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Effect of the Use of a Cream with Leucine and Lactic Acid Associated with Electrostimulation in Contouring and Facial Tonus: A Randomized Clinical Controlled Trial

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Abstract: Evidence has supported the use of leucine as a promising agent for the maintenance of muscle tone. This study aimed to assess the combined effect of leucine and cream-based lactic acid (novel cosmetic product), associated with the use of surface electrical stimulation to improve contour and facial tonus in women. A total of 23 women were randomly allocated into two groups: Experimental (EG)—use of the leucine-based cream and lactic acid + electrostimulation for facial toning (mean intensity 13 Hz and protocol in progression); and placebo (GP)—use of the placebo cream (without the addition of leucine and lactic acid) + stimulation with the same protocol as the EG. Each group used their cream daily and underwent the intervention protocol three x/week with stimulation for 40 min, for a total of 8 weeks. Three main outcomes were reported: angular variation of facial contour by means of photogrammetry, muscle tone through the electromyographic activity of the masseter and zygomatic muscles during rest and functional tasks of biting and smiling. A significant effect of the intervention and between the groups was obtained for the experimental group against the placebo group for facial contour and muscle tone. An increased muscular activity of the masseter (average 28%) when smiling, and a reduction of zygomatic activity (in average 41%) when biting were found. The use of cream containing leucine and lactic acid combined with electrostimulation contributes to the improvement of facial contour and muscle tone when biting and smiling.

Keywords: facial aesthetics; cosmetics; exercise; muscle tone; antiaging; electrical stimulation; facial contour; leucine; lactic acid

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Citation: da Silva, C.S.M.; Costa, G.A.N.; Aguiar, A.F.; Camargo, M.Z.; Fernandes, K.B.P.; Oliveira, M.R.; da Silva, R.A. Effect of the Use of a Cream with Leucine and Lactic Acid Associated with Electrostimulation in Contouring and Facial Tonus: A Randomized Clinical Controlled Trial. *Cosmetics* **2022**, *9*, 36. https://doi.org/10.3390/cosmetics9020036

Academic Editor: Enzo Berardesca

Received: 5 February 2022
Accepted: 17 March 2022
Published: 24 March 2022

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1. Introduction

With the new scenario of an increasing aging population, many people have been concerned with aesthetic body care. Women, in particular, seek professionals in the area for treatment and prevention of the skin aging process [1–3]. The skin is a complex and dynamic organ, displaying the most evident signs of aging.

The aging process reflects the various, individual, intrinsic (biological) and extrinsic (external factors) changes that compromise the skin, as well as physical and social health [1].

In addition to the losses in functional capabilities and the increase of chronic degenerative diseases that come with advancing age [1–3], in facial skin, signs of ptosis, changes in the expression lines and facial contour, presence of wrinkles, muscular flaccidity and decreased muscle tone can be observed [4]. Other important factors associated with advancing age are low self-esteem and lack of quality of life, which can lead to stress, depression, and reduction in an individual's social activities and involvement [5–7].

A popular scientific and social alternative to minimize problems such as muscular flaccidity and decreased muscle tone is the use of leucine [8–11], a cosmetic ingredient that acts directly on improving intramuscular protein synthesis, potentiating the increase of tonus and the contractile response of the muscle during daily living activities [12,13]. Beyond the amino acid leucine, another derived from milk, lactic acid [14,15], may also contribute to the improvement of expression lines, contours, and wrinkles on the face due to the aging process [16]. In fact, both can have a beneficial action in the biological system, in particular for facial health during the aging process [9].

At this time, no study has researched the joint action of these two cosmetic ingredients, leucine and lactic acid, together for external use on the face by cream addition with the aim to improve muscle tone and firmness in women [17,18]. Leucine is often taken orally as a supplement. This product is often used for physical muscular performance in adults [18]. A study [18] evaluating 36 subjects (65–75 years), of whom two groups received L-Leucine orally (one group containing 20% and another group containing 40% of product) and third group received a placebo (lactose containing 0% L-Leucine), showed beneficial effects for the use of L-Leucine supplementation in different variables during a 12-week physical training period. The main variable outcomes measured in this study before and after intervention training were lean tissue mass (LTM) and functional performance related to different tests (e.g., 30 s arm-curl test; 30 s chair-stand test; 6 min walk test and handgrip strength). Specifically, the gains associated with L-Leucine compared to placebo were significant noted in LTM variable (ex: 1.1 $\pm$ 1.1%, $p = 0.003$) and in physical tests such as 30 s arm-curl (ex: 11.0 $\pm$ 11.5%, $p = 0.02$) and 6 min walk test (ex: 8.8 $\pm$ 10.0%, $p = 0.02$). The authors concluded that twice-daily supplementation of L-leucine (mainly containing 40% of product) combined with training stimulus improved lean tissue mass and physical function [18]. These results further support the originality of the present work to generalize these results, but with the use of leucine + lactic acid in a cream for women facing the aging process and aim, for the first time, to determine their impact on contouring and facial tonus.

Another feature often employed for toning the muscles is the use of electrostimulation, called Russian current [19,20]. This technique is used in health as skin-functional and aesthetic physiotherapy or rehabilitation and consists of surface electrostimulation for muscle strengthening [19]. Some evidence suggests that neuromuscular electric stimulation such as the Russian current can enable increased facial muscle tone, leading to an increase in muscle activity and enabling better expression and face contour [21,22]. This feature can also prevent the physiological hypotonia caused by biological changes that occur as we get older [23] or in the presence of degenerative diseases in different experimental populations, such as patients with neurological problems [24]. It would be interesting now to know if the use of electrostimulation combined with leucine and lactic acid can significantly improve muscle tone in adults. This demonstration, using a robust experimental protocol from a clinical trial design is warranted, as well as generalizing the findings to women in this case, since most of the studies on leucine have been on men within a sports or athletic environment.

Thus, the present study aimed to evaluate, for the first time in the literature, the effect of an intervention by means of leucine and lactic acid via facial cream associated with the use of electrostimulation to improve contour and facial tonus in adult women. A second objective was to assess the impact of this intervention on the women's quality of life. Our hypothesis was that the experimental group will present better results in the variables of facial contour and tone than the placebo group (cream without addition of leucine and lactic acid), consequently having a positive influence on quality of life.

2. Methods

2.1. Experimental Design and Participants

This is a longitudinal, descriptive and analytical study of random controlled clinical trial type (Register Number in ReBEC: RBR-6nv3c5; www.ensaiosclinicos.gov.br; accessed date: 15 March 2022. Date of registration: 12 November 2019), according to recommendations of Consort. A total of 50 women were recruited, aged between 30 to 65 years, on a voluntary basis and for convenience in the local community through advertising in social networks, brochures, and through personal contacts. Because cream with addition of leucine and lactic acid is a novel intervention associated to electrostimulation and no information is available on the effects of leucine and lactic acid by cream to improve contour and facial tonus and life quality in women, to support the research team in calculating a sample size, a total of 12 participants were recruited for each group. According to Julious [25], 12 participants are sufficient to assess the feasibility of an intervention and provide baseline data for the development of a subsequent larger study. In the present study, 24 women were included who remained until the final analysis (none dropped out), due to the withdrawal of other recruited women during the initial assessment and who did not meet inclusion and exclusion criteria.

Inclusion criteria for the study were as follows: female sex, healthy body and skin, not presenting obesity (Body Mass Index: BMI < 30 kg/m^2) and Fitzpatrick skin phototype 1 to 4. The exclusion criteria were: presentation any type of physical or mental diseases, which interfere with the use of cream in a continuous form and in the evaluation measures, have carried out some sort of facial surgery, deficiency or absence of cutaneous sensibility, presence of scars, allergies, hematoma and/or abrasions on the face, performing any type of treatment in aesthetic centers during the study period. Women were also excluded from the study with occurrence of tooth pain, pregnancy or lactating diseases with infectious process and/or inflammatory process in the facial region, acne grade ≥ 3, use of pacemakers and metal plates.

All participants were informed about the study and agreed to participate voluntarily according to the terms of free and informed consent including the use the cream and protocol to respect during the study. The project was approved following the standards of the CNS Resolution 466/12 of the National Health Council and the local Ethics Committee (CEP: 1.291.474).

2.2. Randomization of the Sample

The 24 participants were randomly allocated into two groups by means of a numerical sequence placed in an urn and drawn by the participant, guided by an external evaluator to the study. According to randomization, 12 women were allocated in the experimental group (EG), consisting in the use of leucine and lactic acid cream + intervention by facial electrostimulation; while 12 women were allocated in the placebo group (GP) consisting in the use of cream, without addition of leucine and lactic acid, + intervention by facial electrostimulation. Details of the intervention experimental protocol is described in the next section.

2.3. Evaluation

All the data collections occurred in the research laboratory and esthetical clinic at the University, upon appointment. Each evaluation session lasted up to 60 min. The study evaluation procedure consisted of the initial anamnesis through a structured interview to collect: Personal information, general health state, demographic data, anthropometric characteristics, skin analysis, use of medications or supplements, whether the participant is undergoing any aesthetic treatment, familiarization with the experimental protocol and explaining about the satisfaction questionnaire created by the authors, which would be administered at the end of study and was concerned with the feasibility of the study on an intervention with a novel product. A trained aesthetician, blind to the allocation of groups,

performed all the evaluations. Table 1 presents the characteristics of the participants of both groups that participated in the study.

Table 1. Participant characteristics.

	Groups		*t*-Test (*p*-Value)
	Experimental (n = 12)	Placebo (n = 12)	
Age (y)	38 (7)	42 (7)	0.285
Weight (kg)	71 (8)	69 (6)	0.669
Height (m)	1.62 (0.1)	1.60 (0.1)	0.265
BMI (kg/m^2)	26 (3)	27 (2)	0.650
Health perception *	90% (good)	80% (good)	
Medication use	35%	40%	
Supplement use	0%	0%	
Physical activity	20% (regular)	15% (regular)	
Prior aesthetic treatment	40%	20%	
Diseases	20% (metabolic)	20% (metabolic)	
Perception facial beauty *	60% (good)	40% (good)	
Allergies	0%	0%	
Smokers	0%	0%	
Skin type	50% (mixed)	50% (mixed)	

Data are mean ± standard deviation (SD). There were no significant differences between groups ($p > 0.05$). * Descriptive statistics.

2.4. Instruments of Measures (Outcomes)

2.4.1. Facial Contour (Computer-Aided Photogrammetry)

Facial contour was evaluated by angle measurements through computer-aided photogrammetry [26], which is a valid and reliable analysis to evaluate human posture and its body segments, as well as the face [27].

Each participant was placed in a comfortable seated position in front of a non-reflective black background, in accordance with the requirements of the software used for the photo analysis. The images were captured in the previous view (frontal plane), with spherical markers of Styrofoam, with a diameter of 1.5 cm, trapped by means of double-sided tape, in anatomic points, for the subsequent analysis of angles (Figure 1). The markings were fixed in zygomatic, masseter and mentalis [27,28].

The digital camera used for the photos was a 14.1-megapixel Panasonic, positioned parallel to the ground, on an aluminum tripod (Lightweight Tripod) brand VF—WT3510A, with a height of 108 cm in relation to the ground, and at 2.50 m from the participant.

After taking the pictures, the images were digitalized, processed and analyzed by means of ImageJ, v.145 software [29], which was developed for angular and linear analysis [30]. To determine the facial contour and internal facial expressions, measures were computed in linear and angular mode in two ways [28]: (1) measure the distance (in centimeters) and the angle of the nasolabial fold determined by the distance between the lip of the nose, and labial commissure (Figure 1A); and (2) angle between masseter and mentalis (ZMM) obtained by the union of zygomatic, masseter, mentalis points (Figure 1B). Both sides were evaluated, but no significant differences were found (Student's *t*-test, $p > 0.05$), thus the mean between the left and right side was performed to reduce the number of analyses and increase measurement reliability.

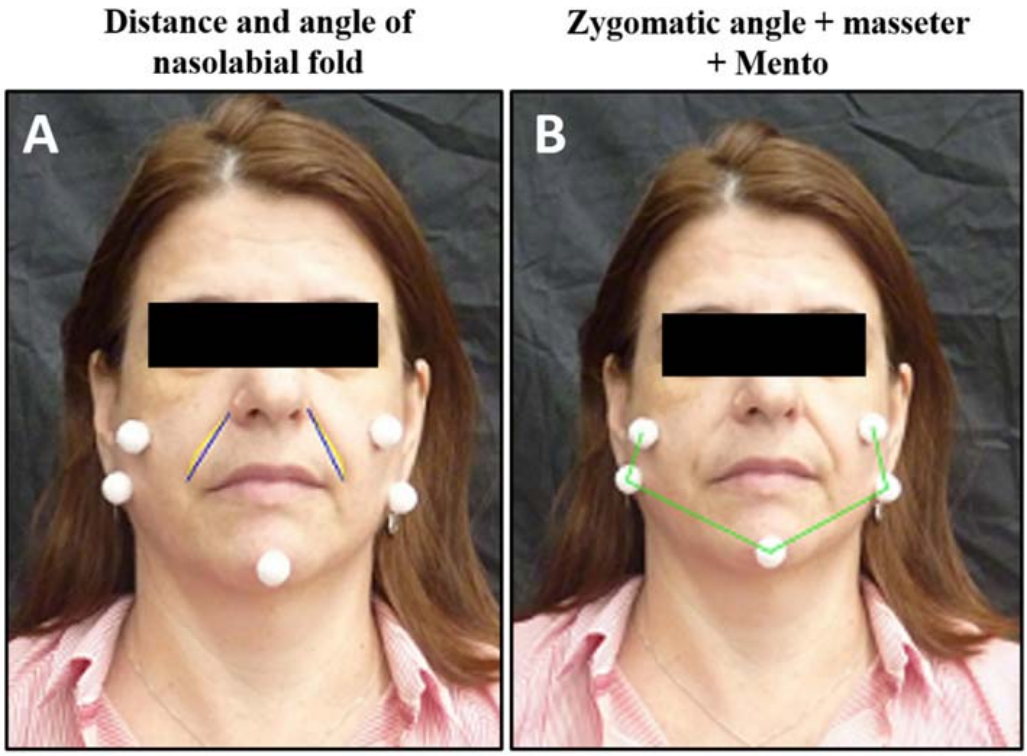

Figure 1. Representative figure showing the distance and angle of nasolabial fold (**A**) and angle between masseter and mento by the union of the zygomatic, masseter, and mento points (**B**) used for facial contour analysis and internal facial expression.

A trained evaluator in the use of photogrammetry (blind to the study) performed all the analyses, while image quality and evaluation criteria were established by a professional in the aesthetics area.

2.4.2. Facial Muscle Tone (Electromyography)

To evaluate facial muscle tone, the parameter of electromyography (EMG) amplitude of the EMG signal in Root Mean Square (RMS) was computed. This parameter shows the amplitude of muscle activity at rest and/or during a submaximal or maximal voluntary contraction [31,32]. Electromyographic signal capture was done using the EMG System of Brazil (brand EMG 430-D, São José dos Campos, SP, Ltda). The EMG signal was captured with four preamplified active circular electrodes (gain: 1000) and filtered in a passband between 10 and 500 Hz, with a sampling frequency of 1000 Hz.

After cleansing the skin with neutral liquid soap, scrubbing, and toning with a tonic astringent to reduce the impedance of the site, the electrodes were placed bilaterally, in relation to the orientation of the muscle fibers, on the masseter and zygomatic muscles [33–35], which are usually used in movements of biting and smiling (Figure 2). The reference electrode was positioned at the styloid process. All EMG signals were processed and treated in routines created in the MATLAB program (Version 7.0; The MathWorks Inc., Natick, MA, USA) to remove the parameter in amplitude of RMS muscle activation to quantify muscle tone during a maximal voluntary contraction (MVC) and an experimental protocol to bite and smile (Figure 2). All measurements were performed by an investigator blinded to the intervention groups.

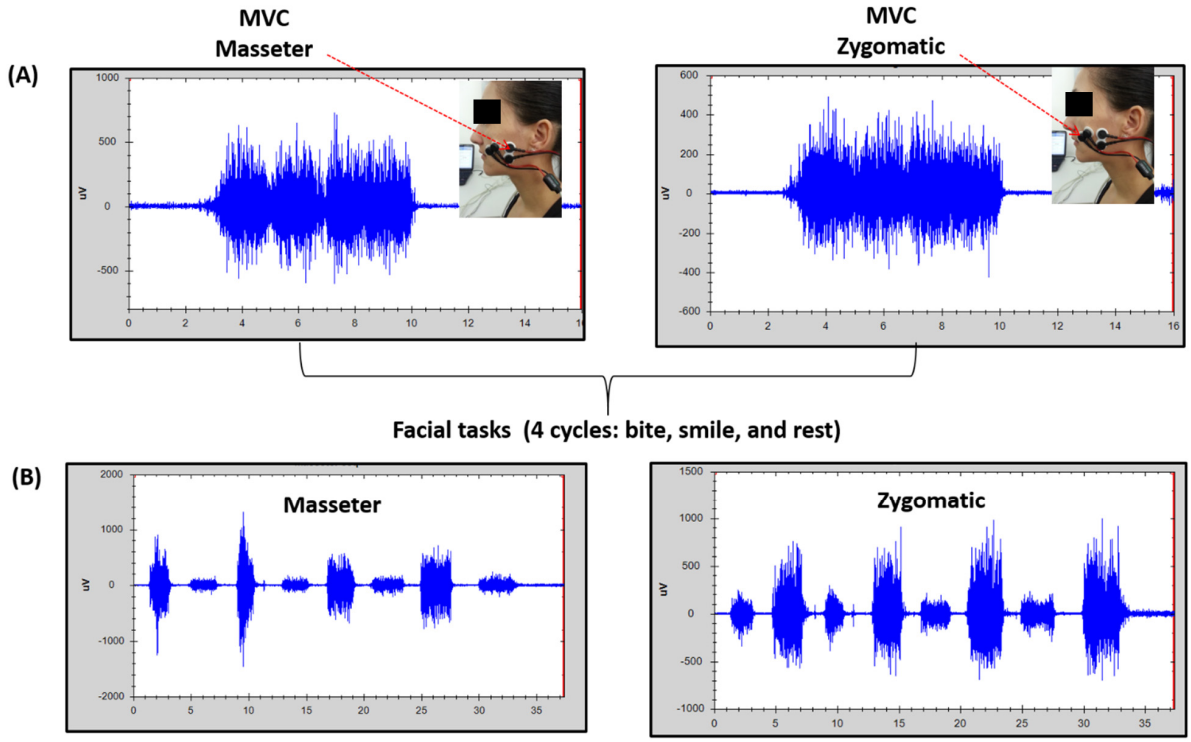

Figure 2. Representative figure showing the maximal voluntary contraction (MVC) of the masseter during bite task (**A-left**) and zygomatic during smile task (**B-right**) during 4 cycles of bite–smile–rest. Electromyography (EMG) signal was obtained during all cycles for maximal muscle activation (RMS_{MAX}).

Three MVCs were requested for each phase of movement, biting (action of the masseter muscle) and smiling (zygomatic muscle) with mouth closed [36], while the electrical signals of the masseter and zygomatic muscles were computed (Figure 2A). The maximum muscle activation peak (RMS_{MAX}) was retained for analysis in the normalization procedure. After MVC and 5 min of rest, all participants underwent 4 cycles of a functional task of bite–relax–smile–relax (2 s each stage), in submaximal contraction [33], to remove RMS amplitude during execution (Figure 2B). The average of 4 cycles was used to determine the RMS_{TASK}: Then, the EMG RMS was normalized for each muscle, based on MVC (RMS_{MAX}) to determine the % of muscle activity (tonus) necessary for the implementation of the experimental task, as in the equation below:

$$\%RMS\ (muscle\ activation) = [(RMS_{TASK}/RMS_{MAX}) \times 100\%]$$

A measure at rest was also applied to differentiate with tonus activity. In addition, no significant difference (Student's *t*-test, $p > 0.05$) was found between the bilateral muscles for both % RMS (muscle activation) and at rest. Thus, the average between the left and right side was performed to reduce the number of analyses and increase measurement reliability [31,32].

2.4.3. Quality of Life (Secondary Outcome)

After the initial structured evaluation, participants answered the short version of the questionnaire on quality of life (WHOQOL-bref), validated and adapted to the Portuguese language [37]. Quality of life has been defined as a perception of the individual in relation to their position in life and in the context of their culture and system of values in which the same is inserted, as well as in relation to their goals, expectations, standards, and concerns [7]. It is a concept of comprehensive scope, affected in complex ways by physical health, psychological state, level of independence, social relationships and characteristics of

the individual's environment [7,37]. For the WHOQOL-bref, every facet is assessed by only one question. This questionnaire is then comprised of four areas of quality of life, being that each area evaluates physical capacity, psychological well-being, social relationships and the individual's environment, totaling 24 questions for a maximum 100% score. In addition to these four areas, the questionnaire has two general questions on overall quality of life. The instrument considers the last 2 weeks experienced by the respondents. The higher the score, the better the quality of life in each domain.

In addition, a self-assessment/evaluation questionnaire will be administered at the end of the intervention. In total, 10 questions were elaborated by researchers and administered to both groups to determine the women's perception before using the cream and of their own concerns regarding the feasibility of using a new product. Responses were categorized as YES or NO. The questionnaire addresses the following questions, to which women should respond:

1. Did you achieve a good general condition of your skin/face after the intervention?
2. Did you feel an improvement in facial muscles?
3. Did you feel a decrease of dilated poles (if any)?
4. Did you notice an improvement in wrinkles and expression lines?
5. Do you feel that your skin/face had a more rejuvenated appearance?
6. Did you feel that your skin was oilier during the intervention?
7. Did you feel that your skin was drier during the intervention?
8. Did you feel that your skin was more hydrated during the intervention?
9. Did you notice an improvement in your spots/acne during the intervention (if any)?
10. Would you buy the proposed product?

2.5. Intervention (Cream + Electrostimulation)

The intervention began with all the participants only after the cream was validated microbiologically to use as standard protocol [38], comprised of base cream with the addition of leucine and lactic acid, and with positive results (see Appendix A including the information about the registration of a novel product in Brazil and main authors of registered cream; a process containing summarized step-by-step information of the cream formulation and also the microbiological validation before application).

The experimental group used the compound hydrant with an addition of leucine and lactic acid, while the placebo group used the same cream without the addition of leucine and lactic acid. Both groups were recommended to use the cream once a day throughout the study period. All participants, in both groups, also received a sunscreen with protection factor 30 for daily use until completion of the study for prevention and to avoid photosensitive reactions with lactic acid (recommendations from Guidelines for the evaluation of the efficacy of cosmetics products by COLIPA: https://colipa.eu/, accessed in date 17 March 2022). No group differences were linked to the use of this sunscreen related to the daily quantity used during the study, and we assumed that it did not affect the study results.

Besides the daily use of cream developed (leucine + lactic acid or placebo), both groups should participate in the electrostimulation sessions, 3x/week, with a total duration of 40 min as complementary intervention. The intervention protocol site included: Hygiene, use of cream, electrode positioning on face (masseter and zygomatic), and contraction and relaxation with current Russian equipment (TONEDERM, Fortis model M40, calibrated by the manufacturer). Figure 3 illustrates all these steps for each participant.

The electrostimulation was performed based on previous protocols, with progression in intensity and duration (Figure 3A,B for illustration purposes) for muscle toning [19,20]. The parameters of the current used in the care service protocol were: frequency modulated = 0–40 Hz; being for a time of rise (rise) = 2 s; time of permanence (cycle on) = 2 s; time of descent (decay) = 1 s; time of rest (cycle off) = 4 s for the first 2 weeks (Figure 3B). Afterwards, these items were adjusted according to progression [20]. The intensity used was based on each participant's tolerance threshold.

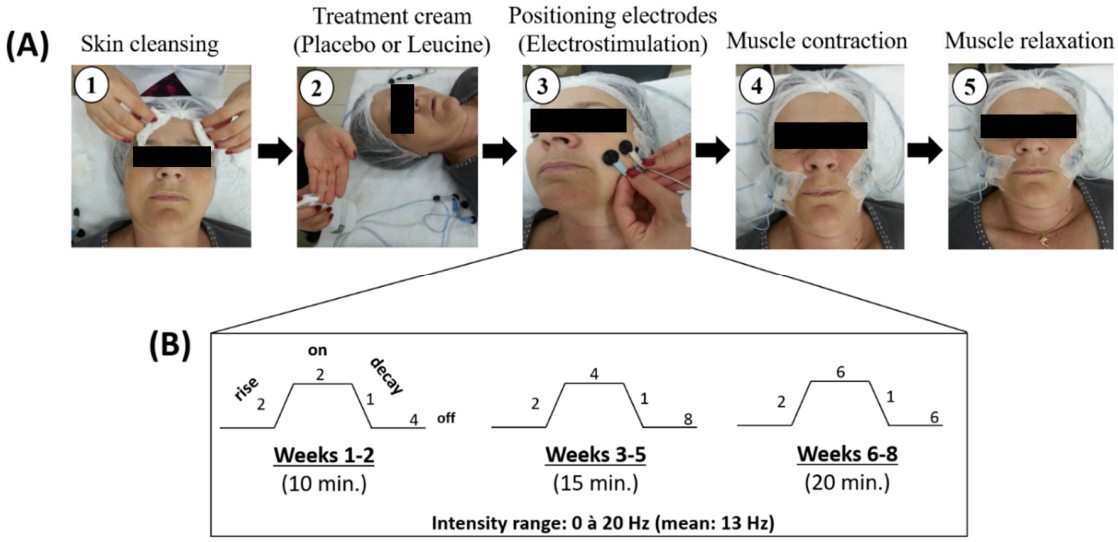

Figure 3. Steps (1 to 5) of intervention (**A**) and current Russian protocol (**B**) during the 8 weeks experimental period (total: 24 sessions).

The intervention lasted for a total of 8 weeks (24 sessions) and was performed by specialized aestheticians. Soon after these 8 weeks, all participants returned to the research laboratory to take the main measurements of the study (quality of life, facial contour, muscle tone and a self-assessment/evaluation questionnaire), according to the same experimental protocol used in the initial evaluation and by the same evaluator to ensure data validity and reliability.

2.6. Statistical Analysis

In general, all data had a normal distribution according to the Shapiro–Wilk test. A t-test for independent samples was used to compare the groups based on their anthropometric characteristics (age, weight, height, and BMI). Previously, the homogeneity of variances was also confirmed by Levene's test for each analyzed variable. The analysis of variance in two factors (two-way ANOVA), with repeated measures, was used to determine the intervention effects (pre- and postintervention) and the differences between the two groups (experimental and placebo) and interactions (Intervention × Groups) in the main measures of the study (facial contour, muscle tone, and quality of life). The data are presented as mean, standard deviation and frequency of findings for some variables, this last such as for the self-structured questionnaire which was used only at the end of study. When necessary, the magnitude of the interventions between the groups was used to determine effect size, in which the values of d [39,40] are suggested from the calculation in the equation below:

$$D = (mpost - mpre/DP)$$

where mpost is the postintervention mean and mpre is the preintervention mean and baseline SD (standard deviation), i.e., the SD of the measured preintervention was used due to being an unbiased estimate of the true variance of elderly women [40]. The magnitude of this effect is characterized by d of Cohen as small, medium and large effect, i.e., d = 0.2 is small, d = 0.5 medium and d = 0.8 large, respectively [39,40]. The statistical significance adopted for all analyses was $p \leq 0.05$ and they were carried out by means of the Statistical Package for Social Sciences (SPSS)v. 20.

3. Results

Both groups presented comparable demographic and anthropometric characteristics, as well as a similar overall health status, as shown in Table 1 (no significant difference between the groups; $p > 0.05$).

3.1. Effect on Facial Contour (Photogrammetry Measure)

Regarding facial contour by means of photogrammetry (Figure 4), significant differences ($p < 0.05$) were found in favor of the experimental group compared to the placebo group. The angle of the nasogenian fold increased by 10° for the experimental group (d = 1.0; $p = 0.043$), while the placebo group increased by only 2° (pre = 141° vs. post = 143°; without intragroup difference: Figure 4A). A significant increase ($p < 0.01$) and superiority to the placebo (GE = 19° vs. GP = 6°) was found in favor of the experimental group (large size of effect in (d = 2.71) to measure the contour of the zygomatic–masseter–mentalis angle (ZMM) obtained by the union of zygomatic, masseter, and mentalis points (Figure 4C). No difference between the groups ($p = 0.678$), nor the intervention ($p = 0.249$) and interaction ($p = 0.424$) effects were found to measure the distance from the nasogenian (Figure 4B).

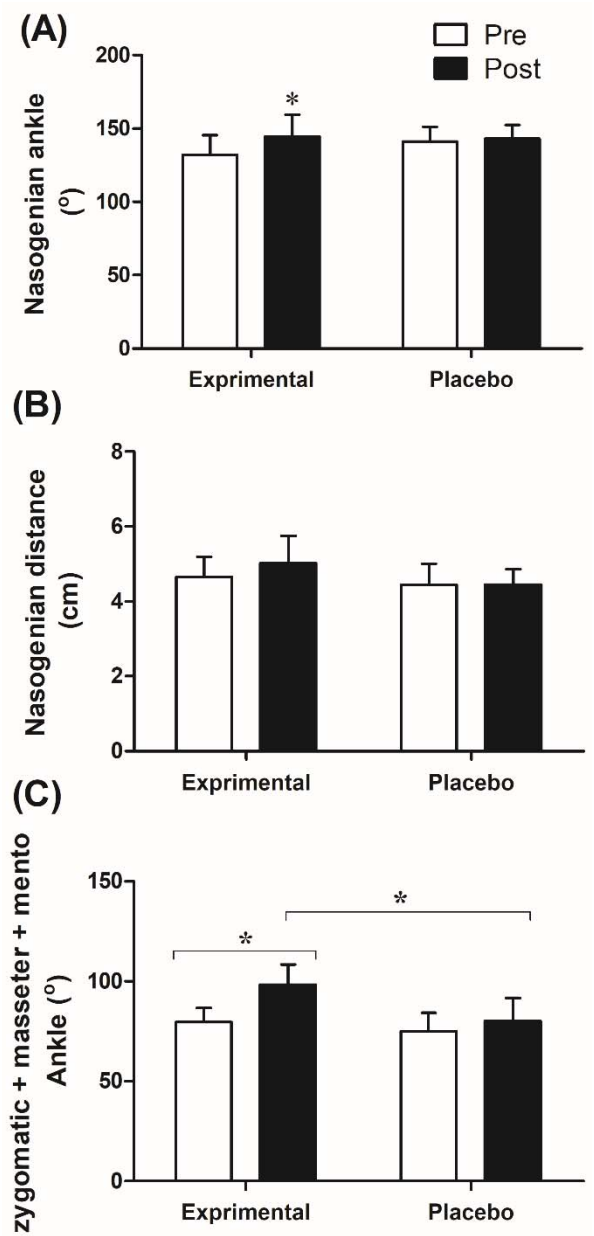

Figure 4. Nasogenian sulcus angle (**A**), nasogenian sulcus distance (**B**) and zygomatic + masseter + mento ankle (**C**). Data are mean and standard error (SE). * $p < 0.01$ compared to preintervention and control groups.

3.2. Effect on Facial Tonus (Electromyography Measure)

Regarding facial tonus (Table 2), there was no significant difference between the groups ($p > 0.05$) and no interaction ($p > 0.05$) was found for muscle activation. Significant differences ($p < 0.01$) were found in favor of both groups (experimental and placebo) for gains in muscle activation (average 28%) postintervention for the masseter muscle during functional task smile (effect size in d = 0.60). Significant differences ($p < 0.01$) were also found for both groups after the intervention in the reduction of muscular activity of the zygomatic (average 41%) during the functional task of biting (great effect in d = 0.80). No change in activation was observed at rest (Table 2).

Table 2. Muscle activation (EMG %) during three facial tasks (rest, smile and bite) before and after intervention.

Muscles	Tasks	Moments	Groups		Two-Way ANOVA (p-Value)		
			Experimental	Placebo	Groups	Intervention	Interaction
Masseter (%)	Bite	Pre Post	72 (17) 70 (14)	68 (11) 64 (12)	0.280	0.514	0.802
Masseter (%)	Smile	Pre Post *	26 (12) 58 (9)	25 (12) 49 (10)	0.238	<0.01 * ES = 0.6	0.345
Masseter (uv)	Rest	Pre Post	5 (2) 6 (2)	4 (2) 5 (2)	0.271	0.297	0.895
Zygomatic (%)	Bite	Pre Post *	85 (11) 49 (16)	86 (6) 40 (14)	0.517	<0.01 * ES = 0.8	0.121
Zygomatic (%)	Smile	Pre Post	34 (12) 32 (9)	35 (8) 33 (12)	0.634	0.546	0.969
Zygomatic (uv)	Rest	Pre Post	4 (2) 3.8 (1)	3 (1) 3.5 (1.5)	0.201	0.625	0.344

Data are mean ± standard deviation (SD). * $p < 0.05$ from pre-to-post-intervention in both groups. *ES*, effect size.

3.3. Effect on Quality of Life and General Perception after Intervention

No effect of the intervention and no difference was observed between the groups for the areas of quality of life investigated in this study (Table 3; $p > 0.05$). Both groups demonstrated a good quality of life, regardless of the use of cream. In general, the groups were similar in responses to questions about the effect of the intervention on the beauty of the face (% of YES as a response similar between them by means of 10 questions), as indicative of improvement, and all participants said that YES, they would purchase the product.

Table 3. Quality of life (WHOQOL-Bref) before and after intervention.

Domains	Moments	Groups		Two-Way ANOVA (p-Value)		
		Experimental	Placebo	Groups	Intervention	Interaction
Physical (%)	Pre Post	70 (14) 73 (13)	70 (9) 70 (10)	0.452	0.725	0.665
Psychological (%)	Pre Post	70 (14) 70 (13)	64 (8) 68 (10)	0.324	0.454	0.798
Social relationships (%)	Pre Post	70 (13) 70 (15)	69 (17) 71 (14)	0.933	0.762	0.998
Environment (%)	Pre Post	60 (13) 70 (12)	58 (6) 61 (6)	0.075	0.275	0.753
Overall QL (%)	Pre Post	70 (12) 70 (11)	65 (8) 67 (7)	0.302	0.443	0.863

Data are mean ± standard deviation (SD). No significant differences were observed (groups, intervention, and interaction; $p > 0.05$). QL, quality of life.

4. Discussion

This was the first study to verify the effect of an intervention by means of leucine and lactic acid via facial cream associated with the use of electrostimulation in improving contour and facial tonus in adult women. The hypothesis was confirmed in part. A significant effect of the intervention and between the groups ($p > 0.01$) in favor of the experimental group compared to the placebo group for facial contour (GE = 19° of increase vs. GP = 6°; =d = 2.71). Muscle tone improved in both groups, but no effect on the quality of life was observed postintervention in favor of one group over another. These results have implications for the use a cosmetic including leucine and lactic acid in cream form. Leucine is one of the amino acids that human cells use to synthesize proteins, with implications for skin health (face) and muscle toning.

Historically, in the scenario of physical activity and health promotion, the modulation of protein synthesis has been made by oral supplementation with amino acids, such as the use of leucine, and has received particular attention as a potential ergogenic resource (ergo—work and gen—production) by capsules for physical performance, muscle toning, and hypertrophy [18,41,42]. This is evidence demonstrating the fundamental role of amino acids, particularly leucine, in regulating anabolic processes involving both muscle protein synthesis and degradation [14]. After an oral administration of leucine + muscular strength training, some studies demonstrated beneficial and therapeutic effects such as reduction in loss of lean mass with the aging process, favoring the healing process, improvement of muscle protein balance in elderly individuals of both sexes, and metabolic pathologies in liver and kidney [15,43]. In addition, use of this product also proved to be beneficial in older people for gains in functional status as well as to improve lean tissue mass [18].

In the present study, the combination of the use of leucine and lactic acid in cream form with electrostimulation had an important effect on the women's facial contour after 8 weeks of intervention, whereas for muscle tone, apparently, the improvement occurred through the stimulus induced by electrostimulation and not necessarily the exclusive use of cream with leucine and lactic acid. Some explanations can be discussed here, although there are limits to comparing this study with previous ones based on oral supplementation (capsules), due to the lack of similarity in the experimental protocols.

It is believed that the improvement in facial contour, characterized by increased pre- versus postintervention angles (Figure 4), is due to an increased volume of muscle mass or enhanced by the use of leucine [40], characterized by a firmer and more contoured face around the zygomatic, masseter, and mentalis muscles. Unfortunately, it is impossible in the present study to identify which mechanism caused the improvement (volume vs. mass), but some theories explain this through an increased availability of amino acids, promoting the transport of the same inside muscle cells, thus stimulating protein synthesis [44,45].

Another possibility is that this effect, by means of leucine specifically, occurs by the increase in the phosphorylation of proteins involved in protein synthesis regulation, including the P70S6k and 4E-BP1, generally available in human skeletal muscle. Thus, the activity of p70S6k induced by combination with electrostimulation in the present study (and not by means of physical exercise as in other studies) [10,46] has contributed to the increase in the volume or muscle mass, which in turn has led to an increase in the facial contour after 8 weeks of intervention. Further studies are needed to explore these hypotheses and identify the real biochemical mechanisms of action and muscular conditions. Until now, these results are positive regarding women's health as a large number of participants in the experimental group reported during the study that they felt that their skin was firmer, more hydrated (combination of lactic acid), and with a reduction of wrinkles in some areas of the face.

On the other hand, this possible effect in increasing the volume or muscle mass that has contributed to the angular contours, was not reflected by a greater increase in muscle activation by means of the experimental group with the use of leucine and lactic acid compared to the placebo group. In fact, most of the studies until now show, in addition to the increase in muscle mass, an increase of mechanical muscle strength for the group

trained with the use of leucine amino acids [10,46]. However, these studies did not utilize features such as surface electromyography which reveals the physiological recruitment in active motor units in the muscle and not necessarily the production of mechanical force involving the active and passive structures of the muscle [47].

Despite the linear relationship between the EMG strength and some muscles, this is not completely true for smaller muscles such as the masseter and zygomatic, due to variations in the biological signals [47]. In other words, the increase of muscle activation is not characterized by increased mechanical force for smaller muscles. Moreover, increased muscle mass or volume does not necessarily translate to greater muscle activation, since this mechanism depends on neural actions and histomorphological changes inside the muscles such as the increase in type II fibers, which are characterized by an increased production of strength and lower resistance to fatigue [48]. The experimental protocol of the present study did not involve a long training or hypertrophy as in prior studies with leucine. It only used electrical stimulation by means of intermittent contractions in a threshold of submaximum muscular contraction in values below 50% of the muscle's maximal activation [19,20], which does not necessarily increase strength, but does improve resistance.

Therefore, it is possible that electrostimulation misled the improvement in muscular endurance and not necessarily in strength in both groups, even with a possible increase in muscular volume in the experimental group. This was evidenced in the activation pattern of the masseter and zygomatic muscles (Table 2). The masseter increased its synergistic participation in the task of smiling with mouth closed (28%), while the zygomatic muscle reduced its workload during the task of biting (41%) specifically in relation to the masseter muscle [36]. Furthermore, in the first 8 weeks of training or muscle stimulation, improvements in activation or strength are characterized by central stimuli (motor control) and not necessarily by hypertrophy or alteration of muscle fiber [48]. All these previously mentioned factors could explain why there is a lack of effect of leucine on the increase in muscle tone mediated by the activation of the facial muscles at rest and during the functional tasks of biting and smiling.

Finally, both groups showed a good quality of life, without any repercussion to this item postintervention. It is worth mentioning that both groups received the treatment involving the use of cream and electrostimulation sessions with the presence of specialized therapists. This brought a sense of safety and motivation to all participants three x/week for a total of 24 sessions, and consequently, contributed to similarity for this item. As in the experimental group, leucine has a peripheral physiological action inside the muscle, it is acceptable to think that no changes have occurred in the neural branches acting in neurotransmitter modulation, such as serotonin, which affects humor, joy, and well-being [49]. In fact, this is the first study that assesses quality of life based on this type of intervention, limiting the possible discussion on the findings.

From a future-oriented perspective, this study is innovative in the sense that the current aging process throughout the world makes it necessary to develop scientifically proven methods of action in cosmetology for the maintenance of facial health and aesthetics [2]. Not all products used in the aesthetics area have scientific support, which limits the generalization of the quality of services and the knowledge of the key benefits of the same in body and facial treatment. Lack of evidence may lead to erroneous thoughts about bad clinical decisions; and induce beliefs established only by experience or guesswork on the part of healthcare professionals. An important reflection of the area is necessary in this sense. The interdisciplinary method developed through this research work contributes to the advancement of science and education in the country and in the pursuit of benefits for the population's health and quality of life.

This study has its limits, as the results cannot be generalized to all women, such as those suffering from some serious metabolic disease, hyperthyroidism or hypothyroidism, or cancer, among others. These also cannot be generalized to men and elderly adults. Measures such as the mechanical strength of the face muscles with the use of dynamometry

were not evaluated, and an ultrasound measurement of muscle volume might have also been useful to correlate with the angular measures of facial contour.

5. Conclusions

The results of this study showed a significant effect of the intervention using a cream with leucine + lactic acid, associated with stimulation, compared to the placebo group in the improvement of facial contour in adult women. Muscle tone improved in both groups and no effect between-groups was observed for the quality-of-life questionnaire. These results have implications for the use of this new product containing lactic acid and leucine in cream form during facial cosmetology care. This cream could contribute to face firmness and/or muscle toning when associated to muscular stimulation.

Author Contributions: Conceptualization, C.S.M.d.S. and G.A.N.C.; methodology, C.S.M.d.S., R.A.d.S., A.F.A., M.R.O., M.Z.C.; software, M.Z.C. and K.B.P.F.; validation, C.S.M.d.S. and G.A.N.C.; formal analysis, R.A.d.S., M.R.O., M.Z.C. and K.B.P.F.; investigation, C.S.M.d.S.; writing—original draft preparation, all authors; writing—review and editing, C.S.M.d.S. and R.A.d.S.; supervision, G.A.N.C.; project administration, C.S.M.d.S., G.A.N.C. and R.A.d.S.; funding acquisition, R.A.d.S. All authors have read and agreed to the published version of the manuscript.

Funding: Rubens A. da Silva is funded by CNPq and FUNADESP (Scholarship on Productivity in Brazil—Level 2/2013 to 2017).

Institutional Review Board Statement: The project was approved following the standards of the CNS Resolution 466/12 of the National Health Council and the local Ethics Committee, Universidade Pitagoras UNOPAR, Londrina, Parana, Brazil (CEP: 1.291.474).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The study did not report any data.

Acknowledgments: We gratefully acknowledge the assistance of the physical therapy students at UNOPAR for data analysis.

Conflicts of Interest: The authors of this study declare no conflict of interest concerning this research.

Appendix A

Cosmetic product

Deposit Number: BR 102016030885-2

Deposit Date: 29 December 2016

National Publication Date: 17 July 2018

International Classification: A61K 8/44; A61K 8/365; A61K 8/368; A61Q 19/00.

CPC Classification: A61K 8/44; A61K 8/365; A61K 8/368; A61Q 19/00.

Title: Cosmetic composition containing leucine and lactic acid for facial muscles.

Holder: *Universidade Pitágoras UNOPAR—Editor and Distributor Educational* S/A, Education and Research Institution. CGC/CPF: 38733648002606.

Address: Av. Americo Deolindo Garla 224—Shop: a-5 piso terreo Pacaembu, Londrina, PR, Brazil (BR), 86079-225.

Inventors: Dr Giselle Aparecida Nobre Costa; Ms Carolina Silveira Martins da Silva.

Validity Period: 20 (twenty) years counted as of 12/29/2016, observing the legal conditions.

Expedited on: 27 July 2021.

Main authors of registered product:

Dre Costa, GN
Professor and researcher from the Food Science and Technology Department. She was an associate professor in the Sciences and milk technology program of UNOPAR and she is a professor at the State University of Londrina (UEL), Londrina, PR, Brazil.

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1 Formulation of Product Related to Article

Two formulations were developed: The base cream without the active leucine and lactic acid used by the placebo group, and a version containing the active leucine and lactic acid used by the experimental group.

The composition of the base formulation is Polawax™NF (Cetearyl Alcohol, PEG-150 Stearate, Polysorbate 60, and Steareth-20), Triglycerides of Capric and Caprylic Acid, BHT, Aqua, Disodium EDTA, Glycerin, Sorbitol, Ammonium Acryloyldimethyltaurate/VP Copolymer, Cyclomethicone, Phenova® (Phenoxyethanol, Methylparaben, Ethyl paraben, Propylparaben, Butylparaben, Isobutyl paraben), Pro-Lipo® Duo (Lecithin, glycerin, alcohol), while the composition of the active formulation was based on the addition of leucine (10.0%; *w/w*) and lactic acid (2.0%; *w/w*). Each active ingredient was weighed separately, then combined in phase 1 until complete dissolution of the waxes, and the phase 2 was separately heated to 80 °C. Phase 2 was poured over phase 1, phase 3 was incorporated and cooled under constant stirring with a spatula until the cream was formed. When the cream reached the temperature of 38 °C, it was added to phase 4 and homogenized. For the formulation with the active ingredients leucine and lactic acid (phase 5), these were added right after the fourth phase of the cream base.

Leucine was added to the Pro-lipo® Duo for phase 5 and taken to constant rotation from 500 to 1000 rpm for 5 min with stirring from Fisaton system (715 WS model). In addition, distilled water was added and stirred for another 5 min with the same agitation, then lactic acid and a preservative were added, and after gently stirring with a spatula, the pH was measured and found to be 5.

Finally, the two formulations were packaged in 80 mL vacuum-sealed containers designed for formulations that are sensitive to the external environment and providing a standardized dose of 0.20 mL per activation, and were labelled.

The formulation was used daily, applied to the whole face and neck area before bedtime during the entire study period. All participants also received an SPF 30 sunscreen for daily use until the end of the study.

2 Microbiological Evaluation from Validation and Formulation

For the microbiological quality evaluation, we used the methodology from the Resolution—RDC #481, on 23 September 1999, Health Ministry of Brazil: https://bvsms.saude.gov.br/bvs/saudelegis/anvisa/1999/res0481_23_09_1999_rep.html, accessed on 15 March 2022. This procedure was applied in the Type II analysis of product in accord with the Resolution. This procedure requires count: Mesophiles: 10^3 CFU/g; maximum limit: 5×10^3 CFU/g; and absence of pathogens (such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* spp.) in g or mL of the final product. All analyses

were performed in duplicate, using 5g of the formulation sample diluted in 45 mL of sterile peptone water (0.1%). Dilutions were also plated from 10^{-1} to 10^{-4}.

The analysis from total Mesophiles was done on PCA (Plate Count Agar) incubated at 35 °C/72 h.

P. aeruginosa—Agar base at 37 °C/48 h was used.

S. aureus—Mannitol agar at 37 °C/24 h was used.

E. coli—EMB (Methylene Blue Eosin) agar at 37 °C/24 h was used.

Salmonella spp.—Lactosate broth incubated at 35°/24 h was used, followed by MacConkey agar at 37°/24 h.

The mesophiles count averaged 3.5×10^1 CFU/g, while for pathogenic microorganisms, no growth of typical colonies was observed; therefore, all microbiological analyses indicated that the product was suitable for use.

Formulation 1: The mesophile count averaged 3.5×10^1 CFU/g.

Formulation 2: The mesophile count averaged 2×10^1 CFU/g.

Biobase SPF 30: The mesophile count averaged 1.5×10^1 CFU/g.

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