A Double-Blind Randomized Trial of Fish Oil to Lower Triglycerides and Improve Cardiometabolic Risk in Adolescents

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Objectives To determine the efficacy of 4 g/day fish oil to lower triglycerides and impact lipoprotein particles, inflammation, insulin resistance, coagulation, and thrombosis.

Study design Participants (n = 42, age 14 ± 2 years) with hypertriglyceridemia and low-density lipoprotein (LDL) cholesterol <160 mg/dL were enrolled in a randomized, double-blind, crossover trial comparing 4 g of fish oil daily with placebo. Treatment interval was 8 weeks with a 4-week washout. Lipid profile, lipoprotein particle distribution and size, glucose, insulin, high-sensitivity C-reactive protein, interleukin-6, fibrinogen, plasminogen activator inhibitor-1, and thrombin generation were measured.

Results Baseline lipid profile was total cholesterol 194 (5.4) mg/dL (mean [SE]), triglycerides 272 (21) mg/dL, high-density lipoprotein cholesterol 39 (1) mg/dL, and LDL cholesterol 112 (3.7) mg/dl. LDL particle number was 1614 (60) nmol/L, LDL size was 19.9 (1.4) nm, and large very low-density lipoprotein/chylomicron particle number was 9.6 (1.4) nmol/L. Triglycerides decreased on fish oil treatment but the difference was not significant compared with placebo (−52 ± 16 mg/dL vs −16 ± 16 mg/dL). Large very low-density lipoprotein particle number was reduced (−5.83 ± 1.29 nmol/L vs −0.96 ± 1.31 nmol/L; $P < .0001$). There was no change in LDL particle number or size. There was a trend towards a lower prothrombotic state (lower fibrinogen and plasminogen activator inhibitor-1; $.10 > P > .05$); no other group differences were seen.

Conclusions In children, fish oil (4 g/day) lowers triglycerides slightly and may have an antithrombotic effect but has no effect on LDL particles. *(J Pediatr 2014;165:497-503)*.

As a consequence of the obesity epidemic, the prevalence of dyslipidemia characterized by increased levels of triglycerides and low levels of high-density lipoprotein (HDL) cholesterol has increased, with an overall prevalence of dyslipidemia of 42%, including increased triglycerides present in obese adolescents who represent 16.9% of the pediatric population.[1] These dyslipidemic traits help characterize the metabolic syndrome, a phenotype associated with premature development of atherosclerosis, future type 2 diabetes mellitus, and premature cardiovascular disease in young adults.[2-4] Adolescents with this dyslipidemia may have a discordant distribution of low-density lipoprotein (LDL) particles predicting risk greater than what would be predicted based on LDL cholesterol level alone.[5] In childhood, obesity-related metabolic derangements are associated with nontraditional risk factors leading to an insulin-resistant, chronic inflammatory, and prothrombotic state.[6]

Fish oil (including docosahexaenoic acid [DHA] and eicosapentaenoic acid [EPA]) has emerged as a controversial treatment with potential benefit in the prevention of atherosclerotic vascular disease and sudden death; however, the underlying therapeutic mechanisms remain unclear. Although some adult clinical trials have shown a benefit of fish oil treatment on certain cardiovascular outcomes, recent meta-analyses, reviews, and clinical studies have questioned the benefit of 1 g/day or lower supplementation.[7-10] Moderate doses of fish oil (4 g/day) are known to lower triglycerides; however, this effect alone may not be sufficient to explain clinical outcomes because the role of triglycerides per se in atherosclerosis progression has not been elucidated.[11]

In children and adolescents, there is a paucity of clinical trial data on treatment of increased triglycerides and on the cardiometabolic effects of fish oil in particular. Therefore, we conducted a multicenter, randomized, double-blind, placebo, crossover trial of 4 g daily of fish oil in adolescents with elevated triglycerides (>150 mg/dL). The primary end point was reduction in triglycerides with secondary aims of characterizing the LDL particle distribution of adolescents with increased triglycerides and assessing the impact of fish oil on lipid particle

ALT	Alanine aminotransferase	EPA	Eicosapentaenoic acid
apo B	Apolipoprotein B	HDL	High-density lipoprotein
AST	Aspartate-aminotransferase	IL-6	Interleukin-6
CRP	C-reactive protein	LDL	Low-density lipoprotein
DHA	Docosahexaenoic acid	PAI-1	Plasminogen activator inhibitor-1
DPA	Docosapentaenoic-n3 acid	VLDL	Very low-density lipoprotein

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distribution and markers of inflammation, insulin resistance, coagulation, and thrombosis.

Methods

This study was approved by the Institutional Review Boards at A. I. DuPont Hospital for Children, Thomas Jefferson University, and Johns Hopkins University Hospital. Parental permission and child assent were obtained before enrollment. At A. I. DuPont Hospital for Children, Thomas Jefferson University, and Johns Hopkins University Hospital, adolescents seen in lipid referral clinics with the following characteristics were recruited: age 10-17 years, fasting triglyceride level of ≥150 mg/dL and <750 mg/dL on 2 separate occasions, and LDL cholesterol level <160 mg/dL. Exclusion criteria were any bleeding abnormality, diabetes mellitus, uncontrolled hypothyroidism, liver disease, allergy to fish/shellfish, chronic use of aspirin or anti-inflammatory agents, taking lipid-lowering medication; LDL cholesterol levels >160 mg/dL, smoking, alcohol use, pregnancy, or participation in another clinical trial. All 42 participants were Tanner 4 or greater. Racial/ethnic composition was white, non-Hispanic (36), white Hispanic (3), black (2), and other (1).

This randomized, double-blind, placebo, crossover trial consisted of two 8-week treatment periods, separated by a 4-week washout. Eligible patients were randomized to receive either fish oil 4 g daily or corn oil placebo during the first 8-week treatment period; patients received the alternate treatment during the next treatment period. GlaxoSmithKline supplied the study drug (containing minimally 1.5 g of DHA and 1.86 g of EPA of ethyl esters) and placebo.

Patients were evaluated at 6 time points: visit 1/baseline (week 0), visit 2/randomization (week 4), visit 3/after treatment 1 (week 12), visit 4/after washout (week 16), visit 5/after treatment 2 (week 24), and visit 6/close out (week 28). Patients were advised to maintain a stable diet and not alter baseline fish consumption. One participant took an oral contraceptive throughout the trial. Any fish oil supplements were discontinued. Advice on a heart-healthy diet was provided. Blood pressure (right arm sitting with appropriate sized cuff, taken 3 times, last measurement used), height, and weight were measured at the beginning of the study, after the first washout period, and close out. Participant phone contact was made during each treatment arm to assess diet stability. A fasting lipid profile was performed at every visit. Red blood cell fatty acid profile and secondary endpoints were measured at all visits except baseline. Secondary endpoints included measures of thrombin generation, high sensitivity C-reactive protein (CRP), interleukin-6 (IL-6), fibrinogen, and plasminogen activator inhibitor-1 (PAI-1). Lipid particle and apolipoprotein B (apo B) measurements were performed at baseline and at the end of each treatment arm. Glucose, insulin, alanine aminotransferase (ALT), and aspartate-aminotransferase (AST) were obtained at baseline and at the end of each treatment arm. Adverse events were recorded at all study encounters. Adverse events were graded mild, moderate, or severe. An independent data- and safety-monitoring physician reviewed all events and safety data biannually.

Lipid, lipoprotein, and lipoprotein particle analysis was performed by a commercial laboratory (Liposcience Inc; Raleigh, North Carolina) as were glucose, insulin, ALT, and AST. Red blood cell fatty acid profile was obtained commercially (OmegaQuant, Sioux Falls, South Dakota) to assess compliance and test the completeness of the washout between study arms. Thrombin generation was measured with a commercially available assay according to the guidelines of the manufacturer (Technothrombin-TGA; Technoclone, Vienna, Austria). Evaluation of thrombin generation was performed automatically via the manufacturer's Technothrombin-TGA evaluation software and calculated as thrombin generation over time.

For analysis, the peak height for thrombin generation, velocity index, or peak rate of thrombin generation (peak

Table I. Demographics and clinical characteristics at visits 1 and 4 with summaries by treatment sequence

Variables	Treatment sequences PT	TP	*P* value
Age, y	14.2 (0.5)	14 (0.4)	.766
Weight, kg			
V1	86 (4.3)	88.8 (3.8)	.604
V4	88 (4.2)	90.4 (4.1)	.712
Height, cm			
V1	166 (2.4)	167 (5.4)	.617
V4	166 (3)	170 (1.9)	.304
Body mass index, kg/m^2			
V1	31 (1)	31 (1)	.737
V4	31 (1)	31 (1)	.768
SBP, mm Hg			
V1	116.3 (3)	116 (3)	.924
V4	119.8 (2)	115 (3)	.232
DBP, mm Hg			
V1	68.9 (2)	72 (2.2)	.356
V4	73.1 (2)	68 (2.7)	.162
Heart rate, bpm			
V1	76.1 (3.0)	70 (3)	.168
V4	72 (2.7)	71 (3)	.734
TC, mg/dL			
V1	195 (6.9)	192 (5.7)	.794
V4	206 (10.8)	202 (6.5)	.743
TG, mg/dL			
V1	280 (25)	260 (22)	.562
V4	264 (36)	261 (27)	.952
HDL cholesterol, mg/dL			
V1	39 (1.5)	40.1 (2.0)	.635
V4	40 (1.9)	41 (1.96)	.679
LDL cholesterol, mg/dL			
V1	106 (4)	110 (6)	.625
V4	109 (5)	114 (6)	.541

bpm, beats per minute; *DBP*, diastolic blood pressure; *PT*, placebo followed by treatment; *SBP*, systolic blood pressure; *TC*, total cholesterol; *TG*, triglyceride; *TP*, treatment followed by placebo; *V1*, visit 1; *V4*, visit 4 precrossover.
Mean (SE) is presented for continuous variables and frequency and percentage is presented for categorical variables. Means between were compared using 2-sample t test and proportion of categorical variables were compared using χ^2 distributions.