"Enhancing Mitochondrial Function and Skin Barrier Repair: A Novel Approach for Male Anti-Aging"

Jing Deng[1], Jianhua Zhang[1,2,*], Shichao Liu[1,2], Wenjiao Guo[1,2]

[1]   N.O.D. topia (GuangZhou) Biotechnology Co., Ltd. Guangdong Guangzhou, 510000, China;
[2]   Simpcare (GuangZhou) Biotechnology Co., Ltd. Guangdong Guangzhou, 510000, China

1. Introduction

Recent studies emphasize the pivotal role of mitochondrial function in maintaining skin health, with a decline in mitochondrial function being closely associated with visible signs of skin aging, such as wrinkles, roughness, and sagging [1]. Interestingly, male facial aging exhibits a pattern distinct from that of females, with men typically showing earlier and more severe signs of aging, especially in areas such as the forehead and around the eyes [2-3]. Therefore, enhancing mitochondrial function and promoting the repair of damaged cells are crucial for maintaining skin vitality and preventing the onset of aging in men.

In light of these considerations, an innovative anti-aging complex (AAC) has been developed, characterized by a combination of bioactive compounds with unique synergistic mechanisms. The complex includes thiotaurine and M3 (magnesium aspartate, zinc gluconate, and copper gluconate), all of which have been shown to aid in cellular repair and enhance cellular energy. This study aims to evaluate the efficacy of this anti-aging complex in both in vitro and clinical trials, with a focus on its role in protecting mitochondrial function, enhancing cellular energy, repairing cellular damage, and improving the overall appearance and health of male skin. Given the growing interest in gender-specific approaches to aging and the increasing demand for effective solutions tailored to the unique aging patterns of male skin, this research provides valuable insights into the potential of mitochondrial-targeted therapies for male anti-aging treatments, offering an effective method for maintaining youthful skin in men.

2. Materials and Methods

2.1 Chemicals and reagents

Phosphate-buffered saline (PBS) and ATP assay kits were purchased from Solarbio. DMEM high-glucose medium and fetal bovine serum (FBS) were purchased from Gibco. The dual antibiotics (penicillin + streptomycin) were purchased from Gibco. Complete culture medium (10% FBS + 90% high-glucose DMEM + 1% dual antibiotics) was prepared in-house. 0.25% trypsin was purchased from Beyotime. Mitochondrial fluorescent probe was purchased from Tongren Chemical. Human skin fibroblasts (HSF) were purchased from the Kunming Cell

Bank, Chinese Academy of Sciences. Human immortalized keratinocyte (HaCaT), P17, purchased from North Carolina Biology.

2.2 Skin testing equipment

Assessments included skin elasticity by Cutometer (Courage + Khazaka，Germany), TEWL by Aqua Flux (AF200, BIOX, UK). Facial image by VISIA (Canfield, USA). All equipment and instruments were prepared and operated by trained and experienced testers.

2.3 ATP content assay

The HSF cell suspension ($1\text{-}4\times10^5$ cells/mL) was seeded into a 6-well plate and incubated in a CO_2 incubator for 24 hours. The complete culture medium in each well was aspirated. The blank control (BC) group was replenished with complete culture medium, while the AAC group was treated with complete culture medium containing AAC at a concentration of 0.05% Thiotaurine and 0.0005% M3. After 48 hours of incubation, the cells were collected, and the ATP content was measured AACording to the instructions provided in the ATP assay kit.

2.4 mitochondrial content assay

The HSF cell suspension ($3\text{-}6\times10^5$ cells/mL) was seeded into a 24-well plate and incubated in a CO_2 incubator for 24 hours. The culture medium was discarded, and of PBS was added to each well. Except for the BC group, all other groups were exposed to UVB induction. Following induction, the PBS in each well was discarded. The cell groups were treated AACording to Table 1. The 24-well plate was incubated in a CO_2 incubator for 24 hours. The cells were washed three times with PBS and fixed with 4% paraformaldehyde for 20 minutes. After fixation, the cells were then permeabilized with 0.1% Triton-X100 at room temperature for 10 min. After washing with PBS, non-specific binding sites were blocked with 10% goat serum. The primary antibody was added, and the cells were incubated at 4°C overnight. After three PBS washes, the secondary antibody was added and incubated at room temperature for 30 min. After three more PBS washes, the cells were stained with DAPI in the dark. Fluorescence microscopy was used to observe the cells, and Image-Pro Plus 6.0 image analysis software was used for quantitative analysis.

Table 1.   HSF cell treatment group

Group	Condition	Sample	Treatment time	Test index
Blank control	/	/		
Positive control		/	24 hours	mitochondrial protein
AAC	UVB	0.1% Thiotaurine + 0.0005% M3		

2.5 cell migration assay

Remove the 12-well plate and, using sterile forceps, place the 2-well wound healing insert in the center of the corresponding well. Gently press the insert to ensure it is securely attached to the well. Cells ($4.0\text{-}6.0\times10^5$ cells/70 μL/well) were seeded into the 2-well wound healing insert, with three replicate wells per group. The plate was then returned to the incubator and cultured for 24 hours until cell confluence was reached.

After incubation, the culture plate was removed, and the 2-well wound healing insert was carefully taken out to create a "wound". The wells were washed with PBS to remove detached cells. The blank group was replenished with culture medium containing 0.1%-1% fetal bovine serum, and the experimental groups were replaced with culture medium containing 0.1%-1% fetal bovine serum and the corresponding test substance. Images were captured at fixed positions, recording data at 0 hours, 24 hours, and 48 hours. The healing of the scratch was considered complete when the area was fused by 95%-100% in the negative control group, which was served as the endpoint.

2.6 Clinical trial

A total of 30 middle-aged male subjects with fragile skin barriers (TEWL baseline value ≥ 15 g/m²h) and visible signs of facial aging (F4 baseline value > 5, wrinkles ≥ grade 1) were recruited, aged between 24 and 55 years. The testing temperature: 20.0℃~21.9℃. Humidity: 50%±10%.

Subjects applied the AAC cream on the entire face twice daily for 4 weeks. TEWL, R2, R5, and R7 were measured by non-invasive instruments on weeks 0, 2, 4. Skin reaction scoring, as well as under-eye fine lines, nasolabial folds, and forehead wrinkles, were conducted by a dermatologist.

Table 2. Skin reaction scoring criteria

Reaction	Score
No reaction	0
Slight erythema	1
Erythema with papular or edematous reaction	2
Erythema with vesicular reaction	3
Corrosive reaction (bullae formation, necrosis)	4

2.7 Statistical analysis

All the results expressed as mean ± standard deviation (SD) were statistically analyzed using Student's *T*-test and one-way ANOVA using IBM SPSS Statistics 26 software.

3. Results

3.1 Energy-amplifying benefits: increased mitochondrial and ATP levels

Investigations has been that several mitochondrial functions, i.e., mitochondrial protein synthesis, respiration, and coupling of respiration to ATP synthesis, deteriorate markedly with aging in HSF cells [4]. To investigate the positive impact of AAC on HSF intercellular energy production, we collected fibroblasts after AAC interference and measured their ATP levels. The results revealed that, compared to the blank group, the AAC significantly increased ATP levels by 141.55% ($P < 0.001$) (as shown in Fig. 1). Thus, AAC can directly promote cellular energy production, enhancing the intercellular energy exchange.
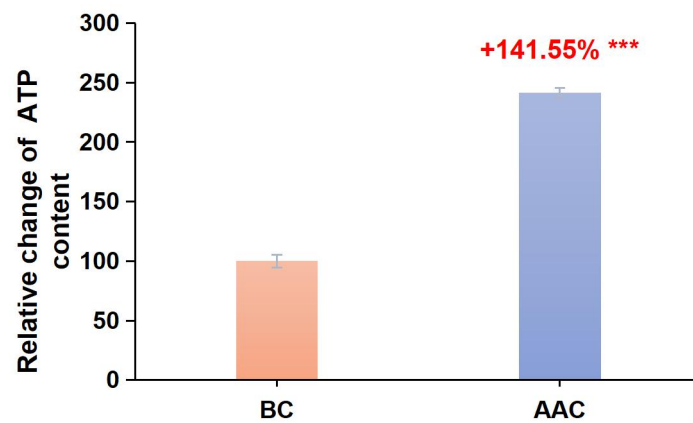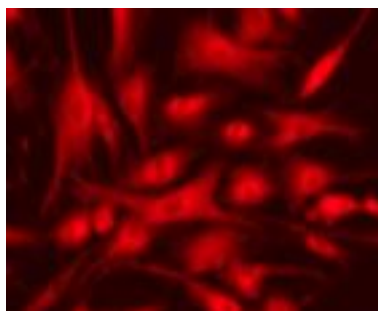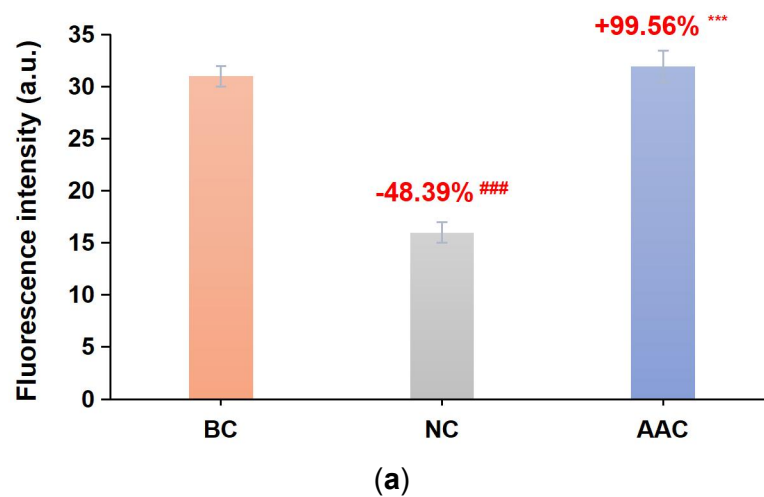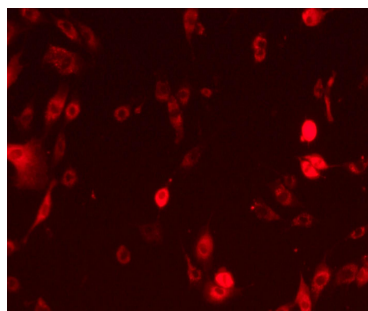
Figure 1. The effect of the AAC on intracellular ATP.

(**a**)

(**b**)   (**c**)   (**d**)
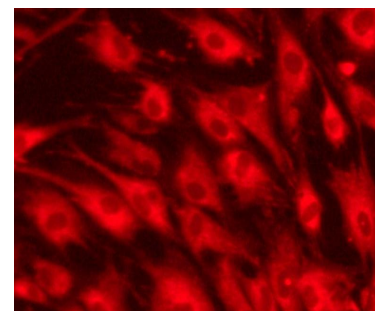
Figure 2. The effect of the AAC on mitochondria: (a) Changes in mitochondrial content in HSF cells; (b-d) Fluorescence microscopic analysis of mitochondrial content in HSF cells. Student's *t* test, [###]$P < 0.001$ vs. BC, ***$P < 0.001$ vs. NC.

Mitochondria are the primary source of energy production in cells. However, mitochondria are influenced by the passage of time and ultraviolet radiation, resulting in mitochondrial damage [5]. Mitochondrial content was determined by immunofluorescence, where greater

fluorescence intensity corresponds to higher mitochondrial protein expression. As shown in Fig. 2a-b, compared to the blank control group, mitochondrial protein content within cells significantly decreased by 48.39% ($P < 0.001$) following UV irradiation. After treatment with AAC, mitochondrial protein content was significantly increased by 99.56% ($P < 0.001$), reaching levels comparable to the blank group, thereby reversing cellular photodamage.

3.2 Promoting skin barrier repair

The epidermis is a self-renewing layer that relies on the subsequent differentiation and migration of the proliferative basal keratinocytes to the cornified keratinocytes in the outermost squamous layer [6]. Importantly, adequate ATP production is essential in cell activation and function in tissue repair [7]. In the aforementioned study, we demonstrated the direct enhancing effect of AAC on cellular energy, suggesting that it may also positively impact skin repair. The results further confirmed that after AAC treatment, the migration rate of keratinocytes was significantly increased by 70.13% ($P < 0.01$) at 24 h and 149.38% ($P < 0.001$) at 48 h (as shown in Fig. 2). Therefore, AAC can effectively repair the skin barrier.
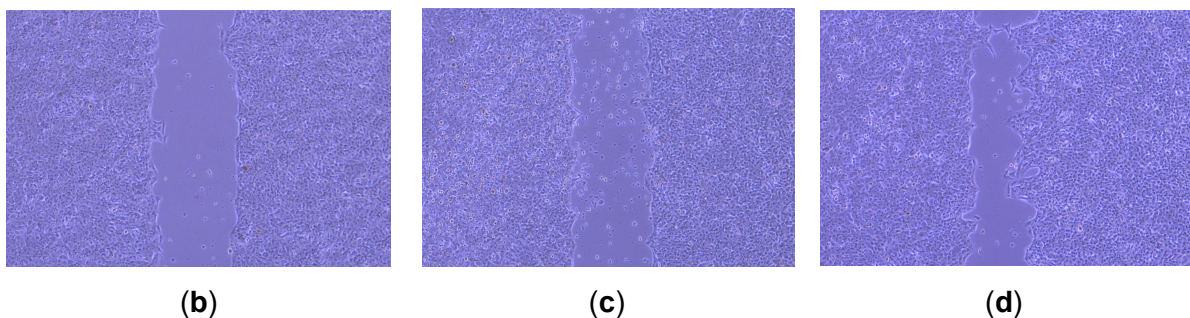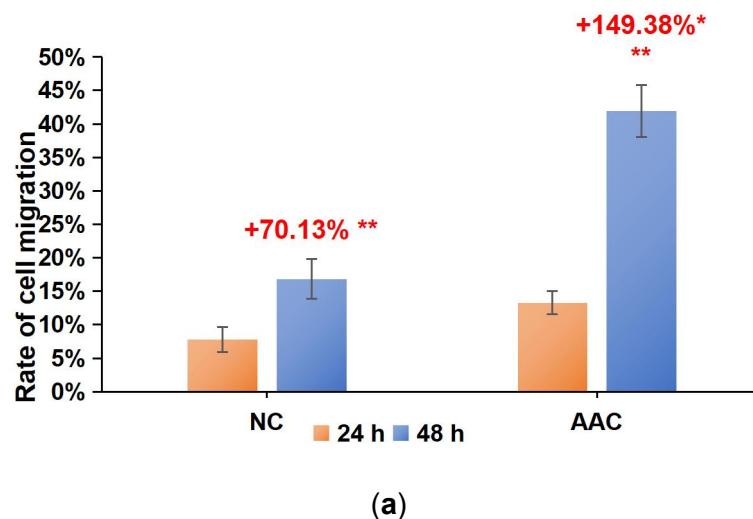

(a)

(b)　　　　　　　　(c)　　　　　　　　(d)

Figure 2. The effect of the AAC on cell migration. (a) Changes in rate of cell migration in HaCaT cells; (b-d) The migration of HaCaT cells at 0 h, 24 h, 48 h respectively. Student's t test, *$P < 0.05$, **$P < 0.01$, ***$P < 0.01$ vs. BC.

3.3 Sustained clinical improvements

We recruited 30 middle-aged men with noticeable signs of aging and fragile skin barriers to explore the clinical efficacy of AAC in improving facial wrinkles, elasticity, and skin barrier function in males. In the clinical trial, significant anti-aging effects were observed within two weeks compared to baseline (as shown in Fig. 3). By the fourth week, notable improvements were observed in TEWL (-14.55%), elasticity (R5: +17.97%, R2: +14.16%, R7: +13.92%), lines under eyes (-20.33%), crow's feet, forehead wrinkles (-21.75%), nasolabial folds (-16.29%), and skin reaction grading (-46.25%), compared to baseline (all $P < 0.05$).
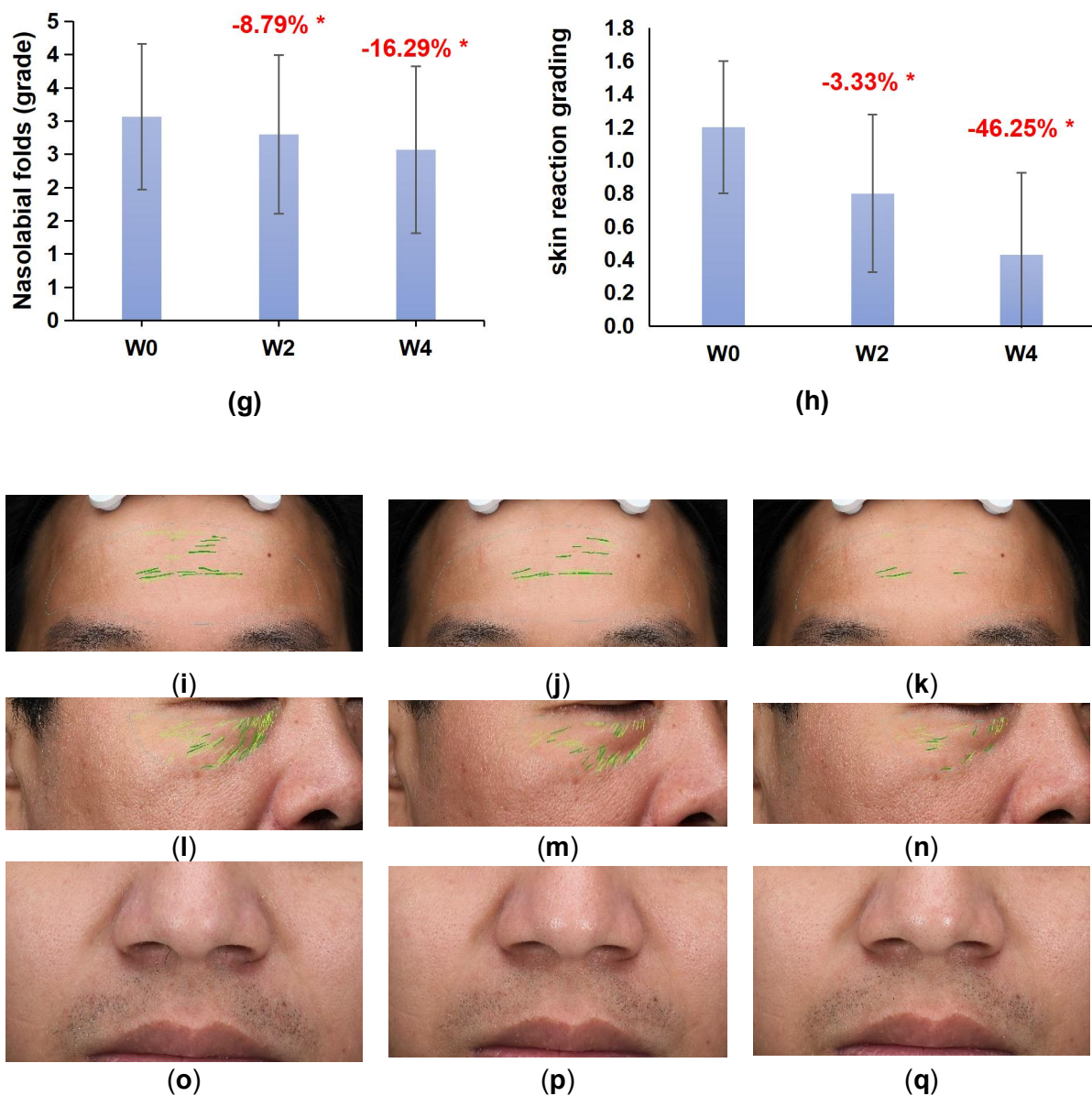

(a)   (b)

(c)   (d)

(e)   (f)
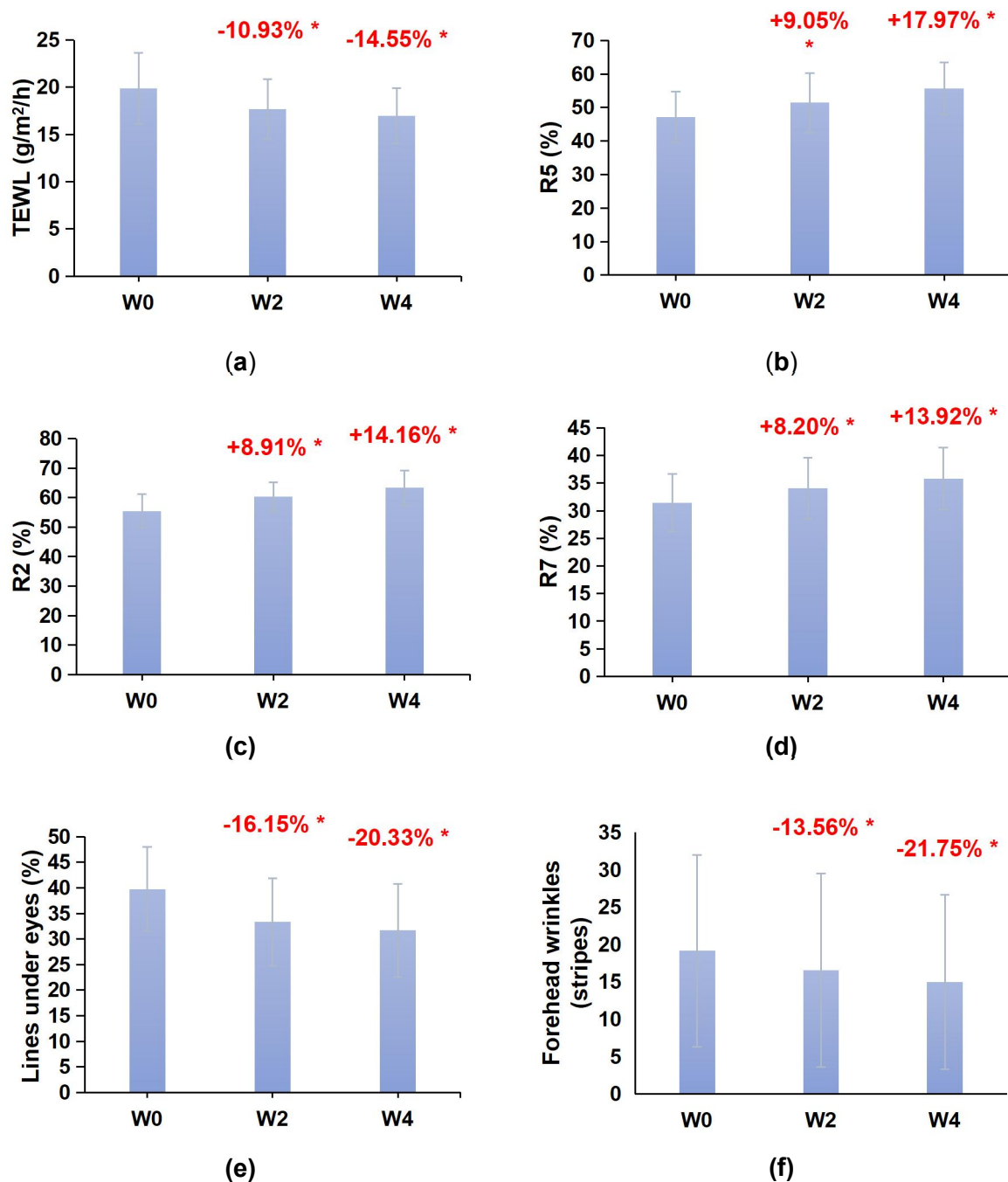
Figure 3. The clinical improvement effects of a cream containing the AAC on signs of male aging: (a) TEWL; (b) R5; (c) R2; (d) R7; (e) Lines under eyes; (f) Forehead wrinkles; (g) Nasolabial folds; (h) skin reaction grading. (i-k) The improvement images of forehead wrinkles on 0, 2, 4 weeks. (l-n) The improvement image of lines under eyes on 0, 2, 4 weeks. (o-q) The improvement image of nasolabial folds at 0, 2, 4 weeks. Student's t test, *$P < 0.05$ vs. W0.

4. Discussion

Studies have shown that men have a greater need for anti-aging interventions compared to women. The skin layer of men is typically thicker, and their antioxidant capacity is weaker, requiring more energy to maintain cellular homeostasis [8]. Additionally, from an aesthetic perspective, men's facial wrinkles are usually more pronounced than those of women, especially in areas such as the forehead, under-eye wrinkles, and nasolabial folds [9]. These features may also make men appear older, with studies confirming that men look, on average, five years older than their actual age [10]. More importantly, men often neglect the importance of skincare, particularly sun protection [11], despite UV radiation being a major cause of premature aging.

Fibroblasts are closely related to skin aging and are a key target for anti-aging interventions. These energy-dependent cells rely heavily reliant on mitochondria to perform essential functions, including maintaining cell vitality, synthesizing the extracellular matrix (ECM), secreting growth factors and cytokines, and promoting cell signaling — processes vital for tissue integrity [12]. When mitochondrial function is compromised or energy level are insufficient, fibroblast function becomes impaired, directly contributing to skin aging, with wrinkles being the most prominent manifestation [13]. Specifically, when the skin is exposed to sunlight, mitochondrial damage primarily occurs in dermal fibroblasts, accompanied by decreased mitochondrial expression, which leads to altered cell functions and accelerated wrinkle formation [6].

Thiotaurine is a potent antioxidant that protects the skin from environmental pollutants, offering significant potential in anti-aging [14-15]. Additionally, M3 (Magnesium Aspartate, Zinc Gluconate, and Copper Gluconate) plays a crucial role in mitochondrial metabolism, optimizing cellular respiration and enhancing energy circulation. By scientifically combining thiotaurine with M3, we have developed a novel anti-aging composition, AAC, which rescues the reduction of fibroblast mitochondria induced by UV radiation and directly increase extracellular ATP levels (as shown in Fig. 1). This demonstrates that AAC can enhances mitochondrial function, generate sufficient ATP, and plays an active role in the prevention and repairing tissue aging. Clinical trials have also demonstrated the efficacy of AAC in anti-aging, significantly improving facial wrinkles and elasticity in men (as shown in Fig. 3).

Another important consideration is that, during the aging process, the skin's barrier function gradually declines, and barrier repair is delayed. When exposed to environmental factors, this can lead to the development of sensitive skin, which forms a vicious cycle and accelerates aging [16-17]. Keratinocytes are the primary cells responsible for constructing the skin barrier. By creating "wounds", we can effectively assess the barrier repair efficacy of active ingredients. This approach helps in understanding how certain compounds can aid in restoring the skin's protective functions, preventing further damage, and enhancing resilience [18]. Interesting, AAC is an effective barrier repair agent that promotes the migration of keratinocytes (as shown in Fig. 2) . It significantly reduces the irritant response of sensitive skin to lactic acid (as shown in Fig. 3), thereby strengthening the skin's barrier function and alleviating sensitivity. This process helps restore and maintain the integrity of the skin barrier, which is crucial in preventing environmental stressors from exacerbating skin sensitivity and aging. These results indicated that AAC has both anti-aging and skin barrier repair effects, significantly improving aging male skin and helping to maintain a youthful condition.

5. Conclusion

The complex directly enhanced cellular energy, effectively protected mitochondria from photodamage, and significantly promoted cell migration, all of which contributed to delaying the aging process. In the clinical trial, the complex significantly improved skin elasticity, reduced wrinkles, repaired the skin barrier, and decreased sensitivity, demonstrating outstanding anti-aging efficacy in practical applications for male skincare.

References

[1] Sreedhar, A., Aguilera-Aguirre, L., & Singh, K. K. (2020). Mitochondria in skin health, aging, and disease. Cell death & disease, 11(6), 444.

[2] Ma, L., Tan, Y., Zheng, S., Li, J., Jiang, C., Chen, Z., & Wang, X. (2017). Correlation study between image features and mechanical properties of Han Chinese facial skin. International Journal of Cosmetic Science, 39(1), 93-100.

[3] Hamer, M. A., Pardo, L. M., Jacobs, L. C., et al. (2017). Lifestyle and physiological factors associated with facial wrinkling in men and women. Journal of Investigative Dermatology, 137(8), 1692-1699.

[4] Greco, M., Villani, G., Mazzucchelli, F., Bresolin, N., Papa, S., & Attardi, G. (2003). Marked aging‐related decline in efficiency of oxidative phosphorylation in human skin fibroblasts. The FASEB Journal, 17(12), 1706-1708.

[5] Perez, A. S., Inada, N. M., Mezzacappo, N. F., Vollet‐Filho, J. D., & Bagnato, V. S. (2024). Ultraviolet radiation inhibits mitochondrial bioenergetics activity. Photochemistry and Photobiology.

[6] Cheng, B., Liu, H., Li, J., & Fu, X. (2021). Skin development and tissue repair and regeneration. Regenerative medicine in China, 119-138.

[7] Sreedhar, A., Aguilera-Aguirre, L., & Singh, K. K. (2020). Mitochondria in skin health, aging, and disease. Cell death & disease, 11(6), 444.

[8] Keaney, T. C. (2016). Aging in the male face: intrinsic and extrinsic factors. Dermatologic Surgery, 42(7), 797-803.

[9] Tsukahara, K., Hotta, M., Osanai, O., Kawada, H., Kitahara, T., & Takema, Y. (2013). Gender‐dependent differences in degree of facial wrinkles. Skin Research and Technology, 19(1), e65-e71.

[10] Flament, F., Prunel, A., Keufer, B., Abric, A., Wang, Y., Reni, A., ... & Delaunay, C. (2021). Changes in facial signs due to age and their respective weights on the perception of age and skin plumpness among differently aged Korean women. Skin Research and Technology, 27(4), 526-536.

[11] Hugo Pacagnelli Infante, V., Melo, M. O., & Maia Campos, P. M. (2022). Impacts of sun protection and skin care habits in the biophysical and morphological properties of young men skin. Journal of Cosmetic Dermatology, 21(10), 5073-5080.

[12] Martic, I., PapAACio, F., Bellei, B., & Cavinato, M. (2023). Mitochondrial dynamics and metabolism across skin cells: implications for skin homeostasis and aging. Frontiers in Physiology, 14, 1284410.

[13] de Araújo, R., Lôbo, M., Trindade, K., Silva, D. F., & Pereira, N. (2019). Fibroblast growth factors: a controlling mechanism of skin aging. Skin pharmacology and physiology, 32(5), 275-282.

[14] Acharya, M., & Lau-Cam, C. A. (2013). Comparative evaluation of the effects of taurine and thiotaurine on alterations of the cellular redox status and activities of antioxidant and glutathione-related enzymes by acetaminophen in the rat. In Taurine 8: Volume 2: Nutrition and Metabolism, Protective Role, and Role in Reproduction, Development, and Differentiation (pp. 199-215). New York, NY: Springer New York.

[15] Egawa, M., Kohno, Y., & Kumano, Y. (1999). Oxidative effects of cigarette smoke on the human skin. International journal of cosmetic science, 21(2), 83-98.

[16] Hashizume, H. (2004). Skin aging and dry skin. The Journal of dermatology, 31(8), 603-609.

[17] Seyfarth, F., Schliemann, S., Antonov, D., & Elsner, P. (2011). Dry skin, barrier function, and irritant contact dermatitis in the elderly. Clinics in dermatology, 29(1), 31-36.

[18] Shaw, T. J., & Martin, P. (2016). Wound repair: a showcase for cell plasticity and migration. Current opinion in cell biology, 42, 29-37.