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Efficacy and safety of oral palmitoleic acid supplementation for skin barrier improvement: A 12-week, randomized, double-blinded, placebo-controlled study

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ABSTRACT

Background: Palmitoleic acid (omega-7) has been reported to be effective primarily for metabolic disorders. Recently, it has been reported to help improve quality of life (QoL) by improving skin symptoms.
Objective: The aim of this randomized, double-blinded, placebo-controlled clinical study is to evaluate the efficacy and safety of oral palmitoleic acid in improving skin barrier, elasticity, and wrinkle formation in adult women.
Methods: In this randomized, double-blind, placebo-controlled clinical study, 90 healthy participants were enrolled and received 500 mg/day palmitoleic acid (intervention) or corn oil without palmitoleic acid (control) for 12 weeks. Skin hydration and transepidermal water loss and skin elasticity, surface roughness, eye wrinkle volume, and wrinkle severity were measured at 6-week intervals to assess the skin barrier function and efficacy in wrinkle improvement, respectively.
Results: After 12 weeks, skin hydration and transepidermal water loss significantly improved in the intervention group compared to the control group. Skin elasticity, surface roughness, eye wrinkle volume, wrinkle severity, and participant-assessed clinical improvement score did not significantly improve compared with the control group.
Conclusion: Oral palmitoleic acid effectively improves the skin barrier function improvement, which may enhance QoL in aging adults.

1. Introduction

Since 1929, dietary oils have been known to affect not only growth but also the general physiological functions of the body, including skin homeostasis, and several oils, including omega-3, omega-6, and evening primrose oil, have been used in adjuvant therapy for inflammatory skin diseases, such as atopic dermatitis (AD), to increase skin hydration by repairing the skin-barrier function

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[1–5]. Palmitoleic acid (C16:1) is a common omega-7 (ω-7) monounsaturated fatty acid constituting approximately 7% of the visceral fatty acids produced by Alaskan pollock [6] and has beneficial effects on human metabolism through promotion of cardiac growth, proliferation of pancreatic β-cells, regulation of lipogenesis and fatty acid desaturation in adipocytes, and alleviation of inflammation [7].

Aging skin becomes dry and saggy, and wrinkles increase, which decreases the quality of life (QoL) [8,9]. Positive effects of oral palmitoleic acid supplementation on the skin included an improvement of the extent and clinical severity of AD [10]. Additionally, palmitoleic acid supplementation significantly improved skin hydration and elasticity in middle-aged women [11]. However, given the paucity of clinical studies, further research is needed to ensure reliability. This study aimed to investigate the efficacy and safety of palmitoleic acid supplementation for improving skin barrier function and skin elasticity, and decreasing wrinkle formation, thereby potentially improving QoL during the aging process.

2. Materials and methods

The study was approved by the institutional review board of Chungang-University Hospital (IRB No. 1982-010-385). This study was conducted to investigate the effects of oral palmitoleic acid supplementation on skin hydration and elasticity. This clinical study was conducted ethically and scientifically in accordance with the tenets of the Declaration of Helsinki.

2.1. Participants

Among the outpatients who visited the Department of Dermatology at the Chung-Ang University Hospital from December 2019 to April 2021, women aged 30–60 years with a grade 3 or more crow's feet lines (CFL; according to Fitzpatrick–Goldman's 9-point classification) were enrolled in the present study [12]. To limit possible confounding effects, individuals were excluded if they met the following criteria: underwent botulinum toxin or dermal filler injection, which might affect crow's feet, such as tissue augmentation within 6 months; had AD or other skin diseases; took other dietary supplements in the past month; had an allergic reaction to related supplements; and had systemic or uncontrolled chronic diseases. The aim of the study and any possible adverse events were explained to all participants by the clinical investigators; all participants voluntarily enrolling in the study signed written consents. Participants could withdraw from the study at will or for medical reasons that were related or unrelated to the treatments. The details of the dropped-out cases have been reported.

We calculated the study sample size based on the following: 1) two-sided testing for all outcome variables, 2) significance determined by $p < 0.05$, 3) 80% power to avoid type 2 errors, 4) a 1:1 ratio of intervention to control participants, and 5) effect sizes for outcome variables based on those reported by Proksch E et al. (2014) [13], which measured effects in the same outcome variables as in this study. Assuming points 1) to 5), the number of samples required for a clinical trial is as follows (two-tailed test).

$$n = \frac{2 \times \left(Z_{\alpha/2} + Z_{\beta}\right)^2 \times \sigma^2}{\Delta^2}$$

(Zα/2: The critical value or quantile at which the area of the right tail of the standard normal distribution is α/2; Zβ: The critical value or quantile at which the area of the right tail of the standard normal distribution is β; α: type I error; β: type II error; Δ: Difference in mean change between the clinically significant test group and the control group).

From the above formula, the sample size (n) per group was obtained as follows.

$$n = \frac{2 \times (1.96 + 0.84)^2 \times 0.125^2}{-0.0882} \approx 32$$

The minimum number of participants for each group was 32. Considering the dropout rate up to 30%, the number of participants who were enrolled per group was set to 45, in a cohort with a total of 90 participants, for the evaluation of efficacy. Participants were recruited through a recruitment guide paper placed in clinics, which specified the inclusion and exclusion criteria.

3. Test products

The palmitoleic acid supplement (7-MEGA™, Wiley Companies in Eastern Ohio, USA) was obtained from Alaskan pollock through distillation, transesterification, molecular distillation, winterization, carbon filter, antioxidation, and packaging. The composition of the capsule is presented in sTable 1, [14]. 7-MEGA™ was used because the palmitoleic acid content was almost double that of sea buckthorn oil [15]. Corn oil was used in the control group, because corn oil has a positive effect on the skin and does not contain palmitoleic acid. Linoleic acid, which is the main component of corn oil, modulates the recovery of the epidermal permeability barrier function of the stratum corneum [16,17] and has wrinkle improvement function when used with oleic acid [18].

3.1. Study design

This randomized, double-blind, placebo-controlled clinical study comprised of participants who visited the study center a total of four times to participate in the study and were screened at Visit 1 (Week −2). Participants were randomized to either the intervention or control group at the second visit (week 0) and the group assignment was not disclosed to investigators. Study data were collected at

three timepoints (weeks 0, 6, and 12). The intervention group was administered 500 mg (one capsule) 7-MEGA™ twice a day (50% palmitoleic acid, 500 mg palmitoleic acid/day), and the control group received a corn oil capsule twice a day (1000 mg corn oil/day). Both capsules looked identical to ensure that the clinical investigators and participants were unaware of the products in the double-blind study.

At week 0, participants were supplied with a full 12-week supply of capsules and instructed on self-administration to take one capsule 30 min after breakfast and dinner. Participants were then trained and asked to record the capsule intake in a diary according to the instructions in the guide and to bring their residual capsules to week 6 and week 12 to check for compliance. The usual diet was maintained, and pollack fish oil-related foods (e.g., omega-7 fatty acid-related foods) were not ingested more than twice a week. This study was conducted independently of the other clinical treatment, and all measurements were obtained only for study purposes. All research team members are clinical providers and staff and were trained for 1 week prior to conducting the study.

3.2. Baseline characteristics and measures

In addition to basic characteristics, such as age and height, we also assessed smoking history, and sleep time per day that could have effects on the skin. Participants were also asked to minimize the following: 1) outdoor activity time per day 2) use of color make-up frequency, and 3) use of sunscreen per week.

3.3. Clinical efficacy evaluation

The capacitance of skin hydration 30–40 μm below the stratum corneum was measured using the Corneometer® (CM 825, Courage & Khazaka Electronic GmbH, Köln, Germany). When a current was sent from the probe, the insulation ion value remaining in the probe head was digitized (AU: arbitrary unit) based on the electrical conductivity of the skin according to the skin-water content [19]. Therefore, the higher the skin hydration level, the higher the measured value. The measurement range of the Corneometer® was 0–120 AU.

Transepidermal water loss (TEWL) was evaluated by Tewameter® TM 300 (Courage & Khazaka Electronic GmbH, Köln, Germany) and reflects the degree of symptoms of dermatitis. This method has been used in several studies as an index to measure the degree of recovery after damage to the skin barrier [19].

Skin elasticity was measured using a Cutometer® MPA 580 (Courage & Khazaka, Electronic GmbH, Köln, Germany) on the left and right sides, and the whole face. Specifically, the vertical deformation of the skin was determined at a constant negative pressure of 450 mbars for 2 s: after the negative pressure was released, the skin was permitted to relax for 2 s. The effects of oral supplementation on skin elasticity were investigated by monitoring the changes in R2:Ua/Uf (gross elasticity), R5:Ur/Ue (net elasticity), and R7:Ur/Uf (biological elasticity). The closer the measurements are to 1, the better the elasticity. The skin hydration, TEWL, and skin elasticity of each left and right periorbital area were measured at weeks 0, 6, and 12, and the average value was calculated additionally.

The skin surface roughness and eye wrinkle volume were measured using an optical 3D *in vivo* skin measurement device PRIMOS^{lite} (GFM, Teltow, Germany). Which can calculate surface parameters and volume. Ra (average roughness), Rmax (maximal roughness), Rp (maximal profile peak height), Rv (maximal profile valley depth), Rz (vertical distance from the highest peak to the lowest valley within five sampling lengths), and wrinkle volume were measured at weeks 0, 6, and 12 [20]. For all roughness parameters, higher values indicate a rougher surface. The roughness parameters and eye wrinkle volume were measured on the left and right sides of the face, and the average value was calculated.

Skin hydration, TEWL, skin elasticity, skin surface roughness, and eye wrinkle volume were measured three times by trained research team members and the average value was used in the analyses.

The clinical photographs of the participants were taken using an imaging tool JANUS ® (PIE, Gyeonggi-do, Republic of Korea), and grade of CFLs were evaluated by blinded and trained dermatologists through photographs taken at weeks 0, 6, and 12. The overall skin improvement was assessed subjectively by the investigators (dermatologists) and by each participant at weeks 6 and 12 (1, markedly improved; 2, improved; 3, no change; 4, worsened; and 5, markedly worsened).

3.4. Statistical analysis

Results were presented as mean ± standard deviation. The paired *t*-test or Wilcoxon signed rank test was used to analyze the intragroup change values from before to after treatment with regard to skin hydration, TEWL, elasticity, and wrinkle severity values, depending on whether normality was satisfied. For the degree of change between the palmitoleic acid group and the control group at each timepoint, a generalized linear model with the baseline as a covariate was used. Investigator/participant-assessed clinical improvement scores were evaluated by conducting a two-sample *t*-test or Wilcoxon rank sum test based on whether the data satisfied the normality requirement. Adverse reactions in each group were analyzed using the chi-square or Fisher's exact test. Serological and vital values were analyzed using the paired *t*-test or Wilcoxon signed rank test to compare intragroup differences, and intergroup differences were analyzed using a two-sample *t*-test or Wilcoxon rank sum test. Urinalysis results were divided as normal and abnormal, and the McNemar test was performed to compare intergroup differences. Differences with a confidence level of 95% ($p < 0.05$) were considered statistically significant. Data were statistically analyzed with SAS® (version 9.4, SAS Institute, Cary, North Carolina, USA).

4. Results

A total of 92 healthy female participants were initially enrolled; however, two participants were excluded due to abnormal serum thyroid-stimulating hormone levels, and the remaining 90 participants were randomly assigned to either the intervention or control group at the second visit. Between week 0 and week 6, two participants were withdrawn due to poor compliance in the control group. Between week 6 and week 12, five participants were excluded due to poor compliance (one from the intervention and four from the control group), and two participants from the control group withdrew from the study; two participants from the control group were excluded due to schedule violation. In total, the intervention group lost one participant whereas the control group lost 10 participants.

A total of 79 healthy female participants (intervention group n = 44, control group n = 35; age 42–59 years) with CFL grade 3 or higher were included in the final study analysis (Fig. 1). The demographic and baseline characteristics of the participants did not show statistically significant intergroup differences (Table 1).

4.1. Clinical efficacy

Compared with baseline, skin hydration significantly increased at Week 6 in both groups, without significant differences between intervention and control groups. However, after 12 weeks, skin hydration of the intervention group significantly increased as compared with the skin hydration of the control group on the left, right periorbital area, and average values (Fig. 2).

The TEWL of intervention group significantly decreased as compared with the control group at weeks 6 and 12 on the left periorbital area. On the right side and in the average value, the TEWL significantly decreased compared with control group at only Week 12 (Fig. 3).

The R2 skin elasticity parameter in the intervention group tended to increase more compared to the control group at week 6 and week 12, but this was not statistically significant on the whole face. The R5 and R7 showed the same pattern (sFig. 1).

All skin surface roughness parameters (Ra, Rp, Rv, Rmax, and Rz) and both wrinkle volumes of the intervention group tended to decrease more as compared to the control group at week 6 and week 12, but the difference was not statistically significant (sFigs. 2 and 3). While the left, right, and average CFL wrinkle grades of the intervention group showed a decreasing tendency at week 6 and week 12, they did not significantly differ from the control group (sFig. 4). Objective measurements were described in sTable 2.

The clinical improvement score was evaluated by the investigators and participants week 6 and week 12 (Fig. 4, sTable3). The investigator-assessed clinical improvement scores did not significantly differ between the groups. At week 12, the scores of the palmitoleic acid and control groups were 2.20 (±0.41 s.d.) and 2.51 (±0.51 s.d.), respectively, which indicated that the score in the palmitoleic acid group significantly improved ($p = 0.0043$). The participant-assessed clinical improvement scores did not differ significantly between the groups.

4.1.1. Adverse effects

In evaluating safety during the test period, two participants in the intervention group and one in the control group reported adverse effects (sTable 4). All adverse effects were mild gastrointestinal disturbances (dyspepsia) which completely resolved during the study period without any treatment, but could not be considered unrelated to supplementation. No serious adverse reactions occurred in both groups, and no dropouts due to adverse reactions were recorded.

There were no significant changes in complete blood count and blood chemistry tests, including aspartate aminotransferase, alanine aminotransferase, total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglyceride.

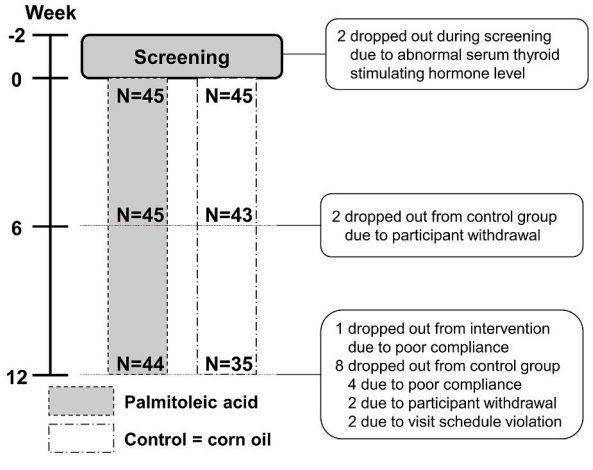

Fig. 1. Study design of 12-week, double-blind, placebo-controlled study.

Table 1
Baseline characteristics and measures.

Demographics	Palmitoleic acid n = 44	Corn oil n = 35	Total N = 79	p-value
Age (years)				0.7976
Mean ± SD	51.36 ± 4.15	51.11 ± 4.44	51.25 ± 4.25	
Min, Max	42, 58	43, 59	42, 59	
Height (cm)				0.1887
Mean ± SD	160.06 ± 4.49	158.67 ± 4.79	159.44 ± 4.64	
Min, Max	150.20, 170.00	148.00, 170.00	148.00, 170.00	
Outdoor activity time/day n(%)				0.4010
<3 h	29 (65.91)	19 (54.29)	48 (60.76)	
3–5 h	14 (31.82)	13 (37.14)	27 (34.18)	
>5 h	1 (2.27)	3 (8.57)	4 (5.06)	
Color makeup frequency/week n(%)				0.9028
0	21 (47.73)	16 (45.71)	37 (46.84)	
1–2	15 (34.09)	12 (34.29)	27 (34.18)	
3–4	6 (13.64)	4 (11.43)	10 (12.66)	
5–7	2 (4.55)	3 (8.57)	5 (6.33)	
Use of sunscreen frequency/week n(%)				0.2302
0	7 (15.91)	4 (11.43)	11 (13.92)	
1–2	12 (27.27)	15 (42.86)	27 (34.18)	
3–4	22 (50.00)	11 (31.43)	33 (41.77)	
5–7	3 (6.82)	5 (14.29)	8 (10.13)	
Smoking history n(%)				0.4430
Non-smoker	44 (100.00)	34 (97.14)	78 (98.73)	
Ex-smoker	0 (0.00)	1 (2.86)	1 (1.27)	
Smoker	0 (0.00)	0 (0.00)	0 (0.00)	
Average sleep time /day n(%)				0.0799
<5 h	5 (11.36)	3 (8.57)	8 (10.13)	
5–8 h	38 (86.36)	26 (74.29)	64 (81.01)	
>8 h	1 (2.27)	6 (17.14)	7 (8.86)	

SD: Standard deviation.

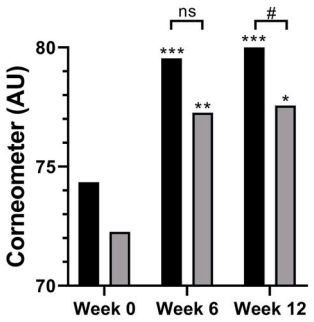

Fig. 2. Change in average skin hydration measured by Corneometer® from the baseline at each visit (black bar = palmitoleic acid group, gray bar = control group, *p < 0.05, **p < 0.01, ***p < 0.001 compared to baseline, #p < 0.05 compared to control group, ns = no significant difference).

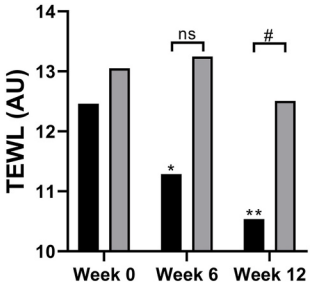

Fig. 3. Change in average TEWL measured by Cutometer Tewameter® from the baseline at each visit (black bar = palmitoleic acid group, gray bar = control group, *p < 0.05, **p < 0.01, #p < 0.05 compared to control group, ns = no significant difference).

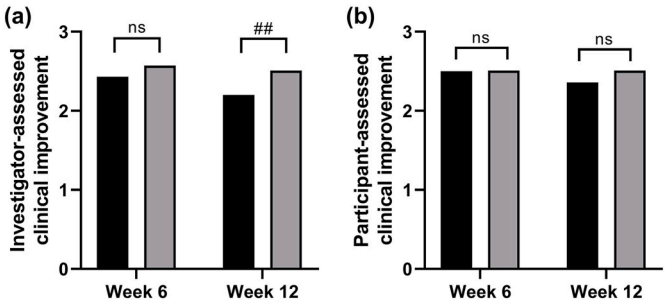

Fig. 4. Clinical improvement score at week 6 and week 12 (a) assessed by investigators and (b) subjects (black bar = palmitoleic acid group, gray bar = control group, $^{\#\#}p < 0.01$ compared to control group, ns = no significant difference).

5. Discussion

In this study, skin hydration and TEWL significantly improved, both within and between groups, at week 6 and week 12 with oral palmitoleic acid supplementation. Skin elasticity, surface roughness, wrinkle volume, and wrinkle severity showed significant intragroup, but not intergroup, improvement.

The results of this study are consistent with those of prior studies [8–11]. Authors expect this study to expand our knowledge of the efficacy and safety of palmitoleic acid supplementation for improving skin function in aging women.

Palmitoleic acid may provide various benefits for the skin. In an *in vitro* study, the addition of palmitoleic acid to human keratinocyte cell lines (HaCaT) increased the expression of silent information regulator 1, which dose-dependently regulates matrix metalloproteinase (MMP)-1 activity. Moreover, H_2O_2-induced ROS generation and MMP-1 expression decreased, whereas the expression of pro-collagen type 1 and elastin increased [21]. When palmitoleic acid is added to UV-B-irradiated HaCaT and human dermal fibroblasts, the expression levels of cyclooxygenase-2, MMP-3, c-Fos, and c-Jun decrease, whereas the expression of type 1 procollagen increases [22]. This provides a theoretical background for the ability of palmitoleic acid to improve skin elasticity and wrinkle formation.

In a UV-B-induced photoaging hairless mouse model, 4-week palmitoleic acid supplementation decreased skin thickness, TEWL, and epidermal thickness and increased the water content of the stratum corneum; moreover, the expression levels of the photoaging markers MMP-3 and c-Jun decreased [23]. Similarly, the effect of palmitoleic acid has been confirmed in cell and preclinical animal experiments.

As humans age, sebum and sweat secretion decreases, which makes the skin drier. Xerosis is the most common cause of pruritus and increases dysmorphic concerns and higher general anxiety as compared to healthy individuals and this decreases the QoL [8,9,24–26]. In addition, epidermal thickness decreases as dermal papillae flatten whereas dermal thickness decreases due to reduction of collagen and elastin fibers in the aging skin, with a resultant increase in wrinkle formation and a decrease in elasticity [25]. Wrinkle formation reduces self-esteem and QoL [9,25]. This study demonstrated the efficacy of palmitoleic acid for the improvement of skin barrier function of the aging skin. Thus, palmitoleic acid may improve QoL in aging individuals.

5.1. Strengths and limitations

The strength of this study is that bias was minimized through the sufficient sample size and randomized double-blind study design. Some limitations of this study need attention. First, although the skin barrier function was significantly improved in this study, subjective symptoms such as dryness and pruritus as well as QoL were not evaluated. This makes it difficult to directly correlate the efficacy of palmitoleic acid with real-life effects. Second, it is difficult to generalize the results to all age and sex groups because the participants were women of a specific age. Third, only one participant dropped out in the intervention group, whereas 10 participants dropped out in the control group, resulting in an imbalance. The investigators determined there was no systematic bias, but rather a coincidence that the majority of drop-outs were assigned to the control group. This determination is further supported by randomization and the fact that no participant or investigator knew which group participants were assigned to.

Future research with larger cohorts and longer supplementation in the general population, for the evaluation of real-life effects including QoL, may be able to determine whether there are additional significant outcomes.

6. Conclusion

This study evaluated whether skin barrier, skin elasticity, and wrinkle formation improved after palmitoleic acid supplementation and showed that palmitoleic acid is a safe and effective intervention for restoring the skin-barrier function and may effectively improve skin elasticity and wrinkle formation and thereby increased the QoL.

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Author contribution statement

Young Gue Koh: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
Joon Seok: Analyzed and interpreted the data; Wrote the paper.
Jae Wan Park: Performed the experiments.
Ka Ram Kim: Contributed reagents, materials, analysis tools or data.
Kwang Ho Yoo, Beom Joon Kim: Conceived and designed the experiments.
Yong Joon Kim: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author, upon reasonable request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix ASupplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e16711.

Appendix. B

Supplementary table 1. Palmitoleic acid supplementation main compounds

Molecular formula	Name	mg/g	%
C14:0	Myristic	4.4 ± 5.0	0.04
C16:0	Palmitic	257.3 ± 27.1	25.7
C16:1 n-7	Palmitoleic	535.6 ± 10.9	53.5
C20:5	Eicosapentaenoic (EPA)	5.6 ± 6.4	0.06

Supplementary table 2. Skin hydration, TEWL, elasticity, surface roughness, eye wrinkle volume, wrinkle severity assessment

	Palmitoleic acid n=44	Corn oil n=35	p-value[$]
Skin hydration (Corneometer, AU)			
Average	mean ± s.d.	mean ± s.d.	
Baseline (visit 1)	74.35±9.25	72.26±10.51	
Week 6 (visit 2)	79.55±7.88	77.26±10.31	
Change from baseline	5.20±9.06	5.00± 8.35	0.4835
p-value	0.0004**	0.0033[#]	
Week 12 (visit 3)	83.20± 7.20	77.56± 11.33	
Change from baseline	8.86± 9.94	5.30± 10.83	0.0146
p-value	<.0001**	0.0215[#]	
Left			
Baseline (visit 1)	73.53± 9.98	71.90± 10.79	
Week 6 (visit 2)	79.50± 8.31	77.53± 9.87	
Change from baseline	5.97± 10.49	5.63± 9.55	0.4762
p-value	0.0005**	0.0015[#]	
Week 12 (visit 3)	82.56± 7.20	77.89± 11.04	
Change from baseline	9.03± 10.30	6.00± 12.19	0.0356
p-value	<.0001**	0.0195[#]	
Right			
Baseline (visit 1)	75.16± 9.37	72.63± 11.48	
Week 6 (visit 2)	79.60± 8.53	76.98± 11.12	
Change from baseline	4.44± 8.51	4.36± 8.19	0.5379
p-value**	0.0012	0.0034	
Week 12 (visit 3)	83.84± 8.30	77.23± 12.01	

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(*continued*)

	Palmitoleic acid n=44	Corn oil n=35	p-value[$]
Change from baseline	8.68± 10.36	4.60± 10.70	0.0095
p-value**	<.0001	0.0157	
TEWL (g/h/m^2)			
Average			
Baseline (visit 1)	12.46± 3.88	13.05± 6.28	
Week 6 (visit 2)	11.29± 3.56	13.25± 5.69	
Change from Baseline	-1.17± 4.88	0.20± 4.16	0.0616
p-value	0.0172[#]	0.7776**	
Week 12 (visit 3)	10.54± 2.22	12.51± 5.08	
Change from baseline	-1.92± 3.85	-0.54± 5.05	0.0193
p-value	0.0012[#]	0.5315**	
Left			
Baseline (visit 1)	12.61± 3.26	13.12± 6.01	
Week 6 (visit 2)	11.12± 2.96	13.37± 5.11	
Change from Baseline	-1.50± 3.89	0.25± 4.52	0.0110
p-value#	0.0027	0.6589	
Week 12 (visit 3)	10.65± 2.29	12.35± 4.82	
Change from baseline	-1.96± 3.42	-0.77± 5.17	0.0405
p-value**	0.0004	0.3867	
Right			
Baseline (visit 1)	12.32± 5.02	12.98± 7.01	
Week 6 (visit 2)	11.47± 4.74	13.13± 6.56	
Change from baseline	-0.85± 6.33	0.15± 4.35	0.2235
p-value	0.1620[#]	0.8440**	
Week 12 (visit 3)	10.43± 2.63	12.67± 5.75	
Change from baseline	-1.88± 4.96	-0.31± 6.09	0.0230
p-value	0.0229[#]	0.7621**	
Skin elasticity (Cutometer, Ua/Uf)			
R2 Average (Ua/Uf)			
Baseline (visit 1)	0.59± 0.13	0.57± 0.14	
Week 6 (visit 2)	0.64± 0.10	0.61± 0.08	
Change from baseline	0.05± 0.13	0.04± 0.14	0.1928
p-value	0.0207**	0.3579[#]	
Week 12 (visit 3)	0.63± 0.12	0.60± 0.10	
Change from baseline	0.04± 0.15	0.04± 0.14	0.4438
p-value	0.0927**	0.5684[#]	
R2 Left (Ua/Uf)			
Baseline (visit 1)	0.62± 0.15	0.58± 0.15	
Week 6 (visit 2)	0.65± 0.11	0.62± 0.09	
Change from baseline	0.03± 0.15	0.04± 0.14	0.3015
p-value	0.1049[#]	0.0964**	
Week 12 (visit 3)	0.64± 0.13	0.60± 0.10	
Change from baseline	0.02± 0.16	0.02± 0.15	0.3586
p-value	0.4575**	0.9872[#]	
R2 Right (Ua/Uf)			
Baseline (visit 1)	0.56± 0.13	0.55± 0.15	
Week 6 (visit 2)	0.62± 0.11	0.59± 0.08	
Change from baseline	0.06± 0.13	0.04± 0.16	0.2398
p-value	0.0042**	0.5127[#]	
Week 12 (visit 3)	0.62± 0.12	0.61± 0.11	
Change from baseline	0.06± 0.16	0.05± 0.16	0.6796
p-value	0.0189**	0.2508[#]	
R5 Average (Ua/Uf)			
Baseline (visit 1)	0.50± 0.18	0.48± 0.19	
Week 6 (visit 2)	0.59± 0.16	0.56± 0.15	
Change from baseline	0.09± 0.17	0.08± 0.18	0.4733
p-value**	0.0008	0.0195	
Week 12 (visit 3)	0.60± 0.21	0.55± 0.19	
Change from baseline	0.10± 0.20	0.07± 0.20	0.2894
p-value**	0.0014	0.0583	
R5 Left (Ua/Uf)			
Baseline (visit 1)	0.51± 0.20	0.49± 0.19	
Week 6 (visit 2)	0.59± 0.17	0.58± 0.17	
Change from baseline	0.08± 0.18	0.09± 0.17	0.9989
p-value**	0.0083	0.0039	
Week 12 (visit 3)	0.61± 0.24	0.53± 0.20	
Change from baseline	0.10± 0.21	0.04± 0.21	0.1343
p-value**	0.0035	0.2236	
R5 Right (Ua/Uf)			

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	Palmitoleic acid n=44	Corn oil n=35	p-value[$]
Baseline (visit 1)	0.48± 0.17	0.48± 0.21	
Week 6 (visit 2)	0.59± 0.17	0.54± 0.15	
Change from baseline	0.11± 0.17	0.06± 0.22	0.2083
p-value**	0.0002	0.1100	
Week 12 (visit 3)	0.59± 0.20	0.57± 0.21	
Change from baseline	0.11± 0.21	0.09± 0.22	0.6547
p-value**	0.0013	0.0214	
R7 Average (Ua/Uf)			
Baseline (visit 1)	0.35± 0.12	0.34± 0.13	
Week 6 (visit 2)	0.40± 0.10	0.38± 0.09	
Change from baseline	0.05± 0.11	0.05± 0.11	0.4718
p-value**	0.0050	0.0235	
Week 12 (visit 3)	0.40± 0.12	0.37± 0.11	
Change from baseline	0.05± 0.13	0.03± 0.13	0.2164
p-value**	0.0144	0.1715	
R7 Left (Ua/Uf)			
Baseline (visit 1)	0.37± 0.13	0.34± 0.13	
Week 6 (visit 2)	0.41± 0.11	0.40± 0.10	
Change from Baseline	0.04± 0.12	0.05± 0.11	0.8312
p-value**	0.0231	0.0091	
Week 12 (visit 3)	0.41± 0.14	0.36± 0.11	
Change from baseline	0.05± 0.14	0.02± 0.13	0.1609
p-value**	0.0319	0.3823	
R7 Right (Ua/Uf)			
Baseline (visit 1)	0.34± 0.11	0.33± 0.13	
Week 6 (visit 2)	0.39± 0.10	0.37± 0.09	
Change from baseline	0.06± 0.11	0.04± 0.13	0.2666
p-value**	0.0020	0.0812	
Week 12 (visit 3)	0.39± 0.11	0.37± 0.11	
Change from baseline	0.06± 0.14	0.04± 0.13	0.3646
p-value**	0.0100	0.0822	
Surface roughness			
Ra Average (μm)			
Baseline (visit 1)	6.77± 1.66	7.29± 1.88	
Week 6 (visit 2)	6.14± 1.91	6.76± 2.13	
Change from baseline	-0.64± 1.67	-0.53± 1.59	0.4864
p-value	0.0011[#]	0.0564**	
Week 12 (visit 3)	5.87± 1.88	6.67± 2.62	
Change from baseline	-0.90± 1.28	-0.62± 1.97	0.3544
p-value	<.0001	0.0711	
Ra Left (μm)			
Baseline (visit 1)	7.02± 2.08	7.40± 2.29	
Week 6 (visit 2)	6.24± 2.21	6.83± 2.34	
Change from baseline	-0.78± 2.11	-0.57± 1.74	0.4094
p-value	0.0047[#]	0.0590**	
Week 12 (visit 3)	6.18± 2.24	6.91± 3.22	
Change from baseline	-0.84± 2.06	-0.50± 2.37	0.3794
p-value**	0.0097	0.2222	
Ra Right (μm)			
Baseline (visit 1)	6.53± 1.96	7.18± 2.36	
Week 6 (visit 2)	6.03± 2.24	6.69± 2.65	
Change from baseline	-0.50± 1.87	-0.49± 2.56	0.5801
p-value#	0.0099	0.0704	
Week 12 (visit 3)	5.56± 2.26	6.44± 2.61	
Change from baseline	-0.97± 1.67	-0.74± 2.38	0.3410
p-value**	0.0004	0.0734	
Rmax Average (μm)			
Baseline (visit 1)	38.80± 11.29	43.86± 11.71	
Week 6 (visit 2)	35.11± 13.54	40.08± 14.72	
Change from baseline	-3.69± 10.51	-3.78± 14.04	0.5857
p-value**	0.0246	0.1200	
Week 12 (visit 3)	34.87± 12.15	38.79± 16.45	
Change from baseline	-3.93± 10.22	-5.07± 16.20	0.7687
p-value**	0.0145	0.0727	
Rmax Left (μm)			
Baseline (visit 1)	39.98± 13.24	45.62± 16.34	
Week 6 (visit 2)	34.94± 12.24	39.62± 15.84	
Change from baseline	-5.04± 12.28	-6.00± 15.52	0.5098
p-value**	0.0094	0.0286	

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	Palmitoleic acid n=44	Corn oil n=35	p-value[$]
Week 12 (visit 3)	36.04± 14.34	39.77± 20.03	
Change from baseline	-3.94± 15.19	-5.85± 18.26	0.8429
p-value	0.0926**	0.0518[#]	
Rmax Right (μm)			
Baseline (visit 1)	37.62± 14.15	42.11± 15.73	
Week 6 (visit 2)	35.28± 19.46	40.54± 17.55	
Change from baseline	-2.34± 13.86	-1.57± 21.78	0.4826
p-value**	0.2687	0.6721	
Week 12 (visit 3)	33.70± 15.70	37.81± 16.06	
Change from baseline	-3.92± 11.28	-4.29± 20.33	0.5619
p-value**	0.0261	0.2199	
Rp Average (μm)			
Baseline (visit 1)	21.00± 7.83	24.98± 9.74	
Week 6 (visit 2)	19.50± 7.82	23.13± 11.06	
Change from baseline	-1.51± 7.30	-1.84± 11.24	0.4145
p-value**	0.1779	0.3387	
Week 12 (visit 3)	19.23± 7.23	21.50± 9.11	
Change from baseline	-1.77± 6.29	-3.47± 10.77	0.7731
p-value**	0.0687	0.0650	
Rp Left (μm)			
Baseline (visit 1)	20.62± 7.78	26.51± 12.73	
Week 6 (visit 2)	19.29± 7.23	23.80± 15.71	
Change from baseline	-1.33± 8.06	-2.71± 13.83	0.6543
p-value	0.0616[#]	0.2545**	
Week 12 (visit 3)	19.96± 8.29	22.20± 12.71	
Change from baseline	-0.66± 10.70	-4.31± 13.64	0.8930
p-value**	0.6828	0.0703	
Rp Right (μm)			
Baseline (visit 1)	21.39± 11.99	23.44± 11.07	
Week 6 (visit 2)	19.70± 13.15	22.46± 9.60	
Change from baseline	-1.68± 9.94	-0.98± 12.78	0.4683
p-value	0.2091[#]	0.6538**	
Week 12 (visit 3)	18.51± 11.56	20.81± 8.12	
Change from baseline	-2.88± 8.39	-2.63± 13.13	0.4973
p-value	0.0279**	0.4592[#]	
Rv Average (μm)			
Baseline (visit 1)	21.57± 7.40	23.65± 6.37	
Week 6 (visit 2)	19.15± 7.47	21.31± 7.16	
Change from baseline	-2.42± 6.58	-2.33± 6.42	0.5231
p-value**	0.0190	0.0387	
Week 12 (visit 3)	18.30± 6.57	21.18± 9.48	
Change from baseline	-3.27± 5.84	-2.47± 7.86	0.3258
p-value**	0.0006	0.0721	
Rv Left (μm)			
Baseline (visit 1)	23.43± 11.03	24.86± 9.24	
Week 6 (visit 2)	19.75± 9.91	20.76± 6.70	
Change from baseline	-3.68± 9.62	-4.10± 8.29	0.8361
p-value#	0.0054	0.0088	
Week 12 (visit 3)	19.73± 9.56	21.47± 10.39	
Change from baseline	-3.70± 9.73	-3.39± 9.47	0.6036
p-value**	0.0154	0.0417	
Rv Right (μm)			
Baseline (visit 1)	19.71± 6.81	22.43± 8.11	
Week 6 (visit 2)	18.55± 8.45	21.86± 10.48	
Change from Baseline	-1.16± 8.04	-0.57± 11.44	0.3087
p-value**	0.3441	0.7716	
Week 12 (visit 3)	16.87± 7.50	20.88± 10.26	
Change from baseline	-2.83± 6.51	-1.55± 9.68	0.1869
p-value**	0.0060	0.3514	
Rz Average (μm)			
Baseline (visit 1)	25.82± 6.48	28.05± 7.36	
Week 6 (visit 2)	23.62± 7.94	26.29± 9.28	
Change from baseline	-2.20± 5.56	-1.76± 6.76	0.6119
p-value	0.0011[#]	0.1325**	
Week 12 (visit 3)	22.36± 7.46	25.40± 10.25	
Change from baseline	-3.47± 4.19	-2.65± 7.62	0.4931
p-value**	<.0001	0.0478	
Rz Left (μm)			
Baseline (visit 1)	26.55± 8.63	28.79± 9.86	
Week 6 (visit 2)	23.91± 8.88	26.54± 10.32	

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	Palmitoleic acid n=44	Corn oil n=35	p-value[$]
Change from baseline	-2.63± 7.04	-2.25± 8.10	0.5187
p-value	0.0042[#]	0.1091**	
Week 12 (visit 3)	23.31± 8.94	26.34± 12.88	
Change from baseline	-3.23± 7.16	-2.45± 9.03	0.5094
p-value	0.0010[#]	0.1177**	
Rz Right (μm)			
Baseline (visit 1)	25.10± 7.50	27.30± 9.05	
Week 6 (visit 2)	23.33± 9.52	26.03± 11.29	
Change from Baseline	-1.77± 6.95	-1.27± 10.36	0.5779
p-value#	0.0197	0.1253	
Week 12 (visit 3)	21.40± 8.93	24.46± 9.73	
Change from baseline	-3.70± 5.94	-2.84± 9.13	0.3733
p-value**	0.0002	0.0744	
Eye wrinkle volume (mm^3)			
Average			
Baseline (visit 1)	2.31± 0.48	2.44± 0.67	
Week 6 (visit 2)	2.15± 0.55	2.16± 0.60	
Change from baseline	-0.15± 0.27	-0.28± 0.35	0.1228
p-value	0.0006**	<.0001	
Week 12 (visit 3)	2.08± 0.55	2.22± 0.66	
Change from baseline	-0.23± 0.30	-0.22± 0.33	0.7896
p-value**	<.0001	0.0004	
Left			
Baseline (visit 1)	2.26± 0.51	2.26± 0.51	
Week 6 (visit 2)	2.09± 0.58	2.28± 0.81	
Change from baseline	2.09± 0.58	-0.18± 0.32	0.9589
p-value**	0.0013 0	0.0019	
Week 12 (visit 3)	1.99± 0.56	2.33± 0.88	
Change from baseline	-0.26± 0.32	-0.14± 0.38	0.1232
p-value**	<.0001**	0.0383[#]	
Right			
Baseline (visit 1)	2.36± 0.58	2.42± 0.77	
Week 6 (visit 2)	2.22± 0.67	2.04± 0.54	
Change from baseline	-0.14± 0.38	-0.38± 0.59	0.0267
p-value	0.0212	0.0003	
Week 12 (visit 3)	2.16± 0.67	2.12± 0.67	
Change from baseline	-0.19± 0.44	-0.31± 0.48	0.3266
p-value**	0.0051	0.0007	
Wrinkle severity classification			
Average			
Baseline (visit 1)	6.09± 1.24	6.30± 1.20	
Week 6 (visit 2)	6.00± 1.22	6.14± 1.22	
Change from baseline	-0.09± 0.27	-0.16± 0.36	0.4020
p-value#	0.0625	0.0313	
Week 12 (visit 3)	5.77± 1.30	6.09± 1.23	
Change from baseline	-0.32± 0.54	-0.21± 0.47	0.3353
p-value#	0.0002	0.0156	
Left			
Baseline (visit 1)	6.07± 1.23	6.23± 1.21	
Week 6 (visit 2)	5.98± 1.21	6.09± 1.25	
Change from baseline	-0.09± 0.29	-0.14± 0.36	0.5207
p-value#	0.1250	0.0625	
Week 12 (visit 3)	5.75± 1.28	6.03± 1.25	
Change from baseline	-0.32± 0.56	-0.20± 0.47	0.2874
p-value#	0.0005	0.0313	
Right			
Baseline (visit 1)	6.11± 1.30	6.37± 1.21	
Week 6 (visit 2)	6.02± 1.28	6.20± 1.23	
Change from Baseline	-0.09± 0.29	-0.17± 0.38	0.3498
p-value#	0.1250	0.0313	
Week 12 (visit 3)	5.80± 1.37	6.14± 1.24	
Change from baseline	-0.32± 0.56	-0.23± 0.49	0.4064
p-value#	0.0005	0.0156	

**: Compared within groups; p-value for Paired t-test (p-value < 0.05)

#: Compared within groups; p-value for Wilcoxon signed rank test (p-value < 0.05)

$: Compared between groups; p-value for GLM adjusted baseline (p-value < 0.05)

Supplementary table 3. Clinical improvement assessment by investigator and subjects

	Palmitoleic acid n=44	Corn oil n=35	p-value[&]
By investigators			
Week6 (visit 2)	2.43± 0.50	2.57± 0.50	0.2227
Week12 (visit 3)	2.20± 0.41	2.51± 0.51	0.0043*
By subjects			
Week6 (visit 2)	2.50± 0.55	2.51± 0.51	0.1671
Week12 (visit 3)	2.36± 0.49	2.51± 0.56	0.1598

&: Compared between groups; *p*-value for Wilcoxon rank sum test (*p*-value < 0.05)

Supplementary table 4. Summary of adverse events (safety set)

Adverse events (n, %)	Palmitoleic acid n=45	Corn oil n=45	Total n=90
Dyspepsia	2 (4.44)	1 (2.22)	3 (6.66)

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