Re-Esterified Triglyceride ω-3 Fatty Acids in Dry Eye Disease With Meibomian Gland Dysfunction
A Randomized Clinical Trial

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IMPORTANCE Taking ω-3 supplements has been associated with a reduction in symptoms of dry eye disease (DED) associated with meibomian gland dysfunction (MGD). However, a recent relatively large clinical trial concluded that treating DED with ω-3 consumption was ineffective, potentially warranting additional investigations.

OBJECTIVES To investigate the effect of re-esterified triglyceride (rTG) ω-3 fatty acid supplementation on DED associated with MGD.

DESIGN, SETTING, AND PARTICIPANTS This double-masked, parallel-group, randomized clinical trial was conducted at 7 institutions from September 2020 to January 2023. Patients with DED associated with MGD were included and randomly assigned to the ω-3 group (received 1680 mg of eicosapentaenoic acid and 560 mg of docosahexaenoic acid), whereas those in the grape-seed group received 3000 mg of grape-seed oil daily.

INTERVENTIONS rTG ω-3 Fatty acid supplementation vs grape-seed oil.

MAIN OUTCOME MEASURES The primary end point was the Ocular Surface Disease Index (OSDI) from baseline to 6 and 12 weeks. The safety parameters were visual acuity and intraocular pressure change.

RESULTS A total of 132 patients (mean [SD] age, 50.6 [13.8] years; 103 female [78.0%]) were included in this study. The mean (SD) baseline OSDI scores of the ω-3 and grape-seed groups were 43.5 (16.5) and 44.1 (16.6), respectively. A total of 58 patients (87.9%) and 57 patients (86.4%) in the ω-3 and grape-seed groups, respectively, completed 12 weeks of follow-up. There were no differences in compliance with the dietary supplement intake between groups (ω-3, 95.8% and grape-seed, 95.4%). The OSDI (SD) change from baseline to 6 and 12 weeks was −20.5 (16.0) and −22.7 (15.7), respectively, in the ω-3 group and −15.1 (20.2) and −18.8 (21.7), respectively, in the grape-seed control group (difference at 6 weeks = −5.4; 95% CI, −12.15 to 1.33; P = .12 and at 12 weeks = −3.9; 95% CI, −10.90 to 3.13; P = .28). There were no changes in safety parameters or adverse events related to taking the dietary supplement in either group.

CONCLUSIONS AND RELEVANCE This randomized clinical trial did not show a benefit of the rTG form of ω-3 for ameliorating symptoms of DED associated with MGD, although fewer than 60 participants were evaluated in each group. Any secondary outcomes from this study should be considered for hypothesis generation of future evaluations of the effect of the rTG form of ω-3 on DED associated with MGD.

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Dry eye disease (DED) is a multifactorial disease of the ocular surface characterized by tear film instability and ocular surface inflammation resulting in visual disturbances and ocular discomfort.1–3 DED has been associated with decreased vision-related patient-reported quality of life measurements, which, in turn, may be related to challenges in everyday activities or work productivity.3,4 DED can be classified into aqueous-deficient and evaporative DED, even though many cases of DED are a mix of both of these main categories.3,5 However, in a general clinic-based cohort, more than 80% of individuals with DED had meibomian gland dysfunction (MGD), making it among the most commonly observed underlying conditions among patients with DED.5,7 MGD is associated with evaporative DED, wherein tear evaporation has been documented to increase even when the tear secretion is normal, presumably ascribed to a lack of, or imbalance in, the lipid layer secreted by the MGs.6 MGD is a chronic, diffuse MG abnormality associated with tear film instability and irritation, ocular surface inflammation and disease, and DED related to the closure of the terminal orifices and changes (both qualitative and quantitative) in the meibum.9,10 Managing DED associated with MGD may be challenging for ophthalmologists. Topical eye drops can be helpful for improving signs and symptoms. Other elements of the treatment of DED associated with MGD include warm compresses, eyelid hygiene, topical and systemic antibiotics, and ω-3 supplements.10–14 ω-Fatty acids are strong lipid mediators that play a crucial role in inflammatory regulation by controlling the arachidonic acid (AA) pathway and prostaglandin metabolism.15,16 ω-3 Fatty acid supplementation changes the fatty acid composition of meibum and promotes MG secretion.17,18 Several systematic reviews have supported the possibility that ω-3 supplementation ameliorates the signs and symptoms of DED associated with MGD.19,20 However, a relatively recent randomized clinical trial was not able to show that ω-3 intake was effective for treating DED.21 Studies investigating the potential benefits of ω-3 fatty acids on DED associated with MGD may yield varying findings, perhaps because of the dose or sources of the ω-3 supplements.16,22,23 A randomized clinical trial showed supplementation with the re-esterified triglyceride (rTG) form of ω-3 is effective in treating DED.18 Therefore, this randomized clinical trial aimed to investigate whether the systemic rTG form of ω-3 fatty acid supplementation is effective in treating DED associated with MGD, compared with grape-seed oil supplementation, which has an antioxidant effect,24–26 using antioxidant foods that can be consumed in daily life as a control group.

Methods

Study Design

This randomized double-masked parallel-group trial was conducted at 7 medical institutions in Korea between September 21, 2020, and January 19, 2023, including a 12-week follow-up (trial protocol and case report form available in Supplement 1 and Supplement 2, respectively). The protocol for this study was reviewed and approved by the institutional review board of each institution: Korea University Ansan Hospital, Wonju Severance Christian Hospital, Seoul National University Bundang Hospital, Asan Medical Center, Yonsei University Severance Hospital, Kangbuk Samsung Hospital, and Seoul National University Hospital. This study was compliant with the Health Insurance Portability and Accountability Act and adhered to the tenets of the Declaration of Helsinki. After receiving information about the purpose and possible outcomes of the study, all patients provided written informed consent to participate. Participants received compensation to participate ($22.50 at each visit). The Consolidated Standards of Reporting Trials (CONSORT) reporting guidelines were followed.

Participants

Patients diagnosed with DED with concurrent MGD were eligible to participate in this study. Because South Korea is a monoethnic nation, all participants in the study are of Korean ethnicity. The following criteria were used to define DED: dry eye symptoms persisted for more than 3 months before clinical trial registration, tear breakup time (TBUT) was less than 10 seconds, and Ocular Surface Disease Index (OSDI) score was 23 or higher. When 1 or more of the following signs were noticed, MGD was diagnosed: (1) attempts at digital expression precipitating either no secretion from the MGs or abnormal secretion, (2) the presence of 2 or more telangiectasias at the eyelid edge, and (3) pouting or plugging of 2 or more of the 8 central MG orifices.27–30 Key exclusion criteria were the presence of Sjögren syndrome, the absence of meibomian secretion, and use of eye drops or oral medications that could affect the clinical trial results. A full list of exclusion criteria is provided in eAppendix 1 in Supplement 3.

Randomization, Intervention, and Masking

Participants were assigned to the ω-3 and grape-seed groups in a 1:1 ratio according to a random number table generated using block randomization (Excel (Microsoft Corp)). Investigational dietary supplements for each institution were packaged and labeled by the sponsor (LYS PHARMA) so that researchers and participants were blinded. Allocation concealment was left until the end of the study. Only when an adverse reaction occurred did the researcher decide to drop the...
participant from the study and terminate allocation concealment. In the ω-3 group, participants received ω-3 supplement capsules containing 1680 mg of eicosapentaenoic acid (EPA) and 560 mg of docosahexaenoic acid (DHA) (De3 Omega Benefits [LYS PHARMA]) at a dose of 4 capsules daily.\textsuperscript{18,31} In the grape-seed oil group, participants received grape-seed oil at a dose of 4 capsules daily containing 3000 mg of 100% grape-seed oil (CNS Pharm Korea Co Ltd) in soft gel capsules identical in appearance and taste to those given to the ω-3 group. Both groups were prescribed preservative-free hyaluronic acid, 0.1% or 0.15%, eye drops and instructed to use them as needed to relieve symptoms up to 6 times per day.

**Assessments**

All participants completed a questionnaire querying their medical history and subjective symptoms. Primary outcome measures were the change in mean OSDI score at 6 and 12 weeks from baseline and mean National Eye Institute Visual Functioning Questionnaire 25 (NEI-VFQ-25) \textsuperscript{12-34} score at 12 weeks from baseline. Secondary outcome measures were the changes in mean tear matrix metalloproteinase 9 (MMP-9) immunoassay grade\textsuperscript{35} and ω-3 index (ω-6/ω-3 ratio and AA/EPA ratio) at 12 weeks from baseline, mean NEI corneal and conjunctival staining scores,\textsuperscript{36-38} TBUT, eyelid wiper epitheliopathy grade,\textsuperscript{39} MG expressibility and secretion score\textsuperscript{40,41} at 6 and 12 weeks from baseline, mean upper and lower MG dropout grades\textsuperscript{42} and lipid layer thickness at 12 weeks from baseline. The mean daily frequency of hyaluronic acid eye drop use and compliance with the investigational dietary supplement were also compared as the secondary outcome measures. Detailed assessment methods and the selection of the study eye are provided in Appendix 2 in Supplement 3. To evaluate the safety of the investigational dietary supplement, changes in visual acuity, increase in intraocular pressure of more than 21 mmHg, and the adverse events that occurred during the study period were assessed at each visit.

**Statistical Analysis**

Statistical analyses were performed using SPSS Statistics for Windows, version 20 (IBM Corp). Primary and secondary outcome analyses were performed in a per-protocol population, and the safety evaluation was conducted in an intention-to-treat population. The clinical characteristics and measurement outcomes of the 2 groups were compared using t tests or repeated-measures analysis of variance with the Tukey honest significant difference post hoc test. Statistical significance was defined as $P < .05$ for the primary outcome. All $P$ values were 2-sided, and there was no adjustment for multiple analyses. No imputation was performed for missing data.

**Results**

A total of 132 patients (mean [SD] age, 50.6 [13.8] years; 103 female [78.0%]; 29 male [22.0%]) were included in this study (sample size calculation is provided in Appendix 3 in Supplement 3). The mean (SD) ages of the participants in the ω-3 and grape-seed groups were 51.5 (14.7) years (range, 20-75 years) and 49.7 (13.0) years (range, 24-69 years), respectively. There were 52 women (78.8%) in the ω-3 group and 51 women (77.3%) in the grape-seed group. Demographic characteristics and measurement outcomes at baseline were comparable between groups (Table).

A total of 66 patients were enrolled in each group after screening 134 patients. Of the 132 randomized patients, 16 patients were lost during the follow-up period, and 1 patient withdrew. The mean (SD) baseline OSDI scores of the ω-3 and grape-seed groups were 43.5 (16.5) and 44.1 (16.6), respectively. A total of 58 patients (87.9%) were placed in the ω-3 group, with 57 (86.4%) in the grape-seed group, all of whom completed 12 weeks of follow-up from September 21, 2020 (the date of the first patient's baseline visit) to January 19, 2023 (the date of the last patient's week 12 visit) (Figure 1). There was no difference in mean compliance to the dietary supplement intake between the ω-3 (95.8%) and grape-seed (95.4%) groups during the 12-week treatment.

The mean (SD) OSDI change from baseline to 6 and 12 weeks was −20.5 (16.0) and −22.7 (15.7), respectively, in the ω-3 group and −15.1 (20.2) and −18.8 (21.7), respectively, in the grape-seed group, (difference at 6 weeks = −5.4; 95% CI, −12.15 to 1.33; $P = .12$ and at 12 weeks = −3.9; 95% CI, −10.90 to 3.13; $P = .28$) (Figure 2A). The mean (SD) NEI-VFQ-25 change from baseline to 12 weeks (Figure 2B) was 6.7 (11.8) in the ω-3 group and 3.5 (15.9) in the grape-seed group (difference = 3.2; 95% CI, −2.05 to 8.38; $P = .23$).

No differences in tear MMP-9 immunoassay grade changes between the ω-3 and grape-seed groups after 12 weeks of treatment were noted (eFigure 1 in Supplement 3). Significant differences (Figure 3A) were noted in the mean (SD) ω-6/ω-3 ratio changes between the ω-3 (−1.1 [1.2]) and grape-seed (−0.4 [1.5]) groups after 12 weeks of treatment (difference = −0.7; 95% CI, −1.16 to −0.17; $P = .009$). There was no difference in the mean (SD) AA/EPA ratio changes between groups (−3.1 [6.5] for the ω-3 group vs −0.9 [7.0] for the grape-seed group; difference = −2.2; 95% CI, −4.72 to 0.30; $P = .08$) after 12 weeks (Figure 3B).

No difference in corneal staining score change from baseline to 12 weeks was noted between groups, including −1.6 in the ω-3 group compared with −1.2 in the grape-seed group (difference = −0.4; 95% CI, −1.27 to 0.46; $P = .36$). Differences also were not identified for conjunctival staining score change (eFigure 2 in Supplement 3) from baseline to 12 weeks between groups (difference = −0.9; 95% CI, −1.88 to 0.02; $P = .06$).

The mean (SD) TBUT change from baseline to 6 weeks in the ω-3 group was 1.0 (2.3) seconds compared with 0 (2.0) seconds in the grape-seed group (difference = −1.0 second; 95% CI, 0.22 to 1.82 seconds; $P = .01$). There also were differences in the mean (SD) TBUT change (Figure 3C) from baseline to 12 weeks between the ω-3 (1.5 [2.5] seconds) and grape-seed (0.4 [1.7] seconds) groups (difference = 1.1 seconds; 95% CI, 0.34 to 1.91; $P = .006$). The mean (SD) eyelid wiper epitheliopathy grade change (Figure 3D) from baseline to 12 weeks in the ω-3 group (−0.4 [0.6]) was greater than that in the grape-seed group (−0.1 [0.8]) (difference = −0.3; 95% CI, −0.58 to −0.04; $P = .02$).

The mean (SD) upper eyelid telangiectasia grade change (Figure 4A) from baseline to 12 weeks in the ω-3 group (−0.4
was greater than that in the grape-seed group (−0.1 [0.6]) for a difference of −0.3 (95% CI, −0.60 to −0.06; \( P = .02 \)). The mean (SD) lower eyelid telangiectasia grade change (Figure 4B) from baseline to 6 and 12 weeks in the ω-3 group (−0.3 [0.6] and −0.5 [0.9], respectively) were greater than that in the grape-seed group (−0.4 [0.4] and 0 [0.5], respectively) for a difference in changes of −0.4 (95% CI, −0.63 to −0.10; \( P = .008 \)) and −0.5 (95% CI, −0.80 to −0.11; \( P = .01 \)), respectively.

There were no differences in the upper and lower gland expressibility changes from baseline to 12 weeks between the ω-3 and grape-seed groups. Changes in the upper and lower gland secretion scores from baseline to 12 weeks between the ω-3 and grape-seed groups were also not different (eFigure 3 in Supplement 3). There were no differences in lipid layer thickness change after 12 weeks of treatment between groups (eFigure 4 in Supplement 3).

During the follow-up, there was no difference in upper MG dropout grade change (Figure 4C) between the omega-3 (−0.1) and grape-seed (0.1) groups (difference = −0.2; 95% CI, −0.36 to 0.05; \( P = .15 \)). Conversely, the difference in lower MG dropout grade change (Figure 4D) from baseline to 12 weeks between the ω-3 (−0.1) and grape-seed (0.2) groups was different (difference = −0.2; 95% CI, −0.44 to −0.04; \( P = .02 \)).

No differences were noted in the mean (SD) daily frequencies of eye drop use in the ω-3 (1.6 [2.0]) and grape-seed (1.4 [1.9]) groups during the study period. There were no changes in mean visual acuity from baseline to 6 and 12 weeks (eFigure 5 in Supplement 3) and no case of intraocular pressure elevation above 21 mm Hg in either group. Moreover, there were no adverse events identified related to taking the dietary supplements in either group.

### Discussion

This randomized clinical trial compared the effects of the rTG form of ω-3 and grape-seed oil, respectively, on DED associated with MGD. Although several systematic reviews...
Figure 2. Comparison of Mean Ocular Surface Disease Index (OSDI) and National Eye Institute Visual Functioning Questionnaire 25 (NEI-VFQ-25) Scores Before and After Treatment Between the ω-3 and Grape-Seed Groups

A, OSDI score. B, NEI-VFQ-25 score.

Figure 3. Comparison of Mean ω-6/ω-3 Ratios, Arachidonic Acid (AA)/Eicosapentaenoic (EPA) Ratios, Tear Breakup Time, and Eyelid Wiper Epitheliopathy Before and After Treatment Between the ω-3 and Grape-Seed Groups

have supported the possibility that ω-3 supplementation ameliorates the signs and symptoms of DED associated with MGD, this study did not show a benefit of the rTG form of ω-3 for ameliorating symptoms of DED associated with MGD, although fewer than 60 participants were evaluated in each group. Any secondary outcomes from this study should be considered for hypothesis generation of future evaluations of the role of the rTG form of ω-3 on DED associated with MGD.

Thesefindingsupport arelatively recent randomized clinical trial that also was not able to show that ω-3 intake was effective for treating DED. In a previous Cochrane systematic review of the effect of ω-3 on DED, the mean difference in the OSI change between ω-3 vs placebo was –2.47 (95% CI, –5.14 to 0.19) and for ω-3 vs ω-6 was –11.88 (95% CI, –18.85 to 4.92). This review suggested a possible role of ω-3 supplements in managing DED, even though the evidence was inconsistent and uncertain. Another previous systematic review demonstrated that the effectiveness of ω-3 in alleviating symptoms of DED was consistently statistically significant throughout the analysis.

Previous studies showed that meaningful OSDI score changes for patients with mild to moderate symptoms were 4.5 to 7.3, and for patients with severe symptoms, score changes were 7.3 to 13.4. In this study, although the amount of change in OSDI in each group was greater than the minimal clinically important difference suggested in the previous research, the difference in the OSDI change between groups was smaller than the minimal clinically important difference. This difference may be because no difference in changes exist between ω-3 compared with grape-seed oil. Grape-seed oil theoretically reduces pro-oxidant numbers and enhances antioxidant enzymes, protecting eye tissues from oxidative stress.

In addition, both groups used preservative-free hyaluronic acid eye drops for 12 weeks. Relatedly, there were no differences in several parameter changes between groups, again supporting the conclusion that ω-3 does not ameliorate the symptoms of DED associated with MGD.

The ω-6/ω-3 ratio has been used as an inflammatory marker in humans. Previous studies have shown that the ω-6/ω-3 ratio is among the risk factors for numerous inflammatory disorders. In this study, as the mean baseline ω-6/ω-3 ratios
were 3.4 in both groups, the magnitude of difference in the ω-6/ω-3 ratio changes between groups (~0.7) and the lower bounds of the 95% CI (~0.17) may be clinically relevant, and the rTG form of ω-3 fatty acid supplementation for 12 weeks was not shown to be different from the control group taking grape-seed oil for ameliorating signs of DED by theoretically reducing inflammation in the plasma or ocular tissues.

The magnitude of the difference in the TBUT change from baseline to 12 weeks between the groups was 1.1 seconds, and the lower bounds of the 95% CI was 0.34 seconds. This finding is similar to the findings of previous systematic reviews showing that the mean difference in the TBUT changes between combined ω-3 and ω-6 vs placebo was 0.55 seconds (95% CI, 0.04-1.07 seconds) and that between ω-3 vs placebo was 0.94 seconds (95% CI, 0.61-1.27 seconds). Because short TBUT-type DED accounts for a large proportion of all DED, the mean difference in the TBUT change between groups of 1.1 seconds and the lower bounds of the 95% CI of 0.34 seconds may be clinically relevant. However, as this was a secondary outcome in which no differences were noted for the primary outcome, this secondary outcome should be considered for hypothesis generation of future studies that might determine the clinical relevance of this finding.

In this study, results with the rTG form of ω-3 fatty acid supplementation suggested, for hypothesis generation consideration, greater changes in upper and lower eyelid telangiectasia and eyelid wiper epithelioathy grades than with grape-seed oil. Improvements in telangiectasia and eyelid wiper epithelioathy imply that ω-3 supplementation is effective at reducing inflammation of the eyelid margin. Previous research has shown that eyelid telangiectasia significantly improves after supplementation with 1200 mg of ω-3 fatty acids (450 mg EPA, 300 mg DHA, and 450 mg α-linolenic acid) combined with 0.25% sodium carboxymethylcellulose eye drops and lid hygiene for 3 months in patients with moderate to severe DED.

In this study, despite no differences in upper MG dropout change between groups, there was a difference suggested for the lower MG dropout changes between groups, again as a hypothesis-generating conclusion warranting future clinical trials to determine this efficacy. Previous research has shown that MG dropout was worse in the lower eyelids than in the upper eyelids in patients with obstructive MGD. A more rapid MGD progression in lower-eyelid MGs was proposed. Indeed, in this study, lower MG dropout possibly only was exacerbated in the grape-seed group. Therefore, ω-3 supplementation could not improve MG dropout, but it might prevent worsening of MG dropout in DED associated with MGD if future clinical trials can compare this.

Limitations

There are some limitations in this study. First, the follow-up period was short, and the sample size was relatively small. Second, using a control group that was not a placebo group has limitations in interpreting the clinical relevance of the results. Third, investigational dietary supplements were contained in identical soft gel capsules and packaged in the same bottle to ensure allocation concealment. However, if patients pop a capsule and taste it, they might be able to tell the difference between the 2 medications. This may be a limitation in this trial. Finally, there may be selection bias because in this study per-protocol analysis was performed without imputation.

Conclusions

This randomized clinical trial did not show a benefit of the rTG form of ω-3 for ameliorating symptoms of DED associated with MGD, although fewer than 60 participants were evaluated in each group. Any secondary outcomes from this study should be considered for hypothesis generation of future evaluations of the role of the rTG form of ω-3 on DED associated with MGD. Additional clinical trials would be needed to determine if systemic rTG ω-3 fatty acids may be an effective treatment option for DED associated with MGD.
during the conduct of the study. No other disclosures were reported.

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**Data Sharing Statement:** See Supplement 4.

**References**


