Accepted: 3 January 2017

DOI: 10.1111/jocd.12314

ORIGINAL CONTRIBUTION

WILEY JCD Journal of Cosmetic Dermatology

The efficacy study of the combination of tripeptide-10-citrulline and acetyl hexapeptide-3. A prospective, randomized controlled study

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Summary

Background: Bioactive peptides have beneficial effects on the skin.

Objective: We investigated to evaluate the effect of acetyl hexapeptide-3 and tripeptide-10 citrulline and the possible synergism between these two peptides.

Methods: Twenty-four healthy volunteers were randomized to receive combination of acetyl hexapeptide-3 with tripeptide-10 citrulline (Group G1), tripeptide-10 citrulline (Group, G2), acetyl hexapeptide-3 (Group G3), or neither peptide (Group G4) for 60 days. Skin properties evaluated included skin microtopography, parameters cR2 and cR3, and transepidermal water loss (TEWL) using a skin visioscan and a tewameter, respectively.

Results: After 20 days, the measurements between G1 and G2 groups (cR2 P=.045, cR3 P=.044), G2 and G3 groups (cR2 P=.017, cR3 P=.017), G3 and G4 groups (CR2 P=.022), and G2 and G4 groups (cR3 P=.028) from baseline were significant. After 60 days, measurements between groups G1 and G3 (cR2 P=.016, cR3 P=.025), groups G2 and G3 (cR2 P=.044, cR3= P=.044), and groups G1 and G4 (cR2 P=.025) were significant. After 20 days, changes in TEWL between groups G1 and G3 (P=.03), groups G2 and G3 (P=.045), and groups G3 and G4 (P=.025) were significant. After 40 days, changes between groups G2 and G3 (P=.028) and groups G3 and G4 (P=.01) from baseline were significant.

Conclusion: Our results confirm the antiwrinkle activity of acetyl hexapeptide-3. A significant decrease in TEWL with acetyl hexapeptide-3 treatment is observed. We provided clinical evidence for the antiwrinkle efficacy of tripeptide-10 citrulline and possibly TEWL. The underlying mechanism by which these two peptides can act synergistically was not clear in this study.

KEYWORDS

acetyl hexapeptide-3, antiwrinkle efficacy, skin microtopography, TEWL, tripeptide-10 citrulline

1 | INTRODUCTION

Cosmetic science has shown a great interest in peptides and their benefits in skin, during the last years. Peptides are important in many natural processes with relevance to skin care, such as the modulation of cell proliferation, cell migration, inflammation, angiogenesis, melanogenesis, and protein synthesis and regulation. Peptides have been shown to reduce the amount or appearance of wrinkles and make skin more taut and smooth.

The acetyl hexapeptide-3 or acetyl hexapeptide-8, as it has been recently renamed, is one of the most commonly used peptides for this purpose. It is marketed under the trade name Argireline® by the Spanish company Lipotec.

It was reported that the antiwrinkle effects of acetyl hexapeptide-3 are similar to those of botulinum neurotoxin (BoNT).[1] The acetyl hexapeptide-3 mimics the N-terminal end of SNAP-25 protein. It competes with the natural protein for a position in the SNARE complex, which is essential for muscle contraction.[2] Due to its high toxicity, botulin toxin is under strict medical control. Compared with botulin toxin A, acetyl hexapeptide-3 is much less toxic, having a potency about 4000 times lower than that of botulin toxin. It can be applied topically, such as in a skin cream, rather than by injection. Acetyl hexapeptide-3 has been reported to penetrate through the skin in vitro[3] and to contribute in collagen synthesis of type I in vivo.[4]

Acetyl hexapeptide-3 is an effective anti-aging compound as already mentioned by Blanes-Mira et al.[3] The author, using silicon replicas, observed attenuation of the depth of the wrinkles after 30 days of application, when compared to vehicle.

Furthermore, acetyl hexapeptide-3 proved to be an effective ingredient due to its reduction in anisotropy of face skin and probable action on its mechanical properties.[5]

Acetyl hexapeptide-3 has been also proposed for the daily topical application after the injection of BoNT in treatment of blepharospasm to extend the duration of action of BoNT.[6]

Tripeptide-10 citrulline is also a mimic tetrapeptide of the sequences of decorin that specifically bind to collagen fibrils, improving skin suppleness and providing higher resiliency. It is marked under the trade name Decorinyl® by the Spanish company Lipotec.

Fibrillogenesis is an essential process in tissue formation, but must be controlled and regulated to avoid excessive bundle-like aggregation of collagen. The fibrillogenesis control is the role of decorin, a small leucine-rich proteoglycan, which is associated with collagen fibrils at specific binding sites in the protein core, controlling fibril dimensions, the uniformity of the diameter and their regular spacing.

Aging skin contains a truncated form of decorin, which lacks binding regions with collagen fibrils, producing a negative effect on the elasticity on the skin.

Tripeptide-10 citrulline has proved to regulate fibrillogenesis, control collagen fibril diameter, and increase skin suppleness.[7]

The combination of active ingredients with different or same mode of action such as of growth factor with antioxidants[8] or acetyl hexapeptide-3 with a botox-like peptide, that is, pentapeptide-3[9] for the enhancement of the anti-aging effects, and the possible synergy is a new trend in the cosmetic industry.

As a part of our research on peptides as anti-aging and cosmeceutical agents,[10,11] we thought it would be of interest to examine the possible synergy of acetyl hexapeptide-3 and tripeptide-10 citrulline.

We have used three different assessment methods to examine the effect and safety of the topical treatment of the skin. Two noninvasive, bioengineering, objective techniques proposed by EEMCO Group (European Group in Efficacy Measurement of Cosmetics and Other Topical Products) and subjective self-evaluation. Skin surface morphology was examined by a direct noninvasive method with the instrument Skin VisioscanVC98. Transepidermal water loss (TEWL) measurement can screen ingredients that have effect on barrier function and offer the possibility to monitor in vivo on human skin the effect of topical treatment in an objective and noninvasive way. Additionally, assessment of adverse effects of these two peptides was performed.

2 | MATERIALS AND METHODS

2.1 | Treatment creams

Creams contained identical vehicle. The active creams contained and acetyl hexapeptide-3 (Argireline®) at 10% w/w and tripeptide-10 citrulline (Decorinyl®) at 5% w/w (N. Crallis S.A. Distributor of Lipotec, Athens, Greece).

Placebo ingredients: Aqua, C12-20 acid PEG-8 ester, glyceryl stearate and PEG-100 stearate, cetyl alcohol, glyceryl stearate, propylene glycol, squalane, dimethicone, cyclomethicone xanthan gum, methylparaben, imidazolidinyl urea, propylparaben, BHT.

2.2 | Equipment

Skin surface evaluation: Skin Visioscan VC98 (Courage and Khazaka, Cologne, Germany). TEWL: Tewameter TM300 (Courage and Khazaka).

2.3 | Study design

Twenty-four female volunteers aged between 30 and 60 (mean age: 45±5 years) were included in the study. They were selected according to the following inclusion criteria:

Discontinuation of using any product for skin care during the study. Volunteers were provided a cleansing milk and were only allowed to use a sunscreen if necessary.

2.4 | Signed informed consent

Avoidance of medication such us steroid hormone replacement, birth control pills and vitamin supplements during the study period. At the baseline visit, the participants were instructed not to apply their test cream on the previous 12 hours.

Twenty-four coded 50-g vessels were distributed for use twice daily at the first visit. At each control, used vessels were collected to allow calculation of the amount of cream used.

The participants rested for a 20-minute acclimatizing period before the measurements at a room temperature ranging between 19 and 21°C and RH between 40% and 60%.

The subject was seated in a comfortable armchair with closed eyes to relax. In this position, measurements were taken in the frontal and periorbital regions.[12] Taking measurements on the forehead is a common practice for cosmetic products targeting moderate lines.[13]

2.5 | Statistical design

2.5.1 | Randomization

There are several commonly used methods of randomization. The purpose of randomization remains the same: to validate the assumption that the differences seen in the outcomes are likely due to differences in the treatments and not the baseline characteristics of the volunteers. Common types of randomization methods are the following: simple randomization, block randomization, stratified randomization, and minimization or adaptive randomization.

In our trial, due to small sample size, there is a need for choice block or stratified randomization.

2.5.2 | Factorial trials

Trials can be classified by design. This classification is more descriptive in terms of how patients are randomized to treatment. The most common design is the parallel-group trial. Patients are randomized to the new treatment or to the standard treatment and followed up to determine the effect of each treatment in parallel groups.

Other trial designs include, among others, crossover trials, factorial trials, and cluster randomized trials.

Factorial trials assign patients to more than one treatment comparison group. These are randomized in one trial at the same time, that is, while peptide I is being tested against placebo, volunteers are rerandomized to peptide II or placebo, making four possible treatment combinations in total.

A factorial design allows two treatments to be evaluated with a trial budget for a single comparison, providing both treatments have similar expected benefits.

2.5.3 | Description of experiment

In a balanced 2 × 2 factorial design, this would mean that from a total of *N* individuals, *N*/2 are randomly allocated to receive peptide I and *N*/2 are randomly allocated not to receive peptide I. Correspondingly, *N*/2 individuals are allocated to receive peptide II or to not receive peptide II Overall:

- *N*/4 individuals are allocated to no treatment (control group).
- *N*/4 individuals are allocated to peptide I (acetyl hexapeptide-3) only.
- *N*/4 individuals are allocated to peptide II (tripeptide-10 citrulline) only.
- *N*/4 individuals are allocated to the combination of peptide I + II simultaneously.

The benefit in terms of sample size or power of the factorial trial becomes apparent in the analysis. The usual method is to compare individuals who are randomized to intervention A (peptide I) (ie, those who receive peptide I and those who receive peptide I+II) with those who are not randomized to A (ie, those receiving either peptide II or no treatment at all). Similarly, individuals who are randomized to intervention B (peptide II) are compared with those who are not randomized to B.

Using a factorial trial, it is possible to perform two comparisons simultaneously at the cost of one experiment. In this case, it is possible to compare women who received peptide I with those who did not by comparing row margins. Similarly, by comparing column totals, it is possible to evaluate the effect of using peptide II. (Table 1).

It is also possible to analyze this as a four-way study by investigating each cell of the contingency table separately; however, the number of individuals included in each comparison is reduced, and consequently, the study loses power. All 2 × 2 factorial studies can be laid out in the same format as Table 1.

In our trial, subjects were randomly assigned to one of four treatment regimens: Group 1 (G1) peptide I+II; Group 2 (G2) peptide II; Group 3 (G3) peptide I; and Group 4 (G4) placebo.

2.5.4 | Sample size

The most common technique used to calculate the sample size for a 2 × 2 factorial study is to first think of the study as consisting of two individual two-arm trials. Sample size calculations are carried out for the target effect size of each intervention separately, assuming the same power and level of statistical significance. The final number of individuals that need to be recruited is taken from the comparison that provides the larger sample size—this will ensure enough power to assess the effect of the remaining comparison. Sample sizes are calculated in the usual way for parallel-arm randomized controlled trials, so the power to detect a treatment difference is dependent on the number of individuals in the groups being compared, not on the overall number of individuals in the study.

The above-mentioned calculations are based on the assumption that there is no interaction between interventions A and B; however, this will not necessarily be true. An interaction between interventions means that the effect of treatment A depends on the presence or absence of treatment B (or vice versa). In this case, it is more appropriate to consider the study as a multiple-arm study and ensure enough power to detect the smallest treatment difference among all possible pairwise comparisons. As a result, the trial can be viewed as a four parallel-arm study instead of a factorial trial, depending on the comparison of interest. With the presence of an interaction effect,

TABLE 1 Factorial design of the experiments

Peptide I (Acetyl hexapeptide-3)	Peptide II (Tripeptide-10 citrulline)		
	Yes	No	Total
Yes	Peptide I+II Group G1	Peptide I Group G3	Treated with peptide I Groups G1, G3
No	Peptide II Group G2	Placebo Group G4	No peptide I Groups G2, G4
Total	Peptide II Groups G1, G2	No Peptide II Groups G3, G4	Total women Groups G1, G2, G3, and G4

sample size calculations will depend on the aim of the study. The possibilities are as follows:

- To compare three active treatments with control and to show that any of the treatment combinations is effective compared with the control.
- To compare two active treatments with control and to show that either intervention is effective on its own compared with the control.
- To make six pairwise comparisons between all four groups.

The final sample size for the four-arm study is determined using the same method as above using the largest sample size as the final trial size.

2.6 | Statistical analysis

It is sometimes assumed that a 2 × 2 factorial study can be analyzed by handling the four different treatment groups separately. However, such an analysis lacks power as it excludes a number of individuals and does not take into account the benefits of the factorial design. On the other hand, if the study subsequently finds an unexpected interaction effect, then this might be a viable approach.

The underlying assumption of no treatment interaction in the analysis of conventional factorial studies needs to be validated; it is possible to test for the presence of an interaction by including an interaction term between treatments in a regression model, and comparing the same model without the interaction term. If the interaction term is a significant part of the model, an interaction between treatments exists and the study results must be presented separately for each treatment combination.

In general, however, the analysis should reflect the initial aim and design of the trial when assumptions seem tenable. To incorporate the full potential of a simple 2 × 2 factorial study, all individuals should be included in the analyses.

Advantages of a factorial design

- Cost
  The main advantage of a factorial design is its relative economy: it is possible to evaluate two or more interventions within the same trial at less than the cost of two separate trials, and possibly with only a marginal additional cost to a single trial of one intervention.

- Sample size
  Take, for example, the previously mentioned trial of peptide I+II, in healthy women. If the same study had been performed as a three-arm parallel study with the same sample size, *N*, then a third of the individuals would have been randomized to receive peptide I, a third would have received peptide II and the remaining third would have received neither. In the factorial design used, however, half of the women were randomized to each treatment irrespective of the other treatment. Therefore, a three-arm parallel trial has less power to make comparisons; moreover, to achieve the same power as in a 2 × 2 factorial trial, the three-arm parallel trial would need to randomize 1.5 *N* women. Hence, substantially fewer individuals are needed in a factorial trial than in a multiple-arm parallel study with the same power.

- Exploring interaction effects
  A second, often-quoted advantage is that factorial designs are useful to crudely evaluate the combination of interventions. If the aim of the study is to accurately quantify the interaction effect, many more individuals are required.

2.7 | Study procedure

Skin surface evaluation and TEWL were measured before (D0), during (D20), (D40), and (D60) after the treatment. Self-evaluation was performed by the test subject after the 2-month treatment (D60). Side effects were also evaluated by the volunteers.

2.7.1 | Microtopography of the skin

The microtopography of the skin was analyzed by the direct noninvasive method using the instrument Skin Visioscan VC98. In skin visioscan technique, the image of the skin is taken by a built of CCD camera. cR2 is the cyclic maximum roughness and cR3 is the cyclic average roughness, known as Rmax and Rz parameters, respectively; in DIN norm, they were measured. It is noteworthy that cR3 is not that much influenced by artifacts due to calculation of average.

2.7.2 | Transepidermal water loss (TEWL)

Tewameter was used. In this instrument, the water evaporation gradient, developed from the skin surface, is measured by a probe that is placed perpendicularly on the skin site to be measured. The probe consists of an open cylinder containing two hydrosensors coupled with the two thermistors placed at different distances from the skin surface. At both points, the relative humidity and temperature are measured and the corresponding vapor pressured is calculated. The difference between the vapor pressures at both points along the gradient is directly related to the rate of evaporative water loss through the particular skin site.

2.7.3 | Side adverse events

To evaluate the tolerability and the potential irritant power of the tested peptides, the subjects were asked to answer whether they experienced feeling of warmth in the skin, dryness, stinging, redness, desquamation, dryness, itching, or ocular intolerance during the treatment. These variables were scored on a scale of 1-4 indicating total (4), great (3), moderate (2), slight (1), and none (0).

3 | RESULTS

The main aim of the study was to investigate the possible synergism between the botox-like peptide acetyl hexapeptide-3 and tripeptide-10 citrulline. Therefore, statistical analysis was emphasized on the comparison of the measurements among four groups. The results are summarized in Tables 2 and 3. The significant results were obtained for the frontal region compared with periorbital region. A possible explanation is that for the periorbital region, a big group of muscle fibers is involved (*orbicularis oculi*) but at the intereyebrows zone level there is only one important mimic muscle (*corrugator supercilii*).[9]

3.1 | Microtopography of the skin

3.1.1 | cR2

After 20 days of application, cR2 was decreased in groups G1 and G3. An increase was noticed for cR2 parameter, in groups G2 and G4. The measurements between groups G2 and G4 and groups G1 and G4 were not statistically significant (Figure 1).

TABLE 2 Summary of efficacy results (mean ± SD)

	G1	G2	G3	G4
Skin Visioscan				
CR2				
0 days	91.1±19.1	91.5±16.4	83.8±21.5	82.3±30.1
20 days	84.6±11.5	94.2±11.3	77.6±10.7	85.8±9.4
40 days	84±18.4	82.4±25,6	75±12.3	83.6±11.7
60 days	79.4±1 4.5	80.4±25.4	74±9.9	84.6±15.3
CR3				
0 days	70±15.7	71.1±18,9	68.8±17.7	72.6±26.1
20 days	65.8±15.5	75.2±9.8	65.1±8.6	77.8±8.2
40 days	64.2±9.5	65.6±15.5	66±7.1	74±10.3
60 days	61.6±17.8	64.8±25.2	60.8±6.7	74.5±10.7
TEWL				
0 days	11.7±5.4	10±3.8	8.1±2.8	7±3.8
20 days	15.4±4.5	13.9±8.0	5.7±2.7	10±3.5
40 days	13.8±7.9	11.7±3.6	5.4±2.2	18±6.5
60 days	15.9±4.3	13.4±5.5	7.5±2.4	14.8±9.0

The observed measurements between groups G1 and G2 (P=.045), groups G2 and G3, and groups (P=.017) G3 and G4 (P=.022) from baseline were statistically significant.

Acetyl hexapeptide-3 led to significant decrease in cR2 (G3, −7.4%) compared with tripeptide-10 citrulline (G2, 3.0%) and placebo (G4, 4.3%). Tripeptide-10 citrulline did not improve the microtopography, although the observed increase in cR2 (G2, 3.0) is lower compared with placebo group (G4, −4.6%).

The combination of the peptides (G1) improved at −7.1% the microtopography of the skin with significance in comparison with tripeptide-10 citrulline (G2, 3.0%). As the acetyl hexapeptide-3 or the combination of the peptides led to significant decrease in cR2, whereas tripeptide-10 citrulline resulted in its increase, which seems that acetyl hexapeptide-3 is more effective for this short period.

After 40 days of application, cR2 was decreased in groups G1, G2, and G3, whereas it was increased in Group G1. The changes from baseline were greatest in tripeptide-10 citrulline group (G2) and second greatest in acetyl hexapeptide-3 group (G3), but there were no significant changes from baseline among four groups.

After 60 days of application, cR2 was decreased in groups G1, G2, and G3. An increase, although not statistically significant, was noticed for CR2 parameter, in Group G4. The measurements nor between groups G2 and G4 or groups G3 and G4 were statistically significant (Figure 1).

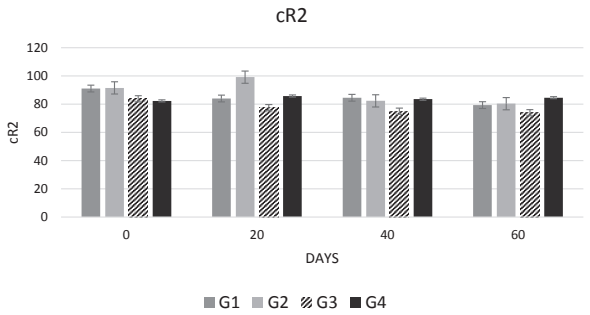

FIGURE 1 Changes in the mean value of cR2 parameter, indicative of fine lines. After 20 days: There were statistically significant changes from baseline between groups G1 and G2 (P=.045) and groups G2 and G3 (P=.017), and groups G3 and G4 (P=.022) After 60 days: There were statistically significant changes from baseline between groups G1 and G3 (P=.016), groups G2 and G3 (P=.044), and groups G1 and G4 (P=.025)

TABLE 3 Changes (%) of the parameters CR2 and CR3

		G1	G2	G3	G4
CR2					
20 days	$\frac{cR2_{20d}-cR2_{0d}}{cR2_{0d}} \times 100$	-7.1^{a}	$3.0^{a,b}$	$-7.4^{b,c}$	4.3^{c}
60 days	$\frac{cR2_{60d}-cR2_{0d}}{cR2_{0d}} \times 100$	$-12.8^{a,c}$	-12.2^{b}	$-11.7^{a,b}$	2.7^{c}
CR3					
20 days	$\frac{cR3_{20d}-cR3_{0d}}{cR3_{0d}} \times 100$	-6^{a}	$5.8^{a,b,c}$	$-5.3^{a,b}$	7.2^{c}
60 days	$\frac{cR3_{60d}-cR3_{0d}}{cR3_{0d}} \times 100$	-12^{a}	-8.8^{b}	$-11.6^{a,b}$	2.6

a, b, c= Significant changes.

The observed measurements between groups G1 and G3 (*P*=.016), groups G2 and G3 (*P*=.044), and groups G1 and G4 (*P*=.025) were statistically significant. The combination of the peptides (G1) improved at −12.8% the microtopography of the skin with significance in comparison with acetyl hexapeptide-3 (G3, −11.7) and the placebo group (G4, 2.7%).

It seems that after 60 days of application tripeptide-10 citrulline (G2 group) led to better results regarding cR2 parameter, whereas after 20 days acetyl hexapeptide-3 (G3 group) was more effective.

3.1.2 | cR3

After 20 days of application, cR3 was decreased in groups G1 and G3. The pattern of the results was similar to that of cR2. Briefly, a statistically significant increase was noticed for cR3 parameter, in groups G2 and G4. The measurements between groups G1 and G4 were not significant (Figure 2).

The changes in cR3 between groups G1 and G2 (*P*=.044), groups G2 and G3 (*P*=.017), and groups G2 and G4 (*P*=.028) were significant.

The combination of the peptides (G1) improved at about 6% the microtopography of the skin with significance in comparison with tripeptide-10 citrulline (G2, 5.8%). Acetyl hexapeptide-3 (G3, −5.3%) led to significant decrease in cR3 compared with tripeptide-10 citrulline (G2, 5.8%). For the period of the first 20 days of application, acetyl hexapeptide-3 seems to make the difference for the cR3 parameter as in the case of cR2 parameter. Tripeptide-10 citrulline (G2, 5.8%) resulted in significant lower increase in cR3 in comparison with placebo (G4, 7.2%).

After 40 days of application, cR3 was decreased in groups G1, G2, and G3, whereas it was increased in Group G4. The changes from baseline were greatest in the combination group (G1) and second greatest in tripeptide-10 citrulline group (G2), but there were no significant changes from baseline among four groups.

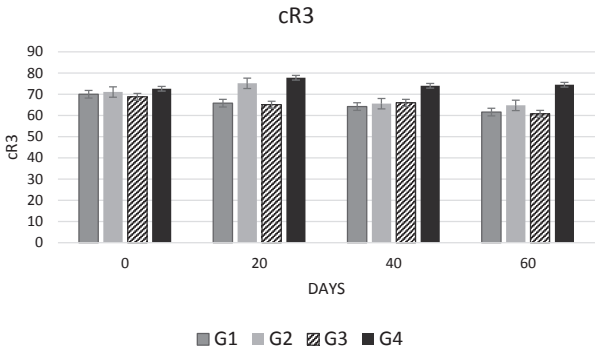

FIGURE 2 Changes in the mean value of cR3 parameter, indicative of fine lines. After 20 days: There were statistically significant changes from baseline between groups G1 and G2 (*P*=.044), groups G2 and G3 (*P*=.017), and groups G2 and G4 (*P*=.028). After 60 days: There were statistically significant changes from baseline between groups G1 and G3 (*P*=.025) and groups G2 and G3 (*P*=.044)

After 60 days, cR3 was decreased in groups G1, G2, and G3. The measurements nor between groups G2 and G4 or groups G3 and G4 were statistically significant (Figure 2).

A statistically significant decrease for cR3 parameter was observed between groups G1 and G3 (*P*=.025) and groups G2 and G3 (*P*=.044). Tripeptide-10 citrulline (G2) decreased significantly cR3 at −8.8% compared with acetyl hexapeptide-3 (G3, −11.6%).

The combination of the peptides (G1 group) improved at about −12% the microtopography of the skin with significance in comparison with acetyl hexapeptide-3 alone (G3, −11.6%). It seems that after a longer period of application, that is, 60 days, the tripeptide-10 citrulline (G2 group) influences on a specific way the microtopography of the skin, regarding parameter cR3.

In conclusion, the change in microtopography for the first 20 days is significant between the groups used the combined peptides (G1) and tripeptide-10 citrulline (G2) and for the total period of 60 days between the groups used the combined peptides (G1) and acetyl hexapeptide-3 (G3) (Table 3).

3.2 | Transepidermal Water Loss (TEWL)

After 20 days of application, TEWL was increased in groups G1, G2, and G4, whereas it was decreased in Group G3. Changes between groups G1 and G3 (*P*=.03), groups G2 and G3 (*P*=.045), and groups G3 and G4 (*P*=.025) were significant (Figure 3).

After 40 days of application, TEWL was increased in groups G1, G2, and G4, whereas it was decreased in Group G3. Changes between groups G2 and G3 (*P*=.028) and groups G3 and G4 (*P*=.01) from baseline were significant.

After 60 days, TEWL was increased in groups G1, G2, and G4. Decrease was observed in Group G3, although the observed changes were not significant (Figure 3).

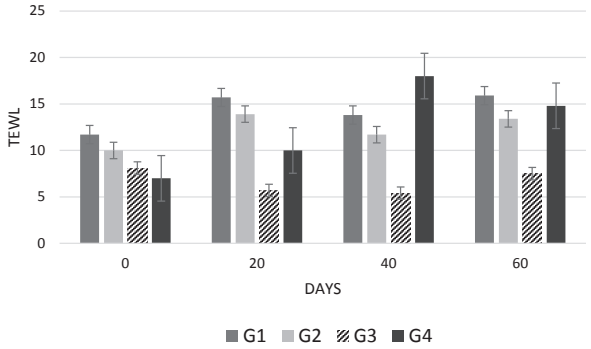

FIGURE 3 Changes in the mean value of transepidermal water loss (TEWL). After 20 days: TEWL was increased in groups G1, G2, and G4, whereas it was decreased in Group G3. Changes between groups G1 and G3 (0-20 days, *P*=.03), groups G2 and G3 (0-20 days, *P*=.045), and groups G3 and G4 (0-20 days, *P*=.025) were significant. After 40 days: TEWL was increased in groups G1, G2, and G4 and decreased in G3. Changes between groups G2 and G3 (0-20 days, *P*=.028) and groups G3 and G4 (0-20 days, *P*=.01) from baseline were significant

The application of acetyl hexapeptide-3 resulted in a significant improving tendency of TEWL for the first 40 days (G3 group). The treatment with tripeptide-10 citrulline alone (G2 group) or with the combination of the peptides (G1 group) increased TEWL, although the increase was much lower compared with placebo (G4 group).

To the best of our knowledge, there is no evidence in the bibliography regarding the correlation of the tested peptides with TEWL. Acetyl hexapeptide-3 (G3 group) seems to contribute significantly to the retention of the TEWL. The significant decrease in TEWL after 40 days of treatment with the peptide may be associated with the observed increase in the moisturization of stratum corneum reported by Tadini et al.[5]

As it could be expected, the greatest increase in the TEWL was observed in G4 group. The placebo cream was poor in emollients and humectants, as our target was to focus on the estimation of the influence of the "pure" peptides on the microtopography of the skin.

3.3 | Side adverse events

Neither of subjects of G1, G2, G3, and G4 groups who used the tested formulations reported feeling of warmth in the skin, stinging, redness, desquamation, itching, or ocular irritation. Dryness (score 3) was reported by the subjects of G4. The base of placebo cream was poor in emollients, because the main target of study was to evaluate the efficacy of the peptides. The results of the side adverse effects questionnaire are in a relative compliance with the results regarding TEWL measurements.

4 | DISCUSSION

Peptides are bioactive ingredients used in functional cosmetics to help improve parameters of the skin physiology. Regarding acetyl hexapeptide-3, our results are in agreement with the study reported by Blanes-Mira et al.[3] when acetyl hexapeptide-3 at a 10% concentration reduced the depth of wrinkles up to 30% after 30 days of use.

Tripeptide-10 citrulline is a synthetic tetrapeptide that improves the quality of collagen. Clinical studies for the efficacy of the tripeptide-10 citrulline have not been reported in the literature up to now. While the results of our study did not reach efficacy significance in all tested cases, the safety profile and the trend for improvement in frontal lines in the group using tripeptide-10 citrulline suggest that the peptide is effective in the improvement in skin microtopography especially after a 60-day period of application, whereas acetyl hexapeptide-3 is more effective after the initial period of application.

In our clinical trial, we observed a significant decrease in TEWL with acetyl hexapeptide-3 and a clear trend in the reduction in the increase in TEWL with tripeptide-10 citrulline or the combination of the tested peptides compared with placebo, although not significant. There are studies that report a correlation between supplementation with peptides and improvement in TEWL,[14,15] The water content of the skin depends on the TEWL and the hydration level of the epidermis, contributing to maintain skin health and youthful appearance.[16]

The significant decrease in TEWL after 20 and 40 days of treatment with acetyl hexapeptide-3 may be associated with the observed tendency in the increase in the moisturization of stratum corneum reported by Tadini et al., after 30 days of application of the same peptide. The possible mechanisms of the influence of acetyl hexapeptide-3 and/or tripeptide-10 citrulline on skin barrier have not been defined in our study.

In conclusion, we provided clinical evidence for the efficacy of tripeptide-10 citrulline to improve skin microtopography and possibly TEWL. Our results are in accordance with the studies reported for the clinical efficacy of acetyl hexapeptide-3. Additionally, a clear trend of acetyl hexapeptide-3 in improvement in TEWL has been observed.

Our experiments suggest that the combination of these two peptides could have clinical effects on the skin. It is well known that the tested peptides act by different mechanisms. The underlying mechanism by which these two peptides can act synergistically was no clear in this study. The results confirm that tripeptide-10 citrulline improves the function of dermis, which contains collagen fibrils and needs time to perform its activity. Additionally, the tested peptides did not exhibit primary skin irritation, thus making their use safe.

Further large-scale studies including elderly subjects will also be necessary to establish general opinion about the possible synergism of these two peptides.

ACKNOWLEDGEMENT

We would like to thank the distributor of Lipotec in Greece, N. Crallis S.A. (Athens, Greece) for providing us with acetyl hexapeptide-3 and tripeptide-10 citrulline.

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How to cite this article: Raikou V, Varvaresou A, Panderi I, Papageorgiou E. The efficacy study of the combination of tripeptide-10-citrulline and acetyl hexapeptide-3. A prospective, randomized controlled study. *J Cosmet Dermatol*. 2017;16:271-278. doi:10.1111/jocd.12314.