

Effects of α -lipoic acid on the biomechanical properties of the skin

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Abstract

Background: The Cutometer MPA 580® (Courage and Khazaka, Germany) is a well-established instrument for the accurate and reproducible measurement of the biomechanical properties of the skin. The purpose of this study was to assess the effect of 4 formulations containing 2.5% and 5.0% of α -lipoic acid and ascorbic palmitate or butylhydroxytoluene on skin elasticity and firmness and to assess the equivalence between alternative parameters (Q0, Q1 and Q3) and the traditional parameters R0, R2, R5, R6, all determined with the same cutometer.

Methods: Measure of in vivo firmness and elasticity of the skin was performed using R and Q parameters measured in the same device.

Results: Different statistical analysis were applied to the results obtained from the parameters (Q0, Q1 and Q3) and the traditional parameters R0, R2, of the in vivo measurements after the application of the four formulations during 28 days. A correlation between both types of measurements was demonstrate.

Conclusion: A four-week treatment with a cream containing 5% α -lipoic acid improves the biomechanical characteristics of the skin, thus contributing to the protection against photo-aging. Both methods of measurement proved to be equivalent.

Keywords: Alpha-lipoic acid; Cutometer; Firmness; Elasticity; Skin

1. Introduction

Alpha-lipoic acid, and its reduced form dihydrolipoate, are anti-inflammatory agents with a potent scavenger capacity on hydroxyl, superoxide and peroxy radicals, singlet oxygen and nitric oxide [1].

Alpha-lipoic acid also plays a crucial role in mitochondrial oxidative phosphorylation dehydrogenase process and as modulator of the inflammatory response.

Its low molecular weight (206.3 Da), together with its solubility in organic solvents justify the high absorption levels through the skin, where it exerts the pharmacological effects [2]. The kinetics of cutaneous and subcutaneous distribution after the topical application of α -lipoic acid on hairless mice has demonstrated a swift penetration through the epidermis and a distribution towards the dermis and the subcutaneous tissue after 4 h of topical application [3]. It is known that animals and humans synthesize α -lipoic acid; however, the exact mechanism biosynthesis pathway is not

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yet fully understood [4]. At the cellular level, α -lipoic acid acts as a vitamin-like compound and participates in the mitochondrial citric acid cycle as a co-enzyme of ketoglutarate dehydrogenase and pyruvate dehydrogenase [5].

DNA oxidative damage, particularly mitochondrial DNA (mtDNA) causes an accumulative effect. It has been demonstrated that α -lipoic acid can revert the age-related impairment of the mitochondrial function [6-8]. When the elasticity of the upper skin layer is measured, negative pressure is applied (suction method), which causes a mechanical deformation of the skin. Thus, the device creates negative pressure driving the skin into the opening of the probe and, after a defined time, the skin is released. Inside the probe, the penetration depth is determined by a non-contact optical measuring system. This optical measuring system consists of a light source and a light receptor, as well as two prisms facing each other, which project the light from the transmitter to the receptor. The light intensity varies as a function of the penetration depth. The resistance of the skin to the negative pressure (firmness) and its capacity to return to its original position (elasticity) are recorded in real time during the measurement as penetration depth (expressed in mm) vs. time curves. This method gives information on the elastic and mechanical properties of the skin surface [9-15] and allows the objective quantification of skin aging through the determination of the following parameters [16]:

1.1. R-Parameters

- R0: Extension; skin firmness, which represents the passive behavior of the skin against an external force. Lower values represent higher firmness.
- R2: Gross elasticity, which is the capacity of the skin to return to its original form.
- R5: net elasticity, which is the ratio between immediate recovery and immediate deformation.
- R6: Viscoelastic amount of elasticity, lower values indicate higher elasticity.

1.2. Q-Parameters

A set of parameters developed by Qu [17] has recently been added to the analysis of the skin properties. These curve parameters show the relationship between skin age and the elastic and viscous recovery:

- Q0: Maximum recovery area.
- Q1: Viscous recovery.
- Q3: Elastic recovery.

We have previously demonstrated *in vivo* the effect of α -lipoic acid (0.5%) and ascorbic palmitate (0.2%) in the improvement of the protective capacity of the skin and in the decrease of cutaneous sensitivity [18]. The aim of this study was to analyze the clinical efficacy of formulations containing α -lipoic at 2.5% and 5.0% by *in vivo* measurement of firmness and elasticity of the skin. R and Q parameters measured in the same device were also compared.

2. Material and methods

2.1. Materials and reagents

Ascorbyl palmitate was provided by Hoffmann La Roche (Switzerland), butylhydroxytoluene was from Eastman Chemical Company, USA. Vitamin A (as palmitate) was purchased from DSM (The Netherlands), Vitamin E (as acetate) was from Merck (Germany) and lipoic acid was from Labochim (Laboratorio Chimico Internazionale, Italy.)

Emulsions were prepared with silicone fluid (Dow Corning, Brazil); mineral oil, vaseline (R.A.A.M., Argentina) as oil phase; anionic self emulsifying wax (Flamacer SX, Flamaquímica, Argentina) as surfactant; imidazolidinyl urea (ISP, United Kingdom) as preservative; and 70% sorbitol (water solution), (Unión Química Argentina, Argentina) and demineralized water as hydrophilic phase.

2.2. Preparation of emulsions

The anionic emulsifier was melted in a stainless steel container; then, silicone fluid and mineral oil were added. The mixture was homogenized by slow stirring to avoid the incorporation of air and keeping the temperature between 72 °C and 74 °C. Lipoic acid and butylhydroxytoluene were then added. The mixture was stirred maintaining the temperature until a full dispersion was obtained. Demineralized water, 70% sorbitol and imidazolidinyl urea were mixed in a separate stainless-steel container. This mixture was heated up to 75 °C. Both phases were filtered by gravity filtration. The first mixture was incorporated into the second one and stirred at 900 rpm, for 5 min. The resulting mixture was then cooled and the stirring was slowed down. Vitamins A, E and ascorbic palmitate diluted in water were then incorporated when the mixture reached 45 °C. The composition of emulsions is shown in Table 1.

Table 1 Composition of emulsions

Materials (g/100 g)		Cream			
<i>INCI</i>		A1	B1	A2	B2
Cetearyl alcohol/ sodium lauryl sulfate/ sodium cetearyl sulfate	Anionic self emulsifying wax	9.000	9.000	9.000	9.000
Dimethicone	Silicone fluid	0.750	0.750	0.750	0.750
Paraffinum liquidum	Vaseline	5.750	5.750	5.750	5.750
Imidazolidinyl urea	Imidazolidinyl urea	0.200	0.200	0.200	0.200
Sorbitol	Sorbitol 70%	9.000	9.000	9.000	9.000
Retinyl Palmitate	Vitamin A palmitate	0.120	0.120	0.120	0.120
Tocopheryl acetate	Vitamin E acetate	0.400	0.400	0.400	0.400
Thioctic acid	Lipoic acid	2.500	5.000	2.500	5.000
BHT	Butylated hydroxytoluene	0.020	0.020	-	-
Ascorbyl Palmitate	Ascorbyl Palmitate	-	-	0.020	0.020
Aqua	Deminerlized water	100.000	100.000	100.000	100.000

2.3. *In vivo* study

The *in vivo* study was conducted in accordance with the intent and purpose of Good Clinical Practice regulations described under Title 21 of the U.S Code of Federal Regulations (CFR), the requirements of the Declaration of Helsinki (1964), Amendments Tokyo (1975), Venice (1983), and Hong Kong (1989), and /or CLAIM Standard Operating Procedures.

To participate in the study, ten women, of ages ranging from 35 to 60 years signed an informed consent previously approved by the CLAIM Institutional Review Board. Procedures for recruitment, selection, and inclusion of subjects had previously been established to provide the participants with clear and precise information. After randomization, half of the face of each volunteer was treated twice daily for 4 weeks with the test cream and the other half with the control cream. Self-evaluation by the test subjects and bioengineering methods were carried out.

2.4. Test procedure

The entire study was performed under specific environmental conditions. Temperature and relative humidity were controlled and maintained for each volunteer. Prior to instrumental measurements, subjects were instructed to rest for at least 20 min in a room set at a temperature of $22 \pm 2^\circ\text{C}$ and a relative humidity of $50 \pm 5\%$. The temperature and the humidity were recorded hourly during the study visit.

The Cutometer MPA 580® (Courage and Khazaka, Germany) is a well-established instrument for the accurate and reproducible measurement of the biomechanical properties of the skin. This instrument was used to determine the effects of treatments on the mechanical properties of the skin [19].

3. Results and discussion

3.1. Preliminary statistical analysis

In order to determine whether there were differences between the results obtained (Table 2) before and after 28 days of treatment, the Student's *t* test for paired samples was used when parameters displayed a normal distribution. Otherwise, the equivalent Wilcoxon non-parametric test for paired samples was used. The Shapiro-Wilks test was applied to test normality. Differences were considered significant if $p < 0.05$.

Table 2 Analytical data

Cream A1 Day 0	Parameter	n	Mean	SD	Median
	Firmness				
	R0	10	0.254	0.044	0.245
	Q0	10	48.96	5.317	48.90
Elasticity					
	R2	10	0.951	0.050	0.951
	R5	10	0.863	0.068	0.839
	R6	10	0.671	0.180	0.660
	Q1	10	0.652	0.450	0.9233
	Q3	10	0.081	0.045	0.078
Cream A1 Day 28	Parameter	n	Mean	SD	Median
	Firmness				
	R0	10	0.212	0.043	0.206
	Q0	10	41.797	4.032	40.77
Elasticity					
	R2	10	0.988	0.027	0.995
	R5	10	0.926	0.099	0.915
	R6	10	0.614	0.228	0.601

	Q1	10	0.889	0.048	0.903
	Q3	10	0.165	0.283	0.070
Cream A2 Day 0	Parameter	n	Mean	SD	Median
	Firmness				
	R0	10	0.259	0.055	0.258
	Q0	10	8.224	23.041	0.945
	Elasticity				
	R2	10	0.903	0.048	0.900
	R5	10	0.793	0.060	0.807
	R6	10	0.674	0.141	0.700
	Q1	10	0.867	0.037	0.885
	Q3	10	0.057	0.012	0.056
Cream A2 Day 28	Parameter	n	Mean	SD	Median
	Firmness				
	R0	10	0.210	0.064	0.215
	Q0	10	7.673	21.407	0.918
	Elasticity				
	R2	10	0.955	0.049	0.972
	R5	10	0.890	0.045	0.909
	R6	10	0.574	0.152	0.557
	Q1	10	0.835	0.056	0.835
	Q3	10	0.074	0.012	0.070
Cream B1 Day 0	Parameter	n	Mean	SD	Median
	Firmness				
	R0	10	0.235	0.035	0.237
	Q0	10	38.949	6.030	38.585
	Elasticity				
	R2	10	0.817	0.035	0.821
	R5	10	0.912	0.099	0.880
	R6	10	0.509	0.088	0.508
	Q1	10	0.868	0.227	0.944
	Q3	10	0.093	0.061	0.065
Cream B1 Day 28	Parameter	n	Mean	SD	Median
	Firmness				
	R0	10	0.239	0.024	0.238
	Q0	10	42.979	4.675	42.050
	Elasticity				
	R2	10	0.881	0.032	0.883
	R5	10	0.935	0.078	0.942
	R6	10	0.703	0.279	0.633
	Q1	10	0.887	0.096	0.941
	Q3	10	0.042	0.009	0.041

Cream B2 Day 0	Parameter	n	Mean	SD	Median
	Firmness				
	R0	10	0.244	0.06	0.244
	Q0	10	49.089	10.471	45.775
	Elasticity				
	R2	10	0.965	0.047	0.994
	R5	10	0.823	0.098	0.850
	R6	10	0.672	0.249	0.668
	Q1	10	0.827	0.180	0.869
Q3	10	0.108	0.067	0.099	
Cream B2 Day 28	Parameter	n	Mean	SD	Median
	Firmness				
	R0	10	0.198	0.066	0.199
	Q0	10	41.564	11.615	42.911
	Elasticity				
	R2	10	0.982	0.047	1
	R5	10	0.910	0.026	0.920
	R6	10	0.550	0.223	0.513
	Q1	10	0.932	0.023	0.931
Q3	10	0.124	0.063	0.114	

The results for Cream A1 are shown in Table 3. Significant differences were found in the skin firmness between times, being R0 and Q0 values on day 0 higher than those obtained on day 28 (p = 0.0072 and p = 0.0001, for R0 and Q0, respectively.)

Table 3 Paired analysis of firmness and elasticity obtained with Cream A1

Firmness		T	d.f.	P value
	Parameter R0: day 0 vs. day 28	3.02	9	0.0072
	Parameter Q0: day 0 vs. day 28	5.82	9	0.0001
Elasticity		T	d.f.	P value
	Parameter R2: day 0 vs. day 28	-1.84	9	0.0495
	Parameter R5: day 0 vs. day 28	-1.82	9	0.05
	Parameter R6: day 0 vs. day 28	1.05	9	0.8386
		Sum of ranges+	N	P value
	Parameter Q1: day 0 vs. day 28	22	10	0.7216
	Parameter Q3: day 0 vs. day 28	27	10	0.0248

df: degrees of freedom

The elasticity parameter (R2) presented significant differences between timepoints, with the average obtained on day 28 being higher than that obtained on day 0 (p = 0.0495.) The average R5 value obtained on day 28 was significantly higher than the baseline value; however, a higher number of subjects should be included to verify this result, since the p value obtained was 0.05.

Significant differences were found between day 0 and day 28, for neither R6 ($p = 0.8386$) nor for Q1 values ($p = 0.7216$). Conversely, highly significant differences were found for Q3, whose average value obtained on day 28 was markedly higher than that of day 0 ($p = 0.0248$)

The results obtained with Cream B1 are shown in Table 4. When firmness was evaluated, no significant differences were found for R0 ($p = 0.407$); however, for Q0, significant differences were found between the timepoints evaluated, being the average value obtained on day 0 lower than that of day 28 ($p = 0.0138$.)

Table 4 Paired analysis of firmness and elasticity obtained with Cream B1

Firmness		T	d.f.	P value
	Parameter R0: day 0 vs. day 28	-0.87	9	0.407
	Parameter Q0: day 0 vs. day 28	-2.63	9	0.0138
Elasticity		T	d.f.	P value
	Parameter R2: day 0 vs. day 28	-4.56	9	0.0007
	Parameter R5: day 0 vs. day 28	-0.76	9	0.4642
	Parameter R6: day 0 vs. day 28	-2.08	9	0.0337
		Sum of ranges+	N	P value
	Parameter Q1: day 0 vs. day 28	33	10	0.575
	Parameter Q3: day 0 vs. day 28	44	10	0.092

df: degrees of freedom

Significant differences were obtained for the elasticity parameter between both timepoints, i.e. both R2 and R6 average values obtained on day 28 were higher than the average values obtained on day 0 ($p = 0.0007$ and $p = 0.0337$, for R2 and R6, respectively.) No significant differences were found for R5 ($p = 0.4642$.) Moreover, significant differences were found for neither Q1 nor Q3 ($p = 0.575$ and $p = 0.092$, for Q1 and Q3, respectively.)

Table 5 shows the results obtained with Cream A2. Highly significant differences were found between day 0 and day 28 as regards firmness parameters R0 ($p = 0.005$ and $p < 0.0001$, for R0 and Q0, respectively.) Significant differences were found between day 0 and day 28 for the elasticity parameters R2 and R5, whose average values for day 28 were higher than the ones obtained on day 0 ($p = 0.0107$ and $p = 0.0008$, for R2 and R5, respectively.) No significant differences were found between day 0 and day 28 for R6 ($p = 0.7172$.)

Table 5 Paired analysis of firmness and elasticity obtained with Cream A2.

Firmness		Sum of ranges+	N	P value
	Parameter R0: day 0 vs. day 28	55	10	0.005
	Parameter Q0: day 0 vs. day 28	50	10	<0.0001
Elasticity		T	d.f.	P value
	Parameter R2: day 0 vs. day 28	-2.78	9	0.0107
	Parameter R5: day 0 vs. day 28	-4.41	9	0.0008
		Sum of ranges+	N	P value
	Parameter R6: day 0 vs. day 28	38	10	0.7172
		T	d.f.	P value
	Parameter Q1: day 0 vs. day 28	2.06	9	0.0346
	Parameter Q3: day 0 vs. day 28	-4.67	9	0.0006

df: degrees of freedom

Significant differences were found for Q1 and Q3, being the average value for Q1 on day 0 higher than that obtained on day 28 ($p = 0.0346$) and the average value for Q3 obtained on day 28 higher than the one obtained on day 0 ($p = 0.0006$).

The results obtained with Cream B2 are shown in Table 6. The firmness parameters showed significant differences between day 0 and day 28; on day 0, the R0 value was higher than the average obtained on day 28 ($p = 0.0006$). Q0 also presented highly significant differences between timepoints ($P < 0.0001$).

Table 6 Paired analysis of firmness and elasticity obtained with Cream B2.

Firmness		T	d.f.	P value
	Parameter R0: day 0 vs. day 28		4.68	9
		Sum of ranges+	N	P value
Parameter Q0: day 0 vs. day 28		50	10	<0.0001
Elasticity		T	d.f.	P value
	Parameter R2: day 0 vs. day 28	-0,77	9	0.4610
	Parameter R5: day 0 vs. day 28	-2.996	9	0.0075
		Sum of ranges+	N	P value
	Parameter R6: day 0 vs. day 28	1.04	9	0.3271
	Parameter Q1: day 0 vs. day 28	4	10	< 0.0001
		T	d.f.	P value
	Parameter Q3: day 0 vs. day 28	-2.25	9	0.0256

df: degrees of freedom

No significant differences were found between timepoints for the elasticity parameters R2 and R6 ($p = 0.4610$ and $p = 0.3271$, respectively.) Nevertheless, R5 values were significantly higher on day 0, as compared to day 28 ($p = 0.0075$).

Highly significant differences were found for Q1 ($p < 0.0001$); and significant differences were found for Q3 ($p = 0.0256$).

3.2. Second statistical analysis

In order to determine whether there were differences between the results obtained on day 0 between creams, the Student's *t* test for paired samples was used when parameters displayed a normal distribution. Otherwise, the equivalent Wilcoxon non-parametric test for paired samples was used. The Shapiro-Wilks test was applied to test normality. Differences were considered significant if $p < 0.05$.

A comparison between Creams A1 and B1 and between A2 and B2 are shown in Table 7 and Table 8, respectively. A comparison between Creams A1 and A2 and B1 and B2 are shown in Table 9 and Table 10 respectively.

Table 7 Firmness and elasticity. Paired analysis between Cream A1 vs B1.

Parameter	Day	T	d.f.	P value
R0	0	1.067	9	0.314
	28	-1.373	9	0.203
R2	0	6.45	9	0.0001
	28	9.51	9	0.0001
R5	0	-1.275	9	0.234
	28	-0.249	9	0.809
R6	0	2.40	9	0.0398

	28	-0.828	9	0.429
Q0	0	4.98	9	0.0004
	28	-0.606	9	0.559
Q1		Sum of ranges+	N	P value
	0	17	10	0.285
		T	d.f.	P value
	28	0.083	9	0.935
Q3		T	d.f.	P value
	0	-0.513	9	0.620
		Sum of ranges+	N	P value
	28	51	10	0.017

df: degrees of freedom

Table 8 Firmness and elasticity. Paired analysis between Cream A2 vs B2.

Parameter	Day	T	d.f.	P value
R0	0	0.55	9	0.593
	28	0.38	9	0.713
R2	0	-2.92	9	0.0086
	28	-1.198	9	0.261
R5	0	-0.793	9	0.448
	28	-1.166	9	0.274
R6	0	0.038	9	0.971
	28	0.314	9	0.761
Q0	0	1	10	0.007
	28	6	10	0.028
Q1		Sum of ranges+	N	P value
	0	29	10	0.3124
		T	d.f.	P value
	28	-4.06	9	0.0014
Q3		T	d.f.	P value
	0	-2.45	9	0.0183
		Sum of ranges+	N	P value
	28	-2.62	9	0.0139

df: degrees of freedom

Table 9 Firmness and elasticity. Paired analysis between Cream A1 vs A2.

Parameter	Day	T	d.f.	P value
R0	0	-0.411	9	0.691
	28	0.053	9	0.959
R2	0	2.5	9	0.034
	28	1.695	9	0.125
R5	0	-4.409	9	0.002
	28	1.244	9	0.245
R6	0	-0.04	9	0.969
	28	0.379	9	0.714
Q0	0	54	10	0.007
	28	54	10	0.007
Q1		Sum of ranges+	N	P value
	0	27	10	0.959
		T	d.f.	P value
	28	2.269	9	0.049
Q3		T	d.f.	P value
	0	1.618	9	0.140
		Sum of ranges+	N	P value
	28	29	10	0.878

df: degrees of freedom

Table 10 Firmness and elasticity. Paired analysis between Cream B1 vs B2.

Parameter	Day	T	d.f.	P value
R0	0	-0.576	9	0.579
	28	1.903	9	0.09
R2	0	--7.554	9	<0.0005
	28	-5.754	9	<0.0005
R5	0	1.926	9	0.086
	28	0.882	9	0.401
R6	0	-1.858	9	0.096
	28	1.355	9	0.208
Q0	0	-2.919	9	0.017
	28	0.323	9	0.754
Q1	0	1.573	9	0.150
	28	-1.149	9	0.280
Q3	0	-0.541	9	0.601
	28	-4.154	9	0.002

df: degrees of freedom

As regards R0, significant differences were found neither between Creams A1 and B1 on day 0 ($p = 0.314$) and on day 28 ($p = 0.203$) nor between Creams A2 and B2 on day 0 ($p = 0.593$) and on day 28 ($p = 0.713$). For Creams A1 and A2 no differences were found on day 0 ($p = 0.691$) and on day 28 ($p = 0.959$). Creams B1 and B2 showed no significant differences on both days ($p = 0.579$ and $p = 0.09$, for day 0 and day 28, respectively.)

The R2 value obtained on day 28 was significantly higher than that obtained on day 0 ($p=0.0007$) for both creams. The R2 average values on days 0 and 28 were higher for Cream A1 than for Cream B1 ($p = 0.0001$). Conversely, the R2 average value was lower for Cream A2 than for Cream B2 on day 0 ($p = 0.0086$), whereas no significant differences were found on day 28 between both creams ($p = 0.261$.)

The R2 average value on day 0 was higher for Cream A1 than for Cream A2 ($p = 0.034$), but no significant differences were found on day 28 between both creams ($p = 0.125$.)

Finally, the R2 average values obtained on day 0 and day 28 were lower for Cream B1 than for Cream B2 ($p < 0.0005$.)

As for parameter R5, on day 0, no significant differences were found between Creams A1 and B1 ($p = 0.234$) and between Creams A2 and B2 ($p = 0.448$). On day 28, p values were 0.809 and 0.274 for A1 vs. B1, and A2 vs. B2, respectively.

The average value for R5 on day 0 was higher for Cream A1 than for Cream A2 ($p = 0.002$), but no significant differences were found on day 0 for Cream B1 vs. Cream B2 ($p = 0.086$.) No significant differences were found between Creams A1 and A2 ($p = 0.245$), and Creams B1 and B2 ($p = 0.401$) on day 28.

The R6 average value on day 0 was higher for Cream A1 than for Cream B1 ($p = 0.0398$), but no significant differences were found on day 28 ($p = 0.429$). Between Creams A2 and B2, no significant differences were obtained ($p = 0.971$ and $p = 0.761$, for days 0 and 28, respectively.) No significant differences were found on day 0 between the average value for Creams A1 and A2 ($p = 0.969$) and between the average values for Creams B1 and B2 ($p = 0.096$). The comparison between Creams A1 and A2, and between B1 and B2 also revealed no significant differences on day 28 ($p = 0.714$ and $p = 0.208$, respectively.)

For Q0, significant differences were found on day 0 between Creams A1 and B1 ($p = 0.0004$) and between Creams A2 and B2 ($p = 0.007$); and on day 28, for Creams A2 and B2 ($p = 0.028$). No significant differences were found between Creams A1 and B1 ($p = 0.559$) for this parameter.

Q0 showed significant differences on day 0 between both pairs of Creams A1 and A2 ($p = 0.007$), and B1 and B2 ($p = 0.017$). On day 28, differences were significant between Creams A1 and A2 ($p = 0.007$), but for Creams B1 and B2, differences were not significant ($p = 0.754$.)

For Q1, no significant differences were found between Creams A1 and B1 ($p = 0.285$) and Creams A2 and B2 ($p = 0.3124$) on day 0, and between Creams A1 and B1 ($p = 0.935$) on day 28. Significant differences were found on day 28 between Creams A2 and B2. The average value for Q1 on day 28 was higher for cream B2 than for cream A2 ($p = 0.0014$.)

No significant differences were found between Creams A1 and A2 ($p = 0.959$) and Creams B1 and B2 on day 0 ($p = 0.150$), and for Creams B1 and B2 on day 28 ($p = 0.280$). Significant differences were found on day 28 between Creams A1 and A2 ($p = 0.049$.)

For Q3, Creams A1 and B1 did not present significant differences ($p = 0.620$) on day 0, whereas for Creams A2 and B2 the difference was statistically significant ($p = 0.0183$.) On day 28, differences were significant ($p = 0.017$ and $p = 0.0139$, for A1 vs. B1 and A2 vs. B2, respectively.)

No significant differences were found between Creams A1 and A2 ($p = 0.140$) and Creams B1 and B2 ($p = 0.601$) on day 0, and on day 28 ($p = 0.878$) between Creams A1 and A2. Significant differences were found on day 28 between Creams B1 and B2 ($p = 0.002$.)

3.3. Third Statistical Analysis

Different types of tests were used to analyze similarities between the methods employed.

Cream A1. Parameter R0

3.3.1. Intraclass correlation coefficient

This coefficient estimates the average of the correlations between all the possible orderings of paired observations. The intraclass correlation coefficient (ICC) is defined as the proportion of the total variability due to subject variability.

In this case, an ICC value of 0.945 was obtained. This value was greater than 0.90, which indicates a strong agreement between methods. Therefore, the observed variability can be explained in terms of the differences among the subjects and not in terms of the differences among the testing methods.

3.3.2. Correlation analysis

The coefficient obtained ($r = 0.569$; $p = 0.086$) indicated a weak correlation between methods; probably, a higher value could be obtained if a greater sample size is analyzed. (Figure 1)

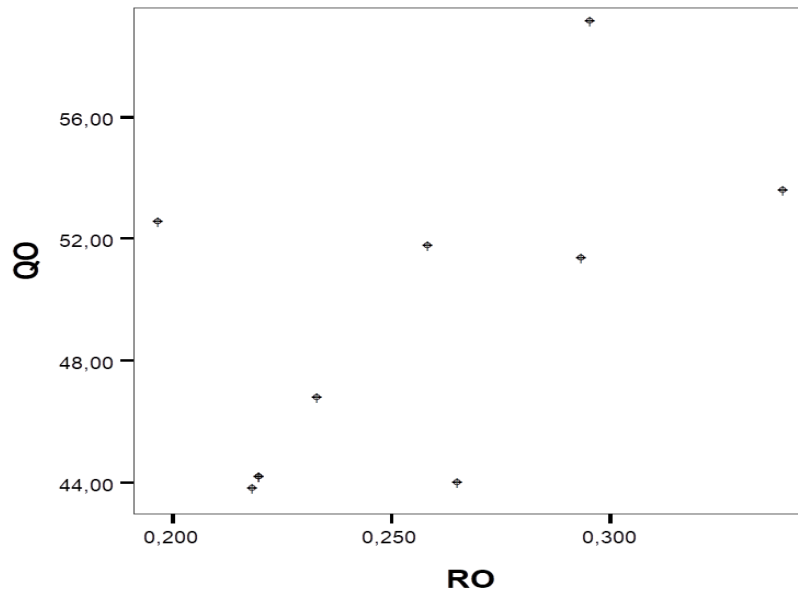


Figure 1 Correlation between the firmness values. RO: skin firmness. Q0: Maximum recovery area.

3.3.3. Conformity between methods of measurement. Analysis of individual differences

To determine the agreement limit and to represent the observed discrepancies graphically, the Bland-Altman method was applied. This method is a graphic representation of the differences between two measurements against its average (Figure 2.) Since in all cases differences were different from 0, no agreement between measurements was obtained.

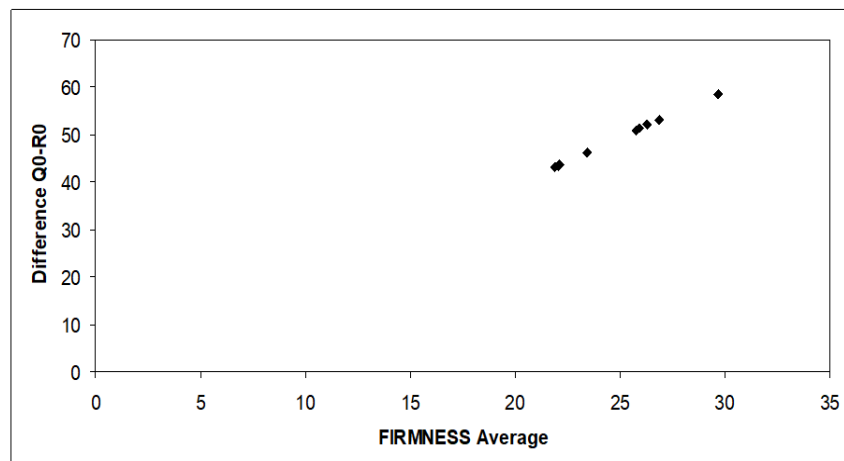


Figure 2 Bland –Altman analysis of differences between the firmness values

Significant differences were obtained for Cream A1 for parameters R0, R2, R5, Q0 and Q3 between the timepoints analyzed. These differences can be clearly observed in the Figure 3. With Cream B1, significant differences were obtained between day 0 and day 28 for R2, R6 and Q0. Cream A2 displayed significant differences between timepoints for parameters R0, R2, R5, Q0, Q1 and Q3. As for Cream B2, significant differences were obtained between day 0 and day 28 for R0, R2, R5, Q0, Q1, and Q3.

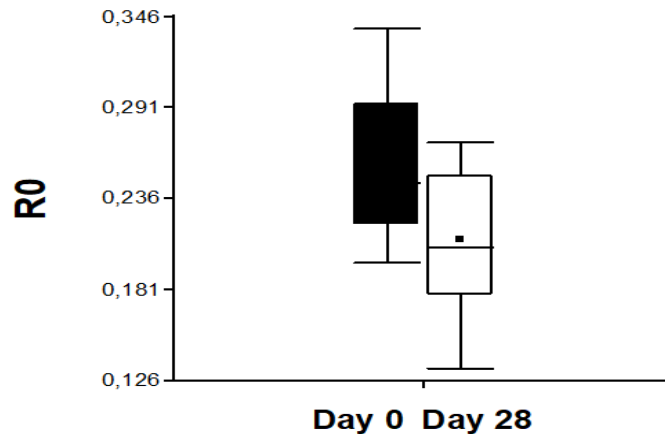


Figure 3 Box Plot graphic for Cream A1: significant differences for parameters R0, R2, R5, Q0 and Q3 between day 0 and day 28.

On day 0, significant differences were observed for Creams A1 and B1 as regards parameters R2, R6, and Q0. The same applied for Creams A2 and B2 for parameters R2, Q0, and Q3.

On day 28, Creams A1 and B1 presented significant differences for R2 and Q3. Differences were also significant between Creams A2 and B2 for parameters Q0, Q1, and Q3.

4. Conclusion

The results obtained in this study allow concluding that a four-week treatment with a cream containing 5% α -lipoic acid improves the biomechanical characteristics of the skin, thus contributing to the protection against photo-aging. Both methods of measurement proved to be equivalent.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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