

Effects of Hydrolyzed Marine Collagen Peptide Supplementation Combined with Phytonutrients on Skin Biophysical Parameters and Extracellular Matrix Biomarkers in Thai Women: A Prospective Clinical Study

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ABSTRACT

Background: Skin aging involves progressive deterioration of extracellular matrix components. This study investigated the effects of hydrolyzed marine collagen peptide supplementation combined with phytonutrients on skin biophysical parameters and dermal biomarkers in Thai women. **Methods:** Sixty-three Thai women aged 40–60 years received daily supplementation (10,000 mg marine collagen peptides, Chlorella, grape seed extract, rice ceramides) for 12 weeks. Assessments included Antera 3D imaging, Cutometer elasticity measurements, Tewameter barrier function evaluation, and Corneometer hydration analysis. A subset (n=20) underwent suction blister biopsy for dermal biomarker analysis.

Results: Significant improvements were observed in Cutometer parameters: R5 net elasticity ($p<0.001$), R6 viscoelasticity ($p=0.018$), and R7 biological elasticity ($p=0.003$). Transepidermal water loss (TEWL) decreased by 12.1% ($p=0.007$). Antera 3D analysis revealed a significant increase in wrinkle width ($p=0.021$), indicating wrinkle flattening. Biomarker analysis showed significant increases in fibrillin-1 (+8.34%, $p=0.005$) and biglycan (+22.91%, $p=0.036$). Glogau photoaging classification improved in 23.8% of participants. **Conclusions:** Marine collagen peptide supplementation combined with phytonutrients significantly improved skin elasticity and barrier function, with molecular evidence of extracellular matrix enhancement in Thai women.

Keywords: Antera 3D, Barrier function, Cutometer, Fibrillin-1, Marine collagen peptides, Skin elasticity

INTRODUCTION

Skin aging is a multifactorial biological process driven by both intrinsic chronological factors and extrinsic insults, most notably ultraviolet (UV) radiation in a process known as photoaging^{1,2}. These mechanisms converge on the dermal extracellular matrix (ECM), leading to profound structural and functional deterioration. A hallmark of aged skin is the progressive decline in type I collagen synthesis by senescent fibroblasts, coupled with increased degradation by matrix metalloproteinases (MMPs), which results in a fragmented and disorganized collagen network^{3,4}. Concurrently, the elastic fiber system, which provides resilience and mechanical integrity, becomes compromised through the degradation of critical structural components, particularly fibrillin-rich microfibrils, leading to a progressive loss of skin elasticity and the development of visible wrinkles⁵. These molecular changes manifest clinically as visible signs of aging, including rhytides, sagging, reduced firmness, and diminished biomechanical integrity⁶. The loss of proteoglycans and hyaluronic acid further exacerbates these changes, resulting in reduced skin hydration and barrier function impairment⁷.

In response to these age-related changes, nutricosmetic strategies employing oral supplementation have gained significant scientific interest as a means to counteract these degenerative

processes from within. Among these approaches, hydrolyzed collagen peptides have emerged as a leading intervention, with a growing body of evidence from systematic reviews and meta-analyses of randomized controlled trials confirming their efficacy in improving skin hydration, elasticity, and reducing the appearance of wrinkles^{8,9}. The therapeutic mechanism is attributed to the high bioavailability of specific bioactive dipeptides, primarily prolyl-hydroxyproline (Pro-Hyp) and hydroxyprolyl-glycine (Hyp-Gly), which are generated during the hydrolysis process. These peptides are efficiently absorbed into the systemic circulation, reach the dermis, and act as signaling molecules that stimulate fibroblast proliferation and enhance the synthesis of hyaluronic acid and new collagen fibers, partly through the activation of STAT3-mediated signaling pathways and upregulation of hyaluronic acid synthase 2 (HAS2)^{10,11}. The bioactive properties of collagen peptides have been demonstrated in both *in vitro* studies using cultured dermal fibroblasts and *in vivo* clinical trials, with consistent improvements observed across multiple skin parameters¹².

Recognizing that skin health is influenced by multiple interconnected biological pathways and that a single intervention may have limited efficacy, recent research has increasingly shifted towards multi-component formulations that provide synergistic benefits. Phytonutrients, such as proanthocyanidins from grape seed extract, offer

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potent antioxidant protection against UV-induced oxidative stress and free radical damage, while extracts from microalgae such as *Chlorella* have demonstrated photoprotective and collagen-restorative properties through the modulation of matrix metalloproteinase expression and upregulation of pro-collagen synthesis^{13,14}. Furthermore, reinforcing the epidermal barrier function is crucial for maintaining skin hydration and preventing transepidermal water loss. Oral intake of plant-derived ceramides has been shown to improve skin barrier function and reduce transepidermal water loss (TEWL) by replenishing depleted stratum corneum lipids, complementing the dermal-focused action of collagen peptides and providing a comprehensive approach to skin rejuvenation¹⁵. This multi-targeted approach addresses both the structural integrity of the dermis and the functional barrier of the epidermis, offering a more holistic strategy for combating skin aging.

This prospective clinical study, therefore, aimed to investigate the efficacy of a novel multi-component formulation containing hydrolyzed marine collagen peptides (10,000 mg daily) combined with synergistic phytonutrients—specifically *Chlorella pyrenoidosa* powder, grape seed extract standardized for oligomeric proanthocyanidins (OPC), and rice-derived ceramides—on a comprehensive set of skin biophysical parameters and molecular extracellular matrix biomarkers in Thai women aged 40–60 years. This demographic and geographic population remain underexplored in the scientific literature regarding such specific multi-component formulations, and the inclusion of dermal biomarker analysis provides mechanistic evidence for the observed clinical improvements. The study employed both non-invasive biophysical measurements (three-dimensional imaging, cutometry, transepidermal water loss, and corneometry) and invasive suction blister biopsy for direct assessment of dermal fluid biomarkers, including procollagen type I, elastin, fibrillin-1, and biglycan, thereby providing a comprehensive evaluation of both clinical and molecular outcomes. However, due to the single-arm, open-label design without a placebo or comparator group, changes observed may be influenced by placebo effects, seasonal variation, lifestyle factors, or regression to the mean, which limits causal inference.

MATERIALS AND METHODS

Study Design, Setting and Ethics Approval

This prospective, single-arm, open-label clinical study was conducted at Chulabhorn International College of Medicine, Thammasat University, Thailand. The study protocol was reviewed and approved by the Human Research Ethics Committee of Thammasat University (Medicine) (Protocol No. MTU-EC-00-5-112/61) and registered with the Thai Clinical Trials Registry (TCTR20190103002). The study was conducted in accordance with the Declaration of Helsinki and International Conference on Harmonisation Guidelines for Good Clinical Practice (ICH-GCP). All participants provided written informed consent prior to enrollment.

Sample Size Calculation

The sample size was calculated based on the primary outcome of net elasticity (Cutometer R5 parameter), using a paired t-test design for pre-post comparison. Based on a previous randomized controlled trial by Kim et al.¹⁶, which demonstrated significant improvements in skin net elasticity (R5) following 12 weeks of low-molecular-weight collagen peptide supplementation (1,000 mg/day) in women aged 40–60 years ($p=0.002$ vs baseline; $p=0.027$ vs placebo), a medium effect size (Cohen's $d=0.4$) was anticipated. Using G*Power software version 3.1.9.7 (Heinrich-Heine-Universität Düsseldorf, Germany) for a two-tailed paired t-test with $\alpha=0.05$ and 80% statistical power, the minimum required sample size was 52 participants. To account for a potential 20% dropout rate, 63 participants were enrolled.

Participants

Eighty Thai women aged 40–60 years were screened for eligibility. Inclusion criteria were: (1) female sex; (2) age between 40 and 60 years; (3) Glogau photoaging classification types I–III; (4) Fitzpatrick skin phototypes I–V; and (5) willingness to comply with study requirements. Exclusion criteria included: (1) dermatological conditions that could affect skin assessment; (2) known allergies to any of the study ingredients; (3) use of topical retinoids, corticosteroids, or other anti-aging products within 4 weeks prior to enrollment; (4) oral collagen or antioxidant supplementation within the previous 3 months; (5) aesthetic procedures within the previous 6 months; (6) BMI ≥ 23 kg/m²; (7) pregnancy or lactation; (8) significant underlying medical conditions; and (9) concurrent participation in other clinical trials.

Intervention

Participants received daily supplementation with Formulation D10 for 12 weeks (Table 1). The supplement was administered as a single daily dose, dissolved in 200 mL of water, and taken in the morning after breakfast. Compliance was monitored through weekly telephone follow-ups and by counting the returned sachets at each study visit. The formulation composition was based on previous clinical trials and safety guidelines: the 10,000 mg dose of hydrolyzed marine collagen peptides was selected based on meta-analyses demonstrating optimal efficacy within the 10–2.5 g/day range¹⁷; *Chlorella pyrenoidosa* (20 mg) and grape seed extract standardized for %30 OPC (60 mg) were included for their documented photoprotective and antioxidant properties within established safe dose ranges; and rice ceramides (20 mg) were selected to reinforce epidermal barrier function based on clinical evidence demonstrating significant improvements in skin hydration and reduction in transepidermal water loss¹⁸. This multi-component formulation provides a comprehensive, evidence-based approach targeting multiple pathways of skin aging through dermal matrix enhancement, epidermal barrier reinforcement, and systemic antioxidant protection¹⁹.

Outcome Measurements

Primary and Secondary Outcomes

The primary outcome of this study was the improvement in skin net elasticity, measured as the R5 parameter (net elasticity representing the elastic portion from relaxation to the elastic portion from suction) using the Cutometer. Secondary outcomes included changes in other biomechanical skin properties measured by Cutometer (R2 gross elasticity, R6 viscoelasticity, and R7 biological elasticity), epidermal barrier function assessed by transepidermal water loss (TEWL), stratum corneum hydration measured by Corneometer, and wrinkle morphometry parameters (depth, width, and volume) assessed by three-dimensional skin surface analysis. Exploratory outcomes involved the quantification of dermal extracellular matrix biomarkers (procollagen type I, elastin, fibrillin-1, and biglycan) from suction blister fluid to assess changes in dermal matrix composition.

Assessment Methods and Procedures

Skin assessments were performed at baseline and weeks 4, 8, and 12 under standardized environmental conditions (temperature: 25–20°C; humidity %60–40) following a 30-minute acclimatization period. Three-dimensional skin surface analysis was performed using Antera 3D CS (Miravex, Dublin, Ireland) to evaluate wrinkle morphometry, including depth, width, and volume²⁰. Skin biomechanical properties were assessed using Cutometer MPA 580 (Courage + Khazaka electronic GmbH, Cologne, Germany), measuring parameters R2 (gross elasticity), R5 (net elasticity), R6 (viscoelasticity), and R7 (biological elasticity)²¹. Transepidermal water loss (TEWL) was

measured using Tewameter TM 300 (Courage + Khazaka)²², and stratum corneum hydration was assessed using Corneometer CM 825 (Courage + Khazaka)²³. All measurements were performed in triplicate at standardized facial locations.

Suction Blister Biopsy and Biomarker Analysis

A subset of 20 participants underwent suction blister biopsy at baseline and week 12 for the collection of dermal interstitial fluid. Blisters were induced on the volar forearm using a negative pressure device (450 mmHg), as previously described²⁴. Blister fluid was collected aseptically and stored at -80°C until analysis. Biomarkers, including procollagen type I, elastin, fibrillin-1, and biglycan, were quantified using commercial enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's protocols.

Statistical Analysis

Data were analyzed using SPSS version 26.0 (IBM Corporation, Armonk, NY, USA). Continuous variables were presented as mean \pm standard error (SE) or median (interquartile range), as appropriate for the data distribution. Normality was assessed using the Shapiro-Wilk test. For normally distributed biophysical parameters, a repeated-measures analysis of variance (ANOVA) with Bonferroni post-hoc correction was used to compare changes over time. For non-normally distributed biomarker data, Wilcoxon signed-rank test was applied for within-group comparisons. Effect sizes were calculated using Cohen's d for parametric data and r for non-parametric data. Statistical significance was set at $p < 0.05$ (two-tailed).

RESULTS

Participant Characteristics and Baseline Demographics

Following the screening process, 17 participants were excluded ($n=10$ due to exceeding the BMI limit; $n=7$ due to scheduling conflicts), resulting in the enrollment of 63 participants (Figure 1). All enrolled

participants completed the 12-week study, yielding a 100% retention rate and ensuring a complete dataset for analysis. The cohort was composed of Thai women with a mean age of 48.92 ± 0.61 years and a mean Body Mass Index (BMI) of 21.74 ± 0.28 kg/m², which is representative of a healthy, non-obese population. At baseline, the participants exhibited a spectrum of photoaging severity, with the majority classified as Glogau Type III (50.8%) and Type II (42.9%), indicating moderate to advanced signs of photodamage. The distribution of Fitzpatrick skin phototypes was predominantly Type III (42.9%) and Type II (27.0%), which is characteristic of the target population in this geographical region. The demographic data, including menstruation status and occupation, are summarized in Table 2, confirming a well-defined and homogeneous study group suitable for assessing the intervention's efficacy.

Wrinkle Morphometry

Objective assessment of periorbital wrinkles using three-dimensional surface analysis revealed a statistically significant, albeit modest, increase in wrinkle width (+1.1%, $p=0.021$) over the 12-week period, while wrinkle depth remained stable (-0.4%, $p=0.397$). This resulted in an elevated width-to-depth (W/D) ratio, which suggests a flattening or plumping effect on the skin's microrelief. Although the parameters for smaller, finer wrinkles (Wrinkle Index and Depth) exhibited consistent decreasing trends of 2-3%, these changes did not achieve statistical significance, suggesting the intervention's primary effect was on the more established, larger wrinkles (Table 3).

Skin Biomechanical Properties

The biomechanical integrity of the skin, a key indicator of youthfulness, was significantly enhanced following the 12-week supplementation period. Cutometer measurements revealed highly significant, time-dependent improvements in several key elasticity parameters (Table 4). Net elasticity (R5), which reflects the skin's ability to return to its original state after deformation, demonstrated a substantial and progressive increase, becoming statistically significant at week 12 ($p < 0.001$). This indicates a marked improvement in the skin's elastic recovery. Similarly, biological elasticity (R7), representing the ratio of immediate retraction to total deformation, showed a significant positive trend ($p=0.003$), confirming an enhancement in the skin's intrinsic elastic properties. The viscoelastic-to-elastic ratio (R6) also improved significantly ($p=0.018$), suggesting a favorable shift towards more elastic and less viscous behavior. In contrast, gross elasticity (R2), which represents the skin's overall distensibility, remained stable throughout the study ($p=0.287$), indicating that the formulation selectively improved the skin's ability to recover its shape rather than altering its fundamental stretchability.

Skin Barrier Function

Over the 12-week study, TEWL decreased progressively and significantly from a baseline of 13.12 ± 0.58 g/m²/h to 11.53 ± 0.44 g/m²/h at the final visit ($p=0.007$), corresponding to a clinically relevant 12.1% improvement (Table 5). This reduction signifies a more robust and efficient skin barrier, capable of retaining moisture more effectively. Conversely, measurements of stratum corneum hydration via corneometry did not show a statistically significant change from baseline ($p=0.733$).

Dermal Extracellular Matrix Biomarkers

Analysis of dermal interstitial fluid, collected via suction blister biopsy from a subset of 20 participants, provided molecular evidence of the intervention's impact on the ECM. The results revealed a statistically significant increase in two key structural proteins (Table 6). Fibrillin-1, a critical component of elastic microfibrils, increased by 8.34% from baseline ($p=0.005$), a change associated with a medium-to-large effect size ($r=0.47$). This suggests a strengthening of the elastic fiber

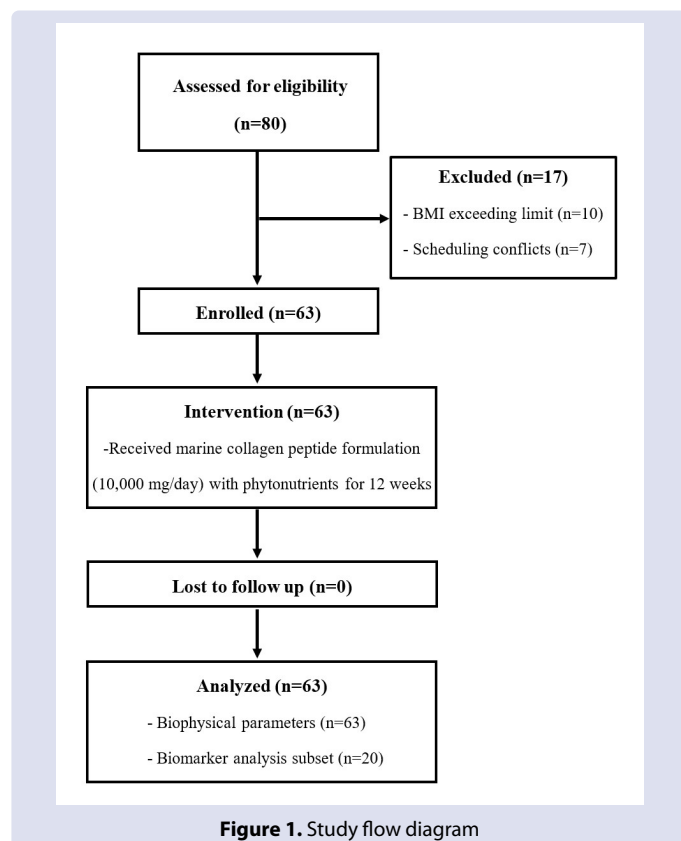


Figure 1. Study flow diagram

Table 1. Formulation D10 Composition

Component	Specification	Amount
Hydrolyzed Marine Collagen Peptides	90% protein, 6% hydroxyproline	10,000 mg
<i>Chlorella pyrenoidosa</i> powder	Whole cell powder	20 mg
Grape Seed Extract (<i>Vitis vinifera</i>)	50:1 extract, 30% OPC	60 mg
Rice Ceramides (<i>Oryza sativa</i>)	Glycosphingolipid complex	20 mg

OPC = Oligomeric Proanthocyanidins.

Table 2. Baseline Demographic and Clinical Characteristics of the Study Participants (n=63)

Variable	Value (n=63)
Age, years (Mean ± SE)	48.92 ± 0.61
BMI, kg/m ² (Mean ± SE)	21.74 ± 0.28
Glogau Classification, n (%)	
Type I / Type II / Type III	4 (6.3) / 27 (42.9) / 32 (50.8)
Fitzpatrick Skin Type, n (%)	
Type II / III / IV / V	17 (27.0) / 27 (42.9) / 15 (23.8) / 4 (6.3)
Menstruation Status, n (%)	
Pre/perimenopausal	37 (58.7)
Postmenopausal	26 (41.3)
Occupation, n (%)	
Indoor work	48 (76.2)

Data are presented as Mean ± Standard Error (SE) or number of participants (n) with percentage (%).

Table 3. Three-Dimensional Morphometric Analysis of Periorbital Wrinkles (n=63)

Parameter	Baseline	Week 12	% Change	p-value
Periorbital Width (mm)	2.442 ± 0.29	2.468 ± 0.22	+1.1	0.021*
Periorbital Depth (mm)	0.228 ± 0.07	0.227 ± 0.07	-0.4	0.397
Width-to-Depth Ratio	10.71	10.87	+1.5	—
Small Wrinkle Index (Ws)	12.62 ± 3.01	12.33 ± 2.68	-2.3	0.241
Small Wrinkle Depth (Ds)	2.45 ± 1.11	2.37 ± 0.98	-3.3	0.245

*p<0.05 indicates statistical significance compared to baseline. Analysis performed using a paired t-test.

Table 4. Time-Course of Skin Elasticity Parameter Improvements (n=63)

Parameter	Baseline	Week 4	Week 8	Week 12	p-value ^a
R2 (Ua/Uf)	0.912 ± 0.03	0.901 ± 0.04	0.927 ± 0.02	0.908 ± 0.05	0.287
R5 (Ur/Ue)	0.582 ± 0.04	0.598 ± 0.05	0.620 ± 0.05	0.675 ± 0.04*	<0.001*
R6 (Uv/Ue)	0.432 ± 0.04	0.431 ± 0.05	0.509 ± 0.05	0.531 ± 0.06*	0.018*
R7 (Ur/Uf)	0.348 ± 0.03	0.356 ± 0.03	0.370 ± 0.02	0.378 ± 0.02*	0.003*

*p<0.05 indicates statistical significance compared to baseline. ^aOverall p-value from repeated measures ANOVA with Bonferroni post-hoc correction.

Table 5. Skin Barrier Function and Hydration Parameters (n=63)

Parameter	Baseline	Week 4	Week 8	Week 12	p-value ^a
TEWL (g/m ² /h)	13.12 ± 0.58	12.76 ± 0.61	12.31 ± 0.53	11.53 ± 0.44*	0.007*
Corneometer (a.u.)	58.41 ± 2.14	59.83 ± 2.36	60.12 ± 2.28	59.67 ± 2.41	0.733

*p<0.05 indicates statistical significance compared to baseline. ^aOverall p-value from repeated measures ANOVA with Bonferroni post-hoc correction. TEWL = Transepidermal Water Loss.

Table 6. Changes in Dermal Fluid Biomarkers (n=20)

Biomarker	Baseline	Week 12	% Change	p-value
Procollagen I (pg/ml)	101.34 (18.72)	110.42 (21.45)	+6.97	0.179
Elastin (pg/ml)	189.23 (34.56)	198.46 (29.87)	+3.21	0.191
Fibrillin-1 (pg/ml)	7467.57 (234.51)	8192.24 (176.67)	+8.34	0.005*
Biglycan (pg/ml)	1.79 (0.38)	2.81 (0.69)	+22.91	0.036*

Data are presented as Median (Interquartile Range). Statistical significance (p<0.05) determined by Wilcoxon signed-rank test.

Table 7. Transition Matrix of Glogau Photoaging Classification from Baseline to Week 12 (n=63)

Baseline	Week 12 Type I	Week 12 Type II	Week 12 Type III	Total
Type I	4	0	0	4
Type II	6 ↑	21	0	27
Type III	0	9 ↑	23	32
Week 12 Total	10 (15.9%)	25 (39.7%)	28 (44.4%)	63

Arrow (↑) indicates an improvement of at least one grade. The total number of participants showing improvement was 15 (23.8%).

network. Additionally, the proteoglycan biglycan, which plays a role in collagen fibril organization, showed a substantial and significant increase of 22.91% ($p=0.036$, effect size $r=0.34$). While the levels of procollagen type I, a precursor to new collagen synthesis, and elastin both demonstrated positive trends (+6.97% and +3.21%, respectively), these increases did not reach statistical significance. These findings point towards a targeted anabolic effect on specific components of the dermal matrix, particularly those involved in elastic fiber integrity and overall ECM organization.

Glogau Classification Changes

Corroborating the instrumental and biomarker data, clinical assessment by dermatologists revealed a visible improvement in the overall appearance of photoaged skin. At the conclusion of the 12-week study, a notable portion of the participants—15 out of 63, or 23.8%—were observed to have improved by at least one Glogau photoaging grade. Specifically, 6 participants (22.2% of the Type II group) transitioned from Type II to Type I, and 9 participants (28.1% of the Type III group) improved from Type III to Type II (Table 7).

Safety and Adverse Events

Throughout the 12-week study period, the intervention was well-tolerated by all participants. No serious adverse events were reported. Minor gastrointestinal symptoms, such as mild bloating or changes in bowel habits, which are occasionally associated with oral collagen supplementation, were not reported. There were no observed allergic reactions or skin sensitivities related to the multi-component formulation, including the marine collagen, *Chlorella*, or grape seed extract. Laboratory safety parameters were not systematically assessed as part of this study protocol, but clinical monitoring confirmed the general safety profile of the daily 10,000 mg collagen dose combined with phytonutrients.

DISCUSSION

This prospective clinical study provides compelling evidence that a 12-week oral supplementation regimen with a multi-component formulation, containing 10,000 mg of hydrolyzed marine collagen peptides alongside synergistic phytonutrients, yields significant and multifaceted improvements in skin health among Thai women aged 40–60 years. The observed enhancements in skin elasticity, epidermal barrier function, and specific dermal extracellular matrix (ECM) biomarkers collectively suggest a coordinated, multi-level mechanism of action that addresses both the structural and functional deficits characteristic of chronological and photo-induced skin aging.

The most pronounced finding was the substantial improvement in skin biomechanical properties, as evidenced by the highly significant increases in net elasticity ($R5$, $p<0.001$) and biological elasticity ($R7$, $p=0.003$). These results are consistent with a growing body of literature from high-quality randomized controlled trials (RCTs) and meta-analyses that confirm the efficacy of hydrolyzed collagen supplementation. For instance, a recent meta-analysis concluded that collagen peptides significantly improve skin elasticity and hydration⁸. Our findings align with trials that reported similar elasticity improvements after 8 to 12 weeks of supplementation²⁵. The 10,000 mg daily dose administered in our study is at the upper end of the clinically effective range of 2.5–10 g/day identified in numerous successful trials, which may account for the robust effects observed^{17,26–28}. The mechanism underlying these improvements is attributed to the high bioavailability of specific bioactive dipeptides, primarily prolyl-hydroxyproline (Pro-Hyp) and hydroxyprolyl-glycine (Hyp-Gly), which act as signaling molecules to stimulate dermal fibroblast proliferation and the synthesis of new collagen and hyaluronic acid^{11,29}. A key and novel finding of this study is the significant upregulation of specific ECM components within the

dermal interstitial fluid. The 8.34% increase in fibrillin-1 ($p=0.005$) is particularly noteworthy. Fibrillin-1 is the foundational protein for the assembly of elastic microfibrils, which are known to be degraded and disorganized in photoaged skin^{5,6}. The observed increase suggests that the intervention actively supports the restoration of the skin's elastic fiber network, providing a molecular basis for the observed improvements in Cutometer-measured elasticity. This is further supported by the significant 22.91% increase in biglycan ($p=0.036$), a small leucine-rich proteoglycan (SLRP) that plays a crucial role in regulating collagen fibrillogenesis and maintaining the structural integrity of the ECM^{30,31}. While the increases in procollagen type I and elastin did not reach statistical significance, likely due to the limited statistical power of the biomarker subset, the positive trends suggest a broader anabolic effect on the dermal matrix. The coordinated upregulation of both a key elastic fiber protein and a collagen-organizing proteoglycan points to a more comprehensive ECM remodeling than would be expected from collagen supplementation alone, highlighting the potential synergistic effects of the multi-component formulation³².

The pattern of increased wrinkle width with stable depth represents a mechanistically meaningful indicator of wrinkle softening. This phenomenon reflects the "plumping effect" of enhanced dermal hydration and increased elastic fiber density. As elastic microfibrils are restored through increased fibrillin-1 expression and the dermis becomes more hydrated through improved barrier function (evidenced by the %12.1 reduction in TEWL), the skin gains improved viscoelasticity and resilience. This increased dermal volume and elasticity causes the wrinkle walls to become more rounded and less sharply defined, resulting in increased width of the wrinkle opening at the skin surface while the maximum depth remains relatively stable. This widening with stable depth is consistent with a "softening" of the wrinkle contour, indicating that the wrinkle has become less severe and more superficial in character, a finding supported by the significant improvements in Cutometer-measured elasticity parameters ($R5$ and $R25$,³³7). The significant %12.1 reduction in Transepidermal Water Loss (TEWL) ($p=0.007$) provides strong evidence of enhanced epidermal barrier function. This effect is likely attributable to the inclusion of rice-derived ceramides in the formulation. Ceramides are essential lipid components of the stratum corneum, and their depletion is directly linked to impaired barrier function and increased TEWL^{18,34}. Clinical studies have consistently shown that oral supplementation with plant-derived ceramides can effectively reduce TEWL and improve skin hydration by replenishing these critical lipids^{15,35}. The combination of dermal-acting collagen peptides with epidermal-focused ceramides represents a holistic, "inside-out" approach to skin health, addressing both the structural foundation in the dermis and the protective barrier of the epidermis. This dual action is likely a key contributor to the overall clinical improvements observed.

The formulation's benefits are further amplified by the antioxidant and photoprotective properties of its phytonutrient components. Grape seed extract, rich in oligomeric proanthocyanidins (OPCs), is a potent antioxidant that can mitigate UV-induced oxidative stress by scavenging free radicals and modulating cellular signaling pathways, such as the JNK/FOXO3a axis^{13,36}. Similarly, extracts from microalgae like *Chlorella* have demonstrated photoprotective effects and the ability to inhibit matrix metalloproteinases (MMPs), the enzymes responsible for collagen degradation¹⁴. This multi-pronged approach—stimulating new ECM synthesis, providing the necessary building blocks, reinforcing the epidermal barrier, and protecting against oxidative damage—creates a synergistic effect that likely surpasses the efficacy of any single ingredient. The clinical relevance of these findings is underscored by the 23.8% of participants who experienced a visible improvement of at least one Glogau photoaging grade. This objective clinical improvement, as assessed by trained dermatologists, provides tangible evidence that the measured biophysical and molecular

changes translate into a meaningful reduction in the visible signs of aging³⁷. These results are particularly significant as they were achieved in a Thai population (predominantly Fitzpatrick skin types III-IV), a demographic that is often underrepresented in nutraceutical research. The findings are consistent with studies in other Asian populations, which have also shown significant benefits from collagen and antioxidant supplementation, suggesting that these interventions are effective across a range of skin phototypes^{38,39}.

Despite the statistically significant findings, the primary limitation of this study is the single-arm, open-label design, which lacks a placebo control group, making it difficult to definitively separate true intervention effects from placebo responses. Consequently, the changes observed may be influenced by placebo effects, seasonal variation, lifestyle factors, or regression to the mean. Without a comparator group, causal inference is limited, and the conclusions drawn regarding the direct attribution of improvements to the intervention must be interpreted with caution. Future research should prioritize large-scale, randomized, double-blind, placebo-controlled trials with extended intervention periods (≥ 24 weeks) to confirm efficacy and assess long-term sustainability. Employing advanced non-invasive imaging techniques, such as multiphoton microscopy, and incorporating transcriptomic or proteomic analyses would provide deeper mechanistic insights. Additionally, studies in diverse populations (including different ethnicities, genders, and lifestyles) and investigations into dose-dependency are needed to broaden the applicability of these findings and optimize future formulations.

CONCLUSION

This prospective clinical study suggests that a multi-component supplement containing hydrolyzed marine collagen peptides, ceramides, and antioxidant phytonutrients may improve multiple facets of skin health in middle-aged Thai women. The formulation demonstrated a multi-level mechanism of action, enhancing skin elasticity, reinforcing epidermal barrier function, and stimulating the synthesis of key dermal matrix proteins. These findings contribute to the growing body of evidence supporting the use of targeted nutraceutical interventions as an effective strategy for combating the complex process of skin aging.

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