Published in final edited form as:

Int J Cardiol. 2013 September 1; 167(5): 2102–2107. doi:10.1016/j.ijcard.2012.05.110.

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 docosahexaenoic 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 biomarker of dietary n-3 fatty acid exposure. Physical activity based on the Paffenbarger questionnaire was coded as < or ≥ 2,000 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 2,000 kcal per week or more in physical activity. These latter findings were noted for DHA but not EPA.

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The authors have no other potential conflicts of interest to disclose.

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

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Background

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 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.

Methods

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 $\geq 180/110$ mm Hg), and extreme obesity (body mass index ≥ 40 kg/m²). 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 minutes), 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.

Heart Rate Variability

Participants were asked to refrain from caffeine for 4 hours, exercise and alcohol for 12 hours, and cold medications for 24 hours 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 1,000 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 $\pm .015$ hertz. 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].

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/3ml 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 methyl-esterified. 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 (mole %).

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 (< 2,000 kcal/week) and high (≥ 2,000 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 (2,016 kcal/week) and its utility in differentiating rates of major cardiac events and all-cause mortality as function of physical activity [27, 28].

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 distributions were trimmed by elimination of the bottom and top 2.5% of the data [29].

Results

As shown in Table 1, the study population was comprised of mid-life adults who, on average, were normotensive and slightly overweight. The low and high physical activity groups were similar in age, body mass index (BMI) and blood pressure, whereas differences in the expected direction were noted for heart rate. With respect to cardiac autonomic control, time-domain measures of HRV (RMSSD and SDNN) were somewhat greater in the high, compared to low, exercise groups, whereas other indices were comparable across

groups. Participants reporting high physical activity tended to have higher circulating levels of EPA and DHA.

Table 2 shows the results of analyses based upon the sum of EPA and DHA. Each linear regression model included covariate adjustment for age, sex, and race. Models 1 through 3 revealed associations between n-3 fatty acids and heart rate, SDNN and RMSSD, and an association between physical activity and both heart rate and RMSSD. Significant n-3 fatty acid–X–physical activity interactions were found for both SDNN and RMSSD (p values 0.02). Among the frequency domain HRV indices, linear regression of high frequency power also revealed a significant n-3 fatty acid–X–physical activity interaction. Similar regression analyses were conducted using respiratory sinus arrhythmia (i.e., power assessed solely in a .3 Hz band encompassing each participant's measured respiratory rate). Here also, the interaction between n-3 fatty acids and physical activity was significant at $P = .01$). To test for the potential effects of extreme values, linear regression models for each of the HRV parameters were repeated after trimming the low and high 2.5% of values. These re-analyses of SDNN, RMSSD, HF and RSA yielded interaction term P values of .06, .01, .01, and .02, respectively.

As displayed in Table 3, regression analyses based upon DHA alone yielded very similar results. DHA was positively related to heart rate, SDNN and RMSSD, and significant interactions were noted between DHA and physical activity with respect to SDNN, RMSSD and high frequency power (p values < 0.01). DHA and physical activity also tended to interact in predicting low frequency/high frequency ratio ($p=0.08$). In contrast, analyses based on EPA revealed a positive association with heart rate and RMSSD, but no interactions between EPA and physical activity (Table 4). Additional covariate adjustment for body mass index did not materially alter the results reported in Tables 2–4.

To investigate the noted interactions, regression analyses of SDNN, RMSSD and high frequency power were conducted separately in the low and high physical activity groups. The findings reported in Table 5 indicate that the sum of EPA and DHA, as well as DHA individually, predicted all three measures of HRV in the high but not the low physical activity groups. Figure 1 displays scatter plots of RMSSD as a function of EPA plus DHA and DHA alone for both low- and high-physical activity groups. This illustrates the positive relationship between n-3 fatty acids and HRV among individuals expending at least 2,000 kcal per week in physical activity.

High frequency power specifically quantifies cardiac parasympathetic control whereas other HRV indices reflect mixed autonomic and possibly other influences. To determine if the findings for SDNN and RMSSD in the high exercise group were driven by cardiac parasympathetic control, analyses were repeated with high frequency power as an additional covariate. The n-3 fatty acids remained significant predictors of both SDNN and RMSSD in the high exercise group.

In additional supplementary analyses treating Paffenbarger scores as a continuous measure, physical activity was unrelated to any index of HRV. Additional sensitivity analyses were conducted to test whether the reported findings might be spurious due to values near the physical activity cut point. Excluding from analyses the 20% of subjects closest to 2,000 kcal/week in energy expenditure did not alter the results. Finally, in separate regression models using measures of HRV collected during paced and unpaced respiration the noted findings were most evident in data collected during paced respiration.

Discussion

This cross-sectional study examined the manner in which n-3 fatty acid exposure and physical activity are related to autonomic control of the cardiovascular system. We found that among 259 community-dwelling and generally healthy adults, the long-chain n-3 fatty acids were associated with the time domain HRV measures of SDNN and RMDD. Additionally, physical activity was found to moderate the association of n-3 PUFAs with time domain HRV and also high frequency power. Specifically, higher levels of n-3 fatty acids in serum phospholipids were associated with greater HRV only in persons expending 2,000 or more calories per week. The results also indicate that among the long-chain fatty acids, DHA is the nutrient most closely related to HRV. By studying generally healthy adults, the study avoids confounding effects of sinus node disease, peripheral neuropathy and prescription medications, and may be interpreted in relation to primary prevention of CVD. Overall, the results suggest that parasympathetic or vagal tone and other, presumably sympathetic influences on sinoatrial function vary in relation to n-3 fatty acid exposure and do so in a manner that is influenced by concurrent exercise habits.

Whereas many clinical studies indicate that long-chain n-3 fatty acids are protective against cardiovascular disease, the mechanisms for such effects are unclear. Increased n-3 fatty acid exposure may lower blood pressure [30], heart rate and catecholamine release, and may improve baroreceptor sensitivity [31–33]. An anti-arrhythmic action has been found in animal studies and some, but not all, clinical trials [34].

The cardiovascular benefits of n-3 fatty acids may be mediated, in part, via the autonomic nervous system. This hypothesis is supported by the current findings. Low values of the particular HRV indices associated with n-3 fatty acids, SDNN, RMSSD and high frequency power, themselves predict greater atherosclerosis progression, cardiovascular disease events and total mortality [35–37]. Associations between those indices and n-3 fatty acids suggest that n-3 fatty acid exposure increases cardiac control by both sympathetic and parasympathetic nervous input.

Regular aerobic activity is a primary component of healthy lifestyle, particular with respect to cardiovascular disease prevention [16]. Its physiologic benefits include lowered blood pressure and resting heart rate, weight control, improved cardiorespiratory fitness and metabolic and lipid parameters, and increased HRV. Effects of exercise on HRV generally involves augmentation of parasympathetic activity in conjunction with reduced cardiac adrenergic activity [17]. Changes in HRV parallel and may well mediate the cardiovascular protection afforded by regular physical activity (viz. ventricular arrhythmias; [38]). Nonetheless, individuals notably differ in their autonomic responses to aerobic activity [39]. Explication of such response heterogeneity has identified age and baseline HRV as moderators. In the presented analyses, physical activity is primarily related to HRV in interaction with n-3 fatty acids, encouraging future consideration of n-3 fatty acid exposure as an additional moderator.

The current findings also offer new interpretations of the existing research on n-3 fatty acids and HRV. Among over 1,000 unselected US adults, self-reported fish consumption correlated positively with RMSSD and high frequency power, whereas these analyses did not distinguish between EPA and DHA intake [40]. Much smaller reports on healthy, young adults suggest that found that serum DHA, but not EPA, is proportional to SDNN though perhaps only among men [14, 41]. The current report corroborates this finding across gender. In contrast, randomized trials supplementing either healthy or heart disease participants with fish oil for 2–4 months have not consistently observed changes in HRV [10, 12–14].

The inconsistency of the above trial findings could be due to a truly absent treatment effect, the small sample sizes employed (generally less than 25 subjects per group), issues related to dose and duration, or the existence of a moderating factor. The current findings indicate that effects of n-3 fatty acids may be specific to DHA and may be moderated by concomitant physical activity. The published trials have not considered physical activity in their analyses and likely studied relatively inactive participants, as many enrolled heart disease patients [10, 12, 14]. An additional, small, 2-x-2 trial randomized obese subjects to DHA-rich fish oil, exercise, both or neither for 12 weeks [11]. Fish oil increased HRV but no interactions between the two interventions were noted. However, the exercise did not affect either heart rate at rest or during exercise, or high frequency power, questioning the adequacy of the exercise intervention.

The neural components regulating cardiovascular function include sympathetic and parasympathetic afferents from dispersed, peripheral mechano- and chemo-receptors, brainstem and hypothalamic nuclei, modulating input from corticolimbic structures, and sympathetic and parasympathetic efferents to the heart and vasculature. While n-3 fatty acid intake and physical activity may directly affect myocardial cells or cardiac beta-receptor function [34], their apparent cardiac autonomic effects likely involves altered central integration of cardiovascular afferent information [17, 38]. Angiotensin II and nitric oxide have been proposed as mediators of the central effects of n-3 fatty acids [17, 38]. Intake of n-3 fatty acids and physical activity appear to also affect cognitive and affective processes [42–44], raising the possibility that corticolimbic modulation of cardiac autonomic control underlies the associations reported here.

The current study is limited by its cross-sectional design and reliance on self-reported physical activity. These considerations may be offset by the relatively large sample and use of serum phospholipid composition to quantify n-3 exposure, thus avoiding recall bias in self-reported diet data. Physical activity was dichotomized in analyses at 2,000 kcal as done elsewhere, and the reported interactions were robust in sensitivity analyses. In conclusion, we observed in generally healthy volunteers a relationship between long-chain, n-3 fatty acids and cardiac autonomic control that was specific to DHA and was moderated by concurrent exercise habits. These findings pertain to the putative role of these health behaviors in primary cardiovascular disease prevention and may inform the design and interpretation of randomized clinical trials.

Acknowledgments

Support: US Public Health Service Awards T32 HL07560, P01 HL40962, and R21 HL081282

MFM, SBM, and JRJ designed the research; MFM, JKY, SBM, and SMC conducted research; MPH and MFM analyzed data and wrote the paper; MFM had primary responsibility for final content. All authors read and approved the final manuscript. MFM received fish oil capsules and matching placebo as a gift from NourishLife LLC for an investigation conducted between 2008 and 2011.

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Fig. 1.

Scatterplots and regression lines of heart rate variability (HRV) in relation to serum phospholipid n-3 fatty acid composition as a function of self-reported physical activity. Panel A displays root mean sum of squares of successive differences (RMSSD) in relation to eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA). Panel B shows the same relationship concerning DHA alone. As is evident, HRV was directly related to n-3 fatty acid content among participants in the high, but not the low, physical activity group. Values were adjusted for age, race and sex.

Table 1Characteristics of study population¹

	Low physical activity ²	High physical activity ²	p value ³
N	127	132	
Gender (% female)	55	48	0.35
Age	45.3 ± 6.6	44.3 ± 6.8	0.20
BMI (kg/m²)	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%)⁴	0.44 ± 0.27	0.51 ± 0.45	0.09
Serum phospholipid DHA (mol%)⁴	1.42 ± 0.63	1.56 ± 0.63	0.05
SDNN (ms)⁴	45.90 ± 14.29	51.68 ± 22.18	0.06
RMSSD (ms)⁴	31.80 ± 16.12	38.46 ± 25.56	0.03
High frequency power (ms²)⁴	42268 ± 46014	47467 ± 79196	0.71
Low frequency power (ms²)⁴	24659 ± 19066	27095 ± 34719	0.49
Low freq:high freq ratio⁴	1.18 ± 1.51	.97 ± 1.02	0.32

¹ Mean values ± standard deviation unless specified otherwise² Low and high physical activity defined as < 2,000 or ≥ 2,000 kcal/week, respectively³ Groups were compared by t-test except in the case of gender for which the Chi-square test for independence was used.⁴ Presented in raw form whereas data were transformed by natural logarithm for statistical analyses.

Linear regression analyses of combined n-3 fatty acids and physical activity as predictors of indices of heart rate variability

Table 2

Model		Heart rate		SDNN		RMSSD		High frequency		Low frequency		Low-to-high frequency ratio	
		β	p	β	p	β	p	β	p	β	p	β	p
1	EPA+DHA	.206	.001	.133	.03	.138	.02	.032	.52	.056	.53	-.008	.89
2	Physical activity	.216	<.001	.095	.12	.133	.03	.015	.78	-.084	.14	-.096	.10
3	EPA+DHA	.178	.003	.122	.04	.122	.04	.031	.55	.049	.39	.007	.91
	Physical activity	.189	.002	.077	.21	.114	.06	.010	.85	-.091	.11	-.097	.11
4	EPA+DHA	.175	.004	.117	.05	.117	.05	.027	.60	.048	.40	.010	.87
	Physical activity	.189	.002	.076	.21	.114	.05	.010	.85	-.091	.11	-.097	.11
	Interaction	.087	.14	.147	.01	.137	.02	.125	.01	.033	.56	-.087	.13

Covariates were age, race, and sex in all models.

SDNN = standard deviation of inter-beat intervals. RMSSD = root mean square of differences in successive interbeat intervals DHA = docosahexanoic acid. EPA = eicosapentaenoic acid.

Table 3
Linear regression analyses of DHA and physical activity as predictors of indices of heart rate variability

Model	Heart rate			SDNN			RMSSD			High frequency			Low frequency			Low-to-high frequency ratio		
	β	p		β	p		β	p		β	p		β	p		β	p	
1	DHA	0.188	0.002	0.124	0.04		0.121	0.04		0.022	0.67		0.027	0.63		-0.012		0.84
2	Physical activity	0.216	<0.001	0.095	0.12		0.133	0.03		0.015	0.78		-0.084	0.14		-0.097		0.10
3	DHA	0.162	0.007	0.114	0.06		0.105	0.08		0.020	0.69		0.039	0.49		0.001		0.99
	Physical activity	0.194	0.002	0.079	0.19		0.118	0.05		0.012	0.82		-0.089	0.12		-0.097		0.11
4	DHA	0.159	0.008	0.109	0.07		0.100	0.08		0.016	0.75		0.039	0.50		0.004		0.94
	Physical activity	0.194	0.001	0.079	0.19		0.118	0.05		0.012	0.82		-0.089	0.12		-0.097		0.10
	Interaction	0.107	0.07	0.169	0.004		0.156	0.007		0.131	0.009		0.016	0.77		-0.104		0.08

Table 4
Linear regression analyses of EPA and physical activity as predictors of indices of heart rate variability

Model	Heart rate			SDNN			RMSSD			High frequency			Low frequency			Low-to-high frequency ratio		
	β	p		β	p		β	p		β	p		β	p		β	p	
1	EPA	0.172	0.007	0.110	0.07		0.126	0.04		0.035	0.49		0.040	0.48		0.016		0.79
2	Physical activity	0.216	<0.001	0.095	0.12		0.133	0.03		0.015	0.78		-0.084	0.14		-0.097		0.10
3	EPA	0.147	0.02	0.100	0.10		0.111	0.06		0.034	0.51		0.051	0.37		0.028		0.64
	Physical activity	0.198	0.001	0.083	0.17		0.119	0.05		0.010	0.84		-0.090	0.12		-0.100		0.10
4	EPA	0.147	0.02	0.100	0.10		0.111	0.06		0.035	0.50		0.052	0.37		0.028		0.65
	Physical activity	0.198	0.001	0.083	0.18		0.119	0.05		0.010	0.85		-0.091	0.11		-0.100		0.10
	Interaction	0.00	0.99	0.040	0.51		0.039	0.50		0.071	0.16		0.067	0.23		-0.025		0.67

Linear regression analyses of n-3 fatty acids as predictors of heart rate variability in low- and high-physical activity groups

Table 5

Independent variable	Dependent variable	Low Physical activity		High Physical activity	
		β	p	β	p
EPA+DHA	SDNN	-0.040	0.64	0.250	0.003
	RMSSD	-0.026	0.75	0.257	0.002
DHA	High frequency power	-0.100	0.15	0.157	0.03
		-0.077	0.36	0.263	0.002
	SDNN	-0.065	0.42	0.260	0.002
	RMSSD	-0.119	0.08	0.155	0.04

Covariates were age, race, and sex in all models.