



Amino Acids Oral Treatment for the Amelioration of Skin, Hair, and Nails Conditions: An Open-Label Study

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Abstract

This study aimed at investigating the effect of a food supplement in ameliorating the skin, hair, and nails conditions. An open-label study on 21 women with mild-to-moderate aging all over the face (including wrinkles/fine lines, loss of skin firmness/elasticity, and normal to dry skin), acute telogen effluvium, and brittle/fragile nails was conducted. Skin (moisturization, elasticity, profilometry, and skin thickness), hair (pull test and phototricogram), and nails (clinical analysis of nail plate status) parameters were measured at baseline, and after 28 and 84 days of intake. The clinical analysis carried out by a board-certified dermatologist and the self-evaluation integrated the instrumental measurements. After 28 (D28) and 84 (D84) days of product intake the skin moisturization (superficial moisturization: +6.0% at D28 and +9.2% at D84; deep moisturization: +4.2% at D28 and +7.6 at D84), elasticity (R0 parameter: -11.5% at D28 and -19.3% at D84; R2 parameter: +7.8% at D28 and +11.9 at D84), profilometry (wrinkle depth: -9.5% at D28 and -14.5% at D84; skin smoothness: -4.9% at D28 and -7.7% at D84) and thickness (+0.11 mm at D28 and +0.24 mm at D84) were improved. At D84 the density of telogen hair was reduced by 9.5%; while at day 28 pull test was not indicative for telogen effluvium. The nail status was improved in most of the enrolled subjects. The instrumental measured effects were perceived by both the dermatologist and the enrolled subjects. Our results highlight the test product efficacy in improving the skin, hair, and nails conditions.



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Introduction

In recent years, oral supplements marketed as “skin, hair, and nail” supplements are becoming popular and trendy treatments in the world of

the skin health.^{1,2} These products are also called “nutricosmetics” and represent the new frontier of the beauty industry.³ The concept behind nutricosmetics is related to their ability to work from the inside

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promoting the beauty from within. This category of “beauty” supplements boosts the Consumer hope to improve the appearance using a capsule, seeking out the “hope in a capsule”. The role of food supplements in correcting/integrating the deficiency of some nutrients, in contributing to maintaining an adequate intake of certain nutrients and in supporting or improving specific physiological functions is widely recognized by both the regulatory bodies and the scientific community.⁴ However, the evidence is sparse for most supplements.^{1,5}

The role of nutrition support in improving skin health is nowadays evidence.⁶⁻⁸ Clinical and epidemiological research have successfully combined nutrition with skin health and aging.⁹ The aging related changes of the skin structures involves the degradation and the alteration of collagen fibers throughout the dermis.¹⁰ Furthermore, amino acids are the building blocks of the most abundant proteins in the skin, like keratin, collagen, and elastin. Their role in maintaining skin health was demonstrated for: water retention in the stratum corneum, protection from UV-induced damage, maintenance of the skin microbiome balance, and wound healing.¹⁰ Glycine, proline, and hydroxyproline (the most abundant amino acids of collagen composition) role in collagen synthesis and animal growth have been reported in different studies.^{11,12} The present study investigated the efficacy of the oral supplementation with a food-supplement containing Glycine, L-Alanine, L-Proline, L-Valine, L-Leucine, L-Lysine, on skin aging.

Regarding the product effect on hair, our attention was focused on transient hair shedding (i.e. acute telogen effluvium, aTE). aTE is a transient, non-scarring shedding of normal club hair.¹³ Usually aTE undergoes to remission in around of 95% of case, with the evidence on examination of shorter re-growing frontal hair.¹³ Even if hair loss is not a life-threatening condition, it is a common complaint and cause of significant emotional distress especially in women.^{4,14} Like for the skin, amino acids are a major constituent of hair fibers. The role of L-lysine was demonstrated to be effective in decreasing the excessive hair shedding in a female population with a mechanism of action that was probably related to its effect in replenishing iron stores.¹⁵

Nail plate brittleness is a common complaint affecting up to 20% of the population. The fragility of the nail plate consist of nails splitting, flaking, and crumbling. Therefore, nails become soft an inelastic.¹⁶ We decide to focus our attention on the “brittle nail syndrome (BNS)” because it is reversible, and it can be improved by the oral intake of bioactives.¹⁷⁻¹⁸

The mixture of amino acids was chosen based on the results of a previous *in vitro* study aimed to clarify, in cultured human fibroblasts, whether the amino acids mixture could produce both collagen and elastin and to optimize the ratio between the amino acids in the mixture.¹⁹ These results were further confirmed by another *in vitro* study, in which the authors demonstrated an upregulation of elastin, fibronectin, and collagen 1 gene expression in cultured human fibroblasts.²⁰ This study was aimed at investigating the clinical efficacy of the amino acid's mixture on skin. Further endpoints included the analysis of hair and nails parameters.

Material and Methods

Study Design, Subjects, and Locations

This monocentric, open label study included twenty-one (N = 21) healthy Caucasian female screened and enrolled by a dermatologist. The study was conducted according to the study protocol approved by the “Independent Ethical Committee for Non-Pharmacological Clinical trials” (ref. 2021/14) under the Declaration of Helsinki principles. Both the informed consent form and the written permission for the picture use, were obtained before the subject enrolment in the study. This study included all the adult ($35 \leq \text{age} \leq 70$ years old) Caucasian female subjects showing the clinical signs of mild-to-moderate aging all over the face (including wrinkles/fine lines, loss of skin firmness/elasticity, and normal to dry skin), acute telogen effluvium, and brittle/fragile nails. The study further included subjects of good general health, with a dermatological anamnesis free of any active skin, nails, and hair diseases (e.g. atopic dermatitis, skin elastosis, etc.). The study excluded pregnant or breastfeeding women, subjects planning a pregnancy, and dermatological diseases or altered conditions in the test area including sensitive or hyper-reactive skin.

Avoiding artificial or natural UV exposure was a constraint during all the study period. Clinic visits were planned at baseline and after 28 (D28) and 84 (84) days of product intake.

The study was conducted by Complife Italia Srl (San Martino Siccomario, Pavia, Italy) from October 2021 to April 2022. Complife Italia is an independent testing laboratory specialized in the *in vitro* and *in vivo* safety and efficacy testing of cosmetics, food supplements and medical devices.

Tested Product

The tested product was a food supplement (Nutrakos Drinkable, Professional Dietetics S.p.A., Milano, Italy) containing Purified Water, Glycine, L-Alanine, L-Proline, L-Valine, L-Leucine, L-Lysine, Trisodium Citrate (E331), Acidifier: Citric Acid (E330), Preservative: Sodium Benzoate. The way and the frequency of use was as follows: during the first two weeks subjects took two stick packs per day, while from week 2 to week 12 subjects continued with 1 stick pack per day. Subjects were also supplied with a base cream to standardize the cosmetic habits. The base cream ingredient list was as follows: Aqua, Helianthus Annuus Seed Oil, Isononyl Isononanoate, Polyglyceryl-3 Methylglucose Distearate, Glyceryl Stearate, Cetearyl Alcohol, Disodium EDTA, Chlorphenesin, O-Cymen-5-OL, Fragrance/Parfum.

Primary and Secondary Endpoints/Outcomes

The evaluation of wrinkle depth, hair shedding and nails conditions was the primary efficacy endpoint. The primary outcomes were the measurement of the wrinkle depth, the percentage of telogen hair and the clinical evaluation of nail status.

The secondary outcomes of the study included the evaluation of the skin roughness, moisturization, elasticity and thickness. These endpoints were measured by instruments but also assessed by dermatologist clinical scoring and/or by the subject's perception.

The measurement process was carried out under temperature ($21 \pm 1^\circ\text{C}$) and humidity ($50 \pm 10\%$) controlled conditions after a 15/20 minutes acclimatation period.

Skin Profilometry

Wrinkle depth and skin roughness (Sa parameter) were measured by a 3D imaging device (PRIMOS^{CR}, Canfield Scientific, Utrecht, Netherlands). The measurement principle of the device is based on the 3D fringe projection technology. The before/after pictures overlapping and matching were regulated electronically by a specific feature of the device integrated software and physically by a stereotactic device (Canfield Scientific, Utrecht, Netherlands). Both the wrinkle depth and the skin roughness were measured in the periocular area ('crow's feet wrinkles'). Sa is inversely related to skin smoothness.

Superficial and Deep Skin Moisturization

The superficial moisturization of the skin (measurement depth: 10-20 μm) was measured by a Corneometer[®] CM 825 (Courage + Khazaka, electronic GmbH, Cologne, Germany).

The deep (500 μm) skin moisturization was measured by a MoistureMeterD (Delfin Technologies Ltd, Bergamo, Italy) method.

Skin Elasticity

The skin elasticity was measured in the cheek area by a Cutometer[®] MPA 580 (Courage + Khazaka, electronic GmbH, Cologne, Germany). The R0 (skin distensibility) and R2 (overall elasticity) parameters were measured.

Skin Thickness

Skin thickness was measured by an Aloka $\alpha 6$ pro-sound (Hitachi) ultrasound machine. The ultrasound machine allows to measure the skin thickness (epidermidis + dermis). In the analyzed region, skin thickness was measured in five points

Phototricogram

Phototricogram was performed according to the TrichoScan[®] supplier recommendations, as follows: i) hair clipping and trimming; ii) removal of the trimmed hair with a sticky tape; iii) hair dyeing; iv) removal of dye remnants using a swab and an alcoholic solution; v) picture taking 48 hours after clipping. Anagen/telogen hair, the total hair number, and hair density were calculated by TrichoScan[®] software.

Clinical Analysis

Skin, hair, and nails parameters were assessed by the dermatologist by a clinical scoring system, as follows:

- Skin wrinkledness at baseline was scored according to the modified Fitzpatrick wrinkle scale,^{21,22} while at the follow-up visits it was scored according to an improvement scale*.
- Skin firmness: 1 Not firm skin (Inelastic, thinned, not dense and relaxed skin with bad resistance to pinching/pulling and a poor elastic recovery after traction), 2 Not very firm skin (Poorly elastic, thinned and less dense skin, initial tissue relaxation with poor resistance to pinching/pulling and a poor elastic recovery after traction) 3 Sufficient firm skin (medium tone, sufficiently full, plump and dense skin, tissues appear slightly relaxed with sufficient resistance to pinching/pulling and a quite good elastic recovery after traction), 4 Firm skin (full and plump skin, tissues don't appear relaxed with good resistance to pinching/pulling and a good elastic recovery after traction, 5 Very firm skin (full and plump skin, tissues are not relaxed with an excellent resistance to pinching/pulling and an excellent elastic recovery after traction).
- Nail status was scored at baseline according to the clinical scoring system reported here below; while at the follow-up visits it was scored according to an improvement scale*:
- Nails hardness: 1 Nails appear brittle, weak, not very resistant and with a tendency to break, 2 Nails appear slightly brittle, weak, with a slight tendency to break, 3 Nails appear compact, hard, and durable.
- Nails hydration: 1 Nails appear dry, flaked, brittle with a tendency to break, 2 Nails appear slightly flaked, brittle with a slight tendency to break, 3 Nails appear hydrated, hard, and resistant.
- Nails smoothness: 1 Nails appear rough, unpolished, and uneven, 2 Nails appear smooth and not very uniform, 3 Nails appear smooth and uniform.
- Nails plate evenness: 1 Nails appear rough and/

or with uneven pigmentation with uneven and uneven surface (striped/wavy), 2 Nails appear slightly rough with uneven and uneven surface (striped/wavy), 3 Nails appear smooth/even, regular, and homogeneous.

* 1 No variation, 2 Slight improvement, 3 Moderate improvement, 4 Remarkable improvement.

Self-Assessment Questionnaire

At D84 subjects compiled a self-assessment questionnaire. The self-assessment questionnaire items investigated the product performance on skin, hair, and nails.

Statistical Analysis

Statistical analysis was carried out by a statistician using NCSS 8 (NCSS, Kaysville, UT, USA). Symmetrically distributed normal data (instrumental measurements) were analyzed by a two-way t test of Student; while non-parametric data (clinical analysis) were analyzed by Wilcoxon test. A p values less than 0.05 was considered statistically significant. Statistical analysis output was reported as follows: *p < 0.05, **p < 0.01, and ***p < 0.001.

Results

Subjects and Product Tolerability

A total of twenty-two (N = 22) female subjects were enrolled in the study. Subjects attended clinic visits at baseline (D0) and after 28 days (D28) and 84 days (D84) of product intake. One subject dropped out for personal reasons (not dependent by the product use). The subjects' baseline demographics are detailed in Table 1. The product was well tolerated. No adverse reactions, neither objective nor subjective, were reported by the dermatologist or by the subject, respectively.

Primary Endpoints: Wrinkle Depth, Hair Loss, Nail Status

The primary endpoints related to the product performances on skin, hair, and nail conditions were measured by means of image analysis on 3D pictures, by phototricogram, and by clinical analysis, respectively. Data are reported in Fig. 1-3.

Table 1: Demographic and clinical characteristics of the study participants at baseline.

Baseline characteristic		Value	Units
Sex	Female	21 (100%)	N (%)
Age		53.6 ± 1.5	Years
Skin type	Normal	9 (47.6%)	N (%)
	Combination	11 (52.4%)	N (%)
Skin moisturization	Superficial	49.7 ± 1.5	Corneometric units
	Deep	40.7 ± 1.1	% of water
Skin elasticity	R0 (skin distensibility)	0.450 ± 0.013	mm
	R2 (overall elasticity)	0.607 ± 0.016	%
Wrinkle depth		263.0 ± 20.0	µm
Skin smoothness (Sa)		46.02 ± 1.77	µm
Skin thickness		1.38 ± 0.04	mm
Pull test		12.8 ± 0.6	Hair no.
Phototricogram	Hair density	182.1 ± 6.8	Hair no.
	Anagen hair density	128.6 ± 5.3 (70.6%)	Hair no. (%)
	Telogen hair density	53.5 ± 2.9 (29.4%)	Hair no. (%)

The “crow’s feet” wrinkle depth was significantly lower ($p < 0.001$) after both 28 (-9.5%) and 84 (-14.5%) days of product intake (Fig. 1a). The decrease of the wrinkle depth was seen also by the dermatologist in most of the subject (62% at D28 and 76% at

D84). A similar trend was observed also for the skin smoothness (Sa) parameter. Sa parameter (Fig. 1b) was significantly ($p < 0.001$) reduced after both 28 (-4.9%) and 84 (-7.7%) days of product intake (Fig. 1b).

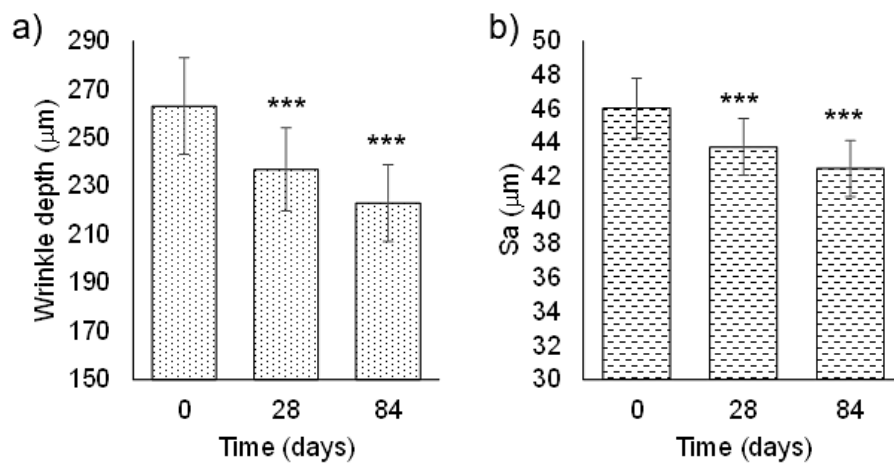


Fig. 1: (a) Wrinkle depth. (b) Skin smoothness. Data are average (± standard error). Above the bars is reported the statistical analysis (vs. baseline), as follows: * $p < 0.05$, ** $p < 0.01$, and * $p < 0.001$.**

The phototricogram analysis (Fig. 2) showed an improvement of the hair density (both total and anagen/telogen density). In particular, the telogen

hair percentage, after 84 days of product intake, was decreased by -9.5% ($p < 0.001$).

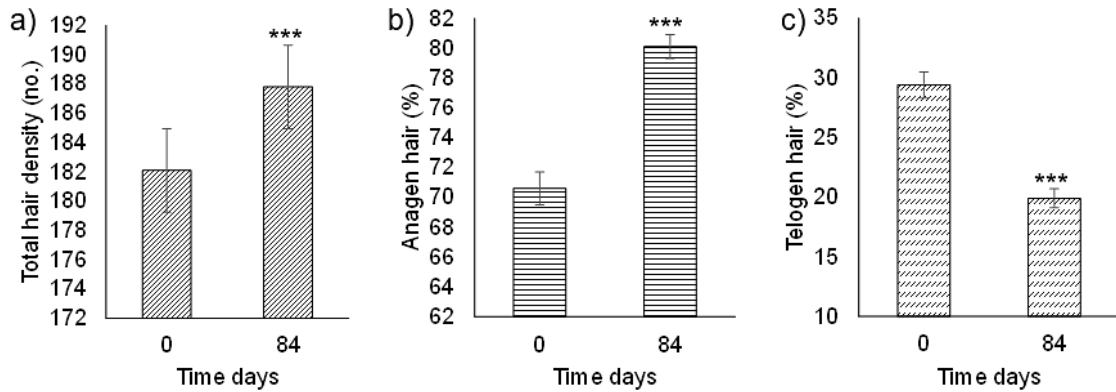


Fig. 2: Phototricogram results. (a) Total hair density. (b) Anagen hair %. (c) Telogen hair %. Data are average (± standard error). Above the bars is reported the statistical analysis (vs. baseline) as follows: * $p < 0.05$, ** $p < 0.01$, and * $p < 0.001$.**

Nail conditions (Fig. 3) were improved (from mild to moderate improvement) both after 84 days of product intake. The responder to treatment were as follows:

62% of subjects for nails hardness, smoothness and evenness, and b) 67% for nails hydration.

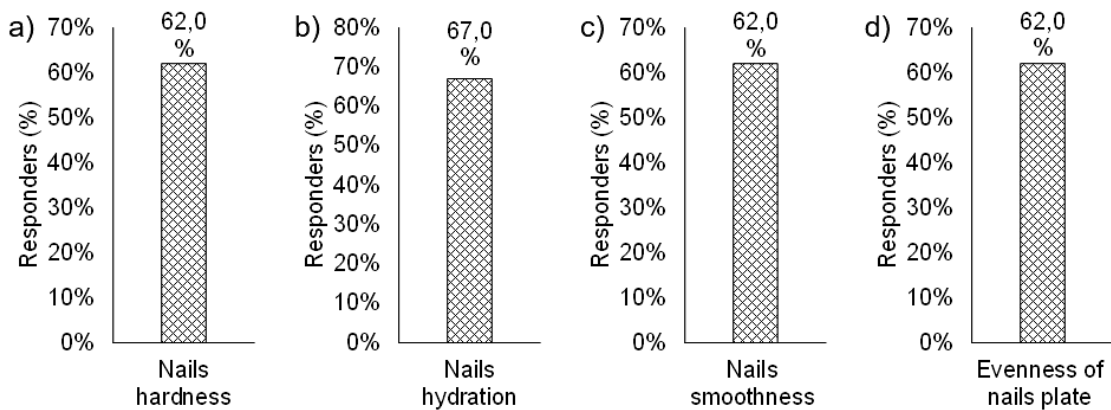


Fig. 3: Nails conditions after 84 days of intake. Data are the percentage of subjects showing an improvement (responders).

Table 2: Secondary endpoints results. Data are average (\pm standard error). In bracket is reported the variation (difference or percentage). The statistical analysis is reported as follows: * $p<0.05$, ** $p<0.01$, and * $p<0.001$. D0 baseline. D28/84 follow-up visit after 28/84 days of product intake. cu corneometric units**

Parameter	D0	D28	D84	Units
Skin moisturization				
Superficial ($\approx 20 \mu\text{m}$)	49.7 \pm 1.5 (+6.0%)	52.6 \pm 1.4*** (+9.2%)	54.1 \pm 1.4***	cu
Deep ($\approx 500 \mu\text{m}$)	40.7 \pm 1.1 (+4.2%)	42.3 \pm 1.0*** (+7.6%)	43.7 \pm 1.0***	% water
Skin elasticity				
R0 parameter	0.450 \pm 0.013 (-11.5%)	0.396 \pm 0.010*** (-19.3%)	0.362 \pm 0.012***	mm
R2 parameter	0.607 \pm 0.016 (+7.8%)	0.652 \pm 0.017*** (+11.9%)	0.677 \pm 0.017***	%
Skin thickness	1.38 \pm 0.04 (+0.11)	1.49 \pm 0.03*** (+0.24)	1.62 \pm 0.04***	mm

Secondary Endpoints

The secondary endpoints as related to the product efficacy are reported in table 2.

The superficial ($\approx 20 \mu\text{m}$) skin moisturization (Table 3) was increased ($p<0.001$) by 6.0% and 9.2% after 28 and 84 days of product intake, respectively. Similar results were obtained for the deep ($\approx 500 \mu\text{m}$) moisturization. The deep skin moisturization was increased ($p<0.001$) by 4.2% and 7.6% after 28 and 84 days of product intake, respectively.

The skin distensibility (R0, skin firmness) was decreased ($p<0.001$) by 11.5% and 19.3% after 28 and 84 days of product intake. An improvement of skin firmness was seen also by the dermatologist in most of the subject (62% at D28 and 76% at D84).

The skin overall elasticity (R2) was increased ($p<0.001$) by 7.8% and 11.9% after 28 and 84 days of product intake. The R2 parameter increase is related to an improvement of the function of the elastic fibers of the skin.

The skin thickness was significantly increased ($p<0.001$) starting from 28 days of product intake

with a mean increase by 0.11 mm. The skin thickness was further increased ($p<0.001$) by 0.24 mm after 84 days of product intake.

Starting from the 28th day of product intake the pull test was not indicative for telogen effluvium. The total number of hair plucked was 9.9 at D28 ($p<0.001$) and 6.3 at D84 ($p<0.001$).

Self-Assessment Questionnaire

The product was scored positively by most of the subjects participating in the study (Table 3). The product efficacy was seen by most of the subjects, as follows: between 1 and 2 months of product intake for skin effects (after 1 months by 42.9% of subjects, after 2 months by 52.4% of subjects, and after 3 months by 4.8% of subjects), between 1 and 2 months of product intake for hair effects (after 1 months by 38.1% of subjects, after 2 months by 38.1% of subjects, after 3 months by 14.3% of subjects, and never by 9.5% of subjects), and between 1 and 2 months of product intake for nails effects (after 1 months by 19.0% of subjects, after 2 months by 52.4% of subjects, after 3 months by 19.0% of subjects, and never by 9.5% of subjects).

Table 3: Self-assessment questionnaire output. Data are the percentage of subjects giving a particular answer.

No.	Item	Completely agree (%)	Agree (%)	Disagree (%)	Completely disagree (%)	Positive answers (%)
Skin						
1	The product improves the overall appearance of my skin	28.6	66.7	4.8	0	95.2
2	My skin looks more radiant	23.8	61.9	9.5	4.8	85.7
3	I fell my skin smoother	23.8	61.9	14.3	0	85.7
4	The appearance of mi fine lines and wrinkles is reduced	23.8	47.6	23.8	4.8	71.4
5	I feel my skin more hydrated	28.6	61.9	9.5	0	90.5
Hair						
6	The product improves the overall appearance of my hair	52.4	33.3	4.8	9.5	85.7
7	My hair is strengthened	42.9	47.6	9.5	0	90.5
8	My hair is stronger and more vigorous	38.1	38.1	23.8	0	76.2
9	The product makes the hair thicker	42.9	33.3	14.3	9.5	76.2
10	The product visibly reduces hair loss	33.3	57.1	9.5	0	90.5
11	The product maintains the health of the hair:	42.9	42.9	14.3	0	85.7
12	I feel the hair less brittle, sparse and thin:	38.1	47.6	14.3	0	85.7
Nails						
13	The product improves the overall appearance of my nails	19	52.4	19	9.5	71.4
14	My nails are strengthened	14.3	52.4	33.3	0	66.7
15	My nails are stronger	14.3	52.4	33.3	0	66.7
16	My nails are more durable	14.3	52.4	33.3	0	66.7
17	My nails are less brittle	14.3	57.1	28.6	0	71.4
18	My nails are less weak	14.3	57.1	28.6	0	71.4
19	The surface of my nails is smoother	14.3	52.4	33.3	0	66.7
20	The product maintains the health of the nails	19	52.4	28.6	0	71.4
Overall satisfaction						
21	Overall, how satisfied are you with the product for its effect on your skin?	28.6	66.7	4.8	0	95.2
22	Overall, how satisfied are you with the product for its effect on your hair?	52.4	33.3	4.8	9.5	85.7
23	Overall, how satisfied are you with the product for its effect on your nails?	19	52.4	19	9.5	71.4
24	Did you like the taste of the product?	Yes	No			
		90.5	9.5			
25	Is the product well tolerated?	100	0			
26	Would you buy the product?	85.7	14.3			

Discussion

The skin, hair and nail health is undermined by different conditions that have a direct impact on subjects' self-esteem and social interactions.^{4,15,23,24} Different aesthetic treatments/procedures and or pharmacological approaches are available to improve skin, hair, and nail appearance, however, subjects can be reluctant to them, due to invasiveness, side effects and cost.

In this study we assessed the efficacy of a food supplement containing amino acids (Glycine, L-Alanine, L-Proline, L-Valine, L-Leucine, L-Lysine) in improving the skin appearance (antiaging effect), in reducing the hair loss in subjects with aTE, and in improving the nail plate conditions in subjects with BNS.

The product showed to be effective in improving a) the aesthetical appearance of the skin (skin wrinkledness) and its conditions (moisturization and thickness), b) in reducing the hair loss in subjects with aTE, and c) in improving the nail status and conditions. Interestingly, the instrumentally measured improvement was also visible to the clinical analysis carried out by the dermatologist and perceived by the subjects. Limitations. The study design was open label while a randomized clinical trial would be the golden standard study design. However, the study design was not influencing data analysis since the study staff was blind to treatment and to Sponsor. Future research may consider greater focus on enhanced the study design (e.g. a randomized clinical trial design).

The intake of Nutrakos Drinkable (Professional Dietetics S.p.A., Milano, Italy) for 84 days was effective in improving the skin appearance (antiaging effect), in reducing hair loss (in subjects with aTE),

and in improving nails appearance (in subjects with BNS). The product intake was well-tolerated. To the best of our knowledge, this is the first study reporting the efficacy of a food supplement containing amino acids (Glycine, L-Alanine, L-Proline, L-Valine, L-Leucine, L-Lysine) in improving skin, hair, and nails appearance.

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Conflict of interest

The author declares no conflicts of interest.

Data Availability Statement

The data presented in this study are available on request from the corresponding author. The data are not publicly available since they are the property of the sponsor of the study (Professional Dietetics S.p.A., Milano, Italy).

Ethics Statement

The study protocol and the informed consent form were approved by the "Independent Ethical Committee for Non-Pharmacological Clinical trials" (ref. 2021/14).

Authors' Contribution

Each author mentioned has significantly and directly contributed intellectually to the project and has given its approval for its publication.

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