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# Effect of fish oil supplementation on serum triglycerides, LDL cholesterol and LDL subfractions in hypertriglyceridemic adults

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**Abstract** *Background and aims:* The well-established triglyceride (TG) lowering effect of fish oil is accompanied by an increase in LDL-cholesterol (LDL-C) concentration. Less is known about the differential impact on LDL particle distribution — the smaller particles being associated with a greater risk for atherosclerosis. We aimed to examine the changes in serum concentrations of four subclasses of LDL particles as well as shifts in LDL phenotype patterns (A, B, AB) among hypertriglyceridemic adults.

*Methods and results:* This was a secondary analysis from a double-blind, parallel design, placebo controlled trial with 42 adults that experienced significant TG lowering and modest increases in total LDL-C concentrations after 12 weeks of 4 g/d EPA + DHA. Reduction in serum TG concentrations (mean ± SEM) was −26 ± 4% (−0.81 ± 10.12 mmol/L), $p < 0.0001$. Total LDL-C concentration increased by 13 ± 3% (+0.31 ± 0.08 mmol/L), $p < 0.0001$. The 12-week changes in concentrations of LDL1, LDL2, LDL3 and LDL4 were +0.06 ± 0.02 mmol/L [+2.2 ± 0.7 mg/dL], +0.07 ± 0.03 mmol/L [+2.6 ± 1.0 mg/dL], +0.16 ± 0.05 mmol/L [+6.3 ± 1.8 mg/dL], and +0.04 ± 0.04 mmol/L [+1.4 ± 1.7 mg/dL], respectively (+20 ± 5%, +64 ± 13%, +26 ± 6%, and +17 ± 9%), $p < 0.05$ for all but LDL4. Changes in LDL phenotype patterns A, B and A/B were negligible and not statistically significant.

*Conclusion:* In this population of hypertriglyceridemic adults, dietary supplementation with fish oil resulted in an increase in total LDL-C concentration which was distributed relatively evenly across the range of smaller and more atherogenic as well as larger and less atherogenic LDL particles.



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## Introduction

Although hypertriglyceridemia commonly affects 30% of the US population [1,2], only 1.3% of those affected use a prescription medication (fenofibrate, gemfibrozil, or niacin) to lower triglycerides (TG) [2]. The most appropriate management approach and the exact timing when to initiate pharmacological treatment is still a source of debate which is especially true for isolated hypertriglyceridemia in the absence of additional cardiovascular risk factors. Extensive evidence supports the cardiovascular benefits of marine omega-3 fatty acids (FA), eicosapentaenoic acid (EPA) and docosahexaenoic (DHA) [3,4]. The American Heart Association recommends 2–4 g/day of EPA/DHA for the treatment of hypertriglyceridemia [5]. Among all cardiovascular benefits of fish oil, the hypotriglyceridemic effect is the most established of fish oil [6]. In comparison to approved TG lowering pharmacological agents, omega-3 FA are highly effective and generally well-tolerated and, if delivered based on evidence, those treatments can be less costly alternatives. However, omega-3 FA are the only TG lowering agent to increase LDL. Because of this concern, the US FDA limited the approval of the only prescription form of omega-3 FA to patients with very high TG concentrations ($\geq$5.7 mmol/L). In recent years, the popularity of fish oil supplements has risen dramatically due to the identification of health benefits beyond the reduction of triglycerides (anti-thrombogenic, anti-inflammatory, hypotensive, anti-arrhythmic) [7]. U.S. sales in omega-3 supplements reached $359.3 million in 2010 [8]. While the TG lowering effects of fish oils are widely recognized, the modest but consistent increase in LDL-C concentration observed with this therapy has received less attention. Given the greater atherogenicity of the smaller LDL particles [9–14], a shift toward larger particles is considered clinically beneficial on the overall lipoprotein profile. Dietary modifications using a low-carbohydrate diet have been shown to decrease serum TG concentrations accompanied by a favorable shift in LDL particle distribution toward fewer small, dense and more large, buoyant LDL particles [15]. It is not clear if the same can be achieved through fish oils. The reported effects on LDL particle distribution have been inconsistent. A PubMed search for randomized controlled trials using the terms "omega-3 FA" and "LDL size" resulted in 7 studies showing improvement in LDL size as evidenced by an increase in LDL particle size or a shift in LDL particle distribution from small, dense LDL particles, to large, buoyant ones [14,16–21]. A similar number of studies, however, did not observe changes in LDL particle size or distribution [22–28]. Finally, two studies found a detrimental effect, manifested as an increase in small, dense LDL particle concentration [29,30]. The difficulty in drawing clear conclusions from these studies lies in the substantial variability in study design, sample size, dosage, LDL particle assessment methodology, and target population. Given the advocated benefits of fish oil supplementation in TG lowering in particular and cardiovascular disease prevention in general, it is clinically relevant to determine whether the commonly observed increase in LDL-C is offset by improvement of LDL particle size among hypertriglyceridemic individuals. If the differential impact on LDL particle size were true of therapies with fish oil, the fish oil induced modest increase in total LDL-C would be of a lesser clinical concern.

## Methods

### Study design

The original study was a double-blinded, parallel design, placebo controlled trial with three active treatment arms to compare the TG lowering effects of the same dose of EPA and DHA provided in three formulations of supplements that differed in the proportion of omega-3 FA present as ethyl esters vs. triglycerides. The active therapy for each of the three fish oil supplementation arms was 4 g/day of combined EPA and DHA provided as: a) 90% TG formulation (TG90), b) 60% TG formulation (TG60), or c) ethyl esters (EE) (i.e., 0% TG). Participants were instructed to take 5 capsules per day, 3 with breakfast, and 2 with lunch or dinner. Each fish oil capsule contained 800 mg of EPA and DHA. The placebo was a soy oil supplement with an identical total fat content. The primary outcome of the original trial was 12-week change in TG concentrations in the active groups vs. placebo. The outcome of primary interest in this secondary analysis is LDL particle distribution, defined as either subclasses LDL1, LDL2, LDL3 and LDL4, or as LDL subclass phenotype patterns A, B, or A/B. Because there was no difference in TG reductions ($p = 0.52$) and LDL-C increases ($p = 0.89$), which occurred across all three treatment arms but not the control group, for the present analysis, the three active groups were collapsed into one category.

### Subjects

Participants were recruited between January and August 2009 through radio and local newspaper advertisements. Eligibility criteria included fasting plasma TG concentration $\geq$1.7 mmol/L but $\leq$7.3 mmol/L, age $\geq$18 y, and a body mass index (kg/m$^2$) below 40. The fasting TG eligibility criterion could be met by either a single value $>$2.3 mmol/L, or, when the initial value was between 1.7 and 2.3 mmol/L, by obtaining a second value and having the average of the two be $\geq$1.7 mmol/L. Exclusion criteria included self-reported history of clinically significant atherosclerosis, diabetes mellitus, malignant neoplasm, psychiatric illness, use of lipid-lowering medications (e.g., niacin, statins), anti-hypertensive drugs (e.g., beta-blockers, thiazides), use of dietary supplements containing omega-3 within the past month, pregnant or lactating, and average alcohol consumption $>$30 g per day. Enrolled participants were instructed to maintain weight and habitual dietary and exercise habits. Participants received counseling and handouts regarding avoidance of omega-3 intake through consumption of fish or other marine products. All study participants provided written informed consent. The study was approved by the Stanford University Human Subjects Committee.

### Data collection and assessment

Participants completed 3-day food records and 7-day Physical Activity Recalls (PAR) [31] prior to randomization and at the end of the study. Dietary intake data were analyzed using Nutrition Data System for Research (NDS-R) software version 2007, developed by the Nutrition Coordinating Center (NCC), University of Minnesota, Minneapolis, MN. Participants and data collectors were blind to treatment assignment. Blood pressure, weight and venous blood samples were collected at four time points: baseline, 4-weeks, 8-weeks and at 12-weeks. Baseline and 12-week visits comprised of two separate visits within a 2 week window.

Measurements of all lipoprotein classes, subclasses and phenotypes were performed by Atherotech. Diagnostics Lab (201 London Parkway, Ste 400, Birmingham, AL 35211) using vertical spin density gradient ultracentrifugation (VAP©Test). Concentrations of TG, LDL-C, LDL1, LDL2, LDL3, and LDL4 were simultaneously and directly measured with LDL1 being the largest, more buoyant and LDL4 the smallest fraction [32]. Based on the predominant LDL size on gradient gel electrophoresis as defined by Krauss et al. [33] subjects were classified as phenotypes A (large particle size; $\geq$26.3 nm), A/B (intermediate particle size; 25.8–26.3 nm), or B (small particle size $<$25.8 nm).

Red blood cell (RBC) concentrations of EPA and DHA were measured in venous blood samples from fasting participants. Blood was drawn into EDTA tubes and centrifuged at 4 °C to separate cells from plasma. The plasma and buffy coat were removed, and RBCs were washed two times with cold saline. Supernatant was removed and RBC layer was frozen at −70 °C until analyzed. An RBC aliquot was thawed and heated at 100 °C for 10 min with methanol containing 14% boron trifluoride. The FA methyl esters thus generated were extracted with hexane and water and were analyzed via gas chromatography using a GC2010 (Shimadzu Corporation, Columbia, MD) equipped with a 100-m capillary column (SP-2560; Supelco, Bellefonte, PA). FA were identified through comparison with a standard FA methyl ester mixture (GLC-727; Nuchek Prep, Elysian, MN). EPA and DHA were measured as percentage of total FA. Harris has defined omega-3 index (OMX-3) expressed as the sum of EPA + DHA as a percent of total identified RBC FA [34] which was used here as a measure of adherence to capsule consumption.

### Statistical analysis

Baseline and 12-week lipid and lipoprotein values were calculated as mean of two separate measurements within 2 weeks. All results are presented as mean ± SD (baseline demographics) or mean ± SEM (lipid and lipoprotein results) unless otherwise indicated. Normality was tested using the Shapiro–Wilk test for the change in TG, LDL, and LDL1-4 for each group. Differences in means for the four LDL particle sizes for the collapsed treatment group from baseline to 12-weeks were determined by matched-pairs *t*-test. Difference in means between groups was calculated with analysis of covariance (ANCOVA) for absolute change from baseline to 12-week. Repeated measures ANOVA were used to test time and treatment interaction between groups. All analyses were performed using SPSS (version 18.0; SPSS Inc., Chicago, IL, USA). Significance was defined as $P < 0.05$ using a two-tailed test.

## Results

### Demographics, enrollment, retention

The enrolled study population was comprised of 46 men and 14 women with a mean age of 52 ± 10 years (range 32–75 years). The ethnicity was predominantly non-Hispanic White ($n = 42$), followed by Asian/Pacific Islander ($n = 12$), Hispanic [5] and other [1]. Of 60 enrolled into the study, a total of 57 participants completed the 12-week protocol, with three participants lost to follow-up (Fig. 1, Table 1).

No changes were detected for diet (Kcals/day; $p = 0.08$), physical activity ($p = 0.38$), or weight for any group ($p = 0.43$) (data not included).

### Adherence

Adherence was determined by pill count and RBC analysis of omega-3 FA performed once at baseline and 12-weeks. Adherence evaluated through pill count in returned bottles was (mean ± SD) 88 ± 6%, 86 ± 9%, 88 ± 8%, and 89 ± 5% for the TG 90, TG 60, EE, and placebo, respectively. Adherence for the collapsed treatment groups was 87 ± 8%. The adherence using OMX-3 was evaluated and presented as percent increase of OMX-3 from baseline. Percent change of OMX-3 from baseline was (mean ± SEM) 105 ± 13%, ($n = 12$) for TG90, 117 ± 15%, ($n = 13$) for TG60, 134 ± 34%, ($n = 15$) for EE, and 21 ± 28%, ($n = 15$) for placebo. For the collapsed treatment group this was 120 ± 14%, $P < 0.001$ for collapsed treatment groups vs. placebo. Data for 5 participants were unavailable as, in addition to the three drop outs, two RBC samples were compromised.

### Triglyceride and total LDL-C concentrations

The results of the lipid parameters are summarized in Table 2.

#### Triglycerides

A decrease in plasma TG concentrations at 12-weeks compared to baseline was observed in all treatment groups but not in the placebo group. The overall reduction in TG (mean ± SEM) was −26 ± 4% for the collapsed treatment group. Change in percent from baseline for TG90 was −19 ± 10%, for TG60 was −27 ± 5%, for EE was −30 ± 6% ($p = 0.52$ for differences among active treatment arms) and for placebo was −2 ± 8%.

#### Total LDL-C

An overall increase in LDL-C concentrations at 12-weeks compared to baseline was observed in all treatment groups but not in the placebo group. The overall increase in LDL-C was +13 ± 3% for the collapsed treatment group. Change in percent from baseline for TG90 was +14 ± 4%, for TG60 was

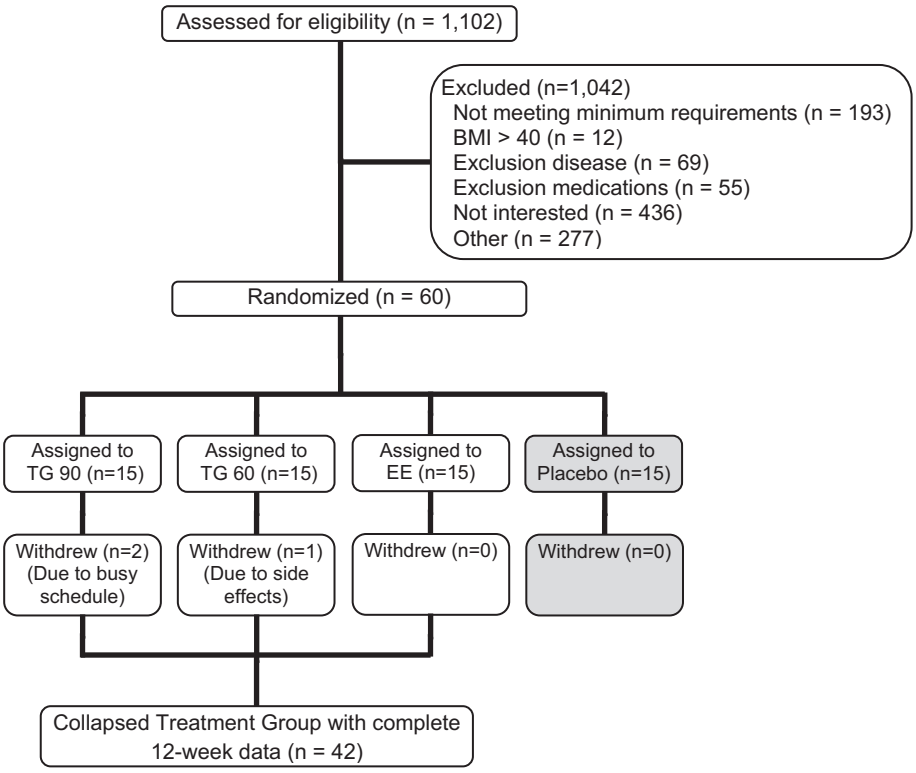


**Figure 1** Participant flow through the trial.

**Table 1** Baseline Participant Characteristics by Group ($n$ = number of participants).

| Characteristics | TG90 ($n$ = 15) | TG60 ($n$ = 15) | Ethyl Ester ($n$ = 15) | Collapsed Rx Groups ($n$ = 45) | Placebo ($n$ = 15) |
|---|---|---|---|---|---|
| **Demographics** | | | | | |
| Age, *y* | 54 ± 7 | 52 ± 10 | 48 ± 11 | 52 ± 10 | 54 ± 11 |
| Female/Male, *n*/*n* | 5/10 | 4/11 | 4/11 | 13/32 | 1/14 |
| **Race/Ethnicity, *n*** | | | | | |
| Non-Hispanic White | 14 | 11 | 7 | 32 | 10 |
| Asian/Pacific Islander | 1 | 3 | 5 | 9 | 3 |
| Hispanic | 0 | 1 | 2 | 3 | 2 |
| Black | 0 | 0 | 0 | 0 | 0 |
| Other | 0 | 0 | 1 | 1 | 0 |
| Education, *y* | 17 ± 2 | 18 ± 2 | 18 ± 2 | 18 ± 2 | 18 ± 2 |
| Body mass index, kg/m$^2$ | 26 ± 4 | 28 ± 4 | 27 ± 4 | 27 ± 4 | 27 ± 3 |
| **Blood pressure, mmHg** | | | | | |
| Systolic | 123 ± 18 | 122 ± 19 | 120 ± 11 | 122 ± 16 | 120 ± 11 |
| Diastolic | 75 ± 10 | 73 ± 12 | 74 ± 9 | 74 ± 10 | 76 ± 6 |
| **Serum lipids[a], mmol/L** | | | | | |
| Total Cholesterol | 5.5 ± 1.0 | 5.3 ± 0.8 | 5.3 ± 1.1 | 5.4 ± 1.0 | 5.7 ± 0.9 |
| LDL Cholesterol[a] | 3.4 ± 1.0 | 3.2 ± 0.7 | 3.1 ± 1.0 | 3.2 ± 0.9 | 3.6 ± 0.7 |
| HDL Cholesterol | 1.1 ± 0.2 | 1.2 ± 0.2 | 1.1 ± 0.3 | 1.1 ± 0.2 | 1.2 ± 0.4 |
| Triglycerides | 2.7 ± 1.5 | 2.5 ± 0.9 | 3.2 ± 1.3 | 2.8 ± 1.3 | 2.6 ± 0.9 |

Values are mean ± SD.

[a] Serum lipid analyses were performed for only the 57 of 60 participants who completed the 12-week protocol, $n = 2$ and $n = 1$ missing from the TG90 and TG60 groups, respectively.

**Table 2** Triglycerides and LDL-Cholesterol at baseline and 12 weeks (Mean ± SEM).

| Lipid Parameters | Collapsed treatment group ($n = 42$) | | *P* value | Placebo ($n = 15$) | | *P* value |
|---|---|---|---|---|---|---|
| | Baseline | 12 wks | | Baseline | 12 wks | |
| Triglycerides, mmol/L | 2.8 ± 0.2 | 2.0 ± 0.2 | <0.001 | 2.6 ± 0.2 | 2.6 ± 0.3 | 0.88 |
| LDL-Cholesterol, mmol/L | 3.2 ± 0.1 | 3.5 ± 0.1 | <0.001 | 3.6 ± 0.2 | 3.6 ± 0.2 | 0.86 |

LDL-C analyses were performed for only 56 of the 60 participants due to missing or compromised samples, $n = 4$ missing from the collapsed treatment group (note: 3 drop-outs and 1 missing/compromised sample).

+14 ± 5%, for EE was +11 ± 6% ($p = 0.89$), and for placebo was +1 ± 4%.

### LDL particle concentrations

For the analyses of specific LDL particles (LDL1, LDL2, LDL3 and LDL4), data are presented for only the collapsed treatment group (Fig. 2). In absolute numbers, the mean concentration of all four particle sizes increased, and the increase was statistically significant ($p < 0.05$) for all but the LDL4 particle class. Change from baseline (mean ± SEM) for LDL1 was +0.06 ± 0.02 mmol/L [+2.2 ± 0.7 mg/dL], for LDL2 was +0.07 ± 0.03 mmol/L [+2.6 ± 1.0 mg/dL], for LDL3 was +0.16 ± 0.05 mmol/L [+6.3 ± 1.8 mg/dL], and for LDL4 was +0.04 ± 0.04 mmol/L [+1.4 ± 1.7 mg/dL].

### LDL phenotype pattern

At baseline, among the 43 participants assigned to the active treatment arms who finished the 12-week protocol, the number and percent of these with a "B" pattern distribution of LDL particles was 36 (84%); 5 (12%) were pattern "A" and 2 (5%) was classified in the "intermediate" or "AB" pattern. At 12-weeks there were minimal changes in these LDL patterns, and the proportion and direction of the changes were almost identical to those in the placebo group: 28% improved; 70% showed no change; 2% worsened in the treatment arms vs. 27%; 67% and 7% in the placebo group.

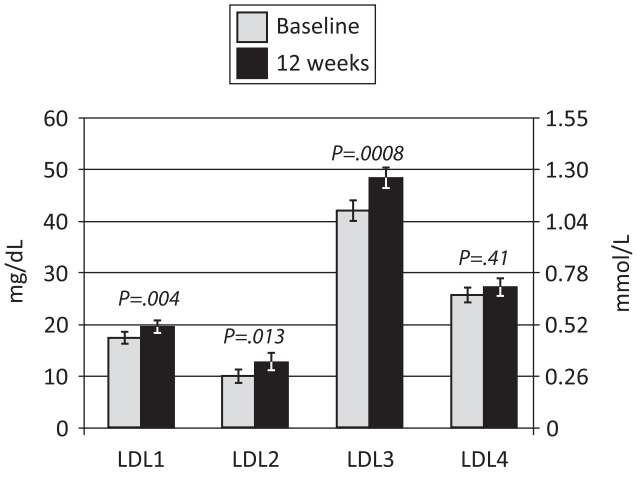


**Figure 2** LDL subfractions at baseline and 12 weeks for the combined treatment groups ($n = 46$). Matched pair *t*-tests.

## Discussion

The primary design using three treatment arms was to compare three formulations of supplements with identical EPA/DHA dose (4 g/day). The treatments for 12 weeks differed only in the proportion of omega-3 FA present as ethyl esters vs. triglycerides. There was no significant difference in the triglyceride lowering effect of the formulations detected. For the present analysis the three active treatment arms were collapsed into one. Participants experienced a decrease in blood TG concentrations of 26%, consistent with the magnitude of effect reported from previous studies. This clinically significant lipid profile improvement was accompanied by a modest increase in LDL-C concentrations of 13%, which is also a finding that replicates past observations. In assessing how this increase in total LDL-C was distributed among different particle size classes of LDL, it was observed that there were significant increases in LDL1, LDL2 and LDL3, and an absolute mean increase in LDL4 that did not achieve statistical significance. In addition, the TG lowering was not accompanied by a shift toward the favorable pattern "A" phenotype.

The overall reduction of TG concentrations in the current study was 26%, which is in line with the range of previous reports for fish oil supplements [35]. It is well known that fish oil effectively lowers blood TG concentrations. Also frequently described is the parallel elevation in LDL as observed in the current study. Although the total LDL-C increase of 13% is higher than the previously reported range of 5–7% from some past studies [36], even higher LDL increases have been reported [18,37]. However, less is known about the effect of fish oil on LDL particle size. Despite the extensive previous work on the lipid-lowering effect of fish oil, only a few studies to date have assessed the effect of fish oil on the LDL subclass distribution, yielding inconsistent results. A review article reported that there was no overall effect on the distribution of LDL size distribution [38]. A conclusive comparison is hampered by the different design, dosing of fish oil, study population and the method used to define LDL particle size or density.

### Impact on lipid profiles of low carbohydrate diets vs. fish oil supplements when targeting elevated TG concentrations

Krauss and others have reported clinically significant reductions in blood TG concentrations by lowering the carbohydrate content of diets, a finding that has been typically accompanied by a shift in LDL particle size from

smaller particles (pattern "B") to larger particles (pattern "A") [15,39]. Although it is not clear whether changes in triglycerides concentrations are directly connected with the shift in LDL pattern, it is possible that different components of the dietary pattern may influence triglycerides and LDL subfractions. High-carbohydrate diets decrease fat oxidation and increase hepatic VLDL secretion and thus result in an increase in triglycerides, whereas the changes in LDL subfractions may be influenced by an increased LDL receptor activity observed with high-carbohydrate diets [15]. Fish oil supplements have a similar if not greater impact on TG lowering than low-carbohydrate diets. Fish oil reduces triglycerides through the inhibition of the synthesis of VLDL and apolipoprotein B [40]. Watson et al. reported that TG concentration is inversely correlated with the size of LDL particles [41]. Hence, it has been hypothesized that a decrease of TG would lead to an increase in LDL size. The minimal effect on LDL size improvement in our study may be attributed to the inability of fish oil to affect insulin resistance [16]. Diets, in contrast, not only are able to modify lipids but also insulin sensitivity which in turn has been acknowledged to lower triglycerides and reduce LDL size [42]. This could explain why Griffin et al. found a decrease of small dense LDL particles despite a lack of impact on insulin sensitivity [16]. Additional factors such as weight loss [14], blood pressure [20], and concomitant cardiovascular disease [21] may contribute to the observed increased LDL size. Our study findings are consistent with a study from Patti et al in 16 non-insulin dependent diabetics with no significant changes in LDL size despite a 45% reduction of triglycerides [28]. Nevertheless, fish oil supplements may provide what for many individuals would be an easier behavior modification to make (i.e., taking dietary supplements vs. changing overall dietary patterns). While the TG lowering effect of these two hygienic/lifestyle measures is similar, the mechanism is quite likely different. Beyond the mechanism being different, the impact on the overall lipid profile is different as well — fish oil supplementation tends to modestly increase total LDL-C concentrations, and does not lead to a substantial shift in distribution of particle size from smaller to larger particles.

### Strengths and limitations

Strengths of the present study included the high retention and adherence rate. An additional strength was the careful selection of a relevant study population of hypertriglyceridemic adults who were otherwise in general good health (i.e., a population likely to consider fish oil supplementation as a treatment for their dyslipidemia). The availability of data from the VAP testing for both LDL particle classifications 1—4, as well as LDL pattern classifications (A, B, A/B) was also a strength. The primary limitation to the study was the relatively small sample size. Notably, this analysis was not the primary intent of the original study, which was intended to examine potential differences in TG lowering for different formulations of fish oil supplementation. No screening for genetic mutations and family history was performed thus participants with underlying familial dyslipidemias cannot be excluded.

## Conclusion

Fish oil supplementation, while having a substantial and highly clinically relevant beneficial impact on lowering blood TG concentrations, also has a modest adverse LDL-C increasing effect. While it is plausible that the adverse increase in total LDL-C might be mitigated to some extent by a shift in LDL particle distribution, it appears this shift is relatively modest, with debatable clinical relevance. Strategies to lower TG concentrations should consider the impact on the overall lipid profile to maximize successful treatment of dyslipidemias.

## Conflict of interest

B. Oelrich, A. Dewell and C. Gardner have no conflicts of interest.

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