiac death, but not total myocardial infarction. This suggests that n-3 fatty acids may specifically protect against ventricular arrhythmias. Nonetheless, trial data regarding effects on sudden cardiac death and ventricular arrhythmias have been mixed, and laboratory studies variously report both pro- and anti-arrhythmic associations with n-3 fatty acids [4,5].

Anti-arrhythmic effects of n-3 fatty acids may be mediated by the autonomic nervous system, and several investigators have examined the association between n-3 fatty acid exposure and heart rate variability (HRV), a non-invasive measure of autonomic control of the heart. In vivo studies have demonstrated that low overall HRV, and particularly low variability in the frequency range of 0.15 to 0.40 hertz (Hz), correspond to low vagal, parasympathetic activity [6]. HRV itself is predictive of less risk of sudden cardiac death in people following acute myocardial infarctions [7–9]. In addition, low HRV indices are prognostic indicators for poor outcomes in people with diabetes mellitus, cardiac transplantation, myocardial dysfunction, ventricular arrhythmias, and end-stage renal disease [6]. Observational studies and randomized trials provide modest support for an

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association between greater n-3 fatty acid exposure and higher HRV [10–15]. However, findings are inconsistent as to which indices of HRV are affected. Additionally, most data were derived from select patient samples, leaving unclear whether n-3 fatty acids are related to HRV in the general population and, by extension, cardiovascular disease prevention.

Exercise is widely regarded as beneficial for cardiovascular health, based on ample evidence that greater habitual physical activity is associated with reduced risk of heart disease [16]. This benefit may be mediated through several mechanisms, such as obesity prevention, preserved insulin sensitivity, reduced vessel atherosclerosis, and increased lactic acid tolerance during ischemic insult. Additionally, physical activity may, like n-3 fatty acids, modulate autonomic control of the cardiovascular system. Exercise lowers resting heart rate while often increasing HRV [17].

However, it is unknown how habitual physical activity and n-3 fatty acid exposure interact as co-determinants of autonomic functioning. Fish oil appears to reduce heart rate and oxygen consumption during exercise [18]. Therefore, the present cross-sectional investigation of 259 healthy adults reports on the association between n-3 fatty acid exposure and standard indices of HRV and on potential interactions with habitual physical activity.

## 2. Methods

### 2.1. Subjects

The data analyzed in this report derive from the University of Pittsburgh Adult Health and Behavior study, which sampled adult volunteers between 35 and 54 years of age from Pittsburgh, Pennsylvania and surrounding communities (predominantly Allegheny County) via mass-mail solicitation. Participants attended several appointments for assessments of basic cardiovascular health parameters and health behaviors, psychosocial questionnaires and interviews, and cognitive testing. Exclusion criteria included a reported history of atherosclerotic cardiovascular disease, chronic kidney or liver disease, cancer, major neurological disorders, current pregnancy, and schizophrenia or other psychotic illness.

A portion of participants enrolled in a further investigation involving additional measures of cardiovascular risk. This protocol excluded persons with diabetes mellitus, severe hypertension (BP ≥ 180/110 mm Hg), and extreme obesity (body mass index ≥ 40 kg/m$^2$). Additionally, persons taking anti-hypertensive, lipid-lowering, anti-arrhythmic, glucocorticoids or psychotropic medications were excluded. Of the 282 participants who met these criteria, 3 had fatty acid chromatogram peaks which were undetectable, leaving 279 subjects. Twenty of these participants did not have complete HRV information due to unwillingness to participate, equipment malfunction, excessive ectopy (≥15 premature beats per 5 min), erratic breathing/holding breath, or deviation from paced breathing. Only subjects who successfully completed both unpaced and paced electrocardiographic (ECG) recordings were included. Data on the remaining 259 subjects were used in analyses.

The study protocol was approved by the Institutional Review Board of the University of Pittsburgh (IRB numbers 0805006, 000535), and informed consent was obtained from all participants in accordance with the University IRB guidelines.

### 2.2. Heart rate variability

Participants were asked to refrain from caffeine for 4 h, exercise and alcohol for 12 h, and cold medications for 24 h preceding the ECG recording. While seated in a temperature and sound-controlled chamber and after a 10-minute rest period, two successive 5-minute, resting ECG recordings were obtained. During the first, patients were instructed to relax and breathe at a comfortable rate. During the second recording period, the subjects' respiratory rate was paced at 11 breaths per second by playing a high-pitched tone during the inhalation period and a low-pitched tone during the exhalation period. The resulting ECG recordings were sampled at 1000 Hz, digitized and analyzed by PSPAT computer software [19]. The program labeled each R wave, and a trained technician reviewed each ECG for accuracy of R-wave identification. The program calculated two time-domain indices of HRV: standard deviation of normal-to-normal intervals (SDNN) and the square root of the mean of the squares of successive normal-to-normal interval differences (RMSSD). The sequential cardiac interbeat interval time series from the selected resting baseline was assessed to determine its component frequencies using a point process analysis developed at the University of Amsterdam, PSPAT [19]. This program yields results similar to a Fourier decomposition, but does not assume a continuous underlying generator function. Conceptually, the analysis is consistent with the integral-pulse frequency-modulation approach used in recent modeling of the neural basis of HRV [20]. High and low frequency HRV was defined as 0.15 Hz–0.39 Hz, and 0.06–0.15 Hz, respectively. Respiratory sinus arrhythmia was defined as power at the participant's respiratory frequency ± 0.015 Hz. The paced and un-paced HRV estimates correlated with one another at r > 0.6 and were averaged prior to data analysis to improve reliability [21].

### 2.3. Serum phospholipid fatty acid composition

A fasting sample of whole blood was centrifuged to separate red blood cells from protein-rich serum. Lipids were extracted and separated from the serum samples using a Strata NH2 500 mg/3 ml column (P/N 8B-S009-HBJ). The phospholipid fraction was collected for analysis.

Quantification of the isolated phospholipids was done by gas chromatography. In order to make the phospholipids suitable for chromatographic analysis, a procedure known as FAME described by Shibahara et al. [22] was used to make the molecules volatile. Briefly, the phospholipids were cleaved into fatty acids and were then methylesterified. Analysis was performed by gas chromatography according to the method described by Yao et al. [23]. For the purposes of this study, we focused on serum phospholipid EPA and DHA (mol%).

### 2.4. Physical activity

Physical activity was assessed using the Paffenbarger physical activity questionnaire [24]. This instrument queries daily walking habits and flights of stairs climbed, as well as exercise, sports and recreational activities. Weekly kilocaloric expenditure in all forms of physical activity was calculated for each participant.

The energy expended in physical activity was interpreted as an estimate given the limitations of the method. The frequency distribution of energy expenditure had a positive skew, and prior research indicates that the relationship between physical activity and health outcomes flattens at higher levels of exercise [25–27]. Because of these considerations and that fact that log transformation would obscure interpretation of levels of physical activity, energy expenditure was categorized as low (<2000 kcal/week) and high (≥2000 kcal/week). This particular cut-point in the Paffenbarger Survey scores was felt to be suitable based on proximity to the median of the current sample (2016 kcal/week) and its utility in differentiating rates of major cardiac events and all-cause mortality as function of physical activity [27,28].

### 2.5. Statistical analysis

All statistical analyses were performed using SPSS (Version 17.0, SPSS Inc., USA). Serum n-3 fatty acid levels and HRV data were natural logarithm transformed for regression analyses to normalize distributions. Race of the subjects was categorized into two groups: Caucasians (N = 231), and other (N = 28). Linear regression models were then created using n-3 fatty acids and physical activity as independent variables, age, race and sex as covariates, and indices of HRV as dependent variables. In each regression model, the interaction between serum n-3 fatty acids and physical activity was tested. The interaction term was calculated by the arithmetic product of the mean-centered n-3 fatty acid variable and dichotomized physical activity (coded as −0.5, +0.5). Distributions of power indices of HRV after log transformation showed extreme values to be present. In order to ensure that results were not unduly influenced by these values analyses were repeated after distributions were trimmed by elimination of the bottom and top 2.5% of the data [29].

**Table 1**
Characteristics of study population[a].

| | Low physical activity[b] | High physical activity[b] | p value[c] |
|---|---|---|---|
| *N* | 127 | 132 | |
| *Gender (% female)* | 55 | 48 | 0.35 |
| *Age* | 45.3 ± 6.6 | 44.3 ± 6.8 | 0.20 |
| *BMI (kg/m$^2$)* | 26.5 ± 4.4 | 26.0 ± 4.1 | 0.38 |
| *Physical activity (kcal/week)* | 1113 ± 493 | 3516 ± 1534 | <0.001 |
| *Heart Rate* | 69.3 ± 9.3 | 64.9 ± 9.0 | <0.001 |
| *Systolic BP* | 114.5 ± 12.8 | 114.0 ± 9.9 | 0.76 |
| *Diastolic BP* | 75.32 ± 9.1 | 75.37 ± 7.9 | 0.96 |
| *Serum phospholipid EPA (mol%)*[d] | 0.44 ± 0.27 | 0.51 ± 0.45 | 0.09 |
| *Serum phospholipid DHA (mol%)*[d] | 1.42 ± 0.63 | 1.56 ± 0.63 | 0.05 |
| *SDNN (ms)*[d] | 45.90 ± 14.29 | 51.68 ± 22.18 | 0.06 |
| *RMSSD (ms)*[d] | 31.80 ± 16.12 | 38.46 ± 25.56 | 0.03 |
| *High frequency power (ms$^2$)*[d] | 42,268 ± 46,014 | 47,467 ± 79,196 | 0.71 |
| *Low frequency power (ms$^2$)*[d] | 24,659 ± 19,066 | 27,095 ± 34,719 | 0.49 |
| *Low freq:high freq ratio*[d] | 1.18 ± 1.51 | 0.97 ± 1.02 | 0.32 |

[a] Mean values ± standard deviation unless specified otherwise.
[b] Low and high physical activity defined as <2000 or ≥2000 kcal/week, respectively.
[c] Groups were compared by t-test except in the case of gender for which the Chi-square test for independence was used.
[d] Presented in raw form whereas data were transformed by natural logarithm for statistical analyses.