

***In vivo* Objectivization Test**

on 35 Probands

over 2 - 4 - 6 Months

Test principle

A total of 35 female volunteers, aged 34-72 (average age: 58 years), participated in the 6-month study.

The anti-wrinkle effect of the **ULI BREITER Antifalten-Creme** and the **ULI BREITER Antifalten-Serum** (hereinafter called ULI BREITER Antifalten-Creme or UB Af-C) was tested as follows:

✍ Profilometry and image analysis

✍ Photos

✍ Clinical examination by a dermatologist

✍ Self-evaluation by participants

This was a blind study in comparison with a placebo and a commercial reference product.

Test plan

The study included 35 female volunteers without allergic reactions and skin diseases in their case histories.

The probands were divided into 2 groups:

- **ULI BREITER Antifalten-Creme** and a placebo cream is applied by 25 persons on both halves of their faces in arbitrary distribution.
- In the same manner, 10 persons apply **ULI BREITER Antifalten-Creme** and a

Results of the study are presented according to the following plan :

A. TEST VERSUS PLACEBO

A.1. Profilometry

- A.1.1. Total area of wrinkles
- A.1.2. Density of deepest wrinkle lines
- A.1.3. Volume of one of the largest wrinkle lines
- A.1.4. Unevenness of the skin
- A.1.5. Average depth of one of the largest wrinkles

A.2. Self-evaluation by probands

A.3. Clinical evaluation by a dermatologist

B. TEST IN COMPARISON TO A COMMERCIAL CREAM WITH VITAMIN C (5 %)

B.1. Profilometry

B.2. Self-evaluation

C. SUMMARY OF THE *IN VIVO* STUDY

ULI BREITER Antifalten-Creme in comparison with a placebo and a commercial product with 5 % vitamin C

A. TEST VERSUS PLACEBO

A.1. Profilometry

An *in situ* polymerizing SILFLO® gel is applied to the epicanthal wrinkles or crow's feet. Stripping the gel will provide a negative impression of the skin's irregularities.

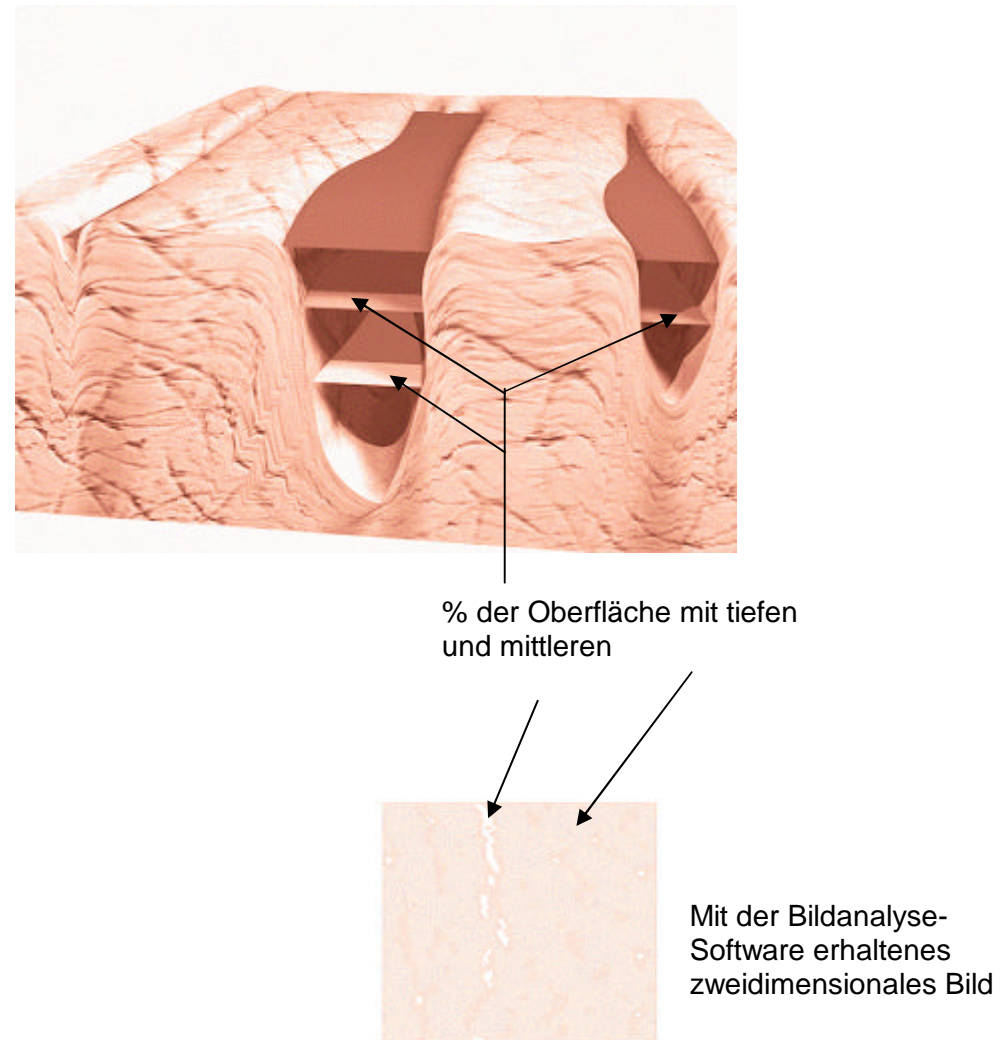
The impressions are illuminated by a flat-angle incident halogen light (25°), and the irregularities thus presented are subjected to image analysis (camera: HITACHI CCTV, software: Mountains

		DEPTH OF WRINKLE (in μm)							
		ULI BREITER Antifalten-Creme				Placebo			
		T0	T2	T4	T6	T0	T2	T4	T6
Average (μm)		120	100	80	60	120	115	117	125
% Difference to T0			-17	-40	-	-	-2	-1	+3
Student's pair test	Comparison Tx /T0	p<0.01 DS	p<0.01 DS	-	-	-	DNS	DNS	DNS
	Comparison UB Af-C /Placebo		T2	T4	T6				
			p<0.01 DS	p<0.01 DS	p<0.05 DS				

DS = Difference significant
DNS = Difference not significant

Die durchschnittliche Tiefe der Falte bei den Freiwilligen, die die **ULI BREITER Antifalten-Creme** testen, liegt an T0 be.

Der Rückgang von - 17 % ist statistisch signifikant im Vergleich zum Rückgang von - 2 % unter Placebo.



For every parameter, the area taken up is measured and expressed as a percentage of the total area.

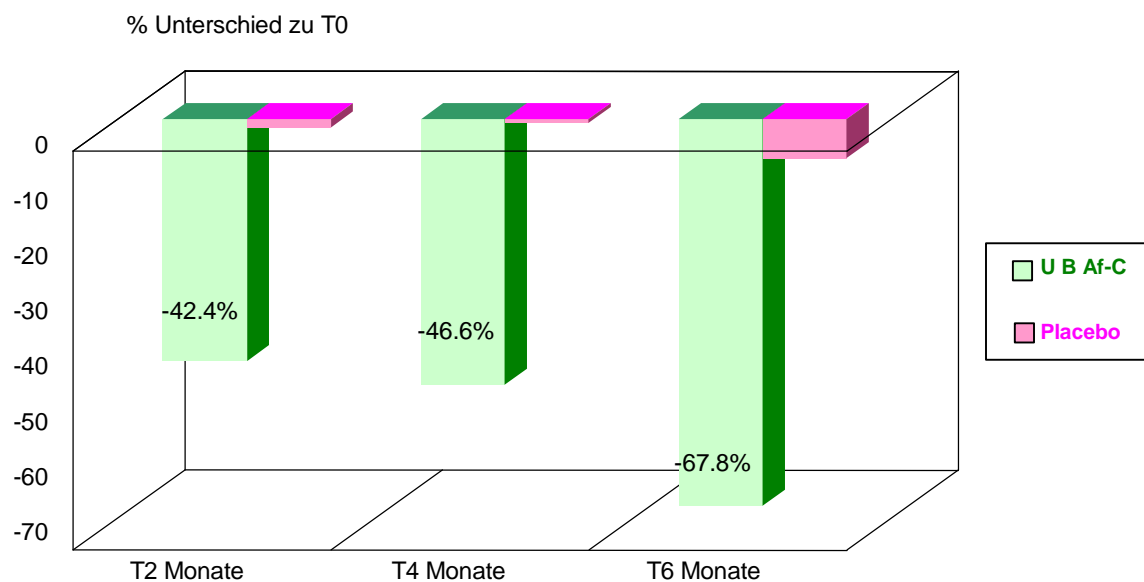
The area taken up by medium-deep wrinkles

becomes smaller : on the average by **- 51 %** (6 wrinkles)

AREA TAKEN UP BY DEEP WRINKLES (%)								
(Rough skin relief > 200µm)								
ULI BREITER Antifalten-Creme					Placebo			
	T0	T2	T4	T6	T0	T2	T4	T6
Average	6.3	3.6	3.4	2.0	4.6	4.6	4.5	4.3
% Difference to T		-42.4	-46.6	-67.8	-	1.4	-0.7	-6.8
Student's pair test	Comparison Tx /T0	p<0.01 DS	p<0.05 DS	p<0.01 DS	-	DNS	DNS	DNS
	Comparison U. B. Af-C/Placebo		T2		T4		T6	
			p<0.05 DS		p<0.05 DS		P<0.01 DS	

DS = Difference significant

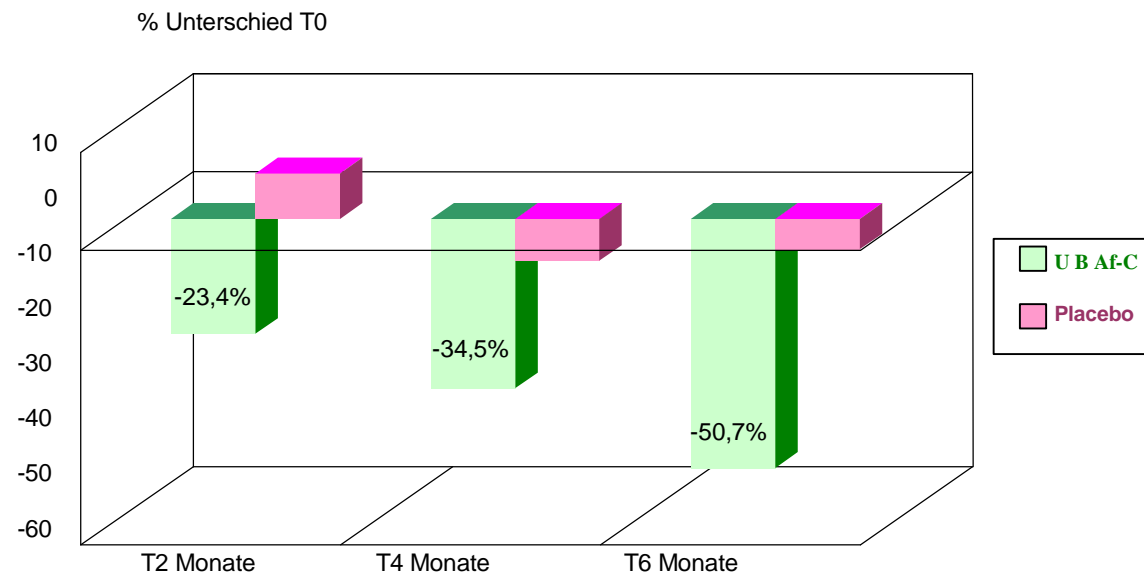
DNS = Difference not significant



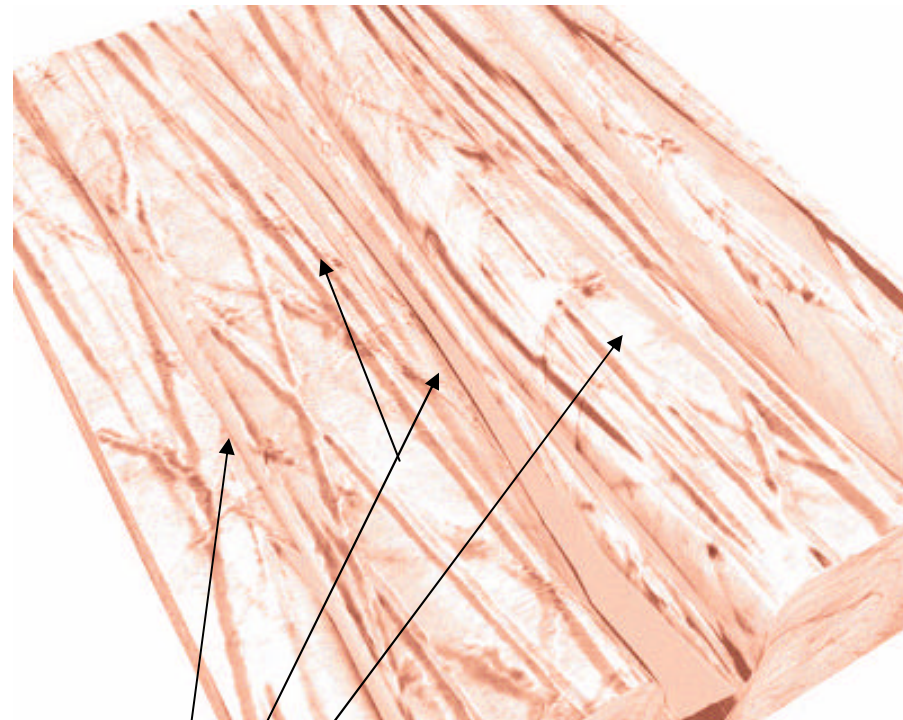
Student's pair test	Comparison Tx / T0	p<0.05 DS	p<0.05 DS	p<0.01 DS	-	DNS	DNS	DNS
	Comparison U B Af-C/Placebo	T2			T4		T6	
		p<0.05 DS			p<0.05 DS		P<0.01 DS	

DS = Difference significant

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This parameter indicates the length of the deepest wrinkles. The sum of the lengths in relation to the examined area results in the density per area unit ($\mu\text{m} / \text{cm}^2$).



Die schraffierten Faltenlinien sind am größten und außerdem am tiefsten. Die Länge der Faltenlinien und deren Summe je Flächeneinheit ($\mu\text{m} / \text{cm}^2$) wird nach Vektorisierung mit der Bildanalyse-Software gemessen.



Mit der Bildanalyse-Software erhaltenes zweidimensionales Bild

Results

ULI BREITER Antifalten-Creme shows a great reduction of the average density whereas the placebo rather has the opposite effect.

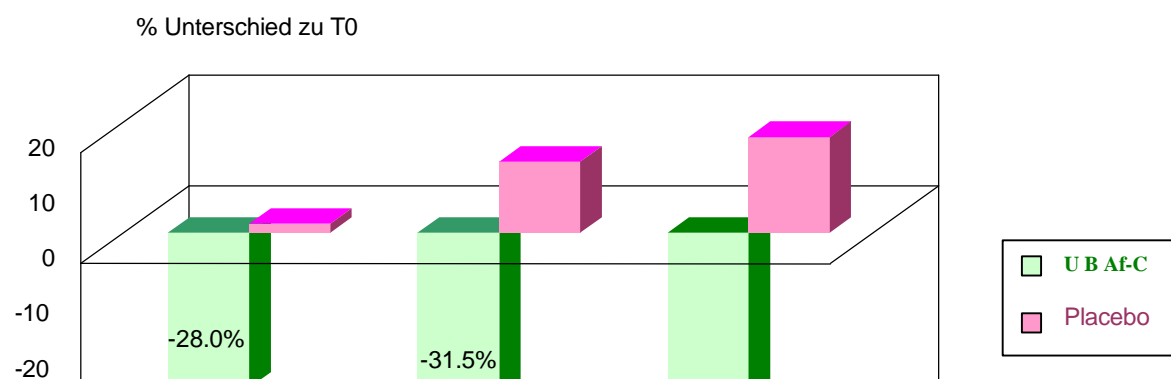
The results of ULI BREITER Antifalten-Creme improve ever more in the course of time: After 2 months, the reduction is 28 %, after 4 months 31 % and after 6 months 47 %.

At the same time, there is an increase in the statistical significance of variations observed after 2, 4 and 6 months versus T0: The p-value drops from 0.01 to 0.002, reflecting the increasing significance of the results. This same tendency is found in a statistical comparison with the placebo (the effects of the placebo are not significant at all times of measurement).

		DENSITY OF WRINKLE LINES (in µm/cm²)							
		ULI BREITER Antifalten-Creme				Placebo			
		T0	T2	T4	T6	T0	T2	T4	T6
Average		186.7	134.4	127.9	99.4	146.0	148.3	164.7	171.1
% Difference to T0		-28.0	-31.5	-46.8		-	1.6	12.8	17.2
Student's pair test	Comparison Tx / T0	p<0.05 DS	p<0.05 DS	p<0.01 DS	-	DNS	DNS	DNS	
	Comparison UB Af-C/Placebo	T2		T4		T6			
		p<0.05 DS		p<0.05 DS		p<0.01 DS			

DS = Difference significant

DNS = Difference not significant



This particularly conspicuous parameter is presented in the following diagram.



With every proband, the volume of one of the deepest wrinkles was measured at the respective times of examination.

The wrinkle became ever smaller in time: after 2 months, it had lost 7 % of its volume, after 4 months 21 % and after 6 months 24 %.

Meanwhile, the placebo remained without effect.

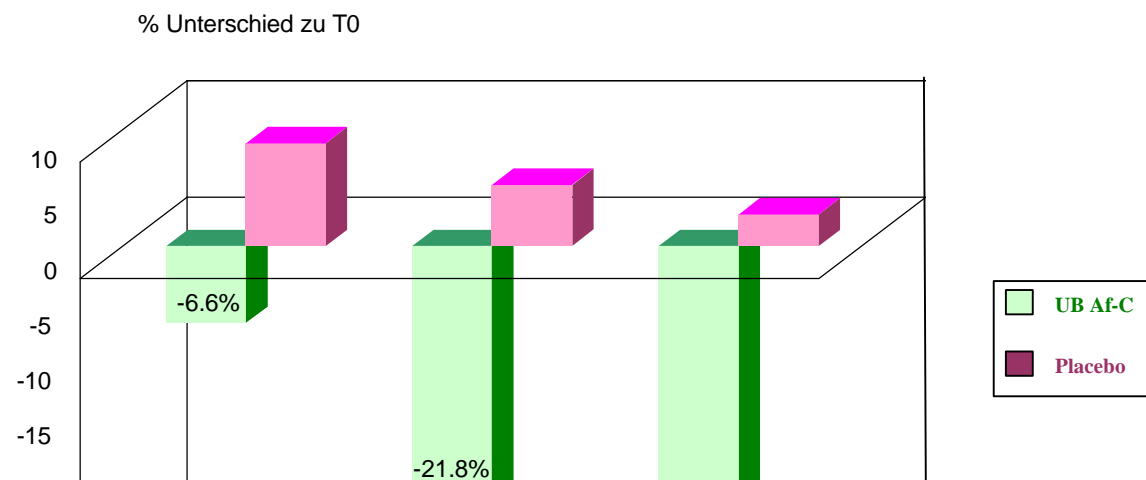
The statistical evaluation confirms that the **effect of the**

Urbreiter Antifalten-Creme becomes ever more pronounced in time

VOLUME OF ONE OF THE LARGEST WRINKLES (in mm ³)								
ULI BREITER Antifalten-Creme					Placebo			
	T0	T2	T4	T6	T0	T2	T4	T6
Average	0.29	0.27	0.23	0.22	0.26	0.28	0.27	0.27
% Difference to T0		-6.6	-21.8	-24.4	-	8.8	5.2	2.7
Student's pair test	Comparison Tx / T0	p<0.05 DS	p<0.01 DS	p<0.01 DS	-	DNS	DNS	DNS
	Comparison UB Af-C/Placebo		T2		T4		T6	
			p<0.05 DS		p<0.01 DS		p<0.01 DS	

DS = Difference significant

DNS = Difference not significant



A.1.4. Unevenness of the skin

This parameter is one of the most frequently examined parameters because it can be measured with various image analysis methods.

The average amplitude of the skin's depressions and bumps provides a global insight regarding the unevenness of the surface examined (surface appearance versus an even surface).

Results

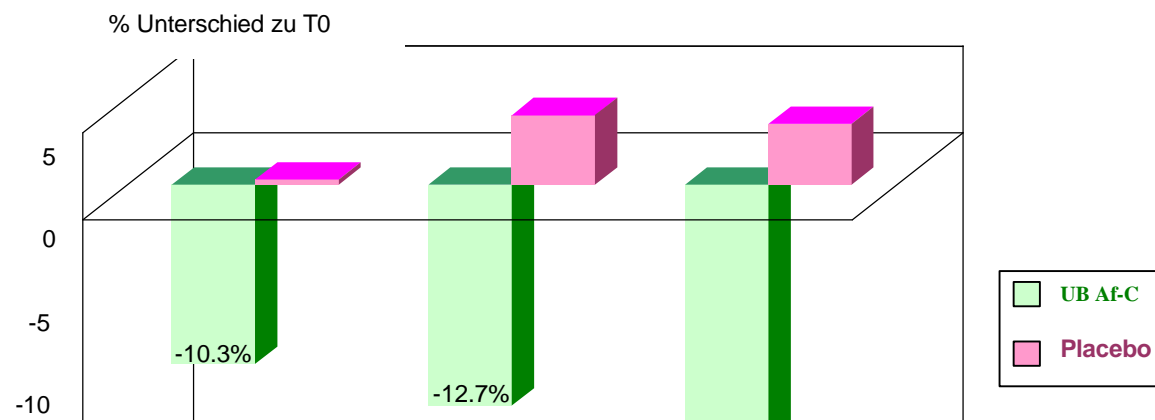
The observed variations of the “unevenness” parameter are a result of the balancing effect of the test product on the skin's surface appearance: it effects overall smoothing.

While the placebo does not reduce the unevenness (very slight, non-significant increase), the **ULI BREITER Antifalten-Creme** is efficacious already after 2 months (- 10 %). The reduction will be ever more pronounced in time and reaches 16 % after 6 months.

UNEVENNESS (in μm)								
ULI BREITER Antifalten-Creme					Placebo			
	T0	T2	T4	T6	T0	T2	T4	T6
Average	29.35	26.33	25.63	24.52	27.69	27.77	28.79	28.66
% Difference to T0		-10.3	-12.7	-16.4	-	0.3	4.0	3.5
Student's pair test	Comparison Tx / T0	p<0.01 DS	p<0.01 DS	p<0.01 DS	-	DNS	DNS	DNS
	Comparison UB Af-C/Placebo		T2		T4		T6	
			p<0.01 DS		p<0.01 DS		p<0.01 DS	

DS = Difference significant

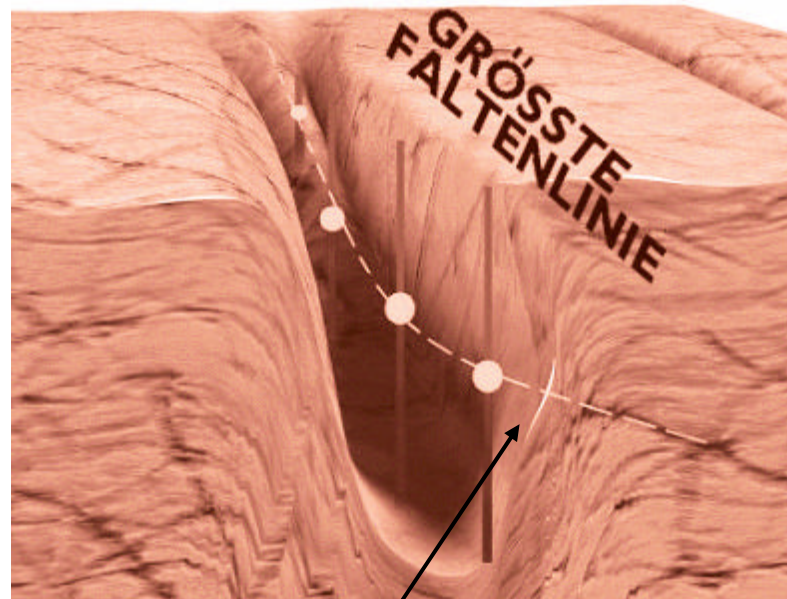
DNS = Difference not significant



A.1.5. Average depth of one of the largest wrinkles

Explanation of this parameter

This parameter is especially interesting because it reflects the improvement achieved in the skin zone with the most wrinkles. This parameter is decisive for evaluating the efficacy of a product.



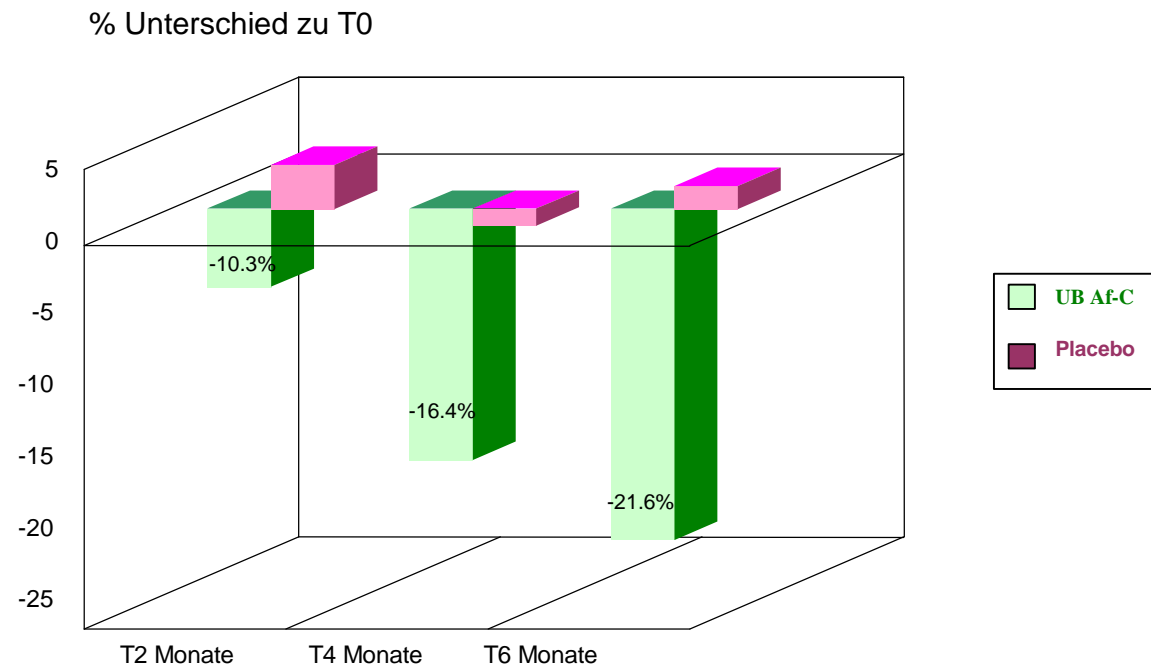
Durchschnittliche Tiefe einer der größten Falten : Die

Bildanalyse-Software berechnet die durchschnittliche Tiefe der "schwarzen Striche" und gibt den

		AVERAGE DEPTH OF ONE OF THE LARGEST WRINKLES (in μm)							
		ULI BREITER Antifalten-Creme				Placebo			
		T0	T2	T4	T6	T0	T2	T4	T6
Average		72.8	69.1	60.9	57.1	68.3	70.2	67.5	69.3
% Difference to T0			-5.1	-16.4	-21.6	-	2.9	-1.1	1.5
Student’s pair test	Comparison Tx / T0	p<0.05 DS	p<0.01 DS	p<0.01 DS	-	DNS	DNS	DNS	
	Comparison UB Af-C/Placebo		T2		T4		T6		
			p<0.05 DS		p<0.01 DS		p<0.01 DS		

DS = Difference significant

DNS = Difference not significant



The average depth of the large wrinkles will be increasingly reduced within the course of the treatment

A.2. SELF-EVALUATION by the probands

After 2, 4 and 6 months, the probands were given a questionnaire for self-evaluation.

The following criteria were evaluated therein:

☞ "Moisturizes the skin for a long time"

☞ "Smoothes the skin"

☞ "Makes the skin softer"

☞ "Reduces wrinkles and fine lines"

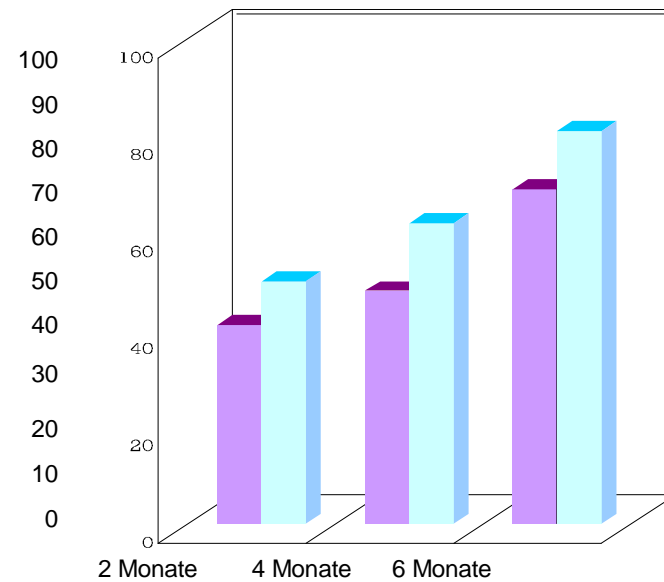
Results

All probands stated that the halves of their faces treated with ULI BREITER Antifalten-Creme and placebo looked differently.

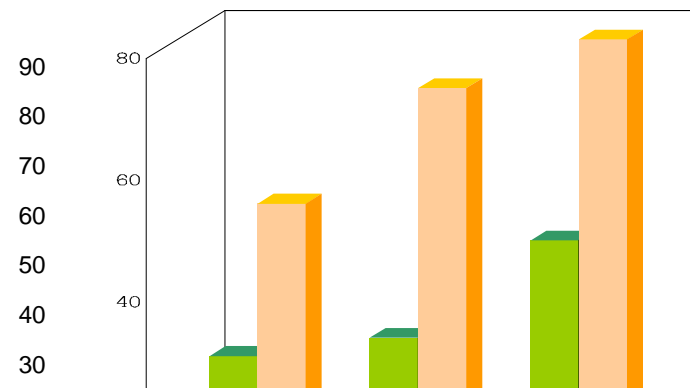
- ? The moisturizing effect of **ULI BREITER Antifalten-Creme** was noticed by 92% of the probands (placebo: 76 %).
- ? The smoothing effect of **ULI BREITER Antifalten-Creme** was recognized by 80% of the probands (placebo: 68 %).
- ? That **ULI BREITER Antifalten-Creme** makes skin softer was stated by 76% of the probands (placebo: 60 %).
- ? That **ULI BREITER Antifalten-Creme** reduces wrinkles and fine lines was stated by 83% of the probands (placebo: 45 %).

Proof of correlation between profilometric data and self-evaluation

As the following graphics show, the results of the image analysis were confirmed by the users' judgments.



% Reduzierung der tiefen Falten (Messung)
 % Bejahung der Frage : „Reduziert die Falten“



A.3. CLINICAL EVALUATION BY A DERMATOLOGIST

A dermatologist was assigned for the clinical evaluation of the changes in the wrinkles' depth, length and density. On T0 as well as after 2, 4 and 6 months, he subjected the half of the face treated with the placebo or with **ULI BREITER Antifalten-Creme** to a careful examination.

The improvement of all parameters giving information on the skin's condition was evaluated with a score from 0-4 for the purpose of data processing:

- ? none = 0
- ? minor = 1
- ? medium = 2
- ? significant = 3
- ? very significant = 4

The great inconsistency of the results at the different times of examination rendered the statistical evaluation difficult. Nonetheless, a positive development of clinical findings was established for the half of the face treated with **ULI BREITER Antifalten-Creme**.

SCORE		After 2 months	After 4 months	After 6 months
Fine lines	UB Af-C	1.55	1.72	1.74
	Placebo	1.82	1.85	1.91
Density	UB Af-C	1.90	1.85	1.55
	Placebo	2.15	2.00	1.65
Depth	UB Af-C	2.05	2.20	2.10
	Placebo	2.25	2.30	2.20





The statistical analysis shows that the difference between the variations of 5 parameters of the halves of the faces treated with **ULI BREITER Antifalten-Creme** and 5% vitamin C was significant to very significant ($0.01 < p < 0.07$) despite the low number of probands ($n = 10$).

Overall, the results of the vitamin C containing product were nearly identical to the placebo (see Section A.1).

It was thus confirmed that ULI BREITER Antifalten-Creme has, *in vivo*, a better efficacy than the reference (vitamin C). The same was already observed in the *in vitro* studies.

B.2. SELF-EVALUATION

The probands were to state whether the two creams (ULI BREITER Antifalten-Creme and VITAMIN C) met the following criteria:

-  "Moisturizes the skin for a long time"
-  "Smoothes the skin"
-  "Makes the skin softer"
-  "Reduces wrinkles and fine lines"

After a treatment period of 6 months, ULI BREITER Antifalten-Creme was judged to be much more efficacious, with regard to smoothing the skin (100 %) and the reduction of wrinkles and fine lines (100 %) than VITAMIN C (50 % and 67 %, respectively).

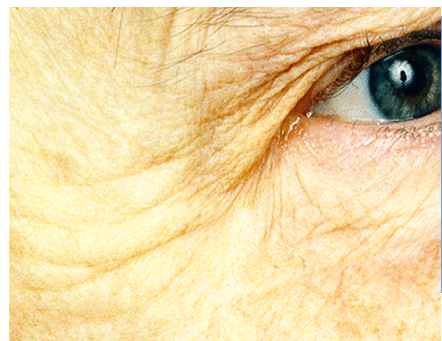
The clinical examination by a dermatologist confirmed the results of the self-evaluation by probands. It was shown that **ULI BREITER Antifalten-Creme** had a greater efficacy on the treated half of the face than the placebo and the commercial cream with vitamin C.

Results of the profilometric study:

- a) The changes established after 2, 4 and 6 months compared with T0 were highly significant with **ULI BREITER Antifalten-Creme**, in comparison with the placebo;
- b) the changes established after 6 months compared with T0 were significant with **ULI BREITER Antifalten-Creme**, versus the commercial cream with 5 % vitamin C.

These effects confirmed by the probands as well as the dermatologist make the skin look smoother (see following photos) :

Vorher



Nachher



It is true that the cosmetic lines on a retinoid base (vitamin A) are very successful, but they irritate the skin which is hardly acceptable with a long-term application.

An "anti-wrinkle product" is, by nature, a product which must be used for a longer period of time. Accordingly, only well tolerable basic substances may be used in the product's formula. A consumer who sees visible results will buy the product regularly.

To the extent that better knowledge was obtained about the physiological processes which result in skin smoothing, new and interesting perspectives opened up, e.g. the biomimetic peptides.

Based on scientific work by KATAYAMA, we examined the pentapeptide sequence described by him as to whether it stimulates the synthesis of collagen and proteins of the extracellular matrix. It concerns the sequence lys-thr-thr-lys-ser – a natural fragment of the C-terminal end of collagen I. We offer it in form of a palmitoyl peptide in a stabilized solution.

ULI BREITER Antifalten-Creme and vitamin C activate the synthesis of the extracellular matrix *in vivo* and *in vitro*, but the intensity of the effect is very different:

Collagen I : ULI BREITER Antifalten-Creme + 117 % versus vitamin C + 42 %

Collagen IV : ULI BREITER Antifalten-Creme + 327 % versus vitamin C + 229 %

Additionally, ULI BREITER Antifalten-Creme activates the production of glycosaminoglycans : + 267 %.

Physiologically, this is effected through the activation of the most important supporting structures of dermis and epidermis.

ULI BREITER Antifalten-Creme can mitigate the ageing process and promote the restoration of the properties of younger skin.

The described results were confirmed in a 6-month *in vivo* blind study in comparison with a placebo. ULI BREITER Antifalten-Creme made wrinkles disappear in time. Results after 6 months:

- ✍ Decrease of the area taken by deep wrinkles by 68 % ;
- ✍ with certain persons even up to 90 % ;
- ✍ Decrease of the density of the largest wrinkles by 47 % ;
- ✍ Decrease of the area taken by the medium wrinkles by 51% ;
- ✍ Decrease of the average depth of the largest wrinkle by 22 %.

A blind test in comparison with a commercial product with 5 % vitamin C confirmed the superior efficacy, as well as the good shelf life and dermal tolerance of ULI BREITER Antifalten-Creme.

ULI BREITER Antifalten-Creme is excellently tolerated. With ULI BREITER Antifalten-Creme, 80 % of the probands detected a visible reduction of their wrinkles.

Clinical examination by a dermatologist confirmed these observations. It showed that the half of the face treated with ULI BREITER Antifalten-Creme was in much better