High dose fish oil supplements are more effective than oily fish in altering the number and function of extracellular vesicles in healthy human subjects: a randomized, double-blind, placebo-controlled, parallel trial

Article

Supplemental Material

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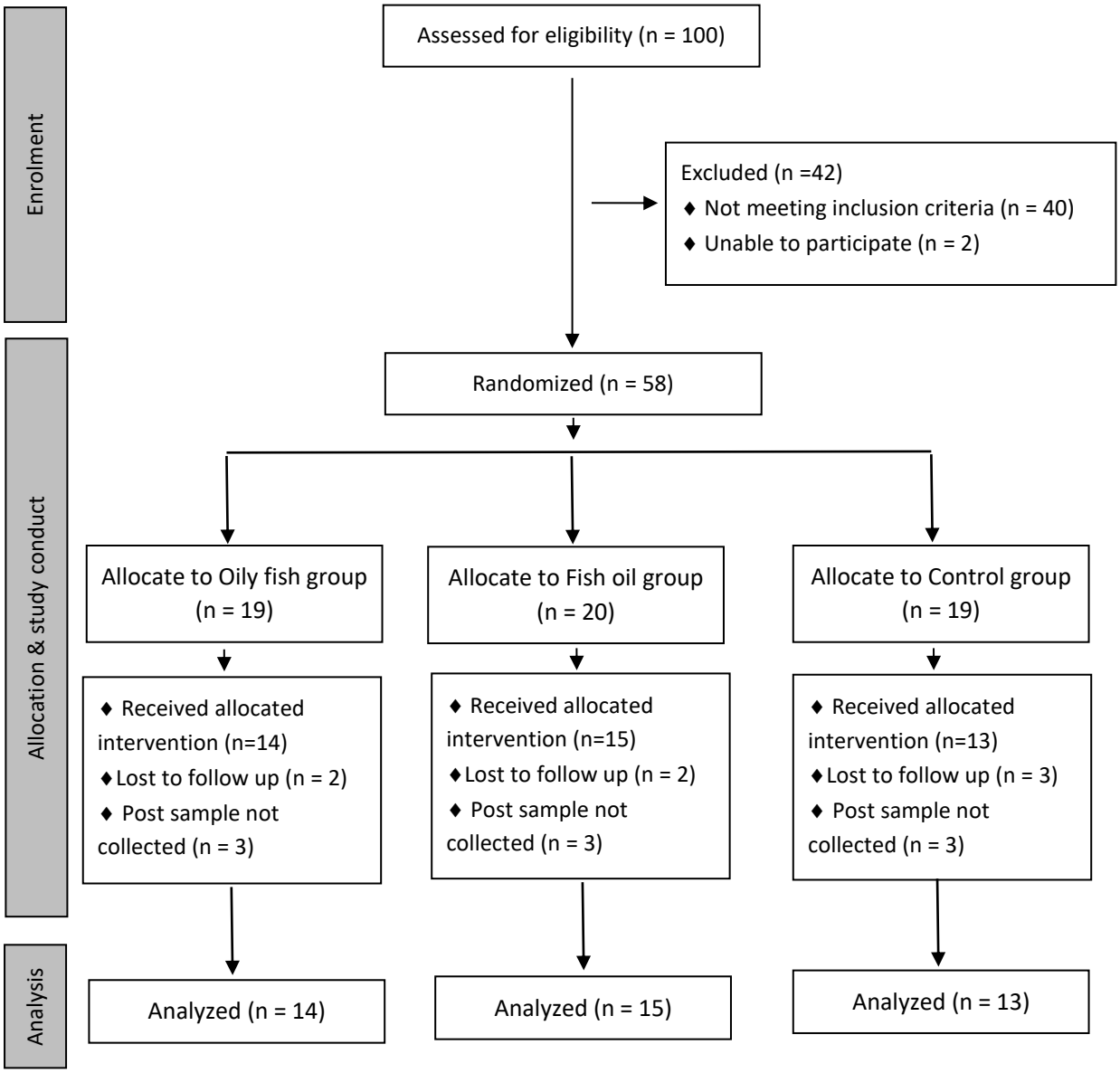
Online Supplementary Material

Supplementary Figures

Supplementary Figure 1. Participant flow



Supplementary Tables

Supplementary Table 1. Scoring tool for recruitment

CVD risk factors	1 point		2 points	
	Males	**Females**	**Males**	**Females**
Total Cholesterol (mmol/L)	5.18-6.21	5.18-6.21	6.22-7.99	6.22-7.99
HDL Cholesterol (mmol/L)	0.91-1.16	1.17-1.29	<90	<1.16
Glucose (mmol/L)	6.00-6.99	6.00-6.99	NA	NA
BMI (kg/m^2)	25.5-29.9	25.5-29.9	30.0-39.9	30.0-39.9
Waist circumference (cm)	>94	>80	>102	>88
SBP (mm Hg)	130-139	130-139	140-159	140-159
DBP (mm Hg)	NA	NA	90-99	90-99
First degree relative diagnosed with MI or T2D (age of diagnosis) (y)	NA	NA	<55 y in male relatives; <65 y in female relatives	<55 y in male relatives; <65 y in female relatives

The scoring tool was based on the Framingham Risk Score system (22), adapted by Chong *et al.* (23) to include a score for family history of MI or T2D. Participants were required to score ≥ 2 points to have an RR ≥ 1.5 of developing CVD, which could be achieved through a combination of CVD risk factors. *MI, myocardial infraction; T2D, type 2 diabetes; NA, not applicable.*

Supplementary Table 2. Effect of fish oil supplements and oily fish on the plasma lipid profile, numbers of EV subtypes, and clot formation and lysis supported by circulating EVs

	Fish oil supplement		Oily fish		Control		P value (treatment)
	Before (wt%)	After (wt%)	Before (wt%)	After (wt%)	Before (wt%)	After (wt%)	
Plasma lipid profile(mmol/L)							
Total cholesterol	4.90±0.28	4.99±0.25	4.30±0.17	4.70±0.17	4.57±0.30	4.79±0.35	0.218
LDL-C cholesterol	2.94±0.23	3.11±0.21	2.55±0.18	2.99±0.15	2.66±0.23	2.84±0.30	0.218
HDL-C cholesterol	1.41±0.05	1.43±0.10	1.15±0.08	1.30±0.08	1.48±0.08	1.54±0.06	0.173
Triacylglycerol	1.21±0.15	0.99±0.07	1.31±0.29	0.89±0.15	0.95±0.15	0.90±0.19	0.988
EV subtype numbers (particles/ ml PFP)							
PS-positive EVs	1.2E+8 ±1.6E+7	6.2E+07±9.3E+6	1.2E+8±1.4E+7	6.9E+7±1.3E+7	1.2E+8±4.1E+7	1.2E+8±4.1E+7	0.976
PDEVs	5.6E+7±9.8E+6	3.39E+7±9.9E+6	7.9E+7±1.3E+7	4.0E+7± 7.1E+6	8.6E+7±4.0E+7	4.2E+7± 7.4E+6	0.567
EDEVs	1.8E+7±2.1E+6	1.2E+7±1.5E+6	1.4E+7±4.3E+6	8.1E+6 ±1.9E+6	1.4E+7±4.5E+6	8.3E+6±1.2E+6	0.387
Clot formation and lysis							
Time to full lysis (min)	468.93±121.91	503.88±128.75	448.54±112.71	477.66±116.51	502.42±99.01	533.00±121.64	0.192
AUC	1073.71±163.68	825.71±133.99	1232.35±183.34	1102.91±179.49	1023.21±199.71	999.37±146.19	0.075

Data are mean ± SEM. Differences in the plasma lipid profile, numbers of EV subtypes, and clot formation and lysis supported by circulating EVs between the three groups were determined using a general linear model, including post-hoc analysis with Bonferroni tests for treatment, period and treatment*time interaction with differences shown at $P < 0.05$. There was no effect of either oily fish or fish oil supplements on the numbers of EV subtypes and plasma lipid profile. *AUC, area under curve; EVs, extracellular vesicles; EDEVs, endothelial-derived extracellular vesicles; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PDEVs, platelet-derived extracellular vesicles; PS-positive EVs, phosphatidylserine positive extracellular vesicles.*

Supplementary Table 3. Associations between fatty acid profiles of circulating EVs with numbers and coagulatory activity of circulating EVs

Fatty acids		EV numbers	EV-dependent thrombin generation (Isolated EVs minus VDP)				EV-dependent clot formation	
			Lag time	Peak thrombin concentration	Velocity index	AUC	Time to full lysis	AUC
Palmitic acid (16:0)	*r*	-.020	.110	-.033	-.119	-.007	.131	.057
	p	.854	.321	.764	.281	.947	.236	.606
Stearic acid (18:0)	*r*	-.064	**.290****	-.003	-.117	.090	.064	.040
	p	.561	**.008**	.981	.289	.418	.562	.721
Oleic acid (18:1, n-9)	*r*	.201	-.055	.126	.186	.102	-.119	.110
	p	.067	.618	.255	.091	.354	.280	.317
Linoleic acid (18:2, n-6)	*r*	-.080	**-.218***	.040	-.062	-.054	-.111	-.056
	p	.469	**.047**	.721	.577	.623	.314	.616
AA (20:4, n-6)	*r*	.124	.164	.114	.270*	.116	-.112	-.092
	p	.261	.137	.303	.013	.294	.312	.403
ALA (18:3, n-3)	*r*	.020	**.233***	-.156	-.122	-.185	.003	.154
	p	.857	**.033**	.156	.269	.091	.981	.161
EPA (20:5, n-3)	*r*	**-.525****	**-.114**	**-.286****	**-.080**	**-.316****	-.013	**-.275***
	p	**<.001**	**.300**	**.008**	**.469**	**.003**	.909	**.011**
DPA (22:5, n-3)	*r*	.017	.149	.081	.149	.121	-.184	.164
	p	.876	.175	.463	.176	.273	.094	.137
DHA (22:6, n-3)	*r*	**-.244***	-.073	-.079	.037	-.210	-.003	-.129
	p	**.025**	.510	.474	.739	.055	.981	.242
Total SFA	*r*	-.028	**.341****	.033	**-.220***	.122	.150	.083
	p	.800	**.001**	.764	**.045**	.267	.172	.452
Total MUFA	*r*	.177	-.043	.128	.209	.098	-.076	.091
	p	.108	.696	.246	.057	.375	.490	.411
Total n-3 PUFA	*r*	**-.492****	-.036	-.153	-.076	**-.317****	-.045	-.085
	p	**<.001**	.744	.165	.494	**.003**	.685	.444
Total n-6 PUFA	*r*	-.055	-.194	.066	.028	.001	-.097	-.092
	p	.617	.078	.550	.803	.991	.378	.403

Pearson's correlation coefficient or Spearman's correlation coefficient was conducted to examine the associations between fatty acid profiles of circulating EVs with numbers and coagulatory activity of circulating EVs**.** *. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed). *AA, arachidonic acid; ALA, alpha-linolenic acid; AUC, area under curve; EPA, eicosapentaenoic acid; EVs, extracellular vesicles; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; SFAs, saturated fatty acids.*

Supplementary Table 4. Associations between fatty acid profiles of RBCs with numbers and coagulatory activity of circulating EVs

Fatty acids		**EV numbers**	**EV-dependent thrombin generation (Isolated EVs minus VDP)**				**EV-dependent clot formation**	
			Lag time	Peak thrombin concentration	Velocity index	AUC	Time to full lysis	AUC
Palmitic acid (16:0)	*r*	-.014	.124	-.026	-.004	-.088	.001	-.046
	p	.899	.268	.814	.975	.431	.990	.683
Stearic acid (18:0)	*r*	**-.221***	.097	.066	-.005	-.078	.161	-.197
	p	**.046**	.388	.554	.967	.487	.149	.077
Oleic acid (18:1, n-9)	*r*	.211	.021	-.003	.107	.072	-.045	**.271***
	p	.058	.852	.977	.339	.520	.685	**.014**
Linoleic acid (18:2, n-6)	*r*	**.270***	-.011	.095	-.101	.161	-.157	.146
	p	**.014**	.918	.396	.365	.149	.158	.190
AA (20:4, n-6)	*r*	.065	.063	.119	.194	.077	.124	-.041
	p	.560	.573	.286	.080	.493	.267	.714
ALA (18:3, n-3)	*r*	.164	-.058	-.023	.062	.017	-.016	.167
	p	.142	.603	.838	.578	.878	.885	.134
EPA (20:5, n-3)	*r*	**-.511****	-.119	**-.301****	-.128	**-.255***	.147	**-.303****
	p	**<.001**	.288	**.006**	.250	**.021**	.189	**.006**
DPA (22:5, n-3)	*r*	**-.444***	-.067	**-.285****	-.132	**-.278***	.126	**-.332****
	p	**<.001**	.550	**.009**	.237	**.011**	.261	**.002**
DHA (22:6, n-3)	*r*	**-.435****	-.172	-.144	-.157	**-.222***	.037	-.187
	p	**<.001**	.123	.197	.158	**.045**	.741	.092
Total SFA	*r*	-.213	.185	.001	.097	-.154	.132	-.139
	p	.055	.096	.920	.385	.166	.237	.213
Total MUFA	*r*	.269*	.065	.022	.107	.088	-.038	**.299****
	p	.015	.559	.846	.340	.431	.733	**.006**
Total n-3 PUFA	*r*	**-.506****	-.164	**-.235***	-.151	**-.279***	.151	**-.261***
	p	**<.001**	.141	**.003**	.175	**.011**	.174	**.018**
Total n-6 PUFA	*r*	**.300****	.004	.189	.041	**.253***	-.094	.060
	p	**.006**	.969	.088	.714	**.022**	.402	.593

Pearson's correlation coefficient or Spearman's correlation coefficient was conducted to examine the associations between fatty acid profiles of RBCs with numbers and coagulatory activity of circulating EVs**.** *. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed). *AA, arachidonic acid; ALA, alpha-linolenic acid; AUC, area under curve; EPA, eicosapentaenoic acid; EVs, extracellular vesicles; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; SFAs, saturated fatty acids.*