

BIOGENINA®* BASED FOOD-SUPPLEMENT:

hair growth enhancer

Fabio Rinaldi, Elisabetta Sorbellini, Paola Bezzola
Dermatologic Dept, Policlinico Multimedia-Milano

Danila Ingrid Marchioretto
Giuliani - Milan

Bioscalin®
Giuliani Retard

con
Biogenina®





BIOGENINA®* BASED FOOD SUPPLEMENT: *hair growth enhancer*

Fabio Rinaldi, Elisabetta Sorbellini, Paola Bezzola
Dermatologic Dept, Policlinico Multimedia - Milan
Danila Ingrid Marchioretto
Giuliani - Milan

Key Words

Spermidine
Polyamines
Food supplements
Telogen Effluvium

INTRODUCTION

The human species has reached a stage in evolution at which body hairs (among which hair represents just a particular form) are not responsible for regulating body temperature anymore, unlike in other animals. This function is carried out in the human body by perspiration and by changes occurring within the diameter of blood vessels. However, we cannot say that hair has no function at all, because it has always played an important aesthetic and psychological role especially when considering its seductive power and sexual appeal, both for men and women. It is well known how even ancient populations such as the Egyptians, the Greeks and the Romans took particular care of their hair and their gods were always featured with long and soft hair. Nowadays nothing has changed: Our current stereotypes of beauty, youth and strength are represented by men and women with healthy and styled hair. Hair is a fast growing element, which needs a healthy body and a balanced supply of nutrients to grow correctly. Any hormonal, blood, metabolic, or psychological changes as well as an imbalanced diet may exert a negative influence on the lifespan of hair; this is why any change affecting hair acts as a signal for general health of a person. Hair growth occurs through different stages within which the hair follicle experiences first strong developments,

followed by metabolic rest periods as well as quite regressive or shedding processes. Such phases are named: anagen, catagen and telogen (1).

Anagen

This step is distinguished by some peculiar changes within the dermal papilla and cells undergo a very intense metabolic activity. The Anagen (or growth) phase lasts 3 to 6 years. During this period hairs grow by 0.3-0.4 mm a day, even though they undergo different growth phases and are not all simultaneously growing. Aminoacids pass from blood to dermal papilla where they are turned into polypeptides. Polypeptides are proteic complexes representing the structural components of hair (especially chera- tines) and determine the final structure of hair.

Catagen

It represents the regressive stage and lasts 2 to 3 weeks. The hair follicle shows some morphological and metabolic changes. During this stage the follicle's length decreases of about one third and the bulb grows smaller, melanocytes stop to synthesize melanine and the dermal papilla becomes atrophic. After all this has taken place the hair falls.

Telogen

Telogen is the rest phase during which the follicle is completely inactive. Hairs stay inside the follicle due to the presence of weak intercellular binds which keep them into the scalp until another anagen stage takes place, or even longer. Telogen phase lasts 2 to 4 months. At the end of all these events, hairs fall. Everyday at least 50 hairs die and they are replaced by new ones due to the fact that the various processes involving different follicles occur in a synchronized way. Hence, the number of total hairs is indeed unchanged. It takes 2 to 6 months to see a complete turnover of all hairs.

CEC Milan, Italy
tel +39.02.4152 943
fax +39.02.416 737
email info.nutrafoods@cec-editore.com
website www.cec-editore.com

VFCI Augsburg, Germany
tel +49.821.325 830
fax +49.821.325 8323
email fischer@sofw.com
website www.sofw.com

However, the balance of hair physiological growing cycle may change in presence of a psychological stress, a metabolic disorder, or a specific lack of micronutrients.

It might happen that follicles currently undergoing the anagen phase turn to the telogen phase, resulting in early hair loss. If the distressing stimulus or the tinged disease continues, hairs can modify their diameter and their length. Therefore, the physiological growth of hair and its life cycle depend on the condition of scalp's skin, on the vitality of the dermal papilla, on the general health (body and mind) of the person and on a balanced intake of nutrients.

Chemical composition of hair is as follows:

- 5% sulphur, 23% oxygen, 15% nitrogen, 7% hydrogen, 50% carbon
- small amounts of fatty acids, traces of metals and minerals (phosphorous, magnesium, sodium, iron, potassium, iodine), whose presence in % varies according to the diet and to the person's general health status.

Men have on average 130.000 hairs on their scalp, whereas women have 150.000 hairs. As we said before, each of us loses about 50 hairs per day, all replaced by new ones. Problems arise when some of these fallen hairs are not replaced. This occurs to a large number of men during their maturity, even if nowadays baldness affects women as well.

Hair and diet

There is a link between hair loss and diet, since hair is made by proteins for 65-95%; among other components are lipids, pigments, oligoelements and water. It is therefore necessary to intake specific nutrients and micronutrients to allow hair to grow correctly.

The diet followed by the majority of Italian families includes a wide variety of all nutritional elements needed for the correct functioning of the human body and therefore for hair growth. However, mistakes are often made and they can lead to an unbalanced diet. Among these common mistakes we can find low or no consumption of fruit and vegetables as well as following very restrictive diets in general. Due to this fact, proteins are sometimes missing, especially those containing sulphur aminoacids, such as methionine and cysteine.

The lack of such aminoacids can lead to hair brittleness, decreased calliper and dry hair. A diet containing low aminoacids, minerals and vitamins may lead to severe hair loss, which can be reversed if a balanced intake of nutritional elements takes place shortly after (2,3).

Spermidine

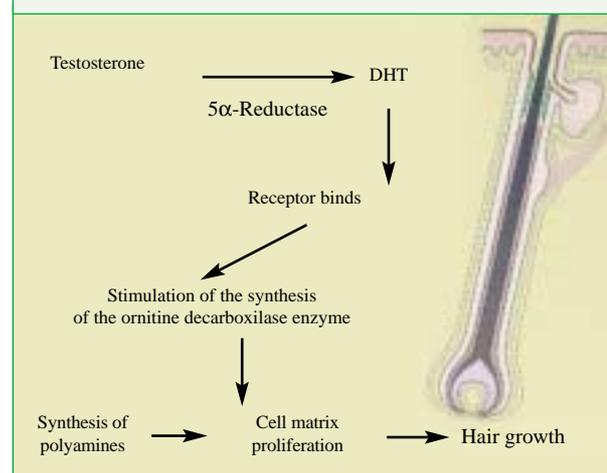
The amine of life

Polyamines, divided into amines of 'life' (spermine and spermidine) and the amines of 'death' (putrescine and cadaverine), are present in all animal and vegetal cells.

They are considered ubiquitous substances and play a major role in all processes involving growth (4-6).

Several research studies carried out in recent years pointed out that aliphatic polyamines, spermine and spermidine, play an important role for the control of all mechanisms of cell growth, division and differentiation (7). Due to this important role, we can assume that they are also responsible for regulating molecular processes leading to ageing and involution of cells. Some important functions of the cells are shown to be altered in aging tissues due to a lower control mechanism usually carried out by aliphatic polyamines, spermine and spermidine. On the contrary, an intake of these polyamines can help the recovery of some metabolic processes usually impaired by ageing (8). Spermidine is contained in milk, to promote cells proliferation in the newborn; It is also contained in the blood, saliva, sperm and urine (9,10). Recent experimental studies, carried out on animals, highlighted that polyamines in general and spermidine in particular can play a major role for hair growth (11-14). To understand the action mechanism of a food supplement containing spermidine, a short overview of the physiology of hair growth is needed (Fig 1). Dermal papilla synthesizes hair chertine (mainly type 19 chertine). The ornithine decarboxilase enzyme (ODC)

Figure 1 *Physiological and biochemical aspects of hair growth*



synthesizes long proteic chains from sulphur aminoacids after activation of the receptor of testosterone or dihydro-testosterone (DHT) on the dermal papilla. ODC promotes the growth of polyaminic chains responsible for proliferation of cellular matrix and, in turn, of hair growth. Obviously, any mechanism affecting one or more of these metabolic processes can stop hair growth by turning the anagen phase into a telogen one and therefore to hair loss. The activation of apoptosis into dermal papilla cells contributes to the development a pathological condition in the hair. To evaluate a food supplement* containing spermidine we performed a double blind, placebo controlled study, enrolling subjects affected by telogen effluvium.

MATERIALS AND METHODS

A clinical trial, double blind, placebo controlled, was performed on 60 healthy and compliant subjects, both men and women, aged 18-60, who had been affected by telogen effluvium for three months before being enrolled in the study. Subjects with similar clinical characteristics were divided into three groups (A,B,C) made up by 20 people each, according to a random list. Group A took capsules containing spermidine alone, Group B took slow release capsules containing the complete food supplement (Bioscalin Giuliani with Biogenina®, Giuliani, Milan), Group C took placebo capsules (Table 1).

The treatment, which lasted two months, implied the intake one capsule a day at breakfast time. A follow-up visit took place 30 days after the end of the treatment. Each subject underwent three visits, namely T0, at the baseline, T1 at the end of the treatment and T2 30 days after the end of the treatment.

The following parameters were considered for each subject:

1 General medical check-up and dermatological visit to assess whether clinical problems were present (this was needed to determine participa-

Table 1 Chemical formula of the food supplement (per cps)

Ingredients	mg
Methionine	300
Vitamin C	90
Vitamin E	15
Calcium pantotenate	9
Zinc (as chelazone)	7.5
Poliphenols from red grape peels	5.0
Vitamin B6	2.0
Copper (as chelazone)	1.25
Spermidine [N-(3-aminopropyl)-1,4-tetramethylenediamine trichlorhydrate]	0.50
Folic acid	0.30
Biotine	0.05

Eccipients: hydroxymethyl,propyl cellulose, calcium phosphate bibasic, microcristalline cellulose, magnesium stearate, silica bioxide

Formula of capsules containing spermidine

- Spermidine 0.50 mg
[N-(3-aminopropyl)-1,4-tetramethylenediamine trichlorhydrate]
- *Eccipients:* calcium phosphate bibasic, microcristalline cellulose, magnesium stearate, silica bioxide

Formula of capsules the placebo

Calcium pantotenate, microcristalline cellulose, magnesium stearate, silica bioxide

tion in the study, concurrent therapies with other drugs etc). Dermatological test to come out with a correct trychological diagnosis and to exclude other concurrent dermatological problems which could jeopardize final result of the study.

- 2 Microscopic assessment of the follicle and of the stem to determine the exact percentage of stems undergoing either the anagen or the telogen phase and to measure the diameter of the stem;
- 3 Pull test: to assess the resistance of hair stem to traction. Results were reported according to the following score system:
2=little or very little resistance
1=moderate resistance
0=high resistance
- 4 Wash test: to assess the number of hairs lost when hair was washed (twice per week). The subject had to count hairs found in the sink at the end of the wash process (average of subjective values reported by all subjects at each wash time).
This value was expressed in integer numbers.
- 5 Haemato-chemical tests: to assess specific deficiencies that could have determined the telogen effluvium in every single subject. Particularly, it seemed to be useful to rule out specific deficiencies of iron and other oligoelements like zinc and magnesium and to evaluate the electrophoresis of haematic proteins. This to exclude specific forms of ipoproteinemy and to check the possible increase of haematic proteins after the intake of the product.
- 6 Possible side effects related to the intake of capsules containing the three specific substances.

* BIOSCALIN GIULIANI con BIOGENINA®
Giuliani, Milan

RESULTS

Dermatological Check Up

The Dermatological check-up following the T2 visit showed a significant decrease in hair loss and better general conditions of the hair structure in 89% of subjects belonging to group A, in 91% of subjects belonging to group B and in 12% of subjects belonging to group C.

Many subjects showed a change of the unguinal lamina, due to dystrophic conditions and fragility of the lamina. These changes in the keratinization process are common in cases of telogen effluvium.

The condition of the unguinal lamina improved at T2 in 76% of subjects for group A, in 78% for group B and 2% for group C.

Microscopic assessment of hair stem

Between T0 and T2 visits, the diameter of the stem increased by 56.2% in group A, by 57.8% in group B and 0.8% in group C. These values are extremely significant. All subjects belonging both to group A and to group B showed an increase of the diameter of the stem at T2.

Trichogram

Even though this test alone cannot be considered the most reliable indicator of the cyclical phase that the hair bulb is undergoing, it is important to quantify in a precise manner the percentages of the various cyclical phases that the hair bulb is undergoing.

The analysis of the data related to changes of the anagen/telogen due to the treatment with the three different products is shown in the following figures. Increases shown during the anagen phase are as follows (*Fig 2*):

- Group A: 17.2% at T2 vs 8% at T1
- Group B: 20.2% at T2 vs 8.1% at T1
- Group C: 7.79% at T2 vs 2.7% at T1

The change of 7.79% occurred during the anagen phase between T0 and T2 in the placebo group could be due to cyclical modifications of some of the hair bulbs.

Instead telogen (*Fig 3*) decreased by:

- 6.67% at T2 in group A (being 9.1% at T0)
- 27.7% at T2 in group B (being 9.6% at T0)
- 4.16% at T2 in group C (being -1.88% at T0)

Haematochemical analysis

No changes were reported in haematochemical values of subjects belonging to group C, whilst a moderate increase of proteins (albumine and alpha 1) was reported by 48% of the subjects belonging to group A.

Instead, subjects belonging to group B showed a slight increase of sideremy, ferritin, red blood cells count, haemoglobin and serum-proteic electrophoresis.

Pull test

Group C score at pull test didn't change (*Fig 4*), whereas resistance to traction increased by 62.9% at T1 and 88.5% at T2 in group A, and by 94.4% at T1 and 88.9% at T2 in group B.

Wash test

Hair loss, assessed both in an objective and *subjective* way by counting hairs found into the sink at the end of each washing (average of subjective values for all subjects at each wash) compared to T0 decreased by (*Fig 5*):

- Group A: 39.1 at T1 and 67.2% at T2
- Group B: 50.6% at T1 and 75.7% at T2
- Group C: 0.2% at T1 and 0.5 at T2

Side effects

Three subjects from group A (5%) reported moderate pyrosis after they took the capsules, this problem was overcome by taking the supplement at meal time.

One subject from group B reported worsening of IBS condition (irritable bowel syndrome) with diarrhea after he took the capsule: This side effect disappeared spontaneously after the subject had taken the fourteenth capsule and did not result in the subject dropping the study.

Two subjects from C group reported moderate pyrosis after they took the capsule presumably due to its jelly cover. Even in this case, they took the capsule at meal time and the problem was overcome. No other side effects were reported during the study.

DISCUSSION

Telogen effluvium is perhaps the most common form of alopecia and the most widespread among teen agers and adults, both men and women, even if it shows to be slightly more common among women. The etiology of telogen effluvium can be normally due to non-specific deficiencies, unbalanced diet, cytotoxic drugs (antibiotics, antiviral agents, antidepressants, chemoterapics, etc.), psychic or physical distress (15). Various new studies have better defined the pathway of death of the dermal papilla of the hair bulb. They also better defined the apoptosis process of the follicle that induces the telogen phase (16).

Different biochemical stimuli, mediated by interleukins and different growth factors, are believed to be responsible for the activation and inactivation of the

Figure 2 Trichogram: anagen phase at T_0, T_1, T_2

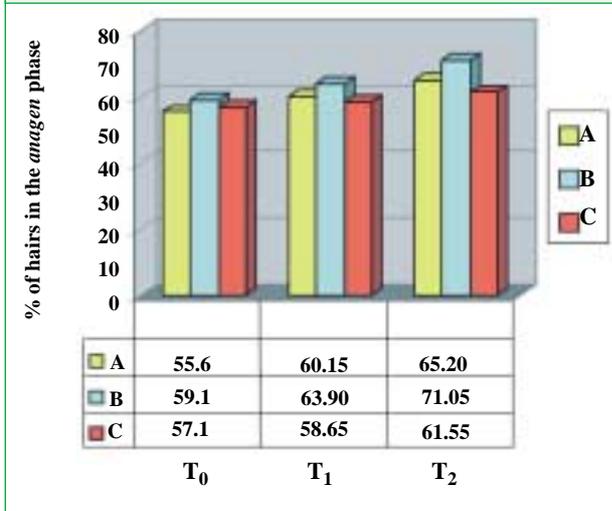


Figure 3 Trichogram: telogen phase at T_0, T_1, T_2

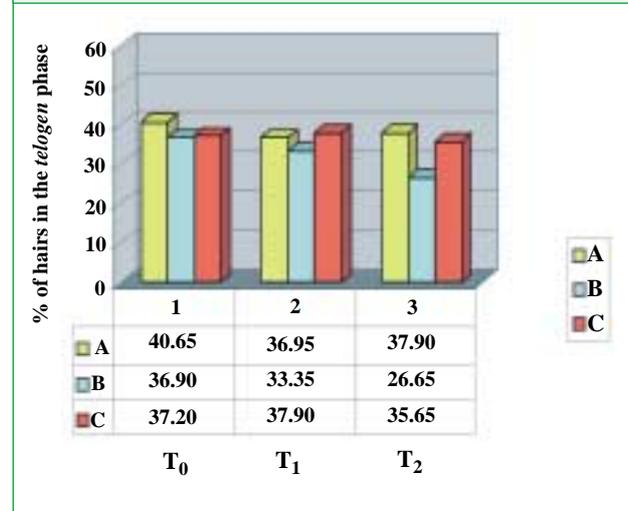


Figure 4 Pull test results at T_0, T_1, T_2

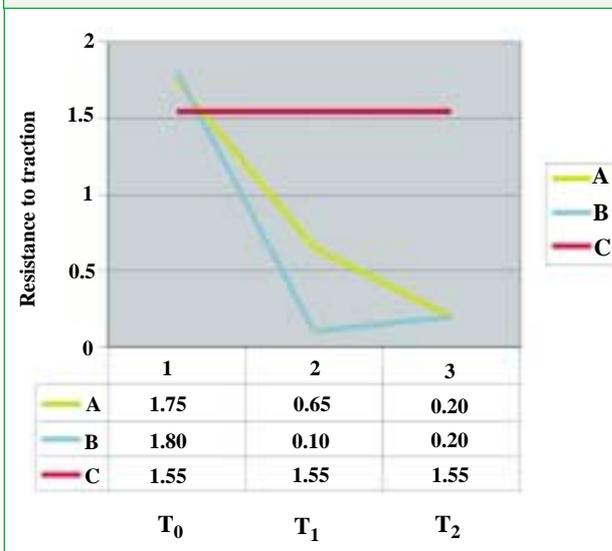
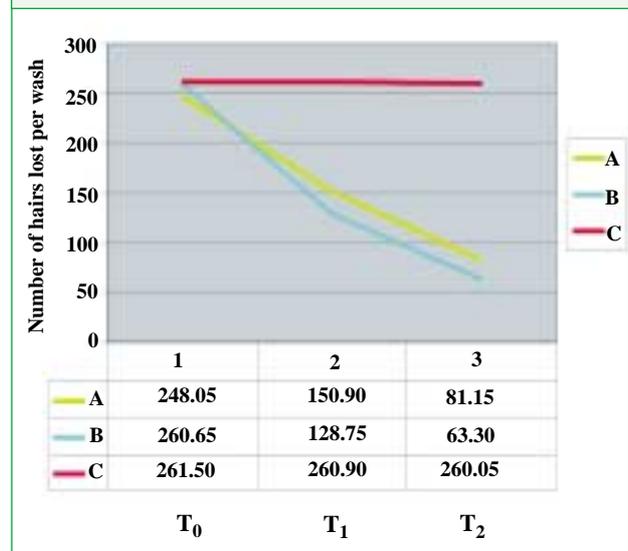


Figure 5 Wash test results at T_0, T_1, T_2



hair follicle. Then it becomes impossible to maintain the anagen phase and therefore the growth process of the stem.

According to this, the maintenance of the anagen phase seems to be the only way to treat any form of alopecia and especially telogen effluvium.

An ideal treatment for the telogen effluvium should focus on controlling the cellular and biochemical homeostasis of the dermal papilla and of the other components of the hair bulb. Such a treatment should try to neutralize (or at least to limit) the various oxidation stimuli that could lead to the change of the anagen phase into telogen, by controlling cellular apoptosis.

Very recent studies demonstrated how important this process is, even in presence of androgenetic alopecia, that therefore would not be due only to the enzymatic mechanism of the 5-alpha-reductase and aromatase.

Therefore, we can infer that a food supplement containing sulphorated aminoacids and oligoelements is helpful for the development of the stem and for hair growth in general.

Such a supplement would be even more effective if it contained a polyamine responsible for the synthesis of the cellular matrix and therefore for hair growth, which could bypass many metabolic processes.

Moreover, if the supplement also contains some agents acting against free radicals, which help counteract the apoptosis, as well as micronutrients essential for hair growth, such supplement can be further expected to improve the control of the telogen effluvium disease. During the double blind study, capsules containing sper-

midine alone and capsules containing spermidine in association with other 'trichogen' substances and placebo capsules were administered.

The results of such study totally confirmed the hypotheses and expectations. Group A subjects (capsules containing spermidine) showed a significant increase of the anagen value and consequently a decrease of the telogen. It is interesting how catagen and anagen are related. When anagen increases catagen decreases. This results in lower hair loss (as demonstrated by the wash test and the pull test) and in an increase of the diameter of the stem due to the chertinization of the dermal papilla. No change was reported as far as the haematochemical values are concerned, due to the fact that spermidine does not interfere with the synthetization of haematic cells and it does not carry oligoelements. However, it was noticed a slight increase of proteins from serum-proteic electrophoresis.

Group B subjects (capsules containing the complete supplement formula) experienced the same changes as group A subjects but the overall effectiveness increased slightly. This was probably due to the intake of oligoelements, vitamins and antioxidants which improve the homeostasis of hair synthesis.

Group C subjects (placebo) showed no change at all. Neither subjective or objective data show improvement in the symptoms of telogen effluvium. This demonstrates how no role is played by psychologic perceptions when treating this type of diseases. It is important to mention that the diameter of the stem increased by 56.2% in group A, and only by 57.8% in group B. This figure is very significant because it demonstrates how important is the role of spermidine alone in stimulating the proteic synthetization within the cell matrix, and therefore for hair growth. The other ingredients included in the capsules administered to group B subjects did not produce any significant improvement for the synthetization of the hair stem. The Wash test and the Pull test are more specific indicators of hair loss and therefore of the progression of telogen effluvium.

In group A, at T1 the number of hairs lost during the wash decreased by 39.1%, in the same group at T2 this percentage decreased to 67.2%. In group B, at T1 the percentage of hairs lost decreased by 50.6% and at T2 this percentage fell down to an amazing 75.7%.

The final formula of the food supplement containing all the micronutrients improved consistently the pathological situation of telogen effluvium.

This is a confirmation of the fact that the oxidation process and the supply of oligoelements and vitamins enhances the effectiveness of the formula. In addition, the resistance to traction improved faster in group B than in group A, even though it reached an equal value for both groups in the long run. The trichogram shows the percentage of changes in the various phases of hair growth. According to existing literature, the normal values for a human trichogram are as follows: 85% of bulbs at anagen phase, 1% at catagen and 14% at telogen.

New research studies found a new phase to be present within hair life cycle: the exogen phase.

This phase occurs right after the telogen and refers to the exact moment in which the hair stem falls. The exogen, morphologically different from telogen, represents the physiological step of the detachment of the stem from various binding systems of dermis. According to these new theories, which seem to be very accredited nowadays, hairs falling during telogen would represent a premature event and therefore a pathological condition of hair loss. The evaluation of the trichogram for this clinical study was carried out considering the morphological differences between telogen and exogen: at T0, only 2.5% of the bulbs could be classified as exogen. Collected data show how capsules containing active ingredients (Groups A and B) have been able to increase the number of bulbs in the anagen phase, with a consequent reduction of telogen. This resulted in a significant improvement of the clinical symptomatology. The microscopic evaluation of a representative sample of hairs collected during the wash test at T2 pointed out the following:

- Group A: 33% of hairs in exogen phase (63% in telogen and 4% catagen)
- Group B: 46% of hairs into exogen phase (52% in telogen and 2% catagen)
- Group C: only 3% of hairs was in exogen phase (91% in telogen and 6% catagen)

Even if these additional data have not been included in the approved protocol, since the exogen phase had not been defined in a standardized manner at the moment of approval, they are highly significant. In fact, at the end of the study, hairs that had fallen were found to be at different stages in the three groups.

The placebo group showed a predominance of hairs in the telogen phase whilst the other two groups showed a significant number of bulbs in the exogen phase (a more 'physiological' phase). The lack of side effects, which could be ascribed to the intake of the capsules, gives us enough confidence to

state that capsules containing active ingredients are safe and at very low risk of side effects.

CONCLUSIONS

This double blind, placebo controlled, clinical study was aimed at evaluating the effectiveness of a food supplement containing either spermidine alone or spermidine in association with other nutrients for the treatment of telogen effluvium. The study was able to demonstrate that spermidine, taken alone or even more in association with other active ingredients, helped to reduce significantly the clinical symptoms and the instrumental values related to the presence of telogen effluvium. The statistical difference resulting from the comparison of the 'active' and the 'placebo' product is highly significant, since the placebo has not produced any changes in either the clinical nor the instrumental symptoms. Moreover, the study, which has been carried out on healthy subjects, has not evidenced any side effects due to the intake of the products tested.

REFERENCES

- 1 **Epstein FH (1999)**
The biology of hair follicles
Nejm **341**(7) 491-497
- 2 **Skolochenko M (2000)**
Crash dieting associated with telogen effluvium
Geriatrics **55**(9) 16
- 3 **Harrap G, Dolphin S, Albiston L (2001)**
The effect of age on hair root amino acid levels in human subjects
Third Intercontinental Meeting of hair Research Societies Abstracts - Tokyo, Japan, 13th-15th June 2001
- 4 **Farriol M, Segovia T, Venereo Y et al (1999)**
Importance of Polyamine: review of the literature
Nutr Hosp **14**(3) 101-113
- 5 **Urdiales JL, Medina MA, Sanchez-Jiménez F (2001)**
Polyamine metabolism revisited
Eur J Gastroenterol Hepatol **13** 1015-1019
- 6 **Okamoto A, Sugi E, Koizumi Y et al (1997)**
Polyamine content of ordinary foodstuffs and various fermented foods
Biosci Biotech Biochem **61**(9) 1582-1584
- 7 **Bardóc S, Duguid TJ, Brown DS et al (1995)**
The importance of dietary Polyamines in cell regeneration and growth
Br J Nutr **73** 819-828
- 8 **Milovic V (2001)**
Polyamines in the gut lumen: bioavailability and biodistribution
Eur J Gastroenterol Hepatol **13** 1021-1025
- 9 **Losser C (2000)**
Polyamines in human and animal milk
Br J Nutr **84**(suppl 1) S55-S58
- 10 **Dorhout B, Van Beusekom CM, Huisman M et al (1996)**
Estimation of 24-hour Polyamine intake from mature human milk
J Pediatr Gastroenterol Nutr **23** 298-302
- 11 **Pietilä M, Alhonen L, Halmekyto M et al (1997)**
Activation of Polyamine catabolism profoundly alters tissue Polyamine pools and affects hair growth and female fertility in transgenic mice overexpressing Spermidine/Spermine N¹-Acetyltransferase
J Biol Chem **272** 18746-18751
- 12 **Pietilä M, Parkkinen JJ, Alhonen L et al (2001)**
Relation of skin polyamines to the hairless phenotype in transgenic mice overexpressing spermidine/spermine N-acetyltransferase
J Invest Dermatol **116**(5) 801-805
- 13 **Alhonen L, Karppinen A, Uusi-Oukari M et al (1998)**
Correlation of polyamine and growth responses to N¹, N¹¹-diethylnorspermine in primary fetal fibroblasts derived from transgenic mice overexpressing spermidine/spermine N₁-acetyltransferase
J Biol Chem **273** 1964-1969
- 14 **Suppola S, Pietilä M, Parkkinen JJ et al (1999)**
Overexpression of Spermidine/Spermine N¹-Acetyltransferase under the control of mouse metallothionein I promoter in transgenic mice: evidence for a striking post-transcriptional regulation of transgene expression by a Polyamine analogue
Biochem J **338** 311-316
- 15 **Headington J (1993)**
Telogen effluvium. New concepts and review
Arch Dermatol **129** 356-363
- 16 **Piérard-Franchimont C, Piérard GE (2001)**
Teloptosis, a turning point in hair shedding biorhythms
Dermatology **203**(2) 115-117



Giuliani S.p.A.

Via Palagi, 2 - 20129 Milano
Tel. +39 02.20541 - Fax +39 02.29401341
www.giulianipharma.com