

Hair loss in older women: supplement to increase scalp hair thickness and reduce hair loss

Floriana Mastropietro¹, Noemi Corasaniti¹, Marco Piacentini¹, Leonardo Celleno^{1,2}

Correspondence to: Marco Piacentini, marcopiacentini@eurofins.com

ABSTRACT

Hair loss and thinning hair are serious problems affecting a large number of women, especially during the menopause. A food supplement containing fermented soy, ornithine, arginine and Tricovel® Tricoage45+ with Bioequolo (Giuliani SpA, Milan, Italy), formulated to promote hair growth in women over the age of 45, was used in a double-blind, placebo-controlled study conducted in healthy adult females going through the menopause. Subjects with hair loss and less than 80% of their hair in the anagen phase were randomized to receive a single daily dose of the study product or placebo. Wash test results showed that hair loss was significantly reduced after the first 8 weeks of treatment with the study product. Hair pull test results also showed significant improvements (fewer hairs removed) in subjects treated with the study product vs. placebo after 8 and 12 weeks of treatment. Moreover, analysis of microscope images revealed a statistically significant increase in the conversion of vellus hair into terminal hair after 12 weeks of treatment with the study product compared with placebo. Self-evaluation by the subjects confirmed these improvements. This study shows that the dietary supplement can decrease hair loss and promote hair growth in women over 45 years of age.

Keywords

Clinical trial
Anti-hair loss supplement
Women
Menopause

Introduction

Hair is an important part of the human body and affects appearance and self-image [1]. Therefore, a reduction in scalp hair can cause psychological trauma and reduce quality of life [2]. Causes of hair loss in women include aging, a poor diet, stress and, in particular, the menopause.

Female pattern hair loss (FPHL) is the most common cause of diffuse hair loss in women. It typically presents as a diffuse reduction in hair density affecting the mid and frontal regions of the scalp with preservation of the frontal hairline, and is characterized by progressive follicular miniaturization and the conversion of terminal hair into vellus hair [3]. The anagen phase of vellus hair is briefer, resulting in the production of shorter and finer hairs [4, 5].

Hair thinning is common in women and increases with age. Two studies in Caucasian women in the UK and USA reported a 3–6% prevalence of reduced hair density in women under 30 years of age, increasing to 29–42% in older women, with a peak at 50 years of age [6, 7]. During the menopause, hair loss is usually a direct result of fluctuating levels of hor-

mones, including androgens which regulate hair growth. The progressive miniaturization of scalp hair is mediated by the conversion of testosterone to dihydrotestosterone (DHT), with the reaction catalyzed by the enzyme 5-alpha reductase. Compared to testosterone, DHT has approximately a fivefold greater affinity for the androgen receptor [8].

The hair follicle is a very complex mini-organ that exhibits high cellular activity and turnover [9, 10]. The high metabolic activity is linked to the supply, uptake and transport of nutrients including trace elements and vitamins. An adequate supply of high-quality amino acids is crucial as hair consists mostly of protein [9, 11, 12]. Accordingly, the body's nutritional status affects the quantity and quality of hair. Moreover, the requirements for certain types and amounts of nutrients change with age and are influenced by lifestyle. For instance, vegans or vegetarians might need extra vitamins, minerals and protein [13]. Consequently, a supplement (Tricovel® Tricoage45+ with Bioequolo) was formulated for use by women over 45 years of age and affected by hair loss.

The supplement contains ingredients essential for the health and tropism of hair, such as amino acids, vitamins (including vitamins H, B9 and B5 which are important for metabolism and hair follicle health), antioxidants (to counteract the formation of free radicals and neutralize their damaging effect on the hair growth cycle), and some trace elements (zinc

¹Eurofins Cosmetics, Clinical Testing, Rome, Italy

²Dermatology Department, Catholic University of the Sacred Heart, Rome, Italy

and copper). The formula also contains soy isoflavones and fermented soy. Soy and its phytoestrogens, including the isoflavones genistein and daidzein, play an important role in hair follicle health [14] due to their estrogenic and antioxidant effects. In particular, a daidzein metabolite, equol, has higher antioxidant activity than isoflavones [15]. In humans, intestinal microflora metabolize dietary soy isoflavones to equol. However, as approximately 70% of Caucasians are unable to produce equol [16], dietary sources of equol are important and include fermented soybean products (like tempeh), where specific strains of bacteria metabolize daidzein into equol [17], thus making it available to individuals unable to metabolize it themselves.

The supplement also contains *Ajuga reptans* extract, titrated at 50% of phenylpropanoids, which has strong antioxidant activity and inhibits 5- α reductase enzyme [18]. The new formulation also contains amino acids involved in the synthesis of polyamines, particularly spermidine, which is important for hair growth regulation [19, 20] and for reducing hair loss especially in severe alopecia, such as chronic telogen effluvium in women [21].

Materials and methods

Subjects

A total of 40 eligible female subjects were enrolled in this double-blind, placebo-controlled study. The study was conducted by the contract research organization Eurofins Cosmetics, Clinical Testing, Rome, Italy.

Inclusion and exclusion criteria

Healthy female subjects aged 45–65 years going through the menopause, suffering from hair loss, and with less than approximately 80% of their hair in the anagen phase were enrolled. The wash and pull tests were used to determine the percentage of anagen hair: if approximately 200 hairs were shed during washing or if more than 18 hairs could be pulled out, then this indicated a high percentage of hair in the telogen phase.

Exclusion criteria included: a personal history of adverse reactions to products similar to the study product, use of minoxidil or finasteride (Propecia®) during the previous 6 months, use of oral or topical cosmetic anti-hair loss treatment during the previous 3 months, surgical correction for alopecia within the previous 12 months, dermatological scalp disorders which could affect interpretation of the results (psoriasis, eczema, inflammatory acne), trichotillomania, previous use of medications which might affect the

results, particularly systemic retinoids within 6 months, topical retinoids within 2 months, topical or systemic anti-inflammatories or antihistamines within 2 weeks, antibiotics within 2 weeks, medication for malignancy (of any kind) within the previous 5 years, desensitization treatment within the previous 6 months, previous, revised or anticipated hormonal treatment, excessive or intensive exposure to natural or artificial sunlight within the previous month or anticipated UV exposure during the study.

Test product

Subjects were randomized in a double-blind design to receive the study product (Tricovel® Tricoage45+ with Bioequolo; Giuliani SpA, Milan) or placebo (Table 1). The placebo consisted of inert tablets with a similar appearance to the study product. Subjects were instructed to take one tablet of the assigned treatment each day at breakfast or lunch, with water.

Study procedures

The study lasted 12 weeks (84 days). The first day of product use was designated 'D1' (Table 2).

Nutritional factors	One tablet	% NRV
Fermented soy	100 mg	–
L-Ornithine	50 mg	–
L-Arginine	50 mg	–
Soy isoflavones	20 mg	–
Pantothenic acid	6 mg	100%
Zinc	5 mg	50%
<i>Ajuga reptans</i> dry extract	2.5 mg	–
Copper	1 mg	100%
Rutin	1 mg	–
Folic acid	200 μ g	100%
Biotin	50 μ g	100%
Resveratrol	0.05 mg	–

NRV Nutrient Reference Value

Table 1 - Nutritional factors in Tricovel® Tricoage45+ with Bioequolo

Activity	D-2	D1	W4	W8	W12
Study product used		√	√	√	√
Wash test	√			√	√
Hair pull test		√		√	√
Hair diameter measurement		√		√	√
Microscopy analysis of scalp		√		√	√
Self-evaluation			√	√	√

Table 2 - Study plan

Wash test

The wash test consisted of washing the hair a week after the last shampoo and counting the number of hairs shed into the wash basin, with gauze placed over the drain. A collection of 250–300 hairs indicated hair loss.

Subjects did not wash their hair for 7 days or brush or comb it for 2 hours before receiving a scalp and hair wash at the study centre.

The wash test was carried out by technicians:

- 2 days before first use of the study product, at D–2
- 8 weeks ±2 days after D1, at W8
- 12 weeks ±2 days after D1, at W12.

Hair pull test

The hair pull test (HPT) consisted of gentle pulling of the hair to bring about shedding of telogen hairs, thus indicating the resistance of hair to traction. The HPT requires gentle pulling using the thumb and the forefinger of about 60 hairs, on three different areas of the scalp, and scoring of hair loss. Technicians at the investigating centre applied the HPT to the frontotemporal, parietal and occipital areas of the scalp of subjects who had not washed their hair for 2 days or brushed or combed it for 2 hours before the test:

- on D1, before the first use of the study product, at D1/t0
- 8 weeks ±2 days after D1, at W8
- 12 weeks ±2 days after D1, at W12.

Scalp analysis using a microscope

Analysis using a microscope was employed to evaluate the condition of the scalp (redness, dandruff, dryness) and identify possible growth of new hairs. Analysis was performed using a DermaScope 50× video camera.

Technicians at the investigating centre examined the fronto-temporal area of the scalp in subjects who had not washed their hair for 2 days or brushed or combed it for 2 hours before the examination.

Analysis using a microscope was carried out:

- on D1, before the first study product use, at D1/t0
- 8 weeks ±2 days after D1, at W8
- 12 weeks ±2 days after D1, at W12.

Self-evaluation questionnaire

The test subjects were interviewed by technicians at the study centre at week 4 (W4), week 8 (W8) and week 12 (W12), and rated their opinion of various statements using a four-point scale where 3=agree, 2=slightly agree, 1=slightly disagree, and 0=disagree (Table 3).

The technician noted on the report form if the test subject was unable to comment. The results were then used to compare the answers of subjects using the study product with those using the placebo.

Statistical analysis

All the data were subjected to statistical analysis using Student’s t-test for repeated measures and for independent samples. The results for the study products in the self-evaluation test were validated by verifying that the difference between the groups was statistically significant.

Ethics

The study was performed in accordance with:

- the general principles of medical ethics in clinical research arising from the Declaration of Helsinki (June 1964) and its successive amendments;

Statements	Answer				Note
	3=agree	2=slightly agree	1=slightly disagree	0=disagree	
Hair is fuller					
Hair is thicker					
Hair is stronger					
Hair is shiner					
Hair is easier to comb					
Hair is less dry					
Hair loss is visibly less					
Hair has started re-growing					
Hair quality has improved					
I am satisfied with the results					

Table 3 - Interview report form

- the international recommendations relating to Good Clinical Practices for conducting clinical trials for drugs ICH E6(R1) of 10/06/1996 (CPMP/ICH/135/95);
- the Directive of the European Parliament and Council 2001/20/EC concerning the harmonization of legislative, statutory and administrative provisions of the member States relating to the application of good clinical practices when conducting clinical trials for drugs for human use – OJ/EC of 01/05/2001;
- the recommendations of COLIPA of August 1997 concerning assessment of human skin compatibility;
- European Directive 95/46/EC on the protection of individuals with regard to the processing of personal data and on the free movement of such data.

Results

Subjects were randomized to receive Tricovel® Tricoage45+ with Bioequolo (N=20) or placebo (N=20).

The mean (\pm SEM) age of subjects was 55 ± 7.32 years in the study product group and 55 ± 5.28 years in the placebo group. No clinical signs attributable to the study products were observed by the investigator during the study. The products were well tolerated, only one drop-out was reported in the placebo group, and no discomfort or adverse reactions were reported by any of the subjects during the study.

Wash test

The mean (\pm SEM) number of hairs shed during the wash test in the placebo group was 294.11 ± 45.27 at baseline, 296.79 ± 45.26 at W8 and 291.00 ± 44.13 at W12. In contrast, the mean number of hair shed at baseline in the Tricoage45+ group was 293.30 ± 41.84 at baseline, decreasing to 270.00 ± 44.70 at W8 and to 250.05 ± 43.22 at W12. These results showed a statistically significant difference in the number of hairs shed at W8 ($p<0.05$) and W12 ($p<0.05$) in subjects treated with Tricovel® Tricoage45+ with Bioequolo. In particular, at W12, 10% of subjects treated with Tricovel® Tricoage45+ with Bioequolo reported a slight reduction in the number of hairs shed, 50% an appreciable reduction, and 40% a large reduction. In contrast, no significant change in the number of hairs shed during the wash test in the placebo group was noted at W8 or W12. In particular, 40% of test subjects in the placebo group reported a slight increase in number of hairs shed, 5% no change, and 55% a slight reduction.

The reduction in hair loss during the wash test in the Tricovel® Tricoage45+ with Bioequolo group was statistically

significant ($p<0.05$) and higher than in the placebo group at W8 and W12 (Fig. 1).

Hair pull test

The mean (\pm SEM) number of hairs pulled out in the HPT in placebo-treated subjects was 21.16 ± 2.41 at baseline, 20.68 ± 2.56 at W8, and 20.42 ± 2.97 at W12. In the Tricovel® Tricoage45+ with Bioequolo group, the mean (\pm SEM) number of hairs pulled out was 22.10 ± 2.53 at baseline, 19.80 ± 2.50 at W8, and 17.40 ± 1.88 at W12.

A statistically significant reduction was seen in the number of hair pulled out after W8 ($p<0.05$) and W12 ($p<0.05$) in subjects treated with Tricovel® Tricoage45+ with Bioequolo. In particular, after 12 weeks of treatment, 20% of subjects treated with Tricovel® Tricoage45+ with Bioequolo reported a slight reduction in the number of hairs pulled out, 45% an appreciable reduction, and 35% a large reduction.

In contrast, there was no statistically significant change in the number of hair pulled out at W8 or W12 in the placebo group: 35% of placebo subjects showed a slight increase, 10% no change, 50% a slight reduction, and 5% a large reduction. The decrease in the number of hair pulled out after Tricovel® Tricoage45+ with Bioequolo supplementation compared with placebo was statistically significant at W8 and W12 ($p<0.05$ and $p<0.05$, respectively) (Fig. 2).

Scalp analysis using a microscope

Scalp analysis using a microscope showed that Tricovel® Tricoage45+ with Bioequolo treatment promoted thicker and fuller hair. Moreover, appreciable conversion from vellus to terminal hairs was noted at W12 in 65% of subjects treated

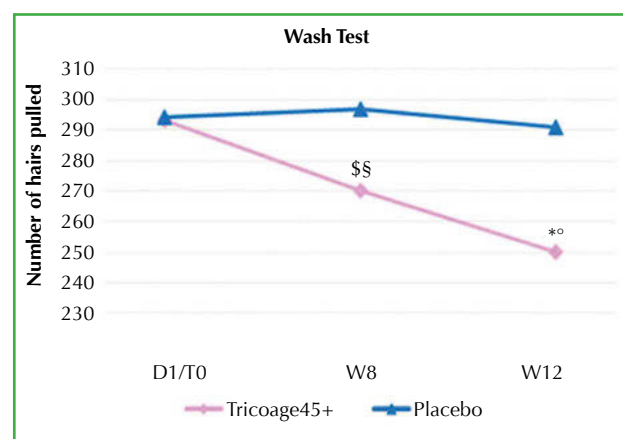


Figure 1 - Wash test results expressed as the mean (\pm SEM) number of hairs shed during the test in the Tricovel® Tricoage45+ with Bioequolo group and the placebo group. $\$p<0.05$, Tricovel® Tricoage45+ with Bioequolo vs. D1; $^{\circ}p<0.05$, Tricovel® Tricoage45+ with Bioequolo vs. D1; $\$p<0.05$, Tricovel® Tricoage45+ with Bioequolo vs. placebo at W8; $^{\circ}p<0.05$, Tricovel® Tricoage45+ with Bioequolo vs. placebo at W12.

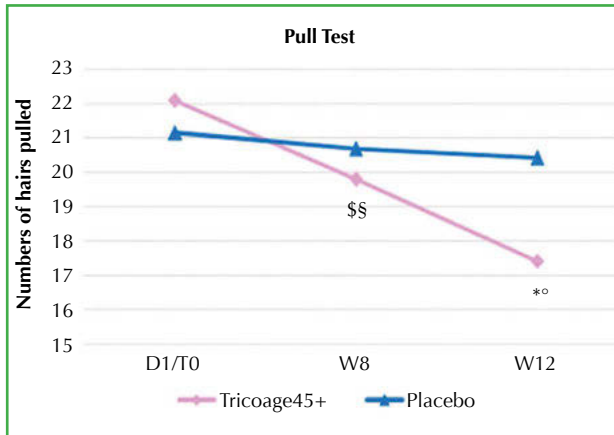


Figure 2 - Number of hair pulled out during the hair pull test (mean±SEM) in the Tricovel® Tricoage45+ with Bioequolo group and the placebo group. $\$p < 0.05$, Tricovel® Tricoage45+ with Bioequolo vs. D1; $^{\circ}p < 0.05$, Tricovel® Tricoage45+ with Bioequolo vs. placebo at W8; $^{*}p < 0.05$, Tricovel® Tricoage45+ with Bioequolo vs. placebo at W12.

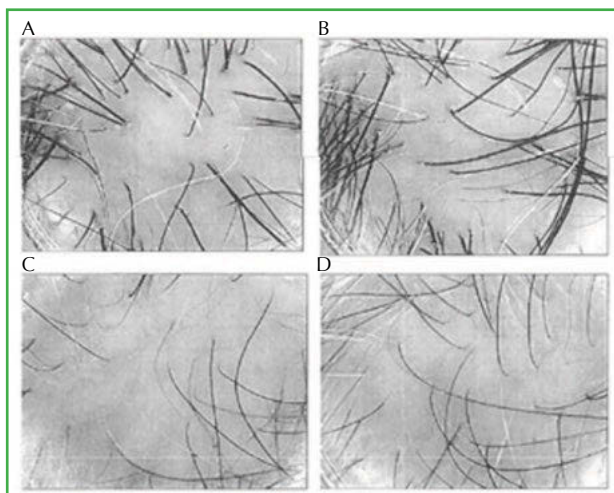


Figure 3 - Representative images from subject treated with Tricovel® Tricoage45+ with Bioequolo (a, b) and placebo (c, d) at the beginning and at the end of the study

with the study product. In contrast, placebo treatment did not result in greater hair thickness (Fig. 3).

Self-evaluation questionnaire

Data collected during the interviews showed that subjects treated with Tricovel® Tricoage45+ with Bioequolo reported better results than subjects in the placebo group (Table 4).

Discussion and conclusions

Poor hair growth and an increase in hair loss in women over 45 years of age is attributed to changes in hormonal status during the menopause, advancing age [22, 23], poor diet, psychological stress or a combination of these factors. Chronological aging cannot be slowed down, but biological aging might possibly be through the use of antioxidants to counteract oxidative stress and the resulting damage to cells. The quantity and quality of hair reflects the nutritional status of the individual, with the amount and variety of nutrients required varying with age. Therefore, the formulation of supplements must take these changing needs into account. This study demonstrated that the studied food supplement (Tricovel® Tricoage45+ with Bioequolo) shows anti-hair loss and hair promoting effects in menopausal women over the age of 45. In particular, the anti-hair loss efficacy of the product (as measured by the wash test) was observed in 90% of test subjects, and resistance of hair to traction (as measured by the HPT) was observed in 80% of test subjects, indicating stronger hair. Moreover, conversion from vellus hair to terminal hair was seen in 65% of test subjects. These clinical effects were confirmed by the results of the self-evaluation questionnaire. These data suggest that a nutritional approach focused on improving hair follicle health can be effective

Statement	Tricoage45+			Placebo		
	W4	W8	W12	W4	W8	W12
Hair is fuller	70%	95%	100%	16%	21%	26%
Hair is thicker	80%	90%	95%	26%	21%	26%
Hair is stronger	75%	95%	95%	21%	32%	32%
Hair is shiner	75%	95%	95%	21%	21%	26%
Hair is easier to comb	70%	95%	100%	21%	16%	32%
Hair is less dry	80%	95%	100%	32%	32%	26%
Hair loss is visibly less	80%	95%	100%	32%	26%	26%
Hair has started re-growing	75%	90%	100%	21%	16%	26%
Hair quality has improved	80%	100%	100%	26%	32%	32%
I am satisfied with the results	95%	100%	100%	26%	32%	37%

Table 4 - Self-evaluation results at week 4 (W4), week 8 (W8) and week 12 (W12) for subjects treated with Tricovel® Tricoage45+ with Bioequolo or placebo

in counteracting hair loss and promoting hair growth. The results suggest that a drug-free approach is possible and effective for treating hair loss and thinning hair in women over 45 years of age.

REFERENCES

- Cash TF (2001) The psychology of hair loss and its implications for patient care. *Clin Dermatol* 19:161–166
- Hadshiew IM, Foitzik K, Arck PC, Paus R (2004) Burden of hair loss: stress and the underestimated psychosocial impact of telogen effluvium and androgenetic alopecia. *J Invest Dermatol* 123:455–457
- Trueb RM (2002) Molecular mechanisms of androgenetic alopecia. *Exp Gerontol* 37:981–990
- Yip L, Rufaut N, Sinclair R (2011) Role of genetics and sex steroid hormones in male androgenetic alopecia and female pattern hair loss: an update of what we now know. *Australas J Dermatol* 52:81–88
- Dinh QQ, Sinclair R (2007) Female pattern hair loss: current treatment concepts. *Clin Interv Aging* 2:189–199
- Birch MP, Messenger JF, Messenger AG (2001) Hair density, hair diameter and the prevalence of female pattern hair loss. *Br J Dermatol* 144:297–304
- Norwood OT (2001) Incidence of female androgenetic alopecia (female pattern alopecia). *Dermatol Surg* 27:53–54
- Kaufman KD (1996) Androgen metabolism as it affects hair growth in androgenetic alopecia. *Dermatol Clin* 14:697–711
- Stenn KS, Paus R (2001) Controls of hair follicle cycling. *Physiol Rev* 81:449–494
- Weinstein GD, Mooney KM (1980) Cell proliferation kinetics in the human hair root. *J Invest Dermatol* 74:43–46
- Gold RJ, Scriver CG (1971) The amino acid composition of hair from different racial origins. *Clin Chim Acta* 33:465–466
- Tea I, Le Guennec A, Frasset-Darrieux M, Julien M, Romek K, Antheaume I, Hankard R, Robins RJ (2013) Simultaneous determination of natural-abundance $\delta^{15}N$ values and quantities of individual amino acids in proteins from milk of lactating women and from infant hair using gas chromatography/isotope ratio mass spectrometry. *Rapid Commun Mass Spectrom* 27:1345–1353
- Dwyer JT (1994) Vegetarian eating patterns: science, values, and food choices – where do we go from here? *Am J Clin Nutr* 59(5 Suppl):1255S–1256S
- Ohnemus U, Uenalan M, Inzunza J, Gustafsson JA, Paus R (2006) The hair follicle as an estrogen target and source. *Endocr Rev* 27(6):677–706
- Setchell KD, Brown NM, Lydeking-Olsen E (2002) The clinical importance of the metabolite equol—a clue to the effectiveness of soy and its isoflavones. *J Nutr* 132(12):3577–3584
- Atkinson C, Frankenfeld CL, Lampe JW (2005) Gut bacterial metabolism of the soy isoflavone daidzein: exploring the relevance to human health. *Exp Biol Med* (Maywood) 230(3):155–170
- Di Cagno R, Mazzacane F, Rizzello CG, Vincentini O, Silano M, Giuliani G, De Angelis M, Gobbetti M (2010) Synthesis of isoflavone aglycones and equol in soy milks fermented by food-related lactic acid bacteria and their effect on human intestinal Caco-2 cells. *J Agric Food Chem* 58(19):10338–10346
- Marzani B, Benedusi A, Giuliani G, Buonocore D, Marzatico F (2009) *Ajuga reptans*: attività biologica dell'estratto secco titolato in fenilpropanoidi. *Cosmetic Technol* 12(1):19–23
- Ramot Y, Pietilä M, Giuliani G, Rinaldi F, Alhonen L, Paus R (2010) Polyamines and hair: a couple in search of perfection. *Exp Dermatol* 19(9):784–790
- Ramot Y, Tiede S, Bíró T, Abu Bakar MH, Sugawara K, Philpott MP, Harrison W, Pietilä M, Paus R (2011) Spermidine promotes human hair growth and is a novel modulator of human epithelial stem cell functions. *PLoS One* 6(7):e22564
- Rinaldi F, Bezzola P, Sorbellini E, Giuliani G (2009) The effect of polyamines on hair cycle clock. *J Plastic Dermatol* 5(2):163–167
- Birch MP, Messenger JF, Messenger AG (2001) Hair density, hair diameter and the prevalence of female pattern hair loss. *Br J Dermatol* 144:297–304
- Tajima M, Hamada C, Arai T, Miyazawa M, Shibata R, Ishino A (2007) Characteristic features of Japanese women's hair with aging and with progressing hair loss. *J Dermatol Sci* 45:93–103