Journal of Skin Health and Cosmetics

Research Article Open Access

Efficacy and Safety of L-Cystine With or Without Natural Keratin (Kera-Diet®) Hydrolysate on Hair and Nails: A Randomized, Placebo-Controlled Clinical Trial in Healthy Women

Vincenzo Nobile[1]*, Joel Duperray[2]*, Enza Cestone[1], Renaud Sergheraert[2] and Francesco Tursi[1]

[1]Farcoderm srl, Complife Group, Via Mons Angelini, 21, 27028 San Martino Siccomario, Pavia, Italy
[2]BCF Life Sciences, Boisel, 56140 Pleucadeuc, France

Abstract

Background: The acute Telogen Effluvium (aTE) and the Brittle Nail Syndrome (BNS) are two medical conditions affecting both males and females. A dietary approach based on aminoacids and/or on protein hydrolysate could be a safe and effective approach in reducing hair loss during acute telogen effluvium and in improving brittle nails conditions.

Objective: In this study, we investigated the efficacy of L-cystine (CYS) associated or not to a commercially available hydrolysate (KDC) of natural keratin obtained from a non-human source (feathers) in improving both aTE and BNS symptoms.

Design: A randomised, parallel group study was carried out for 3 months on 60 female subjects with aTE and BNS. Each subject attended clinic visits at the time of randomization (baseline) and after 45 and 90 days of products use. Anagen/Telogen hair, hair density, pull test, global photography, hair and nail brightness, and nail plate growth rate were measured. At the end of the study, a self-assessment test was carried out.

Result: A statistical significant increase of hair density, hair and nails brightness, and nail plate growth rate were observed both in the CYS and KDC treatment group.

Conclusion: This study demonstrates that L-Cystine alone or a mix Kera-Diet®+L-Cystine, associated with traced elements and specific vitamins at the right dosage can enhance hair and nails conditions, even though human nutrition is more and more balanced.

Keywords: L-Cystine; Hydrolysed keratin; Acute telogen effiuvium; Brittle nail syndrome; Clinical study

Introduction

Acute Telogen Effiuvium (aTE) is triggered when a physiologic stress causes a large number of hair in the growing phase of the hair cycle (anagen) to abruptly enter the resting phase (telogen). The duration interruption of the anagen hair growth varies from 1 to 6 months (3 months average time), although this interruption of growth is not noticed by the patient. When hair re-enter the anagen phase, hair in the telogen phase are extruded from the follicle and hair shedding is noticed by the subject [1-3]. Common triggering events causing aTE are acute febrile illness, severe infection, major surgery, severe trauma, postpartum hormonal changes, hypothyroidism, discontinuing oestrogen-containing medication, crash dieting, low protein intake, malnutrition, heavy metal ingestion, iron/zinc deficiency, and seasonal variation (July to October) [1]. However, clear evidence in supporting such events is lacking [4]. The exact prevalence of aTE is not known, but it is considered to be quite common. aTE can occur in either sex, though women have a greater tendency to experience this condition because of postpartum hormonal changes. Also, women are more disturbed by hair shedding than men and are therefore more likely to seek medical attention [2]. Despite the being benign and mild condition of aTE, the associated psycho-emotional stress may affect the subjects quality of life, and may sometimes lead to secondary morbidity [5-7]. The main factors contributing to psychoemotional stress are inability to style hair, dissatisfaction with appearance, concern about the continuing hair loss and concern about others noticing hair loss [8]. The “brittle nail syndrome” (BNS) is a common condition involving the tendency of nail to peel, chip, split, crack, fray, or layer, to be fragile, thin, ragged, rough, dull, brittle, and/to break easily. This common medical condition affects approximately 30% of women (twice the percentage of men), with a higher prevalence among the elderly. Several factors, including anemia, biotin deficiency, or cysteine deficiency have been postulated as causes of nail brittleness. Another proposed causative or exacerbating factor is nail plate dehydration either from repetitive cycles of hydration and dehydration related to hand washing or from using dehydrating chemicals [9]. Most of the persons affected of BNS considered it an important cosmetic problem, which interfered in daily life activities [10-12]. Because of the slow growth rate of the nail plate (3 mm/month for fingernails and 1.5 mm/month for toenails) and the difficulty of getting the drug active to penetrate the nail tissues, it is usually necessary to wait several months before seeing the efficacy of treatments.This delay often leads to discontinuation of therapy by the patients [13]. BNS is also associated with the presence of depressive

***Corresponding author:** Vincenzo Nobile, Farcoderm srl, Complife Group, Via Mons Angelini, 21, 27028 San Martino Siccomario, Pavia, Italy, Tel: +39-0382-25504; E-mail: vincenzo.nobile@complifegroup.com

Joel Duperray, BCF Life Sciences, Boisel, 56140 Pleucadeuc, France, Tel +33 2 97 26 91 21; E-mail: jduperray@bcf-lifesciences.com

Received: May 05 2024, **Accepted: Jun 12** 2024; **Published:** Jun 17, 2024, DOI: 10.59462/jishc.1.1.105

Citation: Nobile V, Duperray J, Cestone E, Sergheraert R, Tursi F (2024) Efficacy and Safety of L-Cystine With or Without Natural Keratin (Kera-Diet®) Hydrolysate on Hair and Nails: A Randomized, Placebo-Controlled Clinical Trial in Healthy Women. Journal of Skin Health and Cosmetics,1(1):105.

disorders, indicating a possible impact on the quality of life of those who experience them, similarly to what occurs with hair loss perception [14].

In this study, we tested the efficacy on both aTE and BNS of Lcystine (Traced L-Cystine®, BCF® Life Sciences, Boisel, 56140 Pleucadeuc, France) associated or not to a commercially available hydrolysate of natural keratin (Kera-Diet®, BCF® Life Sciences), obtained from a non-human source (feathers).

Methods

Study design

This monocentric, randomised, parallel group, double-blind, placebo-controlled study was carried out in accordance with the Declaration of Helsinki. The study protocol and the informed consent form were approved by the "Independent Ethical Committee for Non-Pharmacological Clinical trials" during its meeting on December 12th 2016. All subjects provided writte informed consent before initiation of any study-related procedures. The study took place at Complife Italia dermatological facilities in San Martino Siccomario (PV), Italy. Complife Italia is an independent testing laboratory for in vitro and in vivo safety and efficacy assessment of cosmetics, food supplements and medical devices.

Subjects

Eligible subjects were all female adults, having ongoing acute telogen effiuvium and brittle nails (not pathological condition). The subjects were of general good health, aged between 30 and 60 years old, had no alimentary/eating disorders (i.e. bulimia, psychogenic eating disorders, etc.), and known history of metabolic syndrome. Exclusion criteria were pregnancy or intention to become pregnant, lactation, food intolerance/allergy, pharmacological treatments known to interfere with the test product or having an effect on metabolism, participation in another similar study, unwillingness or inability to comply with the requirements of the study protocol, history for radiotherapy/chemotherapy treatments, and scalp surgery (e.g. hair transplantation). The study further excluded subjects using food supplements containing active ingredients having an influence on hair loss/growth and on nail plate, oestrogen-progesterone contraception or hormonal treatment therapies within 3 months before starting the study, systemic treatments (e.g. retinoids, anti-mitotic, cytotoxic drugs other than antineoplastic, anti-androgens, androgens, anti-epileptic agents, interferon alpha) affecting the hair growth taken for more than 4 consecutive weeks during the last 24 weeks before inclusion visit, and systemic or local androgenetic alopecia treatment or product, taken or applied (Minoxidil, Aminexil, Finasteride, Dutasteride, cosmetic solution or capsules with vitamin B, zinc, caffeine) for more than 4 consecutive weeks during the last 24 weeks before the inclusion visit, and subjects having excessive and/or fluctuating hair shedding for more than 6 months. Changes in hairstyle or dyeing of the hair were not allowed during all the study period. During all the study period subjects were asked to not cut their nails 7 days before and after each checkpoint. Two (n=2) more subjects per each group were enrolled in order to take into account an anticipate drop-out rate by 10%.

Intervention

The test product was on L-cystine 250 mg and a commercially available hydrolysate of natural keratin 250 mg (Kera-Diet®), obtained from a non-human source and having an amino acid profile similar to the hair. Kera-Diet® amino acids profile consists of 17 amino acids (7.6% Asp, 12.4% Ser, 11.8% Pro, 11.3% Glu, 8.6% Gly, 8.2% Val, 7.7% Leu, 6.4% Arg, 5.5% Cys, 5.1% Thr, 5% Ala, 4.6% Ile, 2.5% Phe, 2% Lys, 0.7% His, 0.3% Tyr, 0.2% Met.) of which 86.5% are in free form (Figure 1). The ingredients were supplied by BCF Life Sciences (Boisel, 56140 Pleucadeuc, France). Subjects were randomly assigned to receive L-cystine (CYS group), Kera-Diet®+L-Cystine (KDC group), or the placebo product (PLA group). The ingredients were formulated in gelatin capsule (Table 1). All the treatment groups were asked to consume four capsules per day (1000 mg/day), two at breakfast and two at dinner, for a total period of 90 days.

Primary and secondary outcomes

The primary endpoints with respect to product efficacy were the measurement nagen/telogen hair and the nail growth speed. Hair resistance to traction (pull testing), hair/nail brightness, and overall hair/nail condition, were secondary efficacy endpoints. The study low and the schedule of assessments chart is reported in Figure 2.

Phototrichogram

Digital images for TrichoScan® analysis (software version 2.3) were taken in the mid vertex area. he area (1.8 cm^2) was clipped evenly using a hair trimmer (Moser, TrichoScan Edition) and short clipped hair were removed by pressing an adhesive strip onto the shaved area. On day 3 (72 ± 2 hours after hair clipping), the clipped hair within the

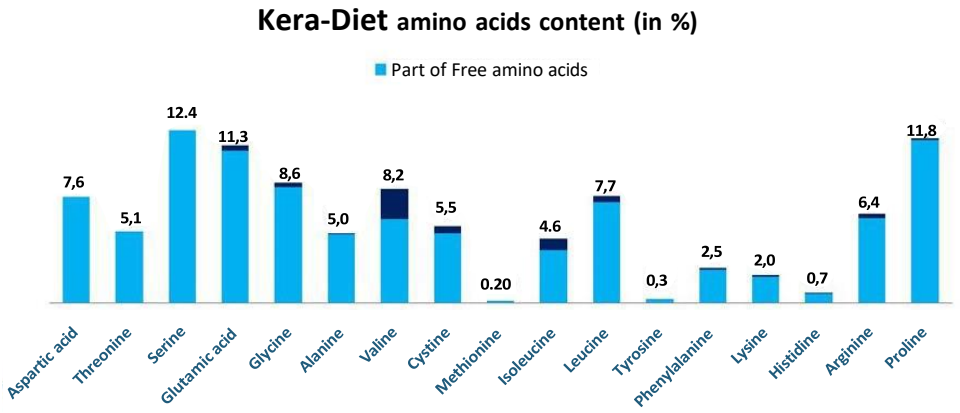


Figure 1: Kera-Diet® aminoacidic composition.

Ingredients	CYS 250 mg L-Cystine	KDC250 mg Kera-Diet®+250 mg L-Cystine	PLA Placebo
Maltodextrin	250	---	524.3
L-Cystine	250	250	---
Kera-Diet®	---	250	---
Magnesium stearate	14	14	14
Zinc sulphate heptahydrate (22% Zn)	11.36	11.36	---
Silice	5	5	5
Vitamin B3 (nicotinamide)	4.5	4.5	---
Vitamin B5 (D-Calcium pantothenate)	3.72	3.72	---
Dry extract of aerial part of Equisetum Arvense	2.5	2.5	---
Copper sulphate pentahydrate	1.48	1.48	---
Vitamin B6 (pyridoxine hydrochloride)	0.6326	0.6326	---
Vitamin B8 (biotin)	0.075	0.075	---

Table 1: Capsules composition: Quantities are reported in mg/capsule.

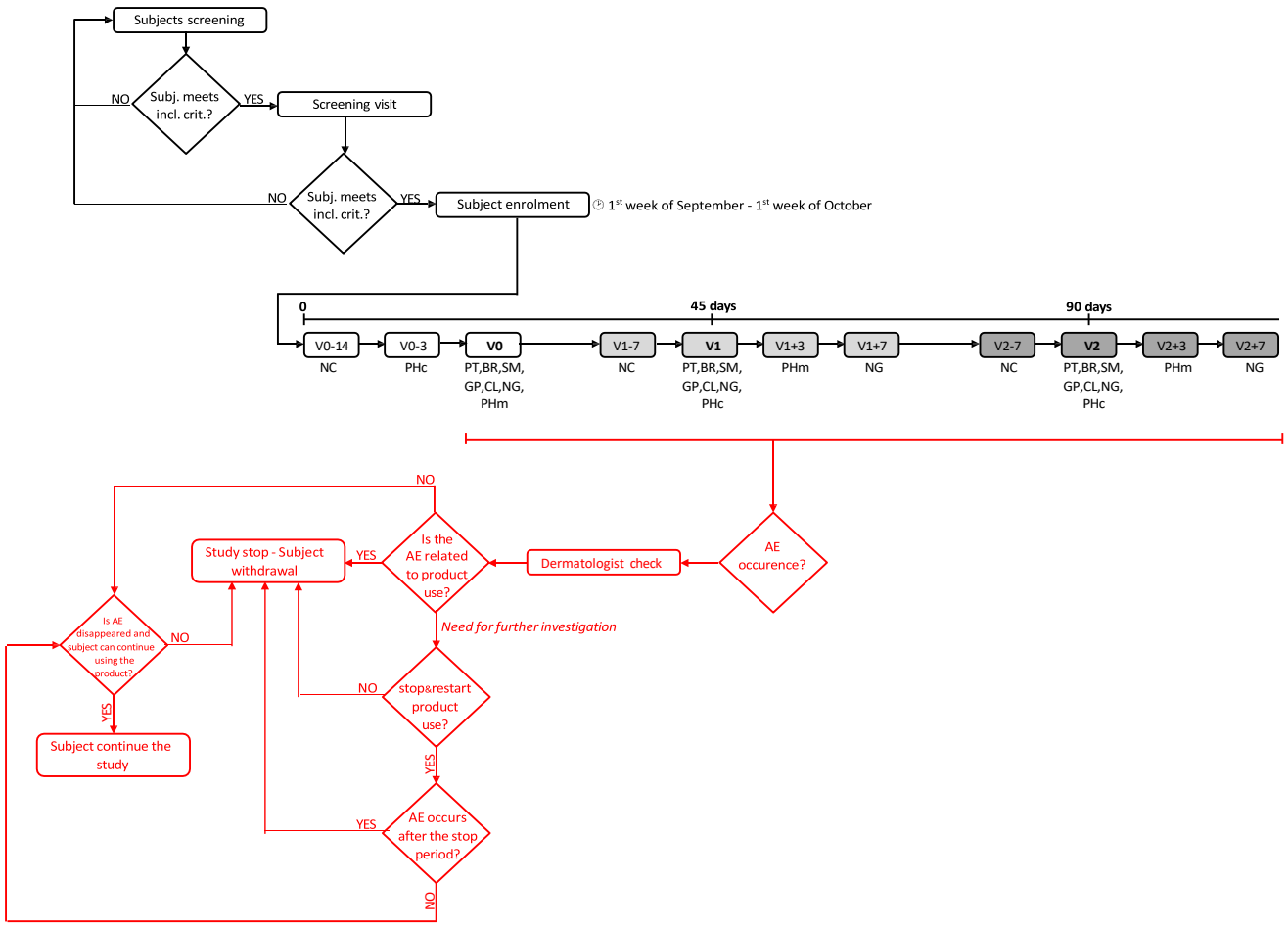


Figure 2: Study flow and schedule of assessment chart. Legend: NC nail cut, PHc Photricogram hair cutting, PT pull testing, BR Brightness measurement, GP Global photography, CL Global photography scoring, NG Nail growth rate, PHm Photricogram hair measurement, SA Self-assessment questionnaire.

target area were died (Goldwell topchic, black 2N, Darmstadt, Germany with Rondo 6% CrèmeOxyd, Coiffeur, Cologne, Germany). After 15 minutes the colored area was thoroughly cleaned with an alcoholic solution (Kodan® Spray, Schülke and Mayr, Vienna, Austria) and digital images were taken using a DermoGenius camera (DermoScan GmbH, D-93055 Regensburg, Germany). To improve the accuracy of taking phototricogram from the same area, a homemade repositioning device was used instead of tattoo land marking.

Pull test

Approximately 20-60 hairs were grasped between the thumb, index and middle fingers from the base of the hairs near the scalp and firmly, but not forcefully, tugged away from the scalp in three different areas (frontal, temporal, and occipital region). Normally, less than three telogen-phase hairs should come out with each pull. If at least three hairs were obtained with each pull or if more than ten hairs total were

obtained, the pull test was considered positive and suggestive of telogen effiuvium.

Global photography scoring

Photographic pictures at the vertex and frontal level were taken under standard lighting conditions, using a professional digital reflex camera NIKON D300/D600 digital (Nital S.p.A., 10024 Moncalieri, To, Italy) camera equipped with a macro-objective (AF-S Micro NIKKOR 60 mm f/2.8G ED), an independent flash system (Kit R1C1) and with cross- and parallel-polarised filters. Pictures were scored using a standardised seven-point rating scale (+3 greatly increased; +2 moderately increased; +1 slightly increased; 0 no change; -1 slightly decreased; -2 moderately decreased; -3 greatly decreased) [15]. This technique had been demonstrated to have excellent reproducibility [16].

Brightness

Hair and nails brightness was measured using a spectrophotometer/ colorimeter CM-700D (Konica-Minolta, 20092 Cinisello Balsamo, MI, Italy). The measured parameter was the 8 gloss (specularly reflected light).

Nail growth

Nail picture were taken at each checkpoint before and after cutting (Figure 2). Nail plate growth was measured, using a morphometric image analysis technique, as the difference between the total nail length after cutting and the total nail length after 14 days from cutting.

Nail conditions

Pictures were scored using a four-point rating scale (1 no effect; 2 mild effect; 3 moderate effect; 4 strong effect).

Self-assessment questionnaire

At the end of the study, subjects were asked to reply to the questions of a self-assessment questionnaire.

Sample size

Sample size was calculated with a two-sided 5% significance level and a power of 80% taking into account a 20% variation of the primary endpoints due to both inter-individual human variability and error in the measurement techniques. Sample size was calculated using PASS 11 statistical software (version 11.0.8 for Windows) running on Windows Server 2008 R2 Standard SP1 64 bit edition (Microsoft, USA). A sample size of 20 subjects per group was necessary given an anticipated dropout rate of 20%.

Randomisation

A restricted randomisation list was created using PASS 11 (version 11.0.8; PASS, LLC. Kaysville, UT, USA) statistical software running on Windows Server 2008 R2 Standard SP1 64 bit Edition (Microsoft, USA) by a biostatistician and stored in a safe place.

Randomisation sequence was stratified using biased coin Efron's algorithm with a 1:1:1 allocation ratio. The allocation sequence was concealed from the in-site study director in sequentially numbered, opaque, and sealed envelopes, reporting the unblinded treatment allocation (based on subject entry number in the study). The A4 sheet reporting the unblinded treatment was folded to make the envelope impermeable to intense light. After acceptance of the subject in the study the appropriate numbered envelope was opened.

An independent technician dispensed either active or placebo products according to the card inside the envelope. The study adhered to established procedures to maintain separation between the investigator and its collaborators and the staff that delivered the intervention. Investigator and its collaborators who obtained outcome measurements were not informed on the product group assignment. Staff who delivered the intervention did not take outcome measurements. Subjects, investigator and collaborators were kept masked to products assignment. The active and the placebo products were in capsule form and identical in appearance. They were prepacked in blisters and consecutively numbered for each subject according to the randomisation schedule. Each subject was assigned an order number and received the capsules in the corresponding prepacked blister.

Statistical methods

Statistical analysis was performed using NCSS 10 (version 10.0.12 for Windows; NCCS, LLC. Kaysville, UT, USA) running on Windows Server 2008 R2 Standard SP1 64 bit edition (Microsoft, USA). Data normality was checked using Shapiro-Wilk W normality test and data shape. Intragroup (vs. baseline) statistical analysis was carried out using repeated measures analysis of variance (RM-ANOVA) followed by Tukey-Kramer post-test. Intergroup (between treatments) statistical analysis was carried out using RM-ANOVA followed by tests for two factor interactions. A p-value<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

The study was conducted between February and July 2017. A total of 60 female subjects were successfully randomized (Figure 3). The population was caucasian. Demographic and baseline characteristics (Table 2) were similar across treatment arms, indicating an unbiased randomization and the absence of covariates. Subjects attended clinic visits at the time of randomization (baseline) and after 45 and 90 days of product use. Data analysis was intention-to-treat and involved all subjects who were randomly assigned.

Subjects' compliance to treatment was assessed by means of product accountability as follows: at each visit, the expected amount of consumed capsule was compared with the amount dispensed minus the amount the subject returned. No major deviations were observed in the treatment regimen. All subjects were included in the safety analysis data set. All the tested products were well tolerated. No adverse reactions occurred during the study period.

Phototrichogram

A statistical significant increase of hair density was observed both

	CYS	KDC	PLA
Sex			
Female	100%	100%	100%
Phototricogram			
%Telogen	21.2 ± 1.0	21.5 ± 0.8	21.0 ± 0.8
%Anagen	78.8 ± 1.0	78.5 ± 0.8	79.0 ± 0.8
Hair density (hair no/cm^2)	193.1 ± 6.1	195.5 ± 4.8	193.2 ± 7.4
Pull test	12.1 ± 0.3	12.7 ± 0.4	12.3 ± 0.3
Hair radiance	3.33 ± 0.32	3.31 ± 0.38	3.33 ± 0.35
Nail growth rate (mm/14 days)	1.25 ± 0.05	1.21 ± 0.05	1.26 ± 0.06
Nail brightness	7.38 ± 0.63	7.11 ± 0.75	7.28 ± 0.51

Table 2: Demographic and baseline characteristics. Data are means ± SE.

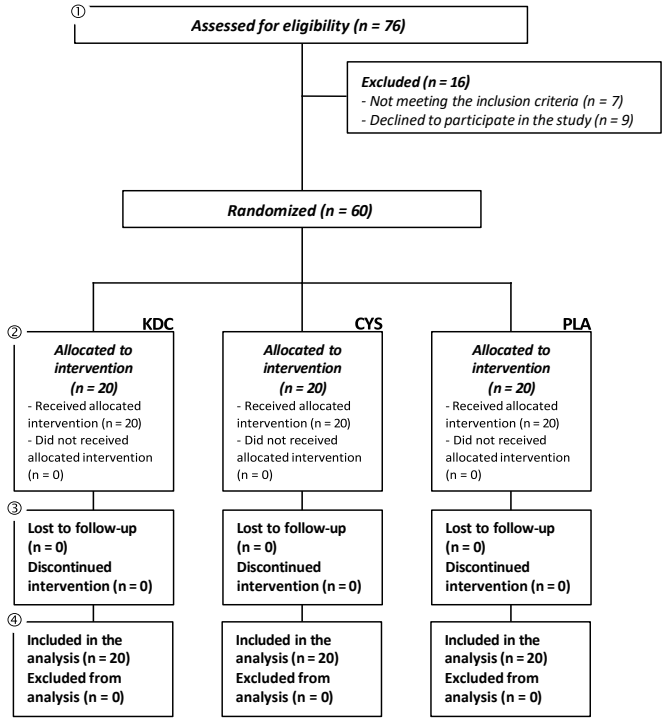


Figure 3: CONSORT 2010 flow diagram. Legend. 1) Enrolment, 2) Allocation, 3) Follow-up, 4) Analysis.

a) Hair density (number/cm²)	**n**	**Mean ± SE**			**Min ÷ Max**		
		KDC	**CYS**	**PLA**	**KDC**	**CYS**	**PLA**
Day 0	20	195.5 ± 4.8a	193.1 ± 6.1a	193.2 ± 7.4a	157.2 ÷ 251.9	152.7 ÷ 252.3	138.3 ÷ 246.2
Day 45	20	198.9 ± 4.8a	198.2 ± 6.1b	194.0 ± 7.1a	160.7 ÷ 247.5	160.1 ÷ 258.5	142.2 ÷ 248.4
Day 90	20	211.3 ± 4.9b	209.4 ± 6.1c	195.0 ± 7.2a	170.3 ÷ 270.4	165.2 ÷ 268.3	145.7 ÷ 256.3
D45-D0	20	3.4 ± 1.0†	5.0 ± 1.2†	0.8 ± 0.8	-4.4 ÷ 11.2	-6.2 ÷ 12.9	-5.5 ÷ 5.9
D90-D0	20	15.8 ± 1.8†	16.3 ± 2.4†	1.8 ± 0.9	-0.3 ÷ 31.3	-3.4 ÷ 40.2	-2.8 ÷ 10.1
b) Anagen hair (%)	**n**	**Mean ± SE**			**Min ÷ Max**		
		KDC	**CYS**	**PLA**	**KDC**	**CYS**	**PLA**
Day 0	20	78.5 ± 0.8a	78.8 ± 1.0a	79.0 ± 0.8a	71.6 ÷ 83.8	71.1 ÷ 84.5	71.6 ÷ 83.7
Day 45	20	82.7 ± 0.7b	83.2 ± 0.7b	78.6 ± 0.7a	74.6 ÷ 89.5	77.9 ÷ 88.8	71.1 ÷ 84.5
Day 90	20	88.2 ± 0.5c	89.1 ± 0.6c	82.2 ± 0.7b	83.6 ÷ 91.9	83.6 ÷ 91.9	74.5 ÷ 87.6
D45-D0	20	4.3 ± 0.5†	4.4 ± 0.6†	-0.4 ± 0.5	0.6 ÷ 8.2	-0.2 ÷ 11.2	-5.5 ÷ 3.2
D90-D0	20	9.7 ± 0.6†	10.3 ± 0.7†	3.2 ± 0.9	4.6 ÷ 13.8	6.0 ÷ 18.2	-1.9 ÷ 12.3
c) Telogen hair (%)	**n**	**Mean ± SE**			**Min ÷ Max**		
		KDC	**CYS**	**PLA**	**KDC**	**CYS**	**PLA**
Day 0	20	21.5 ± 0.8c	21.2 ± 1.0c	21.0 ± 0.8c	16.2 ÷ 28.4	15.5 ÷ 28.9	16.3 ÷ 28.4
Day 45	20	17.3 ± 0.7b	16.8 ± 0.7b	21.4 ± 0.7b	10.5 ÷ 25.4	11.2 ÷ 22.1	15.5 ÷ 28.9
Day 90	20	11.8 ± 0.5a	10.9 ± 0.6a	17.9 ± 0.7b	8.1 ÷ 16.4	8.1 ÷ 16.4	12.4 ÷ 25.5
D45-D0	20	-4.3 ± 0.5†	-4.4 ± 0.6†	0.4 ± 0.5	-8.2 ÷ -0.6	-11.2 ÷ 0.2	-18.2 ÷ -6.0
D90-D0	20	-9.7 ± 0.6†	-10.3 ± 0.7†	-3.2 ± 0.9	-13.8 ÷ -4.6	-5.5 ÷ 3.2	-12.3 ÷ 1.9
d) Pulled hair (hair no.)	**n**	**Mean ± SE**			**Min ÷ Max**		
		KDC	**CYS**	**PLA**	**KDC**	**CYS**	**PLA**
Day 0	20	12.7 ± 0.4c	12.1 ± 0.3c	12.3 ± 0.3c	11 ÷ 16	11 ÷ 16	11 ÷ 16
Day 45	20	9.6 ± 0.5b	8.4 ± 0.5b	12.4 ± 0.5c	5 ÷ 14	3 ÷ 13	10 ÷ 20
Day 90	20	8.3 ± 0.3a	7.2 ± 0.4a	11.0 ± 0.5b	5 ÷ 00	3 ÷ 10	8 ÷ 16
D45-D0	20	-3.1 ± 0.4†	-3.8 ± 0.5†	0.2 ± 0.4	-1 ÷ -7	-1 ÷ -8	4 ÷ -2
D90-D0	20	-4.4 ± 0.4†	-4.9 ± 0.4†	-1.3 ± 0.3	-2 ÷ -8	- ÷ -8	1 ÷ -3

Table 3: Phototricogram and pull testing results. (a) Hair density. (b) Anagen hair. (c) Telogen hair. (d) Pull test results. Significantly different from D0: a<b<c, $p<0.05$. RM-ANOVA followed by Tukey-Kramer post-test.† Significantly different ($p<0.05$) from Placebo. RM-ANOVA followed by tests for two-factor interactions. Data are means ± SE.

in the CYS and KDC treatment groups (Table 3a). The hair density was increased in the CYS treatment group by 5.0 ± 1.2 and by 16.3 ± 2.4, after 45 and 90 days, respectively ($p<0.001$). A similar efficacy profile was seen for the KDC treatment group where hair density was increased by 3.4 ± 1.0 and by 15.8 ± 1.8, after 45 and 90 days, respectively ($p<0.001$). The variation of hair density was not statistically significant in the placebo group ($p>0.05$). Both CYS and KDC hair density variation were statistically significant when compared to the placebo group ($p<0.05$).

A statistical significant increase of anagen hair was observed both in the CYS and KDC treatment groups (Table 3b). The percentage anagen hair was increased in the CYS treatment group by 4.4 ± 0.6 and by 10.3 ± 0.7, after 45 and 90 days, respectively ($p<0.001$). A similar efficacy profile was seen for the KDC treatment group where the percentage of anagen hair was increased by 4.3 ± 0.5% and by 9.7 ± 0.6 after 45 and 90 days, respectively ($p<0.001$). The variation of percentage of anagen hair was not statistically significant in the placebo group ($p>0.05$). Both CYS and KDC variation in the % anagen hair was statistically significant when compared to the placebo group ($p<0.05$).

Specular but opposite results were obtained for the percentage of telogen hair variation (Table 3c).

Pull test

A statistical significant decrease of the number of pulled hair was observed both in the CYS and KDC treatment groups (Table 3d). The number of pulled hair was decreased in the CYS treatment group by 31.1 ± 3.9% and by 40.3 ± 3.5%, after 45 and 90 days, respectively ($p<0.001$).

A similar efficacy profile was seen for the KDC treatment group where the number of pulled hair was decreased by 24.3 ± 3.5% and by 34.0 ± 2.4% after 45 and 90 days, respectively ($p<0.001$). The variation of the number of pulled hair (-10.6 ± 2.6%) was statistically significant in the placebo group after 90 days. Both CYS and KDC variation in the number of puller hair was statistically significant when compared to the placebo group ($p<0.05$). Interestingly, both for CYS and KDC the number of pulled hair after 45 days is not suggestive of telogen effiuvium diagnosis.

Global photography scoring

Figure 4 shows the macroscopic effect of the product in decreasing hair loss. Hair volume and nail conditions were improved both in the CYS and KDC treatment groups. The subjects showing an improvement of hair volume in the CYS treatment group were 5% and 60%, after 45 and 90 days. A similar efficacy profile was seen for the KDC treatment group where the improved subjects were 40% and 75%, after 45 and 90 days.

The improvement was statistically significant for both the CYS and KDC treatment groups when compared to the placebo group (5% and 10% of the subjects, after 45 and 90 days). The subjects showing an improvement of nail conditions in the CYS treatment group were 30% and 65%, after 45 and 90 days. A similar efficacy profile was seen for the KDC treatment group where the subjects improved were 35% and 70%, after 45 and 90 days. The improvement was statistically significant for both the CYS and KDC treatment groups when compared to the placebo group (15% and 20% of the subjects, after 45 and 90 days).

Hair and nails brightness

A statistical significant increase of both hair and nail brightness (Table 4) was observed both in the CYS and KDC treatment groups. Hair brightness in the CYS treatment group was improved by 27.2%

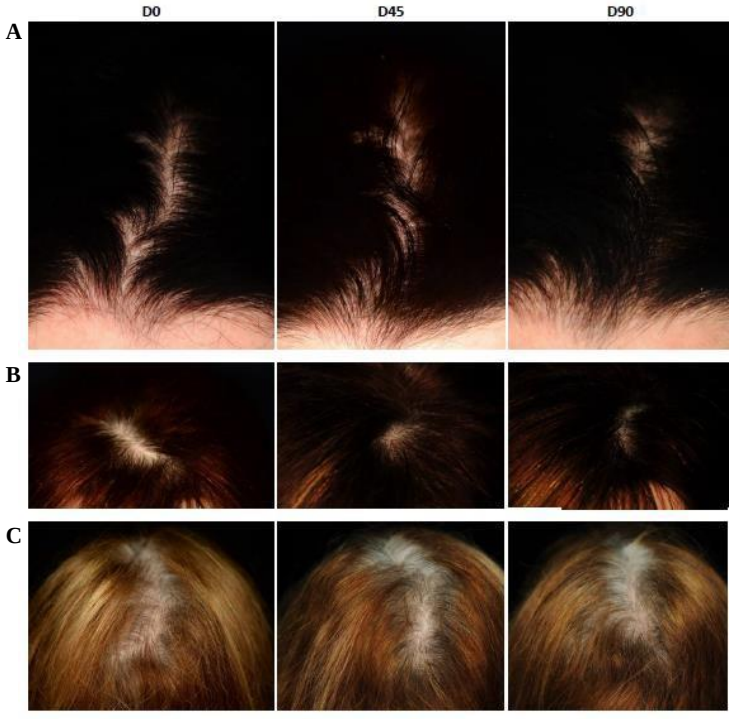
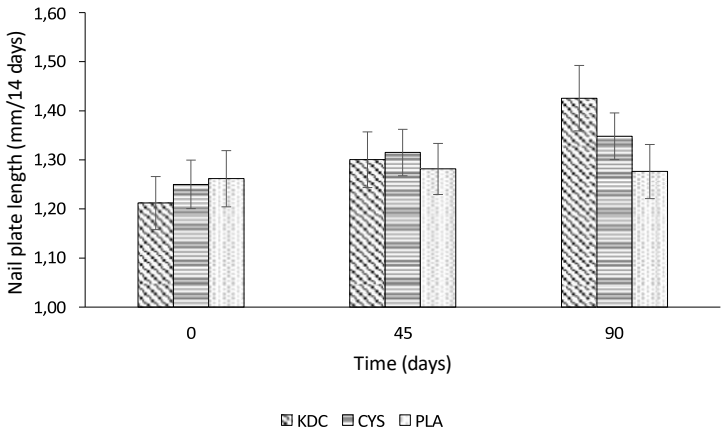


Figure 4: Global photography assessment a) KDC group b) CYS group c) PLA group.

Hair brightness (au)		Mean ± SE			Min ÷ Max		
	n	KDC	CYS	PLA	KDC	CYS	PLA
Day 0	20	3.31 ± 0.38a	3.33 ± 0.32a	3.33 ± 0.35a	1.08 ÷ 6.61	1.03 ÷ 5.64	1.04 ÷ 6.71
Day 45	20	4.08 ± 0.38b	4.29 ± 0.46b	3.28 ± 0.36a	1.55 ÷ 7.17	1.17 ÷ 8.11	1.08 ÷ 6.33
Day 90	20	4.85 ± 0.43c	5.19 ± 0.54c	3.53 ± 0.39a	1.78 ÷ 9.59	1.38 ÷ 9.17	1.17 ÷ 7.63
D45-D0	20	+31.4%†	+27.2%†	-1.90%	2.4% ÷ 96.3%	-5.0% ÷ 70.9%	-24.6% ÷ 18.3%
D90-D0	20	+58.9%†	+56.9%†	+6.40%	3.9% ÷ 132.4%	1.3% ÷ 137.1%	-22.0% ÷ 36.5%
Nail brightness (au)		**Mean ± SE**			**Min ÷ Max**		
	n	**KDC**	**CYS**	**PLA**	**KDC**	**CYS**	**PLA**
Day 0	20	7.11 ± 0.75a	7.38 ± 0.63a	7.28 ± 0.51a	1.26 ÷ 12.24	3.64 ÷ 13.24	4.82 ÷ 12.66
Day 45	20	9.02 ± 0.92b	9.42 ± 1.05b	7.76 ± 0.59a	1.95 ÷ 15.22	5.64 ÷ 24.61	5.00 ÷ 13.45
Day 90	20	10.74 ± 0.99c	10.50 ± 0.66b	8.45 ± 0.69b	2.78 ÷ 17.93	5.88 ÷ 17.57	5.11 ÷ 14.85
D45-D0	20	+28.5%†	+29.6%†	+6.70%	3.3% ÷ 57.3%	-34.2% ÷ 123.4%	-16.1% ÷ 33.9%
D90-D0	20	+58.4%†	+54.5%†	+17.20%	29.0% ÷ 120.6%	-20.4% ÷ 258.0%	-24.1% ÷ 66.1%

Table 4: Hair and nail brightness. Significantly different from D0: a<b<c, p<0.05. RM-ANOVA followed by Tukey-Kramer post-test.†Significantly different (p<0.05) from Placebo. RM-ANOVA followed by tests for two-factor interactions. Data are means ± SE.



Growth rate (mm/14dd)	n	Mean ± SE KDC	CYS	PLA	Min ÷ Max KDC	CYS	PLA
Day 0	20	1.21±0.05^a	1.25±0.05^a	1.26±0.06^a	0.68÷1.59	0.89÷1.62	0.61÷1.60
Day 45	20	1.30±0.06^b	1.32±0.05^b	1.28±0.05^a	0.75÷1.69	0.93÷1.67	0.78÷1.65
Day 90	20	1.43±0.07^c	1.35±0.05^c	1.28±0.06^a	0.79÷2.04	0.98÷1.66	0.69÷1.68
D45-D0	20	+0.09†	+0.06†	+0.02	0.02÷0.15	0.01÷0.13	-0.04÷0.17
D90-D0	20	+0.21†	+0.10†	+0.01	0.08÷0.45	0.02÷0.19	-0.03÷0.08

Figure 5: Nail growth rate. Significantly different from D0: a<b<c, p<0.05. RM-ANOVA followed by Tukey-Kramer post-test. † Significantly different (p<0.05) from Placebo. RM-ANOVA followed by tests for two-factor interactions. Data are means ± SE.

and 56.9%, after 45 and 90 days. A similar efficacy profile was seen for the KDC treatment group where hair brightness was improved by 31.4% and 58.9%, after 45 and 90 days. Both CYS and KDC hair brightness variation was statistically significant when compared to the placebo group (p<0.05).

Nail brightness in the CYS treatment group was improved by 29.6% and 54.5%, after 45 and 90 days. A similar efficacy profile was seen for the KDC treatment group where hair brightness was improved by 28.5% and 58.4%, after 45 and 90 days. Both CYS and KDC hair brightness variation were statistically significant when compared to the placebo group (p<0.05).

Nail growth rate

A statistical significant increase of nail growth rate was observed both in the CYS and KDC treatment groups (Figure 5). The nail growth rate in the CYS treatment group was 0.06 ± 0.01 mm/14days and 0.10 ± 0.01 mm/14days, after 45 and 90 days, respectively (p<0.001). A similar efficacy profile was seen for the KDC treatment group where nail growth rate was 0.09 ± 0.01 mm/14days and 0.21 ± 0.02 mm/14 days, after 45 and 90 days, respectively (p<0.001). The nail growth rate was not statistically significant in the placebo group (p>0.05). Both CYS and KDC nail growth rate was statistically significant when compared to the placebo group (p<0.05).

Self-assessment questionnaire

The complete results of the self-assessment questionnaire are reported in Figure 6. Both CYS and KDC were perceived more effective than PLA. Subjects' answers after 90 days product use are very positive with 70% global satisfaction for L-Cystine for hair and nails.

It should be noted that this percentage is better with the association L-Cystine+Kera-Diet® which obtains 90% of global satisfaction. On the other hand, placebo effect is only 35%; this underlines that all improvement of objectives criteria are enough visible to be perceived by women of CYS and KDC groups.

Discussion

Shining and healthy hair and nails is the attribute of healthy people looking after themselves and taking good care of their body, but for

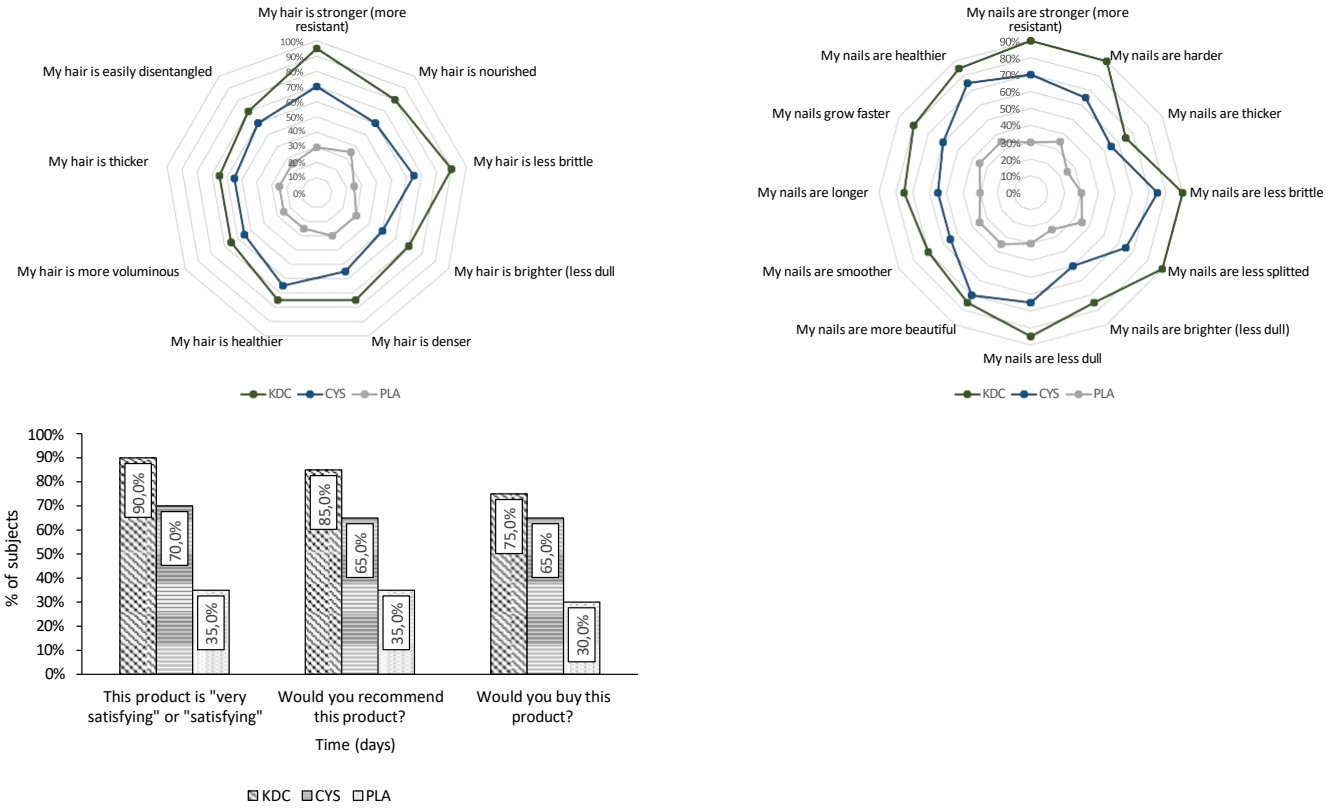


Figure 6: Self-assessment questionnaire. (a) Hair self-assessment questionnaire output. (b) Nail self-assessment questionnaire output. (c) Overall Self-assessment.

women, such properties are also a decoration, which gives them sense of wellbeing.

It is nowadays clear that the nutrients of diet have a direct impact on the structure and growth of both hair and nails. While hair follicles are among the most metabolically active in the body, and hair growth may be impacted by calorie and protein malnutrition as well as micronutrient deficiency, the links are complex. Effects on hair growth, including acute telogen effiuvium, are a well-known effect of sudden weight loss or decreased protein intake [17]. It has also been reported potential associations between nutritional deficiency and chronic telogen effiuvium, androgenetic alopecia, female pattern hair loss, and alopecia areata [18,19].

In recent years, use of food supplements has increased both in Europe and in the USA with many physicians recommending them [20,21]. A survey of health professionals conducted in 2008 showed that 66% of dermatologists (n=300) recommended dietary supplements to patients in relation to skin, hair, and nail health and 79% of clinicians personally used supplements [17]. A search of the keywords “hair loss” within the Vitamins and Dietary Supplements section of an important digital market place, which sells supplements via Internet sales, yields 923 products, many of them being different formulations [22]. While such products contain a variety of nutrients, the review of the medical literature finds a notable lack of evidence supporting their use. In fact, even if some studies are arising in the scientific literature confirming the efficacy of food supplements, some of them used not reliable or standardised methods, and the study design sometimes does not consider the placebo group.

In this study, we investigated the efficacy of two ingredients to be used in food supplements containing, L-Cystine and hydrolysate of natural keratin, minerals, and vitamins. In order to reach this goal, a placebo-controlled, double-blind study was carried out in women showing the clinical signs of aTE and BNS. Our results show clearly that, after 45 days of supplementation, these criteria are significantly enhanced with these ingredients compared to placebo.

Results demonstrate also that the observed effects are always better at 90 days in comparison to the 45 days results, underlining the importance of treatment duration.

The self-assessment questionnaire shows that the improvement, instrumentally measured and clinically evaluated, is visible enough to be perceived by women; answers to self-assessment questionnaire by enrolled subjects after 90 days are very positive, especially for the KDL group, which underline benefits for the customers with this association. This illustrates that it is possible to visually enhance hair and nail health and status, even among healthy and well-nourished people.

Conclusion

In conclusion, this study provides evidence in supporting the hypothesis that L-Cystine or a combination of a hydrolysate of natural keratin (Kera-Diet®) and L-Cystine could represent a safe and effective approach in reducing hair loss during acute telogen effiuvium and in improving brittle nail conditions. Specifically, we have shown that a 90-days intervention period with the test products is beneficial for telogen hair decrease and for increasing nail growth rate. Therefore and more

generally, this study demonstrates that L-Cystine alone or a mix Kera-Diet®+L-Cystine, associated with traced elements and specific vitamins at the right dosage can enhance hair and nail conditions, even though human nutrition is more and more balanced.

References

1. Malkud S (2015) Telogen Effluvium: A Review. J Clin Diagn Res 9:WE01-WE03.
2. Hughes EC, Taneja A (2018) Telogen Effluvium. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing.
3. França K, Rodrigues TS, Ledon J, Savas J, Chacon A (2013) Comprehensive overview and treatment update on hair loss. Journal of Cosmetics, Dermatological Science and Applications 3: 1-8.
4. Kanti V, Messenger A, Dobos G, Reygagne P, Finner A, et al. (2018) Evidence-based (S3) guideline for the treatment of androgenetic alopecia in women and in men - short version. J Eur Acad Dermatol Venereol 32: 11-12.
5. de Koning EB, Passchier J, Dekker FW (1990) Psychological problems with hair loss in general practice and the treatment policies of general practitioners. Psychol Rep 67: 775-778.
6. Cash TF (2001) The psychology of hair loss and its implications for patient care. Clin Dermatol 19: 161-166.
7. Hadshiew IM, Foitzik K, Arck PC, Paus R (2004) Burden of hair loss: stress and the underestimated psychosocial impact of telogen effluvium and androgeneticalopecia. J Invest Dermatol. 123: 455-457.
8. Girman CJ, Hartmaier S, Roberts J, Bergfeld W, Waldstreicher J (1999) Patient-perceived importance of negative effects of androgenetic alopecia in women. J Womens Health Gend Based Med 8:1091-1095.
9. Mackay-Wiggan J, Marji J, Walt JG, Campbell A, Coppola C, et al. (2014) Topical cyclosporine versus emulsion vehicle for the treatment of brittle nails: a randomized controlled pilot study. J Drugs Dermatol 13:1232-1239.
10. van de Kerkhof PC, Pasch MC, Scher RK, Kerscher M, Gieler U, et al. (2005) Brittle nail syndrome: a pathogenesis-based approach with a proposed grading system. J Am Acad Dermatol 53: 644-651.
11. Lubach D, Cohrs W, Wurzinger R (1986) Incidence of brittle nails. Dermatologica 172: 144-147.
12. Gequelim GC, Kubota CY, Sanches S, Dranka D, Mejia MM, et al. (2013) Perception of brittle nails in dermatologic patients: a cross-sectional study. An ras Dermatol 88: 1022-1025.
13. Iorizzo M (2015) Tips to treat the 5 most common nail disorders: brittle nails, onycholysis, paronychia, psoriasis, onychomycosis. Dermatol Clin 33: 175-183.
14. Schmitt JV, Ribeiro CF, Souza FH, Siqueira EB, Bebber FR (2012) Hair loss perception and symptoms of depression in female outpatients attending a general dermatology clinic. An Bras Dermatol 87: 412-417.
15. Leyden J, Dunlap F, Miller B, Winters P, Lebwohl M, et al. (1999) Finasteride in the treatment of men with frontal male pattern hair loss. J Am Acad Dermatol 40: 930-937.
16. Kaufman KD, Olsen EA, Whiting D, Savin R, DeVillez R, et al. (1998) Finasteride in the treatment of men with androgenetic alopecia. Finasteride male pattern hair loss study group. J Am Acad Dermatol 39: 578-589.
17. Mubki T, Rudnicka L, Olszewska M, Shapiro J (2014) Evaluation and diagnosis of the hair loss patient: part I. History and clinical examination. J Am Acad Dermatol 71: 415.e1-e415.e15.
18. Goldberg LJ, Lenzy Y (2010) Nutrition and hair. Clin Dermatol 28:412-419.
19. Finner AM (2013) Nutrition and hair: deficiencies and supplements. Dermatol Clin 31: 167-172.
20. Dickinson A, Shao A, Boyon N, Franco JC (2011) Use of dietary supplements by cardiologists, dermatologists and orthopedists: report of a survey. Nutrition Journal 10: 20.
21. Skeie G, Braaten T, Hjartåker A, Lentjes M, Amiano P, et al. (2009) Use of dietary supplements in the European Prospective Investigation into Cancer and Nutrition calibration study. European Journal of Clinical Nutrition 63: S226-S238.
22. Guo EL, Katta R (2017) Diet and hair loss: effects of nutrient deficiency and supplement use. Dermatol Pract Concept 7: 1-10.