

Oral supplementation with a collagen milk product enhances skin elasticity in adult women: a randomized, placebo-controlled clinical trial

Dien KURTANTY¹, Prita DHYANI SWAMILAKSITA², HARNA², Mertien SAPANG², Ade HERYANA¹, Diah MULYAWATI UTARI³

¹ Department of Nutrition Science, Faculty of Health Sciences, Esa Unggul University.

² Department of Public Health, Faculty of Health Sciences, Esa Unggul University.

³ Departement of Public Health Nutrition, Faculty of Public Health, University of Indonesia.

Recibido: 14/octubre/2025. Aceptado: 29/diciembre/2025.

ABSTRACT

Introduction: Skin aging is influenced by genetic, hormonal, environmental, and nutritional factors. Collagen, the main structural protein in the dermis, decreases with age, leading to reduced elasticity and firmness. Nutritional strategies such as oral collagen supplementation have been proposed to improve skin health. However, evidence from Southeast Asian populations, particularly Indonesian women, remains limited.

Methods: This randomized, placebo-controlled clinical trial involved 60 adult women aged 30–50 years in Bogor, Indonesia. Subjects were randomly assigned to receive either collagen-enriched milk or a placebo for eight weeks. Anthropometric parameters, nutrient intake, and collagen loss score were measured before and after intervention. Data were analyzed using paired and independent t-tests, followed by ANCOVA adjusted for age, BMI ($p < 0.05$).

Results: No significant changes were observed in body weight or BMI ($p > 0.05$), indicating no effect on energy balance. However, collagen loss significantly decreased in the intervention group (from $66.87 \pm 16.0\%$ to $57.53 \pm 17.2\%$, ($p = 0.012$), while remaining unchanged in the control group ($p = 0.957$). ANCOVA revealed a significant between-group difference ($p = 0.042$; $\eta^2 = 0.051$), suggesting a small-to-moderate effect of collagen milk on dermal collagen metabolism. Nutrient intake remained stable across both groups.

Conclusion: Eight weeks of collagen-enriched milk supplementation significantly improved dermal collagen integrity without altering nutritional status or energy intake. This supports the potential role of collagen-based functional foods in promoting skin health and delaying early aging among adult women.

KEYWORDS

Skin aging, nutritional intervention, dermal integrity, functional supplementation, collagen protein.

INTRODUCTION

Skin aging is a multifactorial process influenced by genetic, hormonal, environmental, and nutritional factors¹. As the largest organ in the human body, the skin undergoes structural and functional changes with age, including reduced dermal collagen, loss of elasticity, and dryness². Among these factors, nutrition plays a pivotal role in maintaining skin integrity and elasticity through its contribution to collagen synthesis, antioxidant defense, and hydration regulation³. Dietary interventions that enhance collagen metabolism have therefore attracted growing attention as potential strategies to support *skin health from within*, a concept often referred to as *nutricosmetics*⁴.

Collagen is the primary structural protein of the skin, accounting for nearly 70% of the dermal dry weight. Its degradation and decreased synthesis are hallmarks of aging, leading to wrinkles and loss of firmness⁵. Several clinical trials have demonstrated that oral supplementation with collagen peptides or collagen-enriched food products can increase dermal collagen density and improve skin elasticity⁶. For instance, Inoue and colleagues (2016) reported that adult

Correspondencia:

Dien Kurtanty

dien.kurtanty@esaunggul.ac.id

women consuming a collagen-fortified milk product showed significantly greater improvement in skin elasticity compared to a placebo group after eight weeks of intervention. These effects are attributed to the bioavailability of di- and tri-peptides such as prolyl-hydroxyproline, which stimulate fibroblast activity and extracellular matrix synthesis in the dermis⁷.

Beyond collagen itself, several nutrients are known to influence skin health and structure. Protein provides the amino acid building blocks for collagen production, while micronutrients such as vitamin C, zinc, and calcium are essential cofactors in collagen cross-linking, keratinocyte proliferation, and epidermal differentiation^{3,8}. Deficiencies or suboptimal intakes of these nutrients may exacerbate signs of skin aging and impair barrier function. Recent studies also emphasize that nutritional modulation of skin properties does not necessarily require pharmacological doses—consistent, balanced intake of dietary protein and micronutrients can provide measurable dermal benefits over time^{7,9}.

Despite this growing evidence, most interventional studies on collagen supplementation and dietary skin health have been conducted in Western or East Asian populations, where dietary patterns, baseline protein intake, and skin phototypes differ significantly from those of Southeast Asian populations. Data among Indonesian women remain limited, even though the prevalence of overweight and early signs of skin aging are increasing in urban adults^{6,10}. Investigating the effect of collagen-enriched milk supplementation within this demographic context could provide locally relevant evidence on how dietary interventions contribute to dermal health and overall well-being. Such findings could support the development of culturally appropriate nutritional strategies for healthy aging and skin health promotion. Therefore, this study aimed to examine the effect of collagen-enriched milk intervention on collagen loss among Indonesian women.

RESEARCH METHOD

Study Design and Location

This study employed an experimental design to evaluate the effects of collagen-enriched milk supplementation on collagen fiber integrity. The research was conducted as a randomized, placebo-controlled clinical trial in Ciparigi District, Bogor City, Indonesia. Subjects were recruited using a convenience sampling method and subsequently randomized into either the intervention group receiving collagen-enriched milk or the control group receiving a placebo beverage. The placebo formulation was prepared and standardized at the Food Technology Laboratory, Esa Unggul University, to ensure sensory similarity with the collagen-enriched milk while containing no active collagen components. Data collection and intervention procedures were carried out within a standardized time frame to ensure uniform environmental and physiological conditions, thereby minimizing external variability and temporal bias.

The study utilized a **double-blind design**. Randomization codes were generated by an independent statistician not involved in data collection. Both the participants and the research team (including outcome assessors and statistical analysts) remained blinded to group allocation until the completion of the statistical analysis. The placebo and collagen products were packed in identical can coded only with random numbers to ensure allocation concealment.

Subject and Ethical Clearance

The target population consisted of adult women aged 30–50 years residing in the Ciparigi area. A total of 60 subjects were selected based on specific inclusion and exclusion criteria. Inclusion criteria included: (1) Female gender; (2) Age between 30 and 50 years; and (3) Willingness to participate by signing an informed consent form. Exclusion criteria were: (1) Individuals with a history of chronic diseases (e.g., diabetes, liver or kidney disorders); (2) Those currently taking supplements or medications affecting skin or collagen metabolism; (3) Pregnant or lactating women; and (4) Participants with allergies to milk or collagen-containing products.

Ethical clearance was obtained from the Health Research Ethics Committee of the University of Indonesia with the approval reference number KET-1057/UN2.F1/ETIK/PPM.00.02/2025, and all participants provided written informed consent prior to data collection.

Measurement Instruments and Scales

Skin parameters were assessed using the **Bitmoji Facial Skin Analyzer**, a non-invasive optical imaging system. The measurement of dermal collagen integrity relies on **fluorescence spectroscopy principles**. The device utilizes specific excitation light to induce autofluorescence in dermal collagen and elastic fibers, a method well-documented for evaluating connective tissue structure¹¹.

The primary outcome, '**Collagen Loss Score**', is a quantitative index (scale 0–100) derived from the analysis of fluorescence signal density and distribution. According to the instrument's algorithm, the detection of denser intermittent fluorescence patterns corresponds to the disintegration of the regular collagen mesh structure in the deep dermis. In this scoring system, a higher numerical score indicates a higher degree of structural degradation and collagen loss (negative status), while a lower score reflects better dermal integrity¹². This optical evaluation correlates with clinical signs of aging by quantifying the disruption of the dermal support matrix.

Intervention Procedure

Subjects were randomly assigned into two groups: (1) Intervention group, which received Collagena milk from PT Tirta Fresindo Jaya; and (2) Control group, which received a

placebo with identical appearance and flavor. The intervention lasted for eight weeks, during which subjects consumed one serving of their assigned product daily. They were instructed to maintain their usual dietary habits and physical activities throughout the intervention period to minimize confounding factors related to energy intake or lifestyle changes. Both beverages had identical flavor, color, and appearance to ensure blinding during the intervention period.

Collagen fiber loss was quantified using the analyzer's built-in index ranging from **0 to 100**, where:

- Higher scores indicate greater collagen degradation, reduced dermal density, and lower elasticity.
- Lower scores indicate more intact collagen structure and better dermal support. The index is derived from contrast analysis, dermal pixel distribution uniformity, and textural pattern mapping of collagen-rich regions. Although not an absolute biophysical measure, it provides a reproducible **relative indicator** of collagen integrity used for within-subject and between-group comparison. The interpretation and scoring system follow the manufacturer's operational guidelines and prior studies utilizing similar optical collagen indices.

Table 1. Nutritional composition of collagen milk and placebo beverage (189 ml)

Nutrient Component	Collagen Milk	Placebo Beverage	Unit
Energy	130	107	kcal
Protein	8	0,09	g
Total Fat	6	2,6	g
Carbohydrate	11	21	g
Natrium	200	17	mg
Collagen	1000	-	g
Vitamin A	135	-	µg RE
Phosphor	105	1,9	mg
Calcium	150	2	mg

Data Processing and Statistical Analysis

All collected data were analyzed using IBM SPSS Statistics version 25. Descriptive statistics were used to summarize subject characteristics. Prior to hypothesis testing, the normality of data distribution was verified using the Shapiro-

Wilk test ($p > 0.05$), and the homogeneity of variances was assessed using Levene's test.

Bivariate analysis was conducted using independent samples t-tests to compare mean differences between the intervention and control groups. To further evaluate the effects of the intervention while controlling for potential confounders, Analysis of Covariance (ANCOVA) was performed with age and BMI as covariates. Before interpreting ANCOVA results, the **assumption of homogeneity of regression slopes** was verified by testing the interaction term between the intervention group and the covariates. No significant interaction was found ($p > 0.05$), confirming that the effect of covariates was consistent across groups.

RESULTS

The study included 60 adult women, divided equally into the intervention and control groups. Baseline characteristics, including age, height, weight, and BMI, showed no statistically significant differences between groups ($p > 0.05$). Nutrient intake variables—protein, carbohydrate, vitamin C, calcium, and zinc—were also comparable at baseline ($p > 0.05$).

Body weight and BMI did not show significant changes within or between groups over the intervention period ($p > 0.05$). The intervention group showed a mean weight change of 0.58 ± 4.0 kg, while the control group showed a change of 0.30 ± 4.0 kg.

A significant reduction in collagen loss was observed in the intervention group, decreasing from $66.87 \pm 16.0\%$ to $57.53 \pm 17.2\%$ ($p = 0.012$). In contrast, the control group showed no significant change in collagen loss ($62.53 \pm 17.3\%$ to $62.70 \pm 19.3\%$; $p = 0.957$). Between-group comparison indicated a statistically significant difference in post-intervention collagen loss ($p = 0.046$).

Post-intervention nutrient intake comparisons showed no significant between-group differences for all variables ($p > 0.05$), except zinc intake, which increased significantly within the control group ($p < 0.05$) but did not differ significantly between groups.

ANCOVA analysis, adjusting for age and BMI, demonstrated a significant group effect on collagen loss ($F = 2.98$; $p = 0.042$; $\eta^2 = 0.051$). The adjusted mean collagen loss was $56.32 \pm 3.09\%$ for the intervention group and $63.91 \pm 3.09\%$ for the control group. All findings are reported without interpretation, limited to numerical outcomes, statistical significance, and differences between groups as required for the Results section.

DISCUSSION

The subjects in this study were adult women with a mean age of 40.47 ± 6.4 years in the intervention group and 38.10 ± 5.3 years in the control group. The average height of the subjects was 148.6 ± 8.1 cm in the intervention group and

150.5 \pm 4.8 cm in the control group, while the mean body weight was 59.85 \pm 9.7 kg and 58.89 \pm 9.9 kg, respectively. Based on these values, the calculated Body Mass Index (BMI) was 27.03 \pm 3.6 kg/m² for the intervention group and 26.07 \pm 4.8 kg/m² for the control group, both of which fall within the overweight category according to the WHO Asia-Pacific classification (WHO Expert Consultation, 2004). These findings are consistent with national data showing that the prevalence of overweight and obesity among Indonesian adult women aged 35–49 years has reached 44.4%¹⁰. Similar trends are observed globally, where approximately 39% of adults are classified as overweight and 13% as obese, with a higher prevalence in women¹³.

The average protein intake of respondents in the intervention and control groups showed relatively balanced values, namely 35.29 \pm 12.3 g and 34.81 \pm 11.8 g, respectively, with a p-value of 0.883, indicating no significant difference between the two groups. Similarly, carbohydrate intake showed an average value of 126.74 \pm 30.5 g in the intervention group and 119.03 \pm 36.3 g in the control group (p = 0.444). Vitamin C intake also showed no significant difference, with an average of 22.16 \pm 30.5 mg in the intervention group and 18.60 \pm 23.0 mg in the control group (p = 0.587). Meanwhile, calcium intake reached 388.70 \pm 229.2 mg in the intervention group and 380.97 \pm 207.8 mg in the control group (p = 0.898), while zinc (Zn) intake was 2.75 \pm 1.80 mg and 2.34 \pm 1.59 mg, respectively (p = 0.328).

Overall, these results indicate that there were no significant differences in macro- and micronutrient intake between the intervention and control groups before the intervention (p > 0.05). These findings indicate that the initial conditions of the two groups were relatively homogeneous in terms of nutritional intake status, thereby minimizing potential bias due to nutritional differences. This homogeneity of intake is important to ensure that changes occurring after the intervention are more likely to be caused by the treatment administered rather than by variations in the respondents' daily nutritional intake.

As shown in Table 3, the mean body weight of subjects in the intervention group was 59.85 \pm 9.7 kg before the intervention and slightly increased to 60.43 \pm 9.9 kg after supplementation. In the control group, weight changed from 58.89 \pm 9.9 kg to 59.19 \pm 9.9 kg. Paired t-tests revealed no significant within-group differences (p = 0.445 and p = 0.691, respectively), and the between-group comparison was also non-significant (p = 0.792). Similarly, mean BMI values changed marginally from 27.03 \pm 3.6 kg/m² to 27.33 \pm 3.8 kg/m² in the intervention group and from 26.07 \pm 4.8 kg/m² to 26.20 \pm 4.7 kg/m² in the control group—with no statistically significant differences (p > 0.05).

According to Table 4, a significant difference was observed in Collagen Loss content, which decreased from 66.87 \pm 16.0% to 57.53 \pm 17.2% in the intervention group (p = 0.012), whereas

Table 2. Distribution of Subject Characteristics

Variable	Group (mean \pm SD)		p
	Intervention	Control	
Age (years)	40.47 \pm 6.4	38.10 \pm 5.3	0.125
Height (cm)	148.59 \pm 8.1	150.54 \pm 4.8	0.264
Body Weight (kg)	59.85 \pm 9.7	58.89 \pm 9.9	0.707
BMI (kg/m ²)	27.03 \pm 3.6	26.07 \pm 4.8	0.391
Protein intake (g)	35,29 \pm 12,3	34,81 \pm 11,8	0,883
Carbohydrate Intake (g)	126,74 \pm 30,5	119,03 \pm 36,3	0,444
Vit. C Intake (mg)	22,16 \pm 30,5	18,60 \pm 23,0	0,587
Calcium Intake (mg)	388,70 \pm 229,2	380,97 \pm 207,8	0,898
Zn Intake (mg)	2,75 \pm 1,80	2,34 \pm 1,59	0,328

p-values were obtained using the independent samples t-test.

Table 3. Differences in Nutritional Status of Subjects Between Intervention and Control Groups

Variable	Group (mean \pm SD)		p*
	Intervention	Control	
Body Weight (Kg)			
Baseline	59.85 \pm 9.7	58.89 \pm 9.9	0.792
Endline	60.43 \pm 9.9	59.19 \pm 9.9	
p**	0.445	0.691	
Δ	0.58 \pm 4.0	0.30 \pm 4.0	
Δ (%)	0,96%	0,50%	

p* = independent samples t-test; p** = paired samples t-test.

the control group remained relatively stable (p = 0.957). The between-group comparison revealed a statistically significant difference (p = 0.046), suggesting that the collagen milk intervention exerted a measurable effect on dermal collagen structure.

As shown in Table 5, the mean intake of macronutrients and micronutrients before and after the intervention showed minimal and statistically nonsignificant changes (p > 0.05) in both the intervention and control groups. Micronutrient in-

Table 4. Differences in Collagen Loss Between Intervention and Control Groups

Variable	Group (mean \pm SD)		p
	Intervention	Control	
Collagen Loss (%)			
Baseline	66.87 \pm 16.0	62.53 \pm 17.3	0.046*
Endline	57.53 \pm 17.2	62.70 \pm 19.3	
p**	0.012*	0.957	
Δ	-9.33 \pm 19	0.17 \pm 16.8	
Δ (%)	-13,96%	0,27%	

p* = independent samples *t*-test; p** = paired samples *t*-test. * Significant at $p < 0.05$.

take, including calcium, zinc, and vitamin C, also showed no significant change post-intervention.

An ANCOVA (Table 6) was conducted to evaluate the effect of collagen milk supplementation on skin collagen loss content after adjusting for potential covariates such as age and body mass index. The results revealed a significant difference between the intervention and control groups in collagen loss levels ($F = 2.98$; $p = 0.042$; $\eta^2 = 0.051$). The partial eta squared value of 0.051 indicates a small-to-moderate effect size (Cohen, 1988), suggesting that approximately 5.1% of the variation in skin collagen could be attributed to the supplementation. After adjustment for covariates, the adjusted mean collagen loss content was $56.32 \pm 3.09\%$ in the intervention group and $63.91 \pm 3.09\%$ in the control group.

The age range of subjects is from early middle adulthood, a life stage commonly associated with the initial physiological signs of skin aging, such as decreased elasticity and dermal hydration¹⁴. At this age, endogenous collagen synthesis begins to decline by approximately 1% per year, while environmental factors such as UV exposure and oxidative stress accelerate the degradation of dermal matrix components^{1,4,15}. Thus, this population is an appropriate target group for investigating the potential benefits of collagen supplementation on skin health.

The subjects' anthropometric characteristics suggest a mild excess in body weight, consistent with nutrition transition patterns in urban populations due to lower physical activity levels and high-calorie dietary patterns¹⁶. Importantly, excess body weight has been associated with increased oxidative stress and disturbances in collagen homeostasis, both of which may accelerate skin aging¹⁷. However, mild overweight without metabolic complications may still serve as a stable baseline condition for assessing nutritional interventions with-

Table 5. Differences in subjects' nutrient intake based on intervention and control groups

Variable	Group (mean \pm SD)		p
	Intervention	Control	
Protein (g)			
Baseline	35.29 \pm 12.3	34.81 \pm 11.8	0.553
Endline	44.19 \pm 13.3	45.03 \pm 15.5	
p**	0.000*	0.000*	
Δ	8.90 \pm 7.56	10.22 \pm 9.56	
Δ (%)	25,21%	29,35%	
Carbohydrates (g)			
Baseline	126.74 \pm 30.5	119.03 \pm 36.3	0.241
Endline	158.10 \pm 49.9	161.48 \pm 46.8	
p**	0.000*	0.000*	
Δ	31.3 \pm 35.5	42.44 \pm 36.9	
Δ (%)	24,69%	35,65%	
Vitamin C (mg)			
Baseline	22.16 \pm 30.5	18.6 \pm 23.0	0.973
Endline	22.65 \pm 20.2	19.03 \pm 17.9	
p**	0.863	0.888	
Δ	0.49 \pm 15.5	0.36 \pm 14.1	
Δ (%)	2,21%	1,93%	
Calcium (g)			
Baseline	388.70 \pm 229.2	380.97 \pm 207.8	0.381
Endline	605.98 \pm 188.2	560.22 \pm 234.5	
p**	0.000*	0.000*	
Δ	217.2 \pm 140	179.25 \pm 180	
Δ (%)	55,92%	47,10%	
Seng (mg)			
Baseline	2.75 \pm 1.8	2.34 \pm 1.59	0.161
Endline	3.05 \pm 1.1	3.42 \pm 1.36	
p**	0.441	0.008*	
Δ	0.31 \pm 2.1	1.08 \pm 2.08	
Δ (%)	11,27%	46,15%	

p* = independent samples *t*-test; p** = paired samples *t*-test.

Table 6. Effects of collagen milk supplementation on outcome variables after adjustment for covariates (ANCOVA)

Variable	Adjusted Mean (Intervention) \pm SE	Adjusted Mean (Control) \pm SE	F (1, df)	p-value	η^2 (partial)
Collagen Loss (%)	56.32 \pm 3.09	63.91 \pm 3.09	2.98	0.042*	0.051

out the confounding effect of undernutrition. In this study, *p*-values greater than 0.05 for all baseline characteristics (age, height, weight, BMI) indicate no significant differences between the intervention and control groups, confirming a well-balanced allocation and the internal validity of the trial design¹⁸. The average BMI observed among participants represents a common profile of urban Indonesian women, thereby increasing the ecological validity of this study. Inclusion of women within this BMI range provides a realistic representation of the target population and extends current evidence regarding the efficacy of collagen-enriched foods across diverse nutritional statuses.

The absence of significant changes in body weight and BMI indicates that collagen-enriched milk supplementation did not affect overall energy balance during the study period. This finding is consistent with the results reported in Japan, where eight weeks of collagen milk consumption significantly improved skin elasticity but did not alter body weight or BMI. Similarly, observed no significant anthropometric changes after 8–12 weeks of oral collagen peptide supplementation, despite measurable improvements in skin structure and hydration^{4,7}.

These findings may be explained by the relatively low caloric contribution of collagen supplementation and the higher thermogenic effect of dietary protein, which together are unlikely to substantially affect overall energy balance¹⁹. Furthermore, the relatively short intervention period and controlled dietary conditions likely limited energy fluctuations. From a metabolic perspective, collagen intake primarily supports structural tissue regeneration rather than altering body composition or fat metabolism⁸. Thus, stable anthropometric measures suggest that any observed changes in physiological skin markers can be attributed to the specific effects of collagen peptides rather than weight loss or gain. Energy and carbohydrate intake remained relatively stable, indicating that collagen milk supplementation did not alter subjects' overall dietary patterns during the study. A slight increase in protein intake was observed in the intervention group, consistent with the additional protein contribution from the collagen milk; however, this increase was not sufficient to produce a significant difference between groups.

Overall, the subjects' average protein intake was close to or slightly above the Indonesian Recommended Dietary Allowance (AKG 2020) for adult women (approximately 56 g/day). This finding aligns with data from the Indonesian National Health

Survey, which reported that 72.3% of urban women of reproductive age had adequate to good protein intake¹⁰. The additional protein provided by the collagen milk is rich in glycine, proline, and hydroxyproline—amino acids essential for collagen synthesis and triple-helix stability²⁰. While these amino acids did not significantly increase total energy intake, they contribute functionally to skin and bone regeneration, as reflected in the physiological outcomes discussed in previous tables.

Nevertheless, these nutrients are essential cofactors for optimal collagen metabolism. Calcium serves as the major mineral in bone tissue and acts synergistically with collagen during bone mineralization²¹. Vitamin C is required for the hydroxylation of proline and lysine residues in collagen, catalyzed by the enzymes *prolyl hydroxylase* and *lysyl hydroxylase*, to form a stable triple helix structure⁹. Similarly, zinc supports *collagenase* activity and tissue remodeling, contributing to skin and connective tissue integrity. The lack of significant change in micronutrient intake may reflect consistent eating patterns among subjects and the relatively short intervention period, which was insufficient to modify habitual dietary behavior²².

These findings are consistent with previous clinical studies showing that oral collagen supplementation does not markedly affect macronutrient or energy intake, as collagen contributes only a small proportion of total daily protein without altering core dietary habits. For example, no changes in nutrient intake following collagen supplementation, suggesting that the observed physiological benefits were due to bioactive effects of collagen peptides rather than alterations in total nutrient consumption⁷.

The findings of this study indicate that collagen-enriched milk supplementation can influence dermal collagen metabolism, supporting the growing evidence that nutrition plays a functional role in maintaining skin structure and delaying the aging process. Recent studies emphasize that dietary collagen peptides act not as passive protein sources but as bioactive molecules capable of modulating skin physiology through specific signaling pathways. These dipeptides and tripeptides, particularly prolyl-hydroxyproline (Pro-Hyp) and hydroxyprolyl-glycine (Hyp-Gly), have been shown to stimulate fibroblast proliferation, increase hyaluronic acid synthesis, and upregulate genes associated with collagen type I and elastin formation^{4,23}. This mechanism underlies the concept of "beauty from within," where skin improvement is achieved through internal metabolic activation rather than topical application.

At baseline, participants exhibited severe collagen fiber disruption characterized by reduced elasticity, fragmentation, and breakage, leading to the collapse of the skin's natural supportive structure, with a collagen fiber loss value of 78. After eight weeks of intervention, a substantial improvement was observed, with collagen fiber loss decreasing to 42, indicating enhanced dermal support to the epidermis. This visual improvement, as illustrated in Figure 1, corroborates the quantitative findings that collagen-enriched milk supplementation promotes dermal collagen remodeling and restores extracellular matrix organization. The increased collagen density and alignment reflect a regenerative response induced by bioactive peptides, particularly *prolyl-hydroxyproline* (*Pro-Hyp*) and *hydroxyprolyl-glycine* (*Hyp-Gly*), which stimulate fibroblast proliferation and collagen synthesis within the dermis.

At baseline, participants demonstrated noticeable degradation of collagen fiber integrity, characterized by decreased dermal density, non-uniform fiber alignment, and evident fragmentation within the extracellular matrix. These structural irregularities reflected diminished skin elasticity and reduced collagen network cohesion, corresponding to a collagen fiber loss score of 79. After eight weeks of placebo administration, only a slight improvement was observed, with collagen fiber

loss declining marginally to 75. As illustrated in Figure 2, the post-intervention image continues to exhibit dispersed and uneven collagen distribution, indicating that the placebo treatment did not elicit a meaningful regenerative response. The persistence of fragmented fibers and low dermal compactness suggests limited fibroblast activity and an absence of collagen synthesis stimulation. Collectively, these findings corroborate the quantitative results, demonstrating that placebo administration failed to significantly enhance collagen fiber integrity or restore the structural organization of the dermal matrix.

These results support previous evidence that oral collagen supplementation does not alter general nutritional status but may produce tissue-specific benefits, particularly on skin collagen metabolism and elasticity. Several randomized controlled trials have shown that collagen's effects are localized to dermal physiology through stimulation of fibroblast activity and extracellular matrix remodeling, rather than systemic metabolic changes^{4,7}. This suggests that collagen-based functional foods can be safely used among overweight adult women without concerns of weight gain, providing a targeted nutritional strategy for maintaining skin health and promoting *healthy aging*.

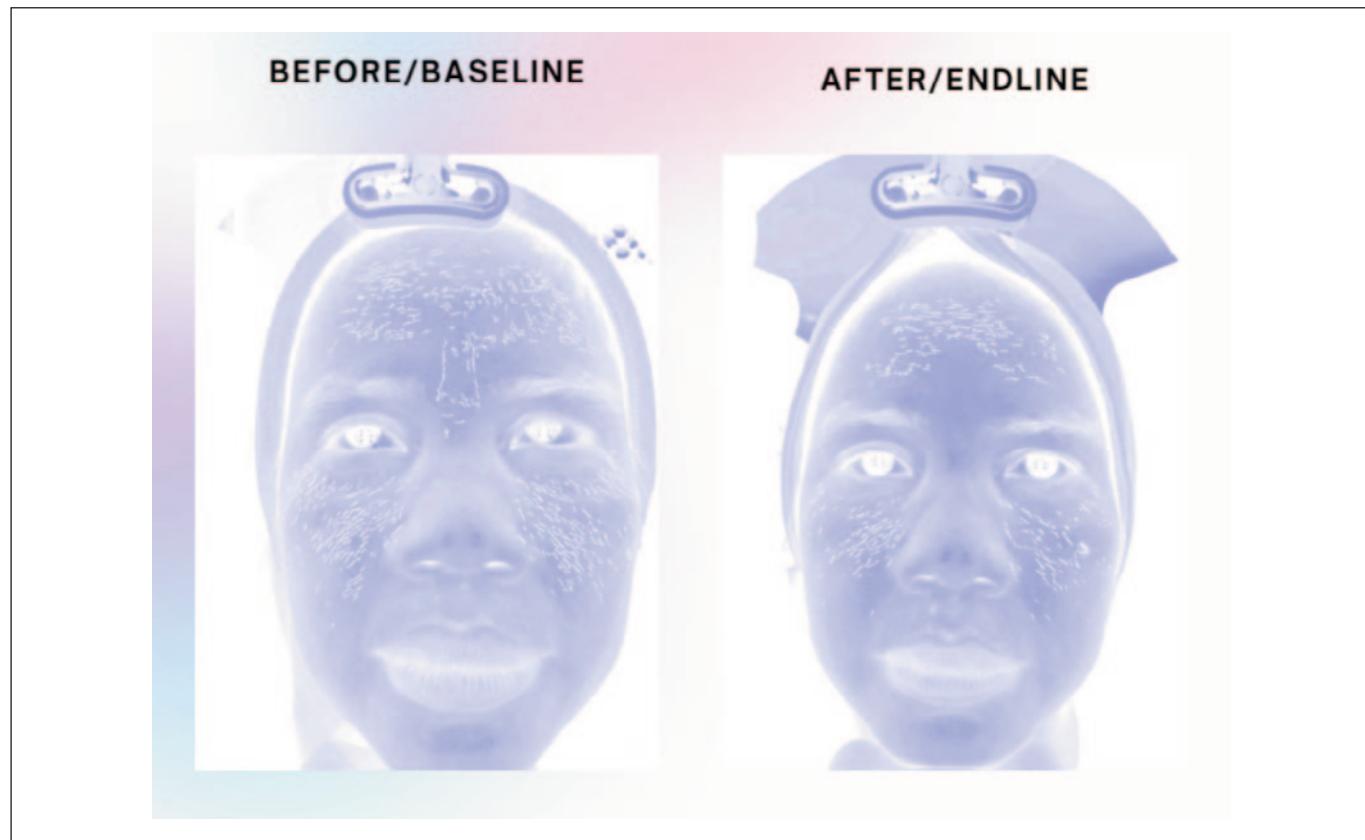


Figure 1. Changes in Collagen Fiber Integrity Observed Before (Baseline) and After (Endline) Eight Weeks of Collagen-Enriched Milk Supplementation



Figure 2. Changes in Collagen Fiber Integrity Observed Before (Baseline) and After (Endline) Eight Weeks of Placebo

Beyond structural effects, collagen supplementation is increasingly viewed as part of a systemic regenerative nutrition approach. Several meta-analyses have demonstrated that hydrolyzed collagen not only improves skin elasticity but also enhances joint function, reduces inflammation, and supports bone metabolism^{6,21}. The shared mechanism involves enhanced extracellular matrix turnover and modulation of oxidative stress through bioactive peptides generated during collagen digestion. Experimental evidence indicates that collagen hydrolysates possess antioxidant properties, contributing to reactive oxygen species scavenging, redox balance, and preservation of extracellular matrix integrity²⁴. These findings expand the implications of collagen supplementation from cosmetic enhancement to connective tissue health and healthy aging.

The outcomes observed in this study also reflect the complex dynamics of collagen remodeling. The temporary decline in collagen loss score in the intervention group, as identified through ANCOVA, may represent the initial phase of extracellular matrix renewal rather than deterioration. Dermal collagen naturally undergoes continuous turnover, which can be accelerated by bioactive peptide supplementation. Long-term trials lasting twelve weeks or more have consistently reported recovery and even improvement in dermal density after early

remodeling phases⁴. These findings suggest that shorter interventions may capture transitional rather than steady-state responses. Understanding the temporal sequence of dermal regeneration is therefore crucial for interpreting short-duration collagen studies.

The efficacy of collagen supplementation also depends on nutritional and physiological context. Adequate intake of vitamin C, zinc, and amino acids such as glycine and proline is essential cofactors for collagen biosynthesis and cross-link stability^{9,22}. The stable micronutrient intake observed in this study suggests that dermal changes are primarily attributable to collagen supplementation rather than dietary confounding. However, interindividual variability—shaped by age, metabolic factors, skin phototype, hormonal status, and glycation burden—remains an important determinant of treatment responsiveness²⁵. Future precision-nutrition research should explore genetic and metabolic predictors of collagen response, including peptide bioavailability, receptor interaction, and extracellular matrix gene polymorphisms.

Overall, this study contributes new insight into the potential of collagen-enriched milk as a functional food for maintaining skin health in Southeast Asian populations, a demographic group underrepresented in previous research dominated by

Western and East Asian cohorts. With the rising prevalence of early skin aging and oxidative stress-related conditions among urban Indonesian women, collagen-based dietary strategies may offer a culturally appropriate, non-pharmacological approach to promote healthy aging. Future investigations should adopt longer follow-up periods, standardized collagen peptide profiles, and combined biomarker analyses such as skin elasticity, dermal density, and oxidative markers to establish mechanistic causality and dose-response relationships.

Physiologically, although the adjusted collagen level appeared lower in the intervention group, this finding may indicate an active dermal remodeling process triggered by collagen peptide stimulation. Previous studies have shown that oral collagen intake enhances collagen turnover, characterized by the breakdown of aged collagen followed by the synthesis of new fibers^{4,8}. During the initial phase of supplementation, this turnover may transiently reduce measurable collagen density before a compensatory increase occurs with longer supplementation periods. Observing a significant increase in dermal collagen density after 12 weeks of bioactive collagen peptide supplementation^{4,7}. Therefore, the temporary reduction observed in this study is more likely to reflect adaptive metabolic remodeling rather than pathological degradation.

The ANCOVA results confirm that collagen milk supplementation exerted a significant effect on skin collagen parameters after adjustment for covariates. This finding supports the concept of "beauty from within," highlighting nutrition-based strategies to enhance skin health and elasticity through internal biological pathways. Although longer intervention periods are needed to confirm sustained collagen improvements, the current results strengthen the evidence that collagen-enriched milk can serve as a functional nutritional strategy for maintaining dermal integrity and delaying visible signs of aging among adult women.

CONCLUSION

The supplementation did not significantly alter subjects' body weight or BMI, confirming that collagen-enriched milk provides targeted physiological benefits without negatively affecting general energy balance or promoting weight gain. The intervention group showed a statistically significant decrease in collagen loss score while the control group remained stable. The ANCOVA analysis confirmed a significant difference in Collagen Loss between the intervention and control groups, even after adjusting for covariates (age, BMI, and protein intake). This suggests that the observed improvement in dermal structure is directly attributable to the bioactive effects of the collagen peptides.

LIMITATIONS OF THE STUDY

This study has several limitations that should be acknowledged. First, the sample size was relatively small ($n = 60$),

which may limit the statistical power and the generalizability of the findings. Second, the duration of the intervention was relatively short, restricting the ability to evaluate long-term effects of collagen supplementation on dermal parameters. Third, the measurement of collagen fiber loss relied on a semi-quantitative optical device, which, despite its practical utility, may introduce variability and does not represent a direct biochemical assessment of collagen structure. Additionally, the study population consisted exclusively of women aged 30–50 years from a single district, which limits the applicability of the results to broader or more diverse populations. Lastly, unmeasured lifestyle factors such as stress, sleep quality, and unreported dietary intake may have contributed to variability in outcomes.

REFERENCES

1. Farage, M. A., Miller, K. W., Elsner, P., & Maibach, H. I. (2008). Intrinsic and extrinsic factors in skin ageing: a review. *International journal of cosmetic science*, 30(2), 87–95. <https://doi.org/10.1111/j.1468-2494.2007.00415.x>
2. Baumann L. (2007). Skin ageing and its treatment. *The Journal of pathology*, 211(2), 241–251. <https://doi.org/10.1002/path.2098>
3. Zague V. (2008). A new view concerning the effects of collagen hydrolysate intake on skin properties. *Archives of dermatological research*, 300(9), 479–483. <https://doi.org/10.1007/s00403-008-0888-4>
4. Proksch, E., Schunck, M., Zague, V., Segger, D., Degwert, J., & Oesser, S. (2014). Oral intake of specific bioactive collagen peptides reduces skin wrinkles and increases dermal matrix synthesis. *Skin pharmacology and physiology*, 27(3), 113–119. <https://doi.org/10.1159/000355523>
5. Varani, J., Dame, M. K., Rittie, L., Fligiel, S. E., Kang, S., Fisher, G. J., & Voorhees, J. J. (2006). Decreased collagen production in chronologically aged skin: roles of age-dependent alteration in fibroblast function and defective mechanical stimulation. *The American journal of pathology*, 168(6), 1861–1868. <https://doi.org/10.2353/ajpath.2006.051302>
6. Pu SY, Huang YL, Pu CM, Kang YN, Hoang KD, Chen KH, et al. Effects of Oral Collagen for Skin Anti-Aging: A Systematic Review and Meta-Analysis. *Nutrients* [Internet]. 2023 Apr 26;15(9):2080. Available from: <https://www.mdpi.com/2072-6643/15/9/2080>
7. Inoue, N., Sugihara, F., & Wang, X. (2016). Ingestion of bioactive collagen hydrolysates enhance facial skin moisture and elasticity and reduce facial ageing signs in a randomised double-blind placebo-controlled clinical study. *Journal of the science of food and agriculture*, 96(12), 4077–4081. <https://doi.org/10.1002/jsfa.7606>
8. Boelsma, E., Hendriks, H. F., & Roza, L. (2001). Nutritional skin care: health effects of micronutrients and fatty acids. *The American journal of clinical nutrition*, 73(5), 853–864. <https://doi.org/10.1093/ajcn/73.5.853>
9. Pullar, J. M., Carr, A. C., & Vissers, M. C. M. (2017). The Roles of Vitamin C in Skin Health. *Nutrients*, 9(8), 866. <https://doi.org/10.3390/nu9080866>

10. Kementerian Kesehatan RI. Kemenkes BKPK. 2023. Survei Kesehatan Indonesia (SKI) 2023. Available from: <https://www.bapakkes.go.id/hasil-ski-2023/>
11. Kollias N, Gillies R, Moran M, Kochevar E, Andreson R. Endogenous skin fluorescence includes bands that may serve as quantitative markers of aging and photoaging. *J Invest Dermatol.* 1998;111(5):776–80. Available from: <https://doi.org/10.1046/j.1523-1747.1998.00377.x>
12. Kirkpatrick N, Hoyng J, Botting S, Weiss J, Utzinger U. In vitro model for endogenous optical signatures of collagen. *Biomed Opt.* 2006;11(5). Available from: <https://doi.org/10.1117/1.2360516>
13. WHO. World Health Statistic 2020 [Internet]. 2020. 92 p. Available from: <https://iris.who.int/bitstream/handle/10665/332070/9789240005105-eng.pdf>
14. Luebberding S, Krueger N, Kerscher M. Skin physiology in men and women: in vivo evaluation of 300 people including TEWL, SC hydration, sebum content and skin surface pH. *Int J Cosmet Sci [Internet].* 2013 Oct 6;35(5):477–83. Available from: <https://onlinelibrary.wiley.com/doi/10.1111/ics.12068>
15. Park S. (2022). Biochemical, structural and physical changes in aging human skin, and their relationship. *Biogerontology*, 23(3), 275–288. <https://doi.org/10.1007/s10522-022-09959-w>
16. Popkin, B. M., Corvalan, C., & Grummer-Strawn, L. M. (2020). Dynamics of the double burden of malnutrition and the changing nutrition reality. *Lancet (London, England)*, 395(10217), 65–74. [https://doi.org/10.1016/S0140-6736\(19\)32497-3](https://doi.org/10.1016/S0140-6736(19)32497-3)
17. Vollmer, D. L., West, V. A., & Lephart, E. D. (2018). Enhancing Skin Health: By Oral Administration of Natural Compounds and Minerals with Implications to the Dermal Microbiome. *International journal of molecular sciences*, 19(10), 3059. <https://doi.org/10.3390/ijms19103059>
18. Shadish W. R. (2010). Campbell and Rubin: A primer and comparison of their approaches to causal inference in field settings. *Psychological methods*, 15(1), 3–17. <https://doi.org/10.1037/a0015916>
19. Westerterp K. R. (2004). Diet induced thermogenesis. *Nutrition & metabolism*, 1(1), 5. <https://doi.org/10.1186/1743-7075-1-5>
20. Shoulders, M. D., & Raines, R. T. (2009). Collagen structure and stability. *Annual review of biochemistry*, 78, 929–958. <https://doi.org/10.1146/annurev.biochem.77.032207.120833>
21. König, D., Oesser, S., Scharla, S., Zdzieblik, D., & Gollhofer, A. (2018). Specific Collagen Peptides Improve Bone Mineral Density and Bone Markers in Postmenopausal Women-A Randomized Controlled Study. *Nutrients*, 10(1), 97. <https://doi.org/10.3390/nu10010097>
22. Nosrati, R., Kheirouri, S., Ghodsi, R., & Ojaghi, H. (2019). The effects of zinc treatment on matrix metalloproteinases: A systematic review. *Journal of trace elements in medicine and biology: organ of the Society for Minerals and Trace Elements (GMS)*, 56, 107–115. <https://doi.org/10.1016/j.jtemb.2019.08.001>
23. Iwai, K., Hasegawa, T., Taguchi, Y., Morimatsu, F., Sato, K., Nakamura, Y., Higashi, A., Kido, Y., Nakabo, Y., & Ohtsuki, K. (2005). Identification of food-derived collagen peptides in human blood after oral ingestion of gelatin hydrolysates. *Journal of agricultural and food chemistry*, 53(16), 6531–6536. <https://doi.org/10.1021/jf050206p>
24. Deng, G., Huang, K., Jiang, X. et al. Developments for collagen hydrolysates as a multifunctional antioxidant in biomedical domains. *Collagen & Leather* 5, 26 (2023). <https://doi.org/10.1186/s42825-023-00131-9>
25. Chen, C. Y., Zhang, J. Q., Li, L., Guo, M. M., He, Y. F., Dong, Y. M., Meng, H., & Yi, F. (2022). Advanced Glycation End Products in the Skin: Molecular Mechanisms, Methods of Measurement, and Inhibitory Pathways. *Frontiers in medicine*, 9, 837222. <https://doi.org/10.3389/fmed.2022.837222>