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The association of serum long-chain *n*-3 PUFA and hair mercury with exercise cardiac power in men

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Abstract

Long-chain *n*-3 PUFA from fish and exercise capacity are associated with CVD risk. Fish, especially large and old predatory fish, may contain Hg, which may attenuate the inverse association of long-chain *n*-3 PUFA with CVD. However, the associations of long-chain *n*-3 PUFA or Hg exposure with exercise capacity are not well known. We aimed to evaluate the associations of serum long-chain *n*-3 PUFA EPA, docosapentaenoic acid (DPA) and DHA and hair Hg with exercise cardiac power (ECP, a ratio of VO_{2max}:maximal systolic blood pressure (SBP) during an exercise test), a measure for exercise capacity. For this, data from the population-based Kuopio Ischaemic Heart Disease Risk Factor Study were analysed cross-sectionally in order to determine the associations between serum long-chain *n*-3 PUFA, hair Hg and ECP in 1672 men without CVD, aged 42–60 years. After multivariate adjustments, serum total long-chain *n*-3 PUFA concentration was associated with higher ECP and VO_{2max} (P_{trend} across quartiles = 0·04 and P_{trend} = 0·02, respectively), but not with maximal SBP (P_{trend} = 0·69). Associations were generally similar when EPA, DPA and DHA were evaluated individually. Hair Hg was not associated with ECP, VO_{2max} or maximal SBP. However, the associations of total long-chain *n*-3 PUFA ($P_{interaction}$ = 0·03) and EPA ($P_{interaction}$ = 0·02) with higher VO_{2max} were stronger among men with lower hair Hg. Higher serum long-chain *n*-3 PUFA concentration, mainly a marker for fish consumption in this study population, was associated with higher ECP and VO_{2max} in middle-aged men from eastern Finland.

Key words: Fatty acids: Exercise capacity: Cohort studies: Cross-sectional study

Low exercise capacity during an exercise test has been established as an independent predictor of risk for total mortality and CVD[1,2]. Exercise cardiac power (ECP), which is defined as a ratio of directly measured VO_{2max}:peak systolic blood pressure (SBP) during exercise test, is an accurate measure for exercise capacity and it is known to be an independent predictor of CVD[3]. The advantage of ECP compared with other exercise capacity measurements is that ECP provides information not only about cardiorespiratory fitness but also considers the differences in cardiovascular resistance and cardiac afterload[3,4].

Although little is known about ECP and risk of CVD, previously in the Kuopio Ischaemic Heart Disease Risk Factor (KIHD) study cohort, lower ECP was associated with increased risk of sudden cardiac death and stroke in men[3,4]. In addition, low cardiorespiratory fitness (VO_{2max}) and increased SBP during exercise were associated with higher risk of cardiovascular events and CVD-related mortality in the KIHD cohort[5–8].

Substantial evidence from epidemiological studies, including KIHD study, demonstrates that long-chain *n*-3 PUFA may reduce the risk of CVD[9–11]. To the best of our knowledge, no previous studies have been conducted to evaluate the association of these fatty acids with ECP. However, a few small supplementation studies have assessed the efficacy of long-chain *n*-3 PUFA on VO_{2max}[12–20] and SBP during exercise[19,21,22], but the findings are inconsistent.

We evaluated the association of serum long-chain *n*-3 PUFA concentrations with ECP, VO_{2max} and maximal SBP during an exercise test among middle-aged and older men from the KIHD cohort. We also evaluated whether high hair Hg concentration, a biomarker for long-term Hg exposure[23], is associated with ECP and whether it could modify the associations with long-chain *n*-3 PUFA, as it has been shown to do with the risk of CVD in the KIHD population[10,11].

Methods

Study population

Subjects were participants of the KIHD, which is a prospective, population-based study designed to investigate risk factors for CVD, carotid atherosclerosis and related outcomes in a randomly selected sample of men from eastern Finland[24]. The baseline examinations were carried out in 1984–1989. Of the 3235 eligible men aged 42, 48, 54 or 60 years who lived in

Abbreviations: DPA, docosapentaenoic acid; ECP, exercise cardiac power; KIHD, Kuopio Ischaemic Heart Disease Risk Factor Study; SBP, systolic blood pressure.

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the city of Kuopio or its surrounding areas, 2682 men (82·9 %) were recruited to the baseline study. The baseline characteristics of the entire study population have been described previously[24]. The KIHD study protocol was approved by the Research Ethics Committee of the University of Kuopio. All subjects gave their written informed consent for participation. From the analyses, we excluded participants with missing data on ECP measurements (*n* 207), a history of CVD (*n* 677) or those with missing data on serum long-chain *n*-3 PUFA (*n* 113) or hair Hg (*n* 13). After exclusions, 1672 men were included in the final analysis.

Measurements

Subjects provided their hair and venous blood samples between 08.00 and 10.00 hours at baseline. Repeat hair samples were collected from twenty-one subjects 4–9 years (mean, 6 years) after baseline examinations to survey the tracking of hair Hg values over time. The subjects were instructed to abstain from alcohol for 3 d and from smoking and eating for 12 h before providing samples. Comprehensive description of the determination of serum lipids and lipoproteins[25], assessment of medical history and use of medications[25], smoking status[25], alcohol consumption[25], resting blood pressure[25] and physical activity[26] have been reported previously. Hypertension diagnosis was defined as SBP/diastolic blood pressure >140/90 mmHg at study visit, clinical diagnosis of hypertension or use of hypertensive medication. Serum C-reactive protein (CRP) was measured using an immunometric assay (Immulite High Sensitivity CRP Assay; DPC). Dietary intakes were assessed using 4-d food recording at the time of blood sampling[27]. Educational status was assessed in years using self-administered questionnaires[27].

Serum fatty acid and mercury measurements

Serum fatty acids were determined in a single gas chromatographic run without pre-separation as described previously[28]. Serum fatty acids were extracted using chloroform–methanol solution. The chloroform phase was evaporated and treated with sodium methoxide, which methylated esterified fatty acids. Quantification was carried out with reference standards purchased from Nu-Check Prep Inc. Each analyte had an individual reference standard, and the internal standard was eicosane. Fatty acids were chromatographed in an NB-351 capillary column (HNU-Nordion) by a Hewlett-Packard 5890 Series II gas chromatograph with a flame ionisation detector (Hewlett-Packard Company, since 1999 Agilent Technologies Inc.). Results for fatty acids were obtained in μmol/l, and in the data analyses proportion of fatty acids from total fatty acids was used. The CV% was 9·4 % for EPA (20 : 5*n*-3), 12·7 % for docosapentaenoic acid (DPA, 22 : 5*n*-3) and 11·9 % for DHA (22 : 5*n*-3). For the serum total long-chain *n*-3 PUFA, we used the sum of EPA, DPA and DHA.

Hair Hg was detected by flow injection analysis-cold vapour atomic absorption spectrometry and amalgamation[29]. The Pearson's correlation coefficient between the original and the repeat measurement collected after 4–9 years was 0·91.

Assessment of exercise cardiac power

A maximal symptom-limited exercise tolerance test was performed between 08.00 and 10.00 hours using an electrically braked cycle ergometer (Medical Fitness Equipment 400 L bicycle ergometer)[30]. The standardised testing protocol comprised of an increase in the workload of 20 W/min with the direct analyses of respiratory gases (Medical Graphics). ECP was measured by the ratio of measured VO_{2max}:peak SBP[3]. VO_{2max} was defined as the highest value for or the plateau on VO_2. Blood pressure was measured every 2 min both manually and automatically during exercise until the test was stopped and every 2 min after exercise. In the present study, we used only manually measured blood pressure values. The highest SBP achieved during the exercise test was defined as the maximum exercise SBP. For safety reasons, all tests were supervised by an experienced physician with assistance from an experienced nurse. Electrocardiography was recorded continuously with the Kone 620 electrocardiograph (Kone)[7,8].

Statistical analysis

The univariate associations between serum EPA + DPA + DHA concentrations and demographic, lifestyle and clinical characteristics at baseline were assessed by means and linear regressions for continuous variables and by the χ^2-test for categorical variables. Correlations between individual long-chain *n*-3 PUFA were evaluated by calculating Spearman's correlation coefficients. Linear regression models were used to determine the association of serum long-chain *n*-3 PUFA with ECP, VO_{2max} and maximum SBP during exercise. The mean values of ECP, VO_{2max} and maximum SBP during exercise in the exposure quartiles were analysed using ANCOVA.

In addition, two models were run to adjust for potential cofounders. Model 1 was adjusted for age (years) and examination year, and model 2 included the variables in the model 1 + BMI (kg/m^2), smoking status (non-smoker, previous smoker, current smoker <20 cigarettes/d and ≥20 cigarettes per d), leisure-time physical activity (kJ/d (kcal/d)), use of drugs for hypertension (yes/no), bronchial asthma (yes/no), LDL-cholesterol and HDL-cholesterol (mmol/l), CRP levels (mg/l) and intakes of energy (kJ/d (kcal/d)), carbohydrates (g/d) and alcohol (g/week). The cohort mean was used to replace missing values in covariates (<0·5 %). Test for linear trend across quartiles was assessed using the median value in each quartile as the continuous variable in the linear regression model. Statistical significance of the interactions on a multiplicative scale was assessed by stratified analysis with hair Hg divided by the median and likelihood ratio tests with a cross-product term. For assessing the clinical significance, we calculated effect sizes based on Cohen's *d* index (the difference between the group means divided by the standard deviation of the comparison category)[31]. All *P* values were two-sided ($\alpha = 0{\cdot}05$). Data were analysed using SPSS software version 21 for windows (IBM Corp.).

Results

Baseline characteristics

Baseline characteristics of the participants are presented in Table 1. Men with higher serum total long-chain *n*-3 PUFA

Table 1. Baseline characteristics according to total serum long-chain *n*-3 PUFA (Mean values and standard deviations; percentages)

	Serum total *n*-3 PUFA quartile								
	Q1 (<3·67) (*n* 418)		Q2 (3·67–4·38) (*n* 418)		Q3 (4·39–5·40) (*n* 418)		Q4 (>5·40) (*n* 418)		
Variables	Mean	SD	Mean	SD	Mean	SD	Mean	SD	$P_{for\ trend}$*
Age (years)	52·0	5·3	52·1	5·2	52·4	5·3	52·7	5·1	0·05
Education (years)	8·9	3·3	8·7	3·4	8·8	3·6	9·6	4·0	0·001
BMI (kg/m^2)	26·6	3·4	26·5	3·1	26·9	3·6	27·0	3·4	0·05
Current smoker (%)	31·1		28·9		30·4		27·5		0·32
Leisure-time physical activity (kJ/d)	556	694	548	669	556	594	653	858	0·03
Leisure-time physical activity (kcal/d)	133	166	131	160	133	142	156	205	0·03
C-Reactive protein (mg/l)	2·27	3·96	2·00	3·03	2·28	3·61	1·91	3·08	0·23
Serum TAG (mmol/l)	1·52	0·93	1·26	0·80	1·16	0·57	1·03	0·51	<0·001
Serum HDL-cholesterol (mmol/l)	1·22	0·28	1·29	0·28	1·33	0·28	1·35	0·31	<0·001
Serum LDL-cholesterol (mmol/l)	3·82	0·92	3·99	0·97	4·08	1·02	4·12	0·96	<0·001
Blood glucose (mmol/l)	4·72	0·96	4·36	0·84	4·73	0·82	4·73	0·90	0·40
Systolic blood pressure (mmHg)	136	17	134	15	134	16	134	17	0·140
Diastolic blood pressure (mmHg)	90	11	89	10	89	10	89	11	0·180
Energy intake (kJ/d)	10 230	2807	10 280	2494	9870	2594	9581	2506	<0·001
Energy intake (kcal/d)	2445	671	2457	596	2359	620	2290	599	<0·001
Carbohydrate intake (g/d)	258	38	254	38	246	39	245	36	<0·001
Alcohol intake (g/week)	57	92	66	103	82	125	85	116	<0·001
Diabetes (%)	5·5		2·9		4·3		4·8		0·99
Hypertension (%)	59·3		53·8		56·5		53·1		0·140
Drug for hypertension (%)	19·4		12·4		12·4		11·7		0·006
Serum EPA (%)†	0·97	0·25	1·29	0·24	1·71	0·30	2·80	1·12	<0·001
Serum DPA (%)†	0·47	0·07	0·53	0·07	0·56	0·78	0·64	0·10	<0·001
Serum DHA (%)†	1·74	0·28	2·20	0·26	2·57	0·29	3·39	0·66	<0·001
Hair Hg (μg/g)	1·18	1·30	1·65	1·78	2·20	2·04	2·71	2·34	<0·001

Q, quartiles; DPA, docosapentaenoic acid.
* Participant characteristics at baseline were assessed by means and linear regressions for continuous variables and the χ^2-test for categorical variables.
† Proportion of all serum fatty acids.

concentrations were more likely to be older ($P=0·05$) and have higher education ($P=0·001$), BMI ($P=0·05$), leisure-time physical activity ($P=0·03$), serum HDL- and LDL-cholesterol concentrations ($P<0·001$), hair Hg concentration ($P=0·05$) and alcohol intake ($P<0·001$). They also had lower carbohydrate intake ($P<0·001$), lower total energy intake ($P<0·001$), lower serum TAG levels ($P<0·001$) and they were less likely to use anti-hypertensive drugs ($P=0·001$).

The mean serum concentrations, as a percentage of all serum fatty acids, were 4·72 (SD 1·60) % for total long-chain *n*-3 PUFA, 1·69 (SD 0·92) % for EPA, 0·55 (SD 0·10) % for DPA and 2·48 (SD 0·73) % for DHA. The correlations between the individual long-chain *n*-3 PUFA were 0·70 for EPA and DHA, 0·56 for EPA and DPA and 0·41 for DHA and DPA. The mean hair content of Hg was 1·94 μg/g and ranged from 0 to 15·67 μg/g.

Serum long-chain n-3 PUFA, hair mercury and exercise cardiac power

The mean ECP was 12·46 (SD 3·07) ml/mmHg. After adjustment for age and examination year (model 1), higher serum total long-chain *n*-3 PUFA concentration was associated with higher ECP (the mean difference between extreme quartiles was 0·42 ml/mmHg (95 % CI 0·03, 0·81, P_{trend} across quartiles = 0·04)). Further multivariate adjustments had little impact on the association (model 2, Table 2). When the fatty acids were investigated individually, generally similar direct associations were observed with EPA, DPA and DHA (Table 2). The effect sizes, based on Cohen's *d* index, were 0·10 for total serum long-chain *n*-3 PUFA, 0·05 for EPA, 0·15 for DPA, 0·20 for DHA and 0·21 for Hg.

Hair Hg concentration was not associated with ECP (Table 2). Although we could not find statistically significant interactions between the long-chain *n*-3 PUFA and hair Hg for ECP ($P_{for\ interaction}=0·15$ for the total long-chain *n*-3 PUFA, $P=0·08$ for EPA, $P=0·47$ for DPA and $P=0·50$ for DHA), we observed statistically significant associations between the long-chain *n*-3 PUFA and higher ECP only in participants with lower hair Hg content (< median 1·30 μg/g) (online Supplementary Table S1).

Serum long-chain n-3 PUFA, hair mercury and VO_{2max}

The mean VO_{2max} was 2545 (SD 559) ml/min. Higher serum total long-chain *n*-3 PUFA concentration was associated with higher VO_{2max} (Table 3). The extreme-quartile difference in the multivariate-adjusted model was 83 ml/min (95 % CI 15, 152, P_{trend} across quartiles = 0·02). The associations were again generally similar with EPA, DPA and DHA (Table 3). The effect sizes were 0·13 for total serum long-chain *n*-3 PUFA, 0·05 for EPA, 0·28 for DPA, 0·15 for DHA and 0·19 for Hg.

We did not find a statistically significant association between hair Hg content and VO_{2max} (Table 3). However, adjusting for hair Hg content modestly attenuated the associations between the long-chain *n*-3 PUFA and VO_{2max} (Table 3). Furthermore, the associations were stronger among those with hair Hg below

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Table 2. Exercise cardiac power (ml/mmHg) in quartiles of serum long-chain *n*-3 PUFA and hair mercury*
(Mean values and 95 % confidence intervals)

	Exposure quartile										
	1 (*n* 418)		2 (*n* 418)		3 (*n* 418)		4 (*n* 418)			Mean difference†	
	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI	$P_{for\ trend}$	Mean	95 % CI
EPA + DPA + DHA (%)		<3·67		3·67–4·38		4·39–5·40		>5·40			
Model 1‡	12·15	11·88, 12·43	12·52	12·25, 12·80	12·59	12·31, 12·86	12·57	12·30, 12·85	0·04	0·42	0·03, 0·81
Model 2§	12·15	11·89, 12·42	12·47	12·21, 12·72	12·65	12·39, 12·91	12·57	12·31, 12·83	0·04	0·42	0·04, 0·80
Model 2 + hair Hg	12·16	11·89, 12·43	12·47	12·21, 12·73	12·65	12·39, 12·91	12·56	12·30, 12·83	0·06	0·40	0·01, 0·80
EPA (%)		<1·13		1·13–1·49		1·50–2·01		>2·01			
Model 1	12·17	11·90, 12·45	12·43	12·15, 12·70	12·75	12·47, 13·02	12·49	12·21, 12·77	0·13	0·32	−0·07, 0·72
Model 2	12·12	11·86, 12·39	12·39	12·13, 12·65	12·76	12·50, 13·02	12·56	12·30, 12·83	0·03	0·44	0·05, 0·83
Model 2 + hair Hg	12·13	11·86, 12·40	12·40	12·14, 12·66	12·76	12·50, 13·01	12·55	12·28, 12·82	0·05	0·42	0·03, 0·82
DPA (%)		<0·48		0·48–0·54		0·55–0·62		>0·62			
Model 1	12·01	11·74, 12·29	12·51	12·39, 12·79	12·51	12·24, 12·78	12·80	12·52, 13·07	<0·001	0·79	0·40, 1·17
Model 2	12·20	11·93, 12·46	12·55	12·29, 12·80	12·44	12·81, 12·70	12·65	12·39, 12·91	0·04	0·46	0·08, 0·84
Model 2 + hair Hg	12·20	11·94, 1·47	12·55	12·29, 12·80	12·44	12·81, 12·70	12·65	12·39, 12·91	0·06	0·44	0·06, 0·83
DHA (%)		<1·97		1·97–2·38		2·39, 2·86		>2·86			
Model 1	12·34	12·06, 12·61	12·43	12·16, 12·71	12·34	12·07, 12·62	12·73	12·45, 13·00	0·06	0·39	0·00, 0·78
Model 2	12·32	12·06, 12·59	12·43	12·18, 12·69	12·40	12·14, 12·66	12·68	12·41, 12·94	0·08	0·35	−0·03, 0·73
Model 2 + hair Hg	12·32	12·07, 12·60	12·44	12·18, 12·70	12·40	12·14, 12·66	12·66	12·40, 12·93	0·11	0·33	−0·06, 0·72
Hair Hg (μg/g)		<0·65		0·65–1·30		1·31–2·54		>2·54			
Model 1	12·31	12·03, 12·59	12·54	12·26, 12·81	12·74	12·47, 13·02	12·24	11·96, 12·52	0·40	−0·07	−0·47, 0·33
Model 2	12·18	11·92, 12·45	12·49	12·23, 12·75	12·74	12·48, 13·00	12·41	12·15, 12·68	0·13	0·23	−0·16, 0·62
Model 2 + EPA + DPA + DHA	12·25	11·99, 12·53	12·51	12·25, 12·77	12·72	12·47, 12·98	12·34	12·07, 12·61	0·89	0·08	−0·32, 0·49

DPA, docosapentaenoic acid.
* The mean values were analysed using ANCOVA.
† Mean difference between the extreme quartiles.
‡ Model 1: adjusted for age and examination year.
§ Model 2: adjusted for model 1 + BMI, current smoking status, leisure-time physical activity, energy intake, carbohydrate intake, alcohol intake, use of drugs for hypertension and C-reactive protein, LDL- and HDL-cholesterol concentrations.

Table 3. Maximum VO_2 (ml/min) in quartiles of serum long-chain *n*-3 PUFA and hair mercury*
(Mean values and 95 % confidence intervals)

	Exposure quartile										
	1 (*n* 418)		2 (*n* 418)		3 (*n* 418)		4 (*n* 418)			Mean difference†	
	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI	$P_{for\ trend}$	Mean	95 % CI
EPA + DPA + DHA (%)		<3·67		3·67–4·38		4·39–5·40		>5·40			
Model 1‡	2470	2418, 2522	2554	2502, 2606	2581	2502, 2606	2575	2523, 2627	0·01	105	31, 178
Model 2§	2483	2435, 2531	2542	2496, 2589	2588	2541, 2635	2566	2519, 2613	0·02	83	15, 152
Model 2 + hair Hg	2490	2442, 2539	2545	2498, 2592	2586	2539, 2633	2557	2510, 2606	0·08	67	−3, 138
EPA (%)		<1·13		1·13–1·49		1·50–2·01		>2·01			
Model 1	2492	2439, 2544	2516	2464, 2568	2612	2560, 2664	2560	2508, 2612	0·04	68	−7, 143
Model 2	2495	2447, 2543	2513	2466, 2559	2609	2563, 2656	2563	2515, 2610	0·03	68	−2, 137
Model 2 + hair Hg	2501	2452, 2550	2517	2470, 2559	2607	2561, 2654	2554	2506, 2603	0·10	53	−18, 124
DPA (%)		<0·48		0·48–0·54		0·55–0·62		>0·62			
Model 1	2441	2389, 2493	2546	2494, 2598	2587	2535, 2639	2606	2555, 2658	<0·001	166	92, 239
Model 2	2476	2428, 2524	2549	2502, 2595	2574	2527, 2621	2581	2534, 2628	0·004	105	37, 173
Model 2 + hair Hg	2481	2434, 2530	2550	2503, 2596	2572	2526, 2619	2576	2528, 2623	0·01	94	24, 163
DHA (%)		<1·97		1·97–2·38		2·39–2·86		>2·86			
Model 1	2489	2437, 2541	2559	2507, 2611	2535	2483, 2587	2597	2544, 2649	0·01	108	34, 182
Model 2	2498	2450, 2545	2557	2511, 2604	2546	2499, 2592	2579	2532, 2626	0·04	81	13, 150
Model 2 + hair Hg	2506	2458, 2555	2559	2513, 2606	2543	2496, 2590	2571	2523, 2619	0·11	65	−6, 135
Hair Hg (μg/g)		<0·65		0·65–1·30		1·31–2·54		>2·54			
Model 1	2514	2461, 2567	2543	2491, 2596	2590	2538, 2642	2532	2479, 2585	0·84	18	−59, 94
Model 2	2495	2447, 2542	2537	2490, 2583	2584	2537, 2630	2565	2516, 2613	0·09	70	0·3, 140
Model 2 + EPA + DPA + DHA	2507	2459, 2556	2540	2493, 2587	2580	2534, 2627	2552	2503, 2601	0·34	44	−28, 117

DPA, docosapentaenoic acid.
* The mean values in the exposure quartiles were analysed using ANCOVA.
† Mean difference between the extreme quartiles.
‡ Model 1: adjusted for age and examination year.
§ Model 2: adjusted for model 1 + BMI, current smoking status, leisure-time physical activity, energy intake, carbohydrate intake, alcohol intake, use of drugs for hypertension and C-reactive protein, LDL- and HDL-cholesterol concentrations.

Table 4. Maximum systolic blood pressure (mmHg) in quartiles of serum long-chain *n*-3 PUFA and hair mercury*
(Mean values and 95 % confidence intervals)

	Exposure quartile										
	1 (*n* 418)		2 (*n* 418)		3 (*n* 418)		4 (*n* 418)			Mean difference†	
	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI	$P_{for\ trend}$	Mean	95 % CI
EPA + DPA + DHA (%)		<3·67		3·67–4·38		4·38–5·40		>5·40			
Model 1‡	204·9	202·3, 207·4	206·6	204·1, 209·2	207·0	204·5, 209·6	207·8	205·2, 210·3	0·14	2·9	−0·7, 6·5
Model 2§	206·1	203·6, 208·7	206·7	204·2, 209·2	206·5	203·9, 209·0	207·0	204·5, 209·5	0·69	0·9	−2·8, 4·5
Model 2 + hair Hg	206·6	204·0, 209·2	206·9	204·4, 209·4	206·3	203·8, 208·8	206·5	204·0, 209·1	0·93	−0·03	−3·8, 3·8
EPA (%)		<1·13		1·13–1·49		1·49–2·01		>2·01			
Model 1	206·4	203·8, 208·9	205·0	202·4, 207·5	207·1	204·6, 209·7	207·9	205·3, 210·4	0·22	1·5	−2·2, 5·2
Model 2	207·5	205·0, 210·1	205·3	202·8, 207·8	206·7	204·2, 209·2	206·7	204·2, 209·3	0·98	−0·8	−4·6, 2·9
Model 2 + hair Hg	207·9	205·3, 210·5	205·6	203·1, 208·1	206·6	204·1, 209·1	206·3	203·7, 208·9	0·63	−1·6	−5·5, 2·2
DPA (%)		<0·48		0·48–0·54		0·54–0·62		>0·62			
Model 1	205·3	202·8, 207·9	205·7	203·2, 208·3	209·1	206·6, 211·7	206·2	203·6, 208·7	0·38	0·9	−2·7, 4·5
Model 2	205·2	202·7, 207·8	205·4	202·9, 207·9	209·1	206·6, 211·6	206·6	204·1, 209·1	0·25	1·3	−2·3, 5·0
Model 2 + hair Hg	205·5	202·9, 208·1	205·4	202·9, 207·9	209·1	206·6, 211·6	206·3	203·8, 208·8	0·40	0·8	−2·9, 4·5
DHA (%)		<1·97		1·97–2·38		2·38–2·86		>2·86			
Model 1	203·7	201·1, 206·2	208·0	205·5, 210·6	207·5	204·9, 210·0	207·1	204·6, 209·7	0·11	3·5	−0·1, 7·1
Model 2	204·8	202·3, 207·4	207·9	205·4, 210·4	207·2	204·7, 209·7	206·4	203·8, 208·9	0·56	1·6	−2·1, 5·3
Model 2 + hair Hg	205·2	202·6, 207·8	208·0	205·5, 210·5	207·0	204·5, 209·5	206·0	203·4, 208·6	0·90	0·8	−3·0, 4·6
Hair Hg (μg/g)		<0·65		0·65–1·30		1·30–2·54		>2·54			
Model 1	206·3	203·7, 208·8	204·9	202·3, 207·5	205·8	203·3, 208·4	209·3	206·7, 211·9	0·03	3·1	−0·6, 6·8
Model 2	207·0	204·4, 209·5	205·1	202·6, 207·6	205·3	202·8, 207·8	209·0	206·4, 211·6	0·11	2·0	−1·7, 5·8
Model 2 + EPA + DPA + DHA	206·9	204·3, 209·5	205·1	202·5, 207·6	205·3	202·8, 207·8	209·1	206·4, 211·7	0·10	2·2	−1·7, 6·1

DPA, docosapentaenoic acid.
* The mean values in the exposure quartiles were analysed using ANCOVA.
† Mean difference between the extreme quartiles.
‡ Model 1: adjusted for age and examination year.
§ Model 2: adjusted for model 1 + BMI, current smoking status, leisure-time physical activity, energy intake, carbohydrate intake, alcohol intake, use of drugs for hypertension and C-reactive protein, LDL- and HDL-cholesterol concentrations.

the median of 1·30 μg/g, although the $P_{\text{for interaction}}$ was statistically significant only for total *n*-3 PUFA ($P=0·03$) and EPA ($P=0·02$), but not for DPA ($P=0·14$) or DHA ($P=0·18$) (online Supplementary Table S2).

Serum long-chain n-3 PUFA, hair mercury and maximal systolic blood pressure during exercise

The mean maximal SBP during exercise was 206·6 (SD 26·5) mmHg. Serum long-chain *n*-3 PUFA were not associated with maximal SBP during exercise (Table 4). Although hair Hg was associated with a trend towards higher maximal SBP after adjustment for age and examination year, further adjustments attenuated the association and it was no longer statistically significant (Table 4). Hair Hg did not modify the associations between the long-chain *n*-3 PUFA and maximal SBP during exercise as well ($P_{\text{for interaction}}=0·23$ for total long-chain *n*-3 PUFA, $P=0·39$ for EPA, $P=0·13$ for DPA and $P=0·26$ for DHA) (online Supplementary Table S3).

Sensitivity analyses

The associations were generally similar when we excluded participants using hypertension medication (*n* 209) from the analyses. For example, the mean ECP in quartiles of serum total long-chain *n*-3 PUFA after excluding those using hypertension medication was 12·20, 12·61, 12·84 and 12·62 ml/mmHg (model 2, $P_{\text{trend}}=0·05$), the mean VO_{2max} 2505, 2582, 2638 and 2585 ml/min ($P_{\text{trend}}=0·05$) and the mean maximal SBP during exercise 207·1, 207·6, 207·1 and 207·7 mmHg ($P_{\text{trend}}=0·83$) (other data not shown).

Discussion

In this cross-sectional study among 1627 middle-aged and older men from eastern Finland, the serum long-chain *n*-3 PUFA were associated with higher ECP and VO_{2max}, but not with maximal SBP during exercise. However, the clinical significance of the associations was quite modest, but this can be expected because exercise capacity is to a large extent determined by genetics and physical activity[32–34]. Furthermore, although hair Hg concentration was not associated with ECP, higher hair Hg concentration modestly attenuated the associations of the long-chain *n*-3 PUFA with VO_{2max} and ECP.

There is little evidence regarding the association between long-chain *n*-3 PUFA and ECP and its components; three small, randomised trials found that VO_{2max} was increased dose-dependently by fish oil supplementation[12,18,20], but this has not been observed in all supplementation studies[13–17,19]. Moreover, in one study, a DHA-rich meal led to lower systemic vascular resistance and to a smaller increase in SBP during exercise compared with a control meal[21], whereas two other studies did not find any effect of fish oil supplementation on exercise-induced blood pressure[19,22]. It has been reported that intake of long-chain *n*-3 PUFA is associated with lower resting blood pressure[35]. We have previously reported a modest, inverse association between long-chain *n*-3 PUFA and resting SBP in the 11-year examination of the KIHD cohort[36,37]. However, in the current study, we could not find such an association. The lack of association might be due to haemodynamic response to exercise, which is not taken into account for SBP at rest[38].

A possible mechanism underlying the beneficial impact of the serum long-chain *n*-3 PUFA on exercise capacity during an exercise test might be explained by the effect of the long-chain *n*-3 PUFA on the vascular endothelial functions[39], such as improvement in vascular reactivity[40,41], increased production of endogenous antioxidant enzymes and decreased inflammatory cytokines[40,41] and bioavailability of endothelial nitric oxide[40,42].

We have previously found that higher hair Hg concentration attenuated the inverse associations of the long-chain *n*-3 PUFA with CVD outcomes in the KIHD cohort[10,36]. In the current study, hair Hg attenuated the associations of the long-chain *n*-3 PUFA with VO_{2max} and ECP, although the interaction was statistically significant only for VO_{2max}. This attenuation may be at least partially explained by the role of Hg on endothelial dysfunction by reduction in nitric oxide bioavailability and nitric oxide synthase expression[43].

The strengths of our study include the use of serum long-chain *n*-3 PUFA and hair Hg as exposures instead of dietary intakes. As serum fatty acids and hair Hg are objective biomarkers for exposure[23,44], their use reduced the bias by misclassification, which would attenuate the associations towards the null. Other strengths include the extensive examination of potential confounders and the large number of participants with the assessment of VO_{2max}, which is considered to be the 'gold standard' for measuring cardiorespiratory fitness[6]. A limitation of this study is that it is based on an ethnically homogenic population of middle-aged and older men, which may limit the generalisability of our results. In addition, the average hair Hg concentrations are somewhat higher in the KIHD cohort compared with other study populations that have reported Hg exposure[45,46]. Therefore, our results may not be generalisable to study populations with lower average Hg exposure.

In conclusion, our results suggest that higher circulating concentrations of long-chain *n*-3 PUFA, mainly a marker of fish consumption in this study population, are associated with higher ECP and VO_{2max} in middle-aged and older men from eastern Finland. As low cardiorespiratory fitness (VO_{2max}) and low ECP are risk factors for CVD[3–6,8], these results could partially explain how long-chain *n*-3 PUFA may reduce the risk of cardiac mortality.

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manuscript; and all the authors critically revised the paper and approved the final version of the manuscript.

There are no conflicts of interest.

Supplementary material

For supplementary material/s referred to in this article, please visit http://dx.doi.org/doi:10.1017/S0007114516002142

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