Supplementary Information (SI) for Food & Function.

Effects of Fish Oil Supplementation on Biomarkers of Vascular Endothelial Function in Middle-Aged and Older Adults: A Randomized Controlled Trial

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Table S1. Summary of Repeated-Measures ANOVA Results for Key Dietary Nutrient Intakes Across Four Intervention Groups and Three Time Points(PP set)

Nutrient & Time	Control(n=47)	FO1(n=51)	FO2(n=52)	FO3(n=51)	P_{group}	P_{time}	$P_{group*time}$
Energy (kcal/d)					0.401	0.001	0.108
Baseline	1290.54±461.47	1466.00±700.40	1419.88±564.06	1331.10±451.19			
6 week	1296.98±441.33	1429.57±476.20	1482.47±405.01	1469.81±554.32			
12 week	1665.58±543.55	1500.71±470.72	1618.41±547.61	1444.89±529.88			
Protein (%E)					0.148	0.940	0.550
Baseline	17.64±4.70	18.33±4.90	20.21±6.30	19.34±5.01			
6 week	18.38±4.31	18.28±4.67	19.02±4.66	19.30±5.38			
12 week	18.37±4.49	19.46±5.01	18.81±5.49	19.00±4.23			
Fat (%E)							
Baseline	24.02±10.47	24.00±9.50	23.97±11.30	24.73±12.10	0.768	0.639	0.587
6 week	23.98±7.43	25.87±11.06	24.96±10.42	25.48±11.25			
12 week	27.26±12.29	23.25±9.98	23.59±10.28	25.86±11.70			
Carbohydrate (%E)					0.710	0.715	0.634
Baseline	58.35±12.13	57.68±11.37	55.82±13.30	55.94±13.76			
6 week	57.63±8.52	55.85±11.85	56.03±11.41	55.21±11.63			
12 week	54.37±12.82	57.30±10.12	57.61±11.17	55.15±11.12			
SFA(g/d)					0.692	0.710	0.670
Baseline	5.09±5.25	5.55±4.74	6.19±7.74	5.95±7.03			
6 week	3.88±4.28	6.42±7.74	5.41±6.79	5.20±5.75			
12 week	5.73±6.43	5.18±4.65	5.44±7.37	4.93±5.31			
MUFA(g/d)					0.898	0.631	0.648
Baseline	7.28±8.11	6.89±6.47	8.20±10.49	8.48±12.76			
6 week	4.85±5.80	8.05±10.34	7.06±9.50	7.46±10.36			
12 week	7.87±9.57	7.25±9.79	6.96±8.85	6.39±10.68			
PUFA(g/d)					0.967	0.423	0.430
Baseline	3.19±3.73	3.36±3.55	3.45±3.43	3.22±3.72			
6 week	2.16±2.28	3.64±4.62	3.38±3.74	3.36±4.42			
12 week	4.63±7.72	3.29±3.80	2.99±3.14	4.04±9.74			
EPA(g/d)					0.466	0.060	0.920
Baseline	0.02±0.05	0.02±0.04	0.03±0.05	0.02±0.03			
6 week	0.01±0.02	0.02±0.05	0.02±0.03	0.02±0.04			
12 week	0.02±0.05	0.03±0.05	0.03±0.08	0.03±0.06			
DHA(g/d)					0.279	0.223	0.996
Baseline	0.02±0.05	0.03±0.06	0.04±0.09	0.02±0.03			
6 week	0.02±0.03	0.03±0.07	0.03±0.05	0.02±0.04			
12 week	0.03±0.05	0.04±0.09	0.04±0.11	0.03±0.06			
ALA(g/d)					0.483	0.643	0.789
Baseline	0.03±0.04	0.03±0.04	0.04±0.04	0.04±0.05			
6 week	0.03±0.03	0.03±0.04	0.04±0.05	0.04±0.04			
12 week	0.03±0.04	0.04±0.04	0.03±0.03	0.05±0.19			

PP, per-protocol; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; ALA, α-Linolenic acid;

Values are mean±SD;

Table S2. Two-way ANOVA Results for the Interaction Between Fish Oil Supplementation and Baseline Omega-3 Index Category on Nitric Oxide and Endothelin-1 Levels

	Per-Protocol Analysis						Intention-to-Treat Analysis					
Outcome	Source	df	MS	F	*P*	ηp²	Source	df	MS	F	*P*	ηp²
NO Absolute Change (μmol/L)	Group	3	5289.717	2.222	0.087	0.033	Group	3	6842.391	2.985	0.032	0.037
	Omega3 Index Category	1	1188.361	0.499	0.481	0.003	Omega3 Index Category	1	1143.007	0.499	0.481	0.002
	Group*Omega3 Index Category	3	6062.410	2.547	0.057	0.038	Group*Omega3 Index Category	3	7871.500	3.434	0.018*	0.043
	Error	193	2380.105				Error	232	2292.346			
ET-1 Absolute Change (pg/mL)	Group	3	21.834	0.477	0.699	0.007	group	3	44.176	0.991	0.398	0.013
	Omega3 Index Category	1	71.552	1.563	0.213	0.008	Omega3 Index Category	1	85.423	1.917	0.168	0.008
	Group*Omega3 Index Category	3	30.668	0.670	0.572	0.010	Group*Omega3 Index Category	3	20.410	0.458	0.712	0.006
	Error	193	45.787				Error	232	44.565			

Note: Absolute Change=(12week-Baseline);Data are presented as results of two-way analysis of variance (ANOVA). The baseline Omega-3 index was dichotomized based on the median value of erythrocyte membrane EPA+DHA as a percentage of total fatty acids in the study population (4.562% for the PP dataset; 4.640% for the ITT dataset): low Omega-3 index group (≤ median) and high Omega-3 index group (> median). The significance level for interaction effects was set at $\alpha = 0.05$. Effect sizes are reported as partial eta-squared (ηp^2); * versus control, $P < 0.05$; **, $P < 0.01$;

Table S3. Simple Effects of Fish Oil Supplementation on the Absolute Change in Nitric Oxide (NO), Stratified by Baseline Omega-3 Index (ITT set)

Omega3 Index Category	Group	n	NO Absolute Change(μmol/L), Mean±SD	Mean Difference(μmol/L) vs. Control (95% CI)	*P*
Low	Control	23	23.03±52.32	-	-
	FO1	39	1.61±50.86	-21.41(-46.21,3.39)	0.090
	FO2	23	0.26±36.89	-22.77(-50.59,5.05)	0.108
	FO3	39	3.29±45.15	-19.74(-44.54,5.06)	0.118
High	Control	37	2.07±41.45	-	-
	FO1	21	-20.71±50.91	-22.78(-48.56,2.99)	0.083
	FO2	37	28.73±59.58	26.66(4.73,48.59)	0.017*
	FO3	21	-0.04±34.95	-2.11(-27.89,23.66)	0.872

Note: This table presents the results of the simple effects analysis following a significant treatment × Omega-3 index interaction ($p < 0.05$) in the two-way ANOVA for the ITT population. The Omega-3 index was dichotomized at the median (4.640%) of the ITT dataset. Mean difference represents the absolute change from baseline in the fish oil group compared to the placebo group within the same Omega-3 index stratum. Abbreviations: CI, confidence interval; ITT, intention-to-treat; SD, standard deviation; * versus control, $P < 0.05$; **, $P < 0.01$;