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ORIGINAL ARTICLE

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A Scientific Approach to Defining, Evaluating, and Treating Pre-Aging With a Cosmetic Regimen Containing a Novel Cosmetic Peptide, Acetyl Dipeptide-31 Amide (AP31)

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ABSTRACT

Background: Patients experiencing signs of pre-aging in their 20s and early 30s desire solutions including topical skincare and cosmetic procedures to help preserve skin quality and function.

Methods: A series of studies was conducted to define, evaluate, and treat pre-aging skin. A total of 180 subjects were assessed to identify clinical features of pre-aging across all Fitzpatrick Skin Types (FSTs). In vitro studies compared the response of young and old dermal fibroblasts to ultraviolet A (UVA) exposure and retinoid treatment and evaluated the beneficial effects of acetyl dipeptide-31 amide, for pre-aging treatment. A 12-week clinical study of 46 patients (mild to moderate photoaging) was conducted to assess the efficacy and tolerability of a skincare regimen to treat pre-aging. Skin glycation index was assessed via UV-fluorescence and cross-polarized images.

Results: Clinical evaluation revealed that the earliest signs of skin aging were consistent across FSTs and included uneven skin tone, fine lines, and roughness. Aging turning points were seen in the mid-twenties in lighter FSTs and mid-thirties in darker FSTs. A reduction in elasticity began in the 20s and collagen content decreased progressively with age. Younger dermal fibroblasts produced more ROS after UVA exposure but were also more responsive to retinoids. Acetyl dipeptide-31 amide demonstrated anti-inflammatory and extracellular matrix (eg, collagen, elastin, etc) building effects. Clinical testing revealed an AM/PM regimen containing acetyl dipeptide-31 amide, sunscreen, and bakuchiol significantly improved early signs of pre-aging and reduced skin glycation index.

Conclusion: These data support the need for and benefit of early intervention to protect and preserve youthful skin.

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INTRODUCTION

Pre-aging is a relatively new term used to describe the early, self-perceived signs of skin aging that occur in the 20s and early 30s.[1] These early changes include dullness, loss of radiance, uneven skin tone, roughness, loss of firmness, dark circles, and fine lines. This young demographic, comprising both Millennials and Generation Z (Gen Z), is heavily influenced by social media, which provides them with a constant stream of information on cosmetic procedures, skincare trends, and ideals for flawless skin. Studies have demonstrated that greater social media use is associated with a heightened desire for cosmetic enhancements.[2] Gen Z, deemed "digital natives," spends more hours on social media as compared to other generations.[3] Therefore it is not surprising that young adults make up one of the fastest-growing markets for injectables like neurotoxins and hyaluronic acid fillers and are a major buying group for skincare.[4,5] According to a 2022 survey of facial plastic surgeons, 75% reported an increased demand for cosmetic procedures in patients younger than 30.[6] However, recently there has been a shift in focus, with Gen Z desiring more preventative interventions as opposed to correction and cosmetic enhancement.[7] Prejuvenation, a term coined by dermatologist Dr Kenneth Arndt in 2013, describes the concept that early intervention can slow or prevent the signs of aging, mitigating the need for reju-

venation later in life. Younger patients, particularly Gen Z's, now make up an increasing number of those seen in the office seeking cosmetic solutions for pre-aging.[7]

While engagement with social media has heightened awareness about skincare and other skin issues, disinformation about sunscreens, skincare products, ingredients, and overall skin strategies prevents many from reaching their skincare goals.[8] In 2024, a survey was conducted by the American Academy of Dermatology of over 1000 adults. Survey results revealed that 52% of Gen Z adults were unaware that sunburn leads to skin cancer and premature skin aging.[9] Thirty seven percent of Gen Zs report they only wear sunscreen when somebody nags them and 32% receive a failing grade of D or F on sun protection knowledge. Of even greater concern is the fact that 28% of Gen Z survey respondents said getting a tan was more important to them than preventing skin cancer, with 70% reporting tanned or darker skin in 2023. There is an opportunity to educate on the importance of prevention in the pre-aging patient population.

In this paper, we will outline the phenotypic changes of pre-aging across diverse phototypes. We will share a novel imaging method that was used to evaluate pre-aging and data that supports the treatment of pre-aging with a scientifically backed skincare regimen based on a novel cosmetic peptide acetyl dipeptide-31 amide.

Intrinsic, Extrinsic, and Pre-Aging

Skin aging is a complex biologic process that results from intrinsic and extrinsic factors.[10-12] Intrinsic aging or natural aging occurs as skin deteriorates over time. Hormones, genetics, metabolic processes, and immune system decline all contribute to pathogenesis.[10] Intrinsic aging results primarily in functional deficiencies in the skin including thinning, fragility, poor wound healing, xerosis, and compromised barrier function.[11] Fine wrinkles, sagging, and crepiness are cosmetic issues associated with intrinsic aging.[10-12]

When skin is exposed to environmental factors extrinsic aging occurs.[11-13] Krutmann defined the exposome as all the environmental factors to which skin is exposed over a lifetime.[14] Exposomal factors include environmental exposures such as solar radiation, pollutants, and changes in temperature and lifestyle factors including cigarette smoke, stress, poor nutrition, and lack of sleep. Extrinsic aging has more profound effects on the phenotype of the skin than intrinsic aging.[11-13] Mottled pigmentation, brown spots, deep course wrinkles, sallowness, telangiectasias, and roughness characterize extrinsic aging. These clinical stigmata occur after years of environmental exposure and are generally more advanced signs of skin aging.[13]

Pre-aging is a unique state of skin aging that precedes aging.[15] It occurs in the 20s and early 30s and the clinical signs of pre-aging are subtle and transient. Textural changes such as dullness, loss of radiance, and roughness predominate and are often among the first signs of pre-aging. Lines and wrinkles may be present with facial movement but disappear quickly at rest. Often, younger consumers perceive the early changes in skin but do not yet view them as aging signs of concern. As such, this population can be motivated to protect youthful skin qualities and prevent decline.

The aging process is a result, in part, of the long-term effects of oxidative damage. The free radical theory of aging was first proposed by Denham Harmon in 1954. Since that time, there has been a great deal of research on the role of oxidative stress in skin aging.[11,12,16,17] Free radicals are generated in intrinsic and extrinsic aging. Under normal circumstances, free radicals are neutralized by innate enzymatic and non-enzymatic antioxidants preventing damaging effects.[18] When free radicals overwhelm the antioxidant system, oxidative stress causes direct damage to DNA, proteins, and cell membranes.[11,12] Redox-sensitive transcription factors like activator protein 1 (AP-1) and nuclear factor-kappa B (NF-kB) are also upregulated by oxidative stress. AP-1 increases the production of matrix metalloproteinases that break down collagen and elastin.[17] Collagen fragments that accumulate in the dermis alter the size and

FIGURE 1. Clinical expert grading shows different patterns and age turning points for different skin phototypes.

	I&II					III&IV					V&VI				
	n=5	n=7	n=4	n=7	n=7	n=18	n=21	n=18	n=20	n=29	n=6	n=8	n=12	n=6	n=9
	18-24	25-30	31-35	36-40	41-50	18-24	25-30	31-35	36-40	41-50	18-24	25-30	31-35	36-40	41-50
Mottled hyperpigmentation	2	3.6	2.8	2.7	4.9	2.2	1.6	2.6	3.4	**3.9***	1	0.9	0.5	1.7	1.3
Overall evenness of skin tone	1.5	2.9	2.5	**2.9***	**4.6***	2.1	1.9	2.4	2.9	**3.9***	1.2	1.4	1.1	1.8	1.4
Dark circles	2	1.3	2.3	3.7	3.4	2.1	1.8	2.1	3.1	**3.5***	1.6	1	1.5	1.5	2.3
Global fine lines	2	2.6	2.5	3.6	**4.6***	1.3	1.6	**2.8***	**3.3***	**3.4***	1	0.7	0.6	1.3	3
Overall photodamage	1.5	2.7	3	**3.3***	**4.6***	1.6	1.7	2.3	**3.2***	**3.8***	0.8	1	0.8	1.3	1.4
Lack of radiance/brightness	1.5	2.6	3	3.4	4	1.9	2.1	2.2	2.7	**3.7***	1.2	0.6	0.8	1.2	1.5
Lack of skin clarity	1.3	2	2	2.7	2.7	1.7	1.6	1.3	2.8	**3.1***	1.6	1.4	1	2.2	1.1
Discrete Pigmentation	1.5	2.6	2	1.7	3.7	2	1.5	1.8	2.4	3	1	1	1.1	1.8	1.4
Tactile surface roughness	1.8	1.6	2.8	2.7	2.4	1.7	2.1	0.9	1.6	2.6	1	0.7	1.7	1.5	1.4
Crow's feet wrinkles	0.3	**2***	**3***	**3.4***	**5.1***	0.8	0.9	**2.2***	**2.4***	**3.5***	0	0.3	0.3	0.3	0.6
Under eye wrinkles	0.3	1.7	**1.8***	**3.4***	**5.1***	0.6	1.1	**1.7***	**2***	**3.6***	0	0.6	0.7	1	**1.1***
Sallowness or yellowing	0.5	1.3	1.3	1.7	**3.1***	0.8	0.9	**1.8***	**2.4***	**2.9***	0.6	0.3	0.9	1.3	1.3
Global skin firmness	0	0.4	0.8	**1.6***	**2.7***	0.2	0.6	0.8	**1.3***	**2.3***	0	0.1	0.2	0.7	0.6
Cheek wrinkles	0	0.4	0.3	1	**2.4***	0	0.2	**0.5***	**0.6***	**1.9***	0	0	0	0	0.3
AVERAGE ALL SIGNS COMBINED	1.1	**2.0***	**2.1***	**2.7***	**3.8***	1.4	1.4	**1.8***	**2.4***	**3.2***	0.8	0.7	0.8	**1.3***	**1.2***
		Age turning point						Age turning point						Age turning point	

Bold numbers indicate a significant difference compared to the 18 to 25 age group, $P<0.01$. 0-9 Scale: 1-3 Mild, 4-6 Moderate, 7-9 Severe. The red color density correlates with increase in the grading of the respective parameter.

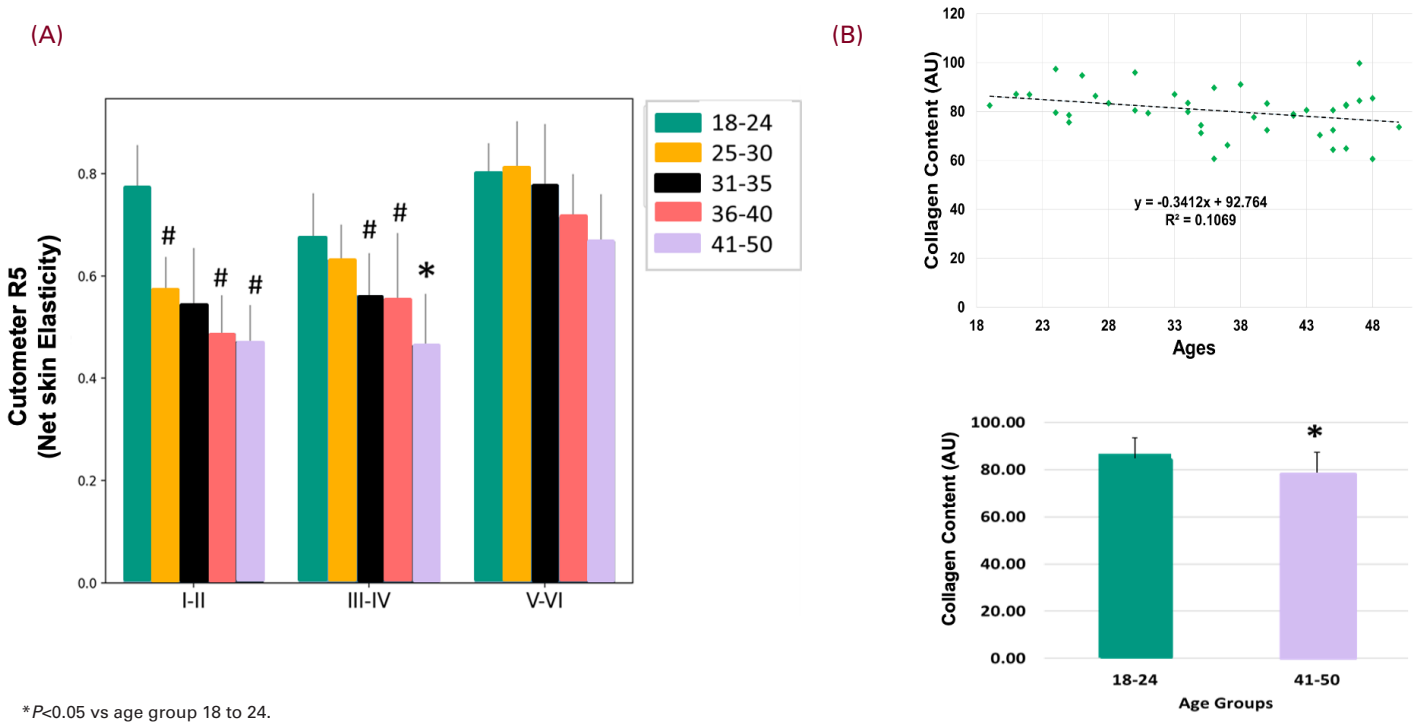
shape of fibroblasts and impair their function by reducing their stretch.[19] These collagen fragments further increase oxidant production, creating a self-perpetuating cycle that ages skin. AP-1 downregulates the transforming growth factor beta (TGF-β) pathway causing a reduction in collagen synthesis.[11] NF-kB upregulates the production of inflammatory mediators and cytokines that contribute to inflammaging.[20] Oxidative stress also accelerates telomere shortening, and mitochondrial dysfunction, and triggers cellular senescence that plays a role in skin aging.[21-23]

Characterizing Pre-Aging Across Diverse Skin Phototypes

To characterize phenotypic changes across age groups and in different skin phototypes, a single-center clinical trial was conducted in North America.[24] The study included 180 female subjects, ages 18 to 50, with Fitzpatrick skin phototypes (FSTs) I-VI. Participants were evaluated by expert clinical grading, instrumentation to measure elasticity, skin hydration, transepidermal water loss (TEWL), and consumer self-assessment. Post-auricular skin biopsies were collected from 42 subjects FSTs I-VI, fixed in formalin and embedded in paraffin. Sections were assessed for histology and stained with Herovici's stain for collagen. Collagen content was assessed by an imaging analysis software ImageJ (NIH, Bethesda, MD).

To analyze the data, subjects were placed into one of three groups according to skin phototype.[24] Group 1 included FSTs I and II, Group 2 included FSTs III and IV, and Group 3 was comprised of FSTs V and VI. Clinical grading revealed that skin phototype strongly influences skin aging with earlier signs of aging seen more and earlier in Group 1 followed by Group 2 and 3, respectively (Figure 1). Skin aging turning points were defined as significant changes in overall skin issues and signs of aging compared to the youngest group. Aging turning points

FIGURE 2. Skin elasticity and collagen content change with age across all skin phototypes I-IV. (A) Significant reduction of skin elasticity observed as early as age group 25 to 30 for skin type I-II and at age group 31 to 35 for skin type III-IV as measured by Cutometer. #P<0.05 vs age group 18 to 24. *P<0.05 vs age groups 18 to 24 and 25 to 30. (B) Skin collagen decrease begins in the twenties across all skin types with ~ 3% decrease in collagen content per decade across all phototypes as evaluated by collagen Herovici special staining in skin biopsies.



*P<0.05 vs age group 18 to 24.

were seen in the mid-twenties in Group 1 while in Group 2 it occurred around age 30 and Group 3 around age 35 (Figure 1). Furthermore, clinical evaluation revealed that the earliest signs of skin aging were consistent across skin phototypes and included uneven skin tone, fine lines, dark circles, and roughness.

Elasticity as measured by the cutometer was significantly decreased with age, especially in skin phototypes I-IV (Figure 2A).[24] There was a significant loss of skin elasticity in Group 1 when comparing 19 to 24-year-olds with 25 to 30-year-olds. Since this loss of elasticity is associated with wrinkle formation and skin sagging, it can be viewed as an "invisible sign" of pre-aging especially in lighter skin phototypes. Within our study, skin hydration and TEWL did not change significantly between the groups with advanced age.

Skin biopsies demonstrated that collagen decline begins in the twenties across all skin phototypes and continues to decline with age (Figure 2B).[24] This decline in collagen was statistically significant when comparing 18 to 24-year-olds with 41 to 50-year-olds (P<0.05). These data are in keeping with previous studies that demonstrated collagen loss begins in the early 20s and continues at a rate of about 1% per year throughout life.[25] A loss of dermal collagen results in structural and functional changes in the extracellular matrix that may lead to loss of firmness and ultimately wrinkles and sagging.

Previously published data sheds added light on the pathogenesis of pre-aging. To evaluate the rate of epidermal cell proliferation non-invasively, skin fluorescence emission was measured on the cheeks of 522 subjects in winter and summer in five geographic locations in the Asia-Pacific region.[26] Similarly, skin

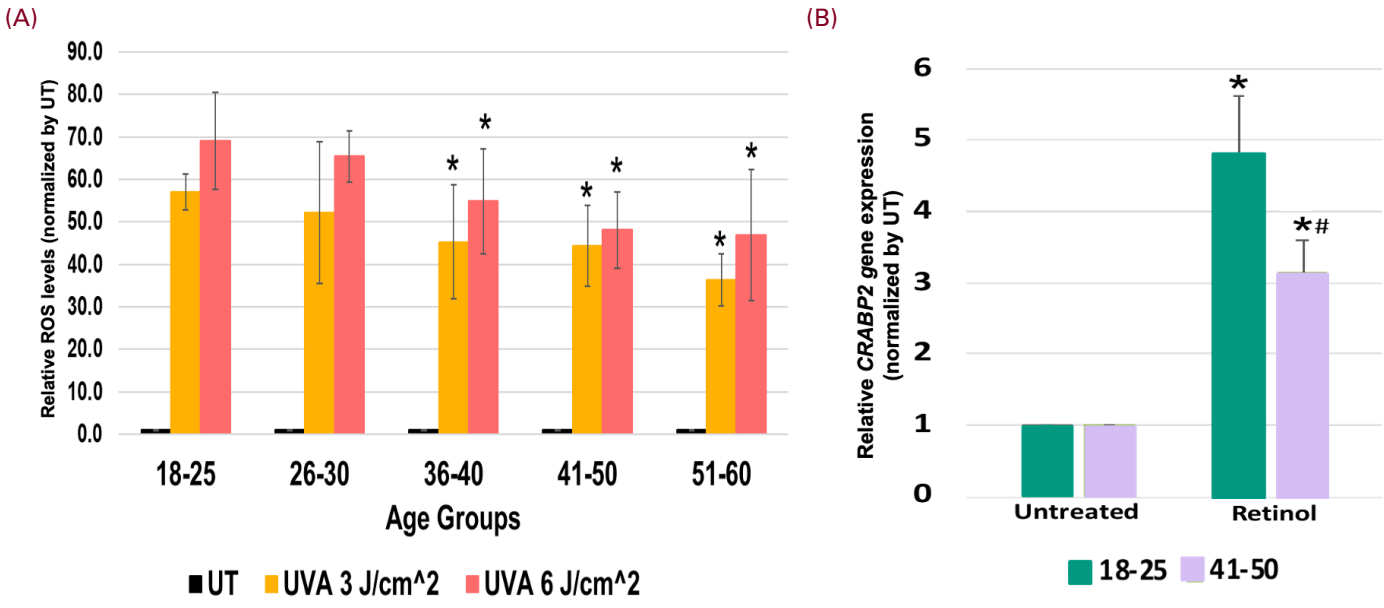
fluorescence was also measured in 80 Caucasians 14 to 75 years old in the United States on the face and sun-protected skin of the upper inner arm. Epidermal proliferation as indicated by tryptophane fluorescence, decreased with age beginning in the twenties but was not influenced by time of year or geographic location. The reduced rate of epidermal cell proliferation was more pronounced in facial skin than in sun-protected arm skin.[26] This reduction in epidermal proliferation may explain the dullness and loss of radiance seen in pre-aging.

In addition, cross-linked fluorescence that occurs because of an accumulation of glycated collagen and elastin was found to increase with age on the face.[26] Glycation occurs when reducing sugars bind non-enzymatically to proteins forming advanced glycation end products (AGEs) via the Maillard reaction.[27-30] Glycated collagen and elastin lose their viscoelastic properties, becoming stiff and rigid. These altered dermal proteins are resistant to degradation by matrix metalloproteinases (MMPs) such that they cannot be repaired or removed. AGE formation is a contributor to skin aging and is accelerated by sun exposure, cigarette smoking, and diet.[28] As previously described, glycated collagen is first observed in the skin at the age of 20 and accumulates at a rate of about 3.7% per year resulting in a 30 to 50% increase by 80 years of age.[28-30] Accumulation of AGEs in the skin causes a loss of elasticity and sagging that is referred to as the "sugar sag." AGEs also result in yellowing and browning of the skin which contributes to an aged appearance.[31] Sallowness is indeed observed as an aging-related parameter, particularly in FSTs I-IV (Figure 1).

Pre-aging Fibroblasts Differ From Aging Fibroblasts

To further delineate the differences between pre-aging and aging, in vitro studies were conducted utilizing human-cultured dermal fibroblasts from subjects 18 to 60 years of age.[32] Fibroblasts from 18 to 25-year-olds demonstrated a greater ability to generate UVA-induced ROS when compared to those of older subjects ages 36 to 60 (Figure 3A). Importantly, fibroblasts from younger subjects also showed greater responsiveness to retinol as indicated by cytoplasmic retinoic acid binding protein

FIGURE 3. Pre-aging fibroblasts differ from aging fibroblasts as measured by ROS generation and CRABP2 expression. (A) Fibroblasts from pre-aging subjects (18-25 years old) have ~1.3 times higher UVA-induced ROS generation than those of aging subjects (36-60 years old). *P<0.05 vs age group18 to 25. (B) Pre-aging fibroblasts (age group 18-25) are ~1.5 times more responsive to retinol-based treatment than aging fibroblasts (age group 41-50).



*P<0.05 vs Untreated (UT). #P<0.05 vs age group 18 to 25.

2 (CRABP2) expression (Figure 3B).[32] These data suggest that young fibroblasts are more vulnerable to exposure to UVA but are also more responsive to treatment. Thus, there is a precedent to protect, preserve, and even boost extracellular matrix components in the pre-aging population.

Developing a Topical Regimen for Pre-aging

In 2019, beauty editors began writing about the importance of taking a proactive approach to saving collagen.[33] This approach includes preserving, protecting, and boosting collagen and is an effective strategy for consumers who want to maintain youthful-appearing skin. This can be achieved by using cosmeceutical skincare and procedures such as ultherapy, microneedling, radiofrequency, chemical peels, red light therapy, and collagen-stimulating injectables such as calcium hydroxyapatite and poly-L-lactic acid.[34,35] The goal of taking this proactive approach is to protect and enhance the skin's ability to produce collagen despite the presence of external aggressors.

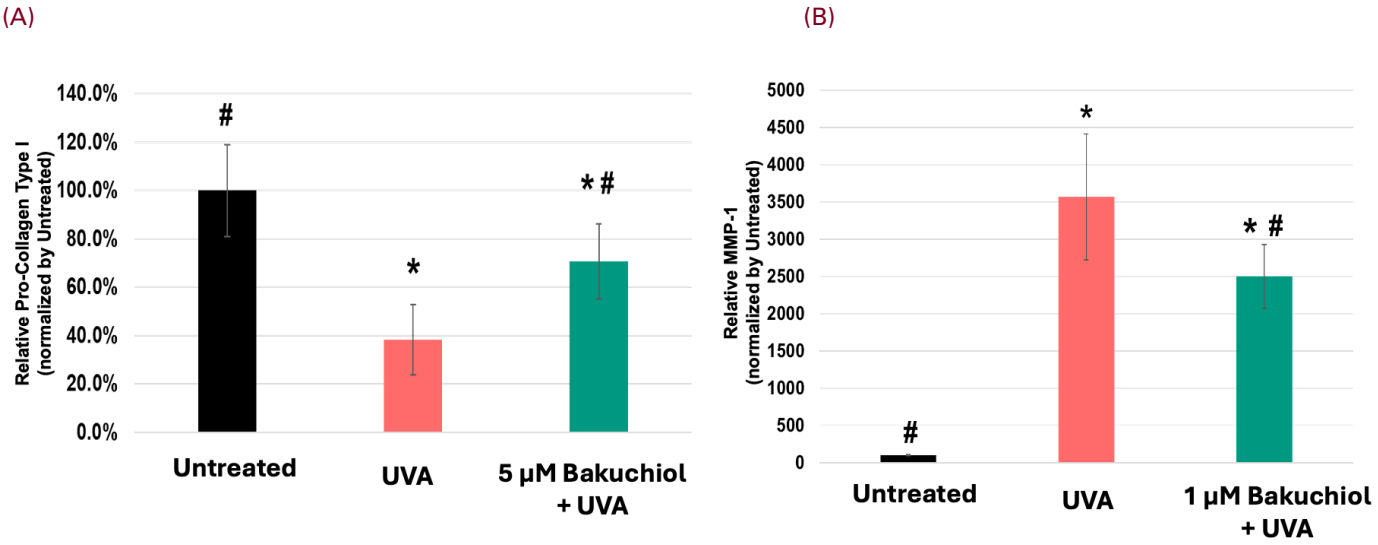
To address pre-aging, a skincare regimen consisting of a day and night moisturizing cream was developed and clinically tested. The daytime product includes acetyl dipeptide-31 amide (AP31) and SPF 30 sunscreens (Neutrogena Collagen Bank SPF Moisturizer) while the night product includes AP31 and bakuchiol (Neutrogena Collagen Bank Moisturizer).

Acetyl dipeptide-31 amide is a novel molecule with a low molecular weight of 229D.[36] This micropeptide is 2 times smaller than most cosmetic peptides and has been shown to penetrate human skin readily. AP31 is multi-functional, gentle, and has anti-inflammatory and anti-aging benefits. Moreover, it was shown to be well tolerated and improve symptoms of eczema in a controlled clinical study.[37] Pre-clinical studies in epidermal keratinocytes indicate that AP31 significantly reduces inflammatory mediators including IL-8 and TNF-α in a dose-dependent fashion.[36] The anti-aging effects of AP31 were investigated in cultured dermal fibroblasts. Fibroblasts treated with 0.01% AP31 showed statistically significant upregulation of expression of five of the skin's natural extracellular matrix components including pro-collagen, hyaluronic acid, elastin, decorin, and fibronectin, compared with vehicle controls.[36] Human skin explants treated with 0.01% AP31 in formulation every 2 days for 8 days confirmed significant increases in collagen III and elastin protein compared with untreated samples (P<0.05). Pro-collagen, elastin, and hyaluronic acid help improve skin hydration and tensile strength making skin plump, elastic, and firmer while decorin and fibronectin assist with collagen matrix assembly making skin smoother and tighter. Gene expression in human skin equivalents demonstrated upregulation of genes involved in barrier function, skin hydration, skin plumping, and epidermal metabolism and a downregulation of genes involved in cellular senescence.[36]

Bakuchiol is a monoterpenoid phenol derived from *Psoralea corylifolia*.[38] Bakuchiol has been described to have antioxidant, anti-inflammatory, anti-microbial, anti-aging, and anti-acne benefits.[38-44] This botanical active is considered a functional mimetic of retinol as it induces gene expression in skin, similar to that induced by retinol.[39] Studies in mature fibroblasts demonstrate that bakuchiol upregulates the expression of types I, II, and IV collagen.[39] Moreover, bakuchiol was also shown to protect against the pro-collagen type I decrease and matrix metalloproteinase-1 (MMP-1) increase that occurs following UVA irradiation of human dermal fibroblasts in culture (Figure 4).

Clinical studies have confirmed the skin benefits of bakuchiol. Draelos et al, demonstrated that a bakuchiol moisturizer was well tolerated and effective in patients with sensitive skin including those with rosacea, eczema, and cosmetic intolerance.[43] Skin hydration measured by corneometry was improved by 16% after 4 weeks of using the bakuchiol-based anti-aging moisturizer. A 12-week, prospective, randomized, double-blind study was conducted comparing once daily application of topical bakuchiol 0.5% cream and retinol 0.5% cream for treating

FIGURE 4. Effects of bakuchiol in UVA-treated human dermal fibroblasts. (A) Bakuchiol showed protection against 3.5 J/cm^2 UVA-induced procollagen type I protein decrease at 4 hours post-UVA irradiation in human dermal fibroblasts. *P<0.05 vs Untreated. #P<0.05 vs UVA. (B) Bakuchiol showed protection against 5 J/cm^2 UVA-induced MMP-1 increase at 72 hrs post-UVA irradiation in human dermal fibroblasts.



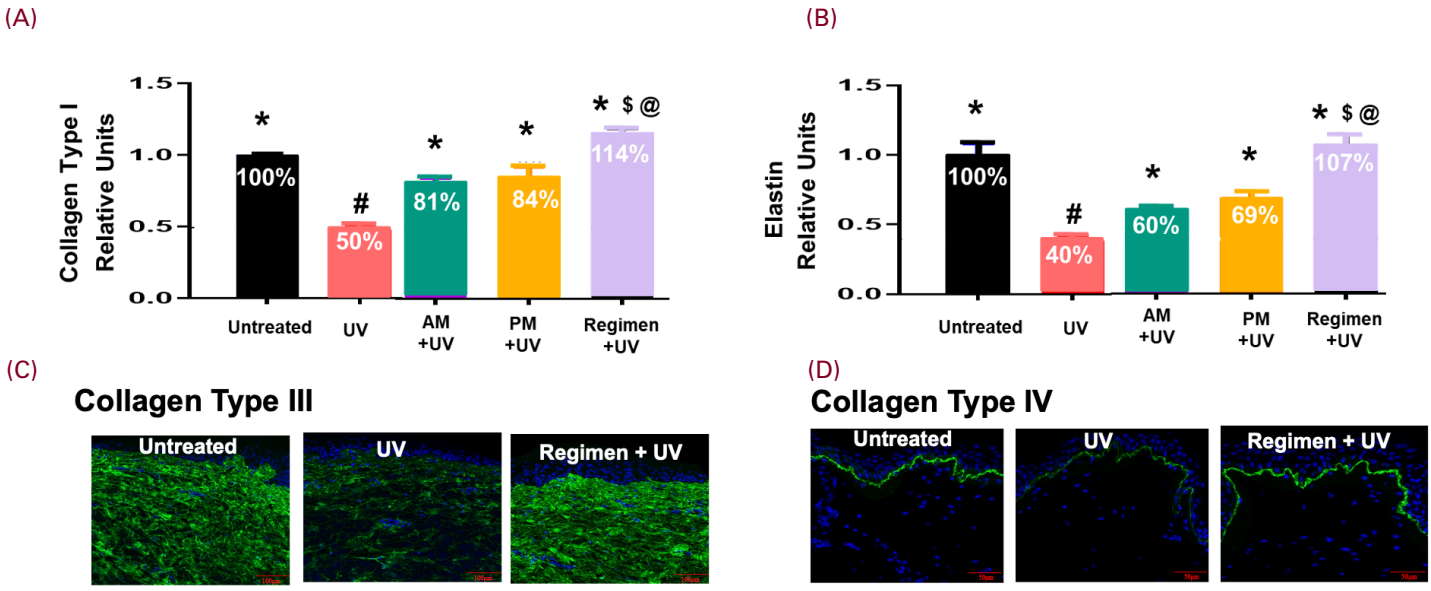
*P< 0.05 vs Untreated. #P< 0.05 vs UVA.

facial photoaging.[44] This study demonstrated that topical bakuchiol produced comparable improvement to retinol in photoaged skin including improvement of wrinkles and hyperpigmentation. The tolerability profile of bakuchiol was superior to that of topical retinol.

Ex Vivo and In Vivo Testing of the AP31 Pre-Aging Regimen

The effects of AP31 were evaluated using human 3D skin equivalents. To mimic product regimen use, human skin explants were topically treated with AP31 + Bakuchiol containing PM moisturizer for 9 hours and removed. The AP31 containing AM facial moisturizer with sunscreen was then applied 30 minutes prior to UV irradiation, and remained on skin for 14.5 hours post-UV; this procedure was repeated for 7 days. For AM or PM-only treatment applications, test products were applied once daily as described above. An immunohistological examination was performed using antibodies against elastin, collagen type I, collagen type III, and collagen type IV. While both AP31 products provided protection against UV-mediated damage, the combined regimen showed statistically significant enhanced protection against UV-induced collagen and elastin degradation compared to baseline measurements and compared to AM moisturizer or PM moisturizer alone. These data highlight the added benefit of using both products together as a regimen to preserve skin's collagen and elastin (Figure 5).

FIGURE 5. Collagen and Elastin protection against UV-induced decline in ex vivo model with Pre-Aging Regimen (AM/PM AP31 formulations). Human skin explants were topically treated with AP31 and irradiated with UV daily for seven days. The treated tissues were immunohistologically stained by antibodies against collagen type I (A), elastin (B), collagen type III (C), and collagen type IV (D). AP31 protects collagen I, III, IV, and elastin from damage induced by solar-simulated UV lights.



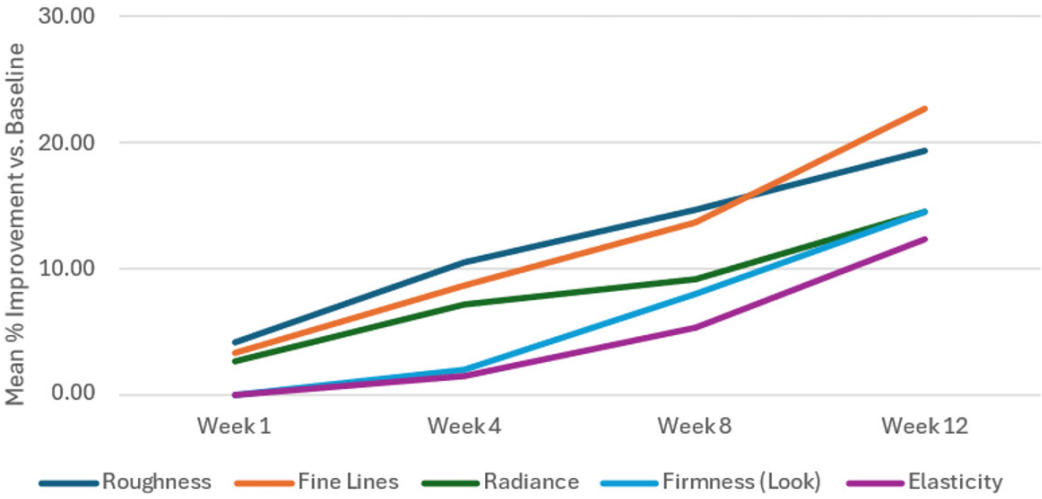
#*P*<0.05 vs Untreated. **P*<0.05 vs UV. $*P*<0.05 vs AM+UV. @ *P*<0.05 vs PM+UV.

The efficacy and tolerability of the AP31-based pre-aging skincare regimen were further evaluated in 46 subjects with mild to moderate photoaged skin. Women aged 25 to 55 with FST I-VI and mild to moderate scores for at least three of six skin parameters including fine lines, wrinkles, lack of firmness, evenness of skin tone, roughness, and lack of radiance were enrolled in a 12-week, single-center clinical study. Subjects were instructed to use the AP31+SPF moisturizer in the morning after cleansing, and the AP31 + bakuchiol moisturizer in the evening. Investigator- and subject-graded tolerance and efficacy assessments were performed at baseline, weeks 1, 4, 8, and 12. Digital Images and videos were obtained using the VISIA-CR Gen 5 system (Canfield Imaging Systems, Fairfield, New Jersey) and with a Canon R5 digital SLR camera (Canon Incorporated, Tokyo, Japan) at baseline, weeks 4, 8, and 12. Tolerance was measured using clinical (edema, erythema, dryness) and subjective (burning/stinging, itching, and tightness) assessments. Efficacy was measured using the following parameters: tactile

surface roughness, lack of clarity, fine lines, under-eye wrinkles, crow's feet wrinkles, look of skin firmness/laxity, feel of skin firmness/laxity, lack of skin elasticity, overall unevenness of skin tone, lack of radiance/brightness, blotchiness, photodamage, and elasticity.

Application of the AM/PM AP31 formulations resulted in significant improvement in early visible signs of collagen decline including roughness, fine lines, radiance, firmness, and elasticity (Figure 6). Clinical grading for roughness, lack of clarity, global fine lines, lack of radiance/ brightness, and elasticity showed improvement at weeks 1, 4, 8, and 12 (P<0.05). Under-eye wrinkles, crow's feet wrinkles, look of skin firmness/laxity, feel of skin firmness/laxity, lack of skin elasticity, and overall unevenness of skin tone showed improvement at weeks 4, 8, and 12 (P<0.05). There was a reduction in overall photo-damage at weeks 8 and 12 (P<0.05) as well as visual improvement in signs of aging (Figure 7A). There was also a significant reduction in skin glycation index after 12 weeks of treatment (P<0.05) using a skin glycation imaging method that combines fluorescence (excitation/ emission of 365/440 nm) and cross-polarized images.

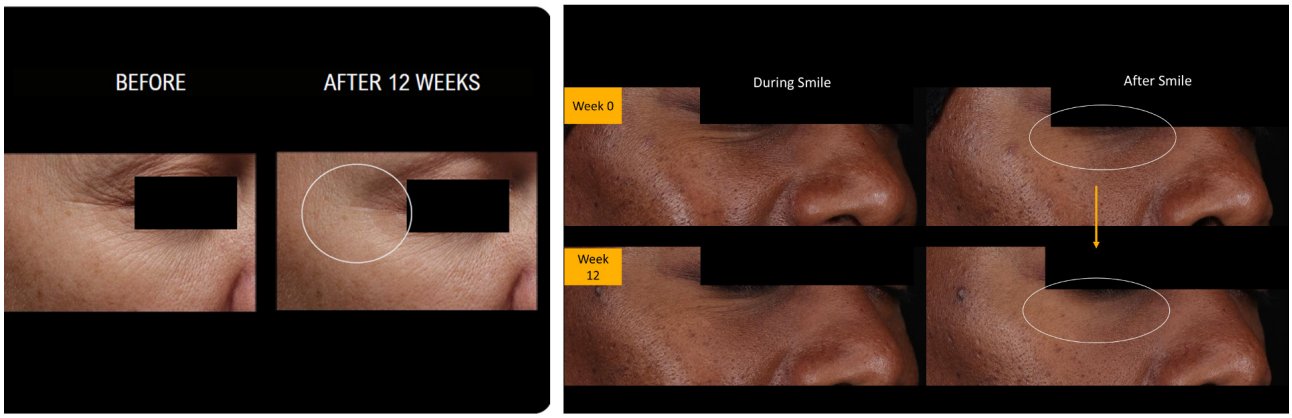
FIGURE 6. Clinically graded improvements in parameters representing collagen decline beginning at week 1 or 4 and continuing through week 12 following twice-daily use of AM/PM AP31 pre-aging formulations.



P<0.05.

FIGURE 7. (A) Visible improvement in skin texture, radiance, and fine lines following 12 weeks, twice daily use of AM/PM AP31 pre-aging formulations, P<0.05. (B) Dynamic facial imaging photography demonstrates improvement in visible expression lines suggesting skin is more elastic with faster recovery after smiling following 12 weeks, twice daily use of AM/PM pre-aging formulations.

(A) (B)



Subjects self-reported significant improvements in the look and feel of their skin as early as the week 1 timepoint, with greater than 70% of subjects reporting improved radiance, tone, and texture. After 12 weeks, 93% of subjects reported improvement in at least three clinical signs of early collagen decline, with 87% of subjects reporting improvement in elasticity and feel of skin firmness($P<0.05$). Additionally, subjects agreed that the product left their skin with a healthy glow and a more luminous appearance, and nearly 70% agreed they would consider delaying getting neuromodulator injections after using these products.

The AP31-based regimen was well tolerated by subjects. Clinical and subjective assessments of tolerability showed early and sustained improvement in dryness/scaling and tightness/dry feeling at weeks 1, 4, 8, and 12 ($P<0.05$). No statistically or clinically relevant increases in irritation signs or symptoms were found at the week 12 time point.

Three adverse events (AE) were reported during the study, of which two were reported with possible relationship to test product or study procedures. No actions were taken with regard to test product usage. One subject reported an eye infection in one eye; 1 subject reported moderate erythema and a skin-burning sensation on the face. No serious AEs (SAEs), or other significant AEs were reported/observed.

Facial Imaging for Pre-Aging

The repetitive movements involved in facial expressions, along with a loss of bone, fat, collagen, and elastin, contribute to one of the most noticeable signs of aging: the formation of wrinkles. Repeated mechanical stress during facial expression causes dynamic wrinkles, and the pattern of expression lines eventually evolves into persistent wrinkles that are present even at rest.[45] A newly developed dynamic facial imaging (DFI) technique was developed to record standardized high-resolution videos of repetitive facial skin movements, such as a smile, and to analyze the appearance of fine lines and wrinkles at different stages of the skin movements. This type of pre-aging sign is subtle because the change is not obvious in the appearance of fine lines and wrinkles at rest, but there are noticeable changes in the appearance of fine lines and wrinkles during and after facial expressions. Current validated scales, such as the Glogau Wrinkle Scale and the Merz Wrinkle System, may not accurately assess these signs of pre-aging.

Utilizing DFI, researchers were able to demonstrate the effects of the AP31-containing regimen on dynamic expression lines occurring during pre-aging. After 12 weeks of use, subjects experienced faster recovery of dynamic expression lines, which can reduce the overall impact dynamic expressions have on the skin (Figure 7B).

CONCLUSION

The pre-aging population represents both a challenge and an opportunity to significantly impact the course of skin aging. The notion that more advanced signs of skin aging can be delayed or perhaps even prevented by early intervention with effective topicals is a novel concept that prompted the research presented here. Characterization of the phenotype of pre-aging across FSTs and identification of aging turning points adds an important perspective to our understanding of pre-aging. Studies demonstrate that early functional changes do occur in pre-aged skin. Changes include a reduction in epidermal proliferation and loss of dermal collagen. Pre-aged skin generates more free radicals after UVA exposure when compared to older skin but is more responsive to the application of retinol. Finally, a clinical trial using an anti-aging regimen consisting of a morning moisturizer with AP31+SPF 30 and an evening moisturizer with AP31+bakuchiol resulted in significant improvement in early visible signs of pre-aging and collagen decline, as well as protection against UV-induced collagen decline. Collectively, these data suggest a paradigm shift supporting the need for early intervention in the pre-aging patient.

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DISCLOSURES

Ramine Parsa, Dara Miller, Thomas Shyr, and Wen-Hwa Li are employees of Kenvue Brands, LLC. Dr Patricia Farris a consultant and advisory board member of Kenvue Brands, LLC. Dr Cheri Frey is a consultant for Kenvue Brands, LLC.

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