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CLA Glutathione and Sodium DNA for Reducing Hair Loss

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ABSTRACT: *A new ingredient blend uses, in one amphiphilic molecule, glutathione's antioxidant activity and conjugated linoleic acid's cell protecting functions to support the reduction/oxidation status and vitality of bulb cells. The blend has proven active in treating excessive hair loss. Some advantages over minoxidil are shown.*

Substances involved in biological cycles of the human body are appealing for cosmetic formulations because they have high tolerability and potential functionality. They are metabolically activated, and when they become deficient, they are compensated for either by reduced cell activity or topical supply of skin nutrients. However, transepidermal delivery may become a limiting factor to their targeted efficacy.

The speed of a material's in-depth delivery and the amount of material absorbed is determined based on the polarity of the molecules and the partition coefficient between the vehicle and the skin's epidermal layers. Formulation strategies designed to establish the simultaneous presence of potentially active substances in a formula are frequently hindered by the "resistant" activity of the skin. Indeed, the skin operates by separating and almost unpredictably delaying the transport of the active substances that are intended to act on the same skin site at the same time.

This paper describes a molecule created by fusing conjugated linoleic acid (CLA)

with glutathione, each of which has intrinsic biologic activity. The peculiarity of CLA glutathione lies in its chemical structure based on the lipophilic linoleic acid and the hydrophilic glutathione tripeptide. The resulting molecule is shown to be capable of bypassing the hurdle of skin transport and to be efficacious in optimizing the co-presence of different actives. In this case, CLA glutathione is combined with sodium DNA for a new approach to the treatment of premature hair loss.

CLA glutathione is capable of bypassing the hurdle of skin transport.

Linoleic Acid

Linoleic acid is an essential fatty acid (EFA). EFAs are fundamental constituents of bio-membranes and precursors of biological substances such as prostaglandins, leucotriens and hydroxylated fatty acids. However, linoleic acid is not synthesized

by the human body and consequently it must be included in the diet. Luckily enough, it is abundant as one of the omega-6 fatty acids found in some vegetable oils, in milk and its derivatives, and in some meats.¹ Deficiency of omega-6 results in numerous conditions, including dry hair and hair loss.² Linoleic acid applied topically on skin has been shown to have anti-inflammatory, acne reduction and moisture retention properties.²

Conjugated Linoleic Acid

Vegetal-derived linoleic acid consists of a C18 linear chain with two double bonds (C18:2n-6). All the possible *cis*- and *trans*- conformational isomers are referred to with the acronym CLA (conjugated linoleic acid). Unsaturation can be positioned at C9 and C11 or at C10 and C12. Highly unsaturated acids, such as arachidonic acid (C20:4n-6), are essential precursors for the biosynthesis of prostaglandins and involved both in inflammatory processes and in their repair. These highly unsaturated acids can be synthesized in the human body from conjugated linoleic acid consumed in foods. Without such external sources, life would be impossible.

Clinical evidence assigns CLA a key role in lipid transport into the blood stream. Consequently, it is used in the treatment of diet-dependent *hyperlipoproteinemia* or *high cholesterol*, and in atherosclerosis prophylaxis or hardening of the arteries.³ CLA also induces a cytostatic or cytotoxic effect on some types of cancer.⁴ Recent evidence also has shown the oral intake of CLA to help reduce fat body mass while at the same time helping to increase lean body

mass in obese or overweight persons⁵⁻⁷ reportedly due to the reduction of the average dimensions of adipocytes.

In common dermatological diseases such as eczema, psoriasis and atopic dermatitis, modifications to EFA metabolism have been observed. In most cases these were due to insufficient intake of linoleic acid and its isomers as initiators of EFA production.⁸

Growing scientific knowledge about CLA involvement in physio-pathologic processes and the studies performed to clarify its biologic mechanism of action have paved the way to a strategy of employing CLA in the cosmetic domain as a lipo-reducing and anticellulite active, as well as a skin moisturizer and barrier organizer.

Glutathione

Glutathione is a tripeptide formed by glutamic acid with cysteine and glycine. It is synthesized in the liver, in yeasts and in plant leaves and performs a cell-protective activity in all animal and vegetal tissues thanks to its two-fold activity as an antioxidant and as a

combination agent for metabolites to be disposed of by the organism.

CLA was the only PUFA capable of inducing glutathione synthesis without modifying the oxidative equilibrium within cells.

In biochemistry, two forms are commonly discussed. The oxidized form (glutathione disulfide) and the reduced form (glutathione) transform one into the other in living organisms with the help of enzymes and the action of reducing/oxidizing agents. Only reduced glutathione is biologically active. It is typically written as GSH because the SH thiol group is the reducing part of the molecule. GSH works in conjunction with endogenous glutathione peroxidase that re-establishes the oxido-reduction mechanism. By forming nonreactive

molecules, it neutralizes hydrogen peroxide as well as oxygenated radicals that have disruptive effects on lipids, proteins, enzymes and cellular DNA.

Synergy between CLA and GSH:

The increase in redox potential in body cells is a cytoprotective strategy against oxidative damage. A recent study⁹ showed that—thanks to its hairpin configuration of the *trans*-10, *cis*-12 isomer—CLA was able to induce the expression of the sub-catalytic unit of the enzyme γ -glutamyl-cysteine-ligase, which is involved in the speed-limiting phase of GSH synthesis. The CLA increase inside the cell leads to augmented GSH content without either secondary induction of ROS or lipo-peroxidation of the cell components.

In studies reported elsewhere,¹⁰⁻¹² the formed GSH successively participates in the synthesis of eicosanoids and, in general, modifies the redox conditions of the cells, thus playing a key role in up-regulating the immune system. Arachidonic acid also has a positive influence on the synthesis of GSH but at a lower level than CLA. Indeed, in the studies cited, GSH formed by arachidonic acid was an average of 16 nmol/mg protein while CLA gave 21 nmol/mg protein. Normal level is between 8 and 12. Conjugated linoleic acid increased by 30% the amount of oxidant (tert-butylhydroperoxide) necessary to induce 50% cell death. Of all the fatty acids tested in these studies,^{9,10,12} these are the only two acids that demonstrate this effect. In fact, only for these two acids was the lipo-peroxidation, measured by the malonyl dialdehyde level after a seven-day treatment period, not increased by a high amount of ROS. However, the amount of GSH formed by arachidonic acid was not sufficient to protect the cells whereas CLA provided a higher amount.

Thus, CLA was found to be the only polyunsaturated fatty acid capable of inducing glutathione synthesis without modifying the oxidative equilibrium within cells.

CLA Glutathione

Based on this understanding of synergy between CLA and glutathione, the authors designed a cell-protection mechanism using those two substances. One molecule carrying both substances provides the double advantage of simultaneous synergistic activity of both

moieties with easy transepidermal delivery, made possible by the amphiphile nature of the compound.

CLA glutathione synthesis: Vegetal-derived CLA, transformed into its acyl chloride, is reacted with GSH at a low temperature (50–60°C) in order to avoid both the thermal decomposition of GSH and the oxidation of CLA. An excess in GSH protects CLA chloride, which easily reacts with GSH on its free amino group thus forming the amide bond. By acidification, CLA glutathione is separated and successively neutralized with potassium hydroxide to pH 7. The structure of CLA glutathione is shown in **Figure 1**.

Functional mechanism: Long-chain polyunsaturated fatty acids are known to give rise, through oxidation reactions, to arachidonic acid, the most abundant precursor of eicosanoids such as prostaglandins, thromboxanes and leucotrienes. These powerful molecules act as mediators in local inflammatory processes. Therefore, supplementing CLA in the cells aids the activation of the arachidonic acid cascade, leading to prostaglandins synthesis that successively will induce the activation of local immune mechanisms.^{9–10}

Arachidonic acid requires oxygen and an electron donor to be transformed into prostaglandins (**Figure 2**). The reducing activity can be insured by GSH. In the case of CLA glutathione and skin, the reducing activity of GSH occurs exactly at the skin depth where it is needed, a fact made possible by the ease of transdermal delivery of the amphiphilic molecule. If pure glutathione is applied to the skin, it does not diffuse in depth, being much too polar and hydrosoluble. The scientific rationale of this functional mechanism is the basis of the unique skin activity of CLA glutathione.

In vivo performance: The use of CLA glutathione was tested in the treatment of alopecia or baldness. This condition is characterized by the onset of a series of irreversible phenomena. First, a progressive involution and miniaturization of the hair follicle is observed, moving then from the subcutaneous layer to the superficial dermal layer, giving rise to smaller and thinner hair. Finally, fibrosis of the connective tissue around the bulb takes place and is frequently accompanied by inflammation. Once the follicle and the surrounding tissues have been modified

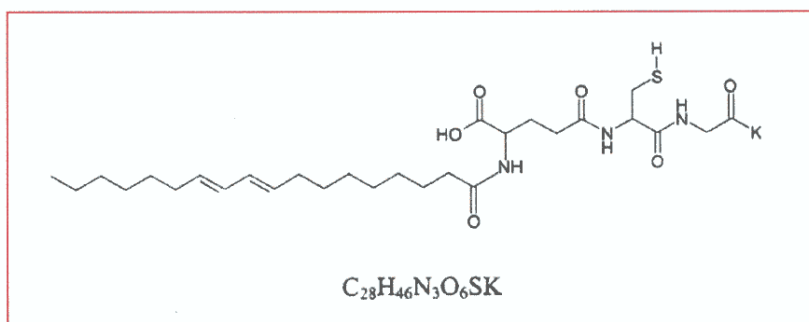


Figure 1. Structure of CLA glutathione

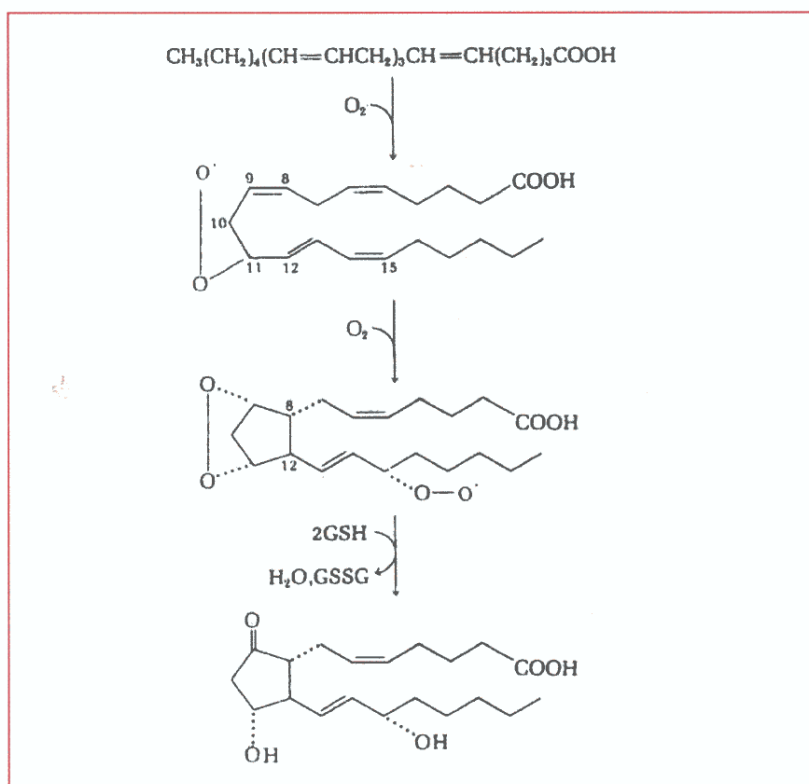


Figure 2. Synthesis of prostaglandin E₂ starting from arachidonic acid

in this way, the hair starts falling out and the bulb is definitively atrophic.

The use of CLA glutathione in scalp-care products could help to prevent these phenomena mostly due to its bipolarity, i.e., the simultaneous presence of the lipophilic fatty chain and the hydrophilic amino acids chain. The anti-free radical activity of GSH could hinder the connective tissue degeneration at the bulb level while the CLA intervenes in the inflammatory process.⁹ Indeed, in the in-use test reported later in this article, CLA glutathione was shown to be effective in both preventing and reducing premature hair loss.

Sodium DNA

Sodium DNA refers to purified, depolymerized and neutralized nucleotide fragments. These fragments, donors of purinic and pyrimidinic bases, are known to be active at the cutaneous tissue level and enter cells by pinocytosis when the skin is under extremely stressful conditions. These fragments are known for their stimulating activity on cell regeneration and capability to counteract the appearance of wrinkles and degradation phenomena related to skin aging.¹³

In skin cells, they participate in the metabolism of DNA cells by bringing

base structures to the synthesis of nucleic acids and their co-factors. This process takes place in cells quite easily during aging, sun exposure, stress, excessive free radical production and other instances of metabolic disequilibrium, just as it happens in the scalp of individuals suffering from alopecia.¹⁴⁻¹⁶

Here, sodium DNA acts as a strong stimulator of cell repair and regeneration of cutaneous tissues. Clinical tests demonstrated that after it has been integrated into the cells, sodium DNA helps to reduce inflammatory symptoms and stimulates the growth and granulation of epithelium tissues.¹⁷⁻²¹

CLA Glutathione and Sodium DNA

From the balanced association of CLA glutathione and sodium DNA, a new functional blend^a was derived. For the purposes of this article, that blend will be called CLASH-DNA. Hair bulb inflammatory processes and skin regeneration are the targets of these two actives.

^a Stimucap (INCI (pending): CLA Glutathione (and) Sodium DNA) is a product of Kalichem Italia Srl, Botticino Sera, Italy.

- The lipo-peptide CLA glutathione provides biological mobility and prevents oxidative damage at the hair bulb level. Its amphiphilic structure creates the base for such mobility, resulting in a more than proportional augmentation of bioavailability and simultaneous presence both of the CLA and the glutathione. The regulation and repair of inflammatory processes at the hair bulb level is given by the CLA actions.
- The DNA nucleotide fraction has a demonstrated activity on skin regeneration.²¹

Their blend 2:1 in water (total solids 6.5-7.5%) is a stable solution (at pH 7). Contrary to glutathione, the blend does not oxidize easily at room temperature or in finished formulations containing usual amounts of antioxidant blends. Such association provides the right environment and conditions for bulb cell equilibrium. No penetration enhancers are required to increase the activity of this blend.

The CLA glutathione molecule is composed of several conjugated forms of linoleic acid and glutathione. It might be

surmised that it reaches its maximum efficiency when, after having been absorbed at a cellular level, it is cleaved from the amino-acid bond. Because of its special molecular configuration, the CLA influences the cells' production of enzymes, which leads to increased GSH production in the cell, and it does this without forming free radicals. The GSH associated with CLA in the molecule assists in forming the arachidonic cascade, which causes the formation of prostaglandin, thus strengthening the local immune system. The arachidonic acid obtained from the CLA, in order that it may be converted into prostaglandin PGE₂, requires oxygen and an electron donor, both of which are guaranteed by the GSH. Consequently, the activity of CLA glutathione increases the redox level inside cells and avoids their damage, while also acting on the inflammatory mediators of bulb cells.

CLASH-DNA In-use Test

The mechanism of CLASH-DNA and its synergies make it suitable to treat scalp disorders such as alopecia. A study was carried out to evaluate the anti-hair loss efficacy of sample solution based on 0.2% CLA glutathione and 0.2% of sodium DNA, equal to 3% of CLASH-DNA, in comparison with an active solution containing 2% minoxidil as an anti-hair loss functional ingredient at standard use conditions.

Materials and methods: The study was performed in Milan, Italy, from November 2006 to January 2007 on 30 volunteers, men and women between the ages of 25 and 55 affected by alopecia and telogen effluvium: II-III stage of the Hamilton scale for men and I-II stage of the Ludwig scale for women. Fifteen randomly selected volunteers applied the **Formula 1A** (with minoxidil) once daily for three months, while the remaining 15 used **Formula 1B** (with CLASH-DNA).

At the beginning of the study, and after one, two and three months, the following evaluations were carried out, after which all data obtained were analyzed and statistically compared.

Wash test: The hairs lost after standard washing were counted. After two and three months of treatment with the trial solutions, a significant decrease ($p < 0.01$) of the number of hairs lost during washing was recorded for **Formula 1A**. A similar

Formula 1. Test solutions used in the anti-hair-loss efficacy and tolerability tests.

	A	B
Water (<i>aqua</i>)	X	X
Alcohol denat.	X	X
CLASH-DNA (Stimucap, Kalichem), 7% aq	3% w/w	0
Minoxidil	0	2% w/w
PEG-40 hydrogenated castor oil	X	X
PPG-26-buteth-26	X	X
Fragrance (<i>parfum</i>)	X	X

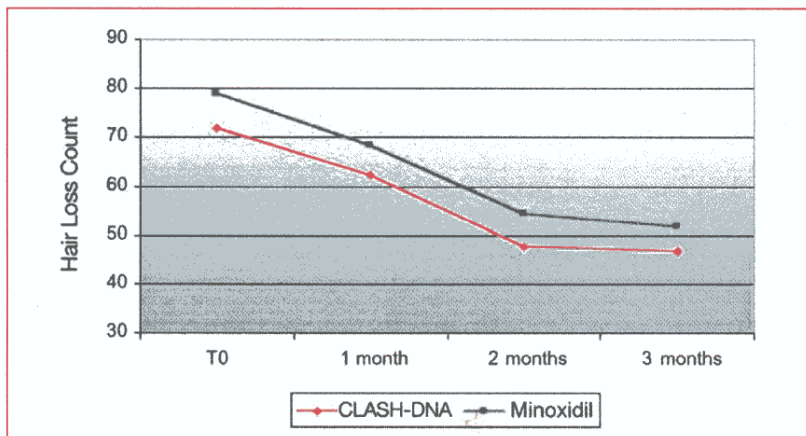


Figure 3. Hair loss count after standard washing (see Table 1 for means and standard deviations)

result (but with $p = 0.01$) was obtained after only a three-month treatment with the **Formula 1B**. In the case of **Formula 1B**, the hair loss reduction was also statistically significant ($p < 0.05$) after two months, but at a lower level of certainty (see **Figure 3**). The means and standard deviations are shown in **Table 1**.

On the whole, **Formula 1A** based on CLASH-DNA performed as well as the minoxidil-based solution, in terms of anti-hair loss activity. However, the notable advantage was achieving this result in a shorter time.

Pull test: The hair traction resistance was determined. A standard traction force was applied to hair locks at three different sites on the scalp. The traction resistance was scored by a semi-quantitative scale, shown in **Table 2**.

After three months of treatment, a statistically significant 40.0% increase ($p < 0.05$) in the hair resistance to traction with **Formula 1A** was measured. With **Formula 1B**, the increase was 17.6%, but the value was not significant ($p > 0.05$) (see **Figure 4**). At two months, both formulas showed increases that were not statistically

Table 1. Means (N=30) and standard deviations in the wash test (count of the number of hairs lost) and the pull test (hair resistance to traction) comparing similar formulations containing either CLASH-DNA or minoxidil

	Wash Test		Pull Test	
	CLASH-DNA	Minoxidil	CLASH-DNA	Minoxidil
T ₀	72.0 ± 49.8	79.0 ± 47.2	1.5 ± 0.9	1.7 ± 0.7
T _{1month}	62.3 ± 50.1	68.3 ± 24.3	1.5 ± 0.8	1.7 ± 0.5
T _{2month}	47.7 ± 38.7	54.3 ± 37.3	1.9 ± 1.0	2.0 ± 0.7
T _{3month}	46.6 ± 37.1	51.6 ± 44.0	2.1 ± 0.6	2.0 ± 0.7
T ₁ - T ₀	-9.7 (-13.4%)	-10.7 (-13.5%)	0.0 (0%)	0.0 (0%)
T ₂ - T ₀	-24.3 (-33.7%)	-24.7 (-31.3%)	0.4 (26.7%)	0.3 (17.6%)
T ₃ - T ₀	-25.4 (-35.3%)	-27.4 (-34.7%)	0.6 (40.0%)	0.3 (17.6%)

Table 2. Semi-quantitative scale used in the evaluation of hair resistance to traction

Score	Number of removed hairs in the three areas
0	>6
1	6-4
2	3-1
3	0

significant ($p > 0.05$). The means and standard deviations are shown in **Table 1**.

Dermatological evaluation: A dermatological evaluation was conducted for the overall tolerability of the product, evaluated on the following scale: 0 = low; 1 = moderate; 2 = average; 3 = good; 4 = very good. Also, the possible onset and intensity of local adverse reactions were analyzed by using a nonparametric scale from 0 to 3, which takes into account the presence/intensity of dandruff and

seborrhea (0 = absent; 1 = weak; 2 = moderate; 3 = severe).

Objective evaluation of the scalp and of the general conditions of the volunteers allowed the researchers to determine good overall tolerability and the absence of local adverse reactions such as seborrhea or dandruff after the application of both solutions. After three months of treatment, a statistically significant increase (+11%, $p < 0.05$) in overall tolerability in subjects treated with **Formula 1A** was observed,

while a nonsignificant increase in overall tolerability was detected in subjects treated with **Formula 1B** (+2.5%, $p > 0.05$).

Subjective evaluation: By filling out a questionnaire on a monthly basis, the volunteers expressed their opinion on efficacy, tolerability and treatment pleasantness of the test product; in particular, they recorded their perceptions about the onset of dandruff, seborrhea, itch or burning sensation. These answers were associated with scores from 0 to 3 with the following scale: 0 = absent; 1 = weak; 2 = moderate; 3 = severe. Overall efficacy, tolerability and treatment pleasantness were scored as follows: 0 = low; 1 = moderate; 2 = average; 3 = good; 4 = very good.

A statistically significant increase in tolerability perceptions for **Formula 1A** was reported after a two- and three-month treatment, while for **Formula 1B** the increase was not statistically significant. In both cases, a statistically significant reduction of itch perception (1A: 83%, $p < 0.05$; 1B: 67%, $p < 0.05$) on the scalp was recorded after a three-month treatment.²²

Conclusions: The evaluations demonstrated that CLASH-DNA could prevent hair loss at comparable or even superior efficacy to the compared product containing minoxidil.

Summary

Considering the described key mechanisms of biological action, it may be stated that reduced glutathione, a tripeptide existing in most living cells, is a powerful antioxidant and a free radical inhibitor.

CLA is necessary for the arachidonic acid cascade but is subject to oxidation. In order to convert CLA initially into arachidonic acid and then into prostaglandins, both oxygen and an electron donor must be present simultaneously.

CLA glutathione is an amphiphilic molecule with a high biological activity.

When CLA glutathione enters in the cell environment the glutathione moiety also provides the electrons necessary for arachidonic acid synthesis.

Thus, CLA glutathione was assumed to be useful in the treatment of excessive hair loss bound to disequilibrium in the redox status and in the alteration of growth factors and cytokines.

In tests on volunteers affected by hair cycle disturbance, CLA glutathione combined with sodium DNA demonstrated efficacy and tolerability at a comparable or even superior level to a reference ingredient. The best results were obtained in terms of hair traction resistance and fast anti-loss efficacy. Moreover, the active blend proved to be well tolerated by the volunteers who found it acceptable.

The combined action of CLA glutathione and sodium DNA in products aimed for scalp treatment may represent a new approach to the treatment of premature hair loss.

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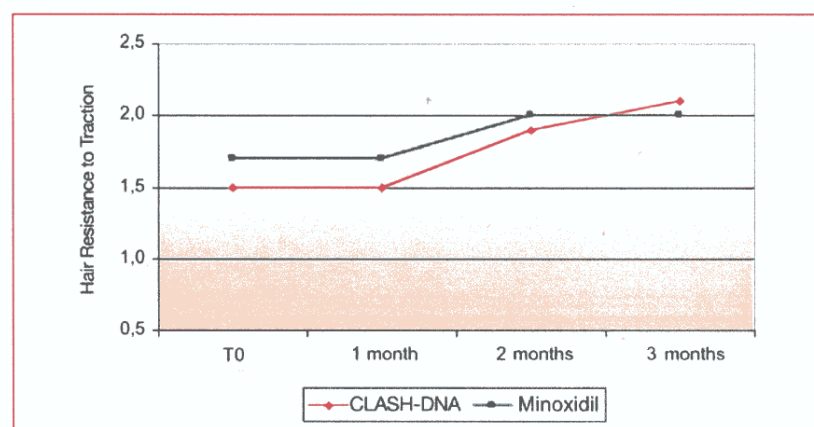


Figure 4. Evaluation of hair resistance to traction (see Table 1 for means and standard deviations)

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