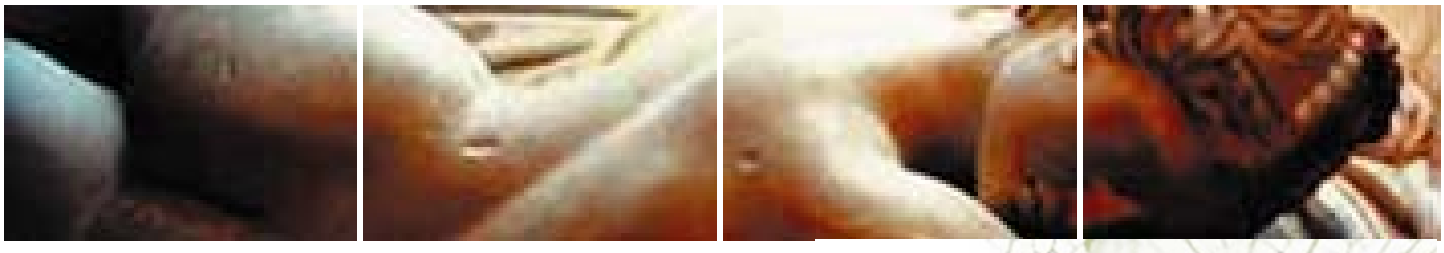
PERFELINE®

Fights Against Cellulite



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1 Introduction

Excessive intake of food and lack of activation of lymphatic drainage due to reduced physical movement lead to accumulation of detrimental substances within cells and tissue. Surplus energy carriers (lipids and sugar) are metabolized as triglycerides and subsequently stored inside the vacuoles of the fat cells (adipocytes). A typical consequence is the buildup of undesired cellulite skin with its often slightly inflamed lumpy depressions and curvatures.
Improving the appearance of cellulite skin or getting rid of it completely is of great interest, especially during the warmer seasons. Of major concern is the care of the areas where most often cellulite builds up such as the upper thigh, the hip and the gluteal region.
In order to achieve a noticeable visible effect, an anti-cellulite active substance has to act upon the fat cells and activate the lymph flow. The objective of such activity is the drainage of connective tissue fluids and of waste products (fats and proteins).
PERFELINE® is the name of the active substance concept presented here. It fulfills the objective and achieves remarkable in-vivo and in-vitro results as described below.

Sleeping Hermaphrodite, Louvre, Paris

2 Active Principle

PERFELINE® acts on three levels as a unique cellulite active substance.

1. The inhibition of phosphodiesterase through caffeine, increases the c-AMP level in cells. In turn, this leads to activation of protein kinase. Once activated, this enzyme phosphorylates triacylglycerol lipase which leads to fat degradation.

2. L-carnitin optimizes the trans-membrane transport of fatty acids for oxidative degradation inside mitochondria.

Fat (Triacylglycerol)

Fat cleavage: ***Caffeine*** inhibits the degradation of cAMP (cyclic-AMP) to AMP which leads to an accumulation of cAMP.

An increased amount of cAMP stimulates the protein kinase to phosphorylate triacylglycerol lipase. As the lipase is activated, it cleaves triacylglycerol (fat) into fatty acids and glycerol.

Fatty acids + Glycerol

Membrane Transport: ***L-Carnitine*** is temporarily linked to activated fatty acids. This is the way fatty acids can cross the mitochondrial membrane and reach the energy generating centers of the cell. Inside the mitochondria the temporary link is established and fatty acids are cleaved (β-oxidation).

Mitochondria

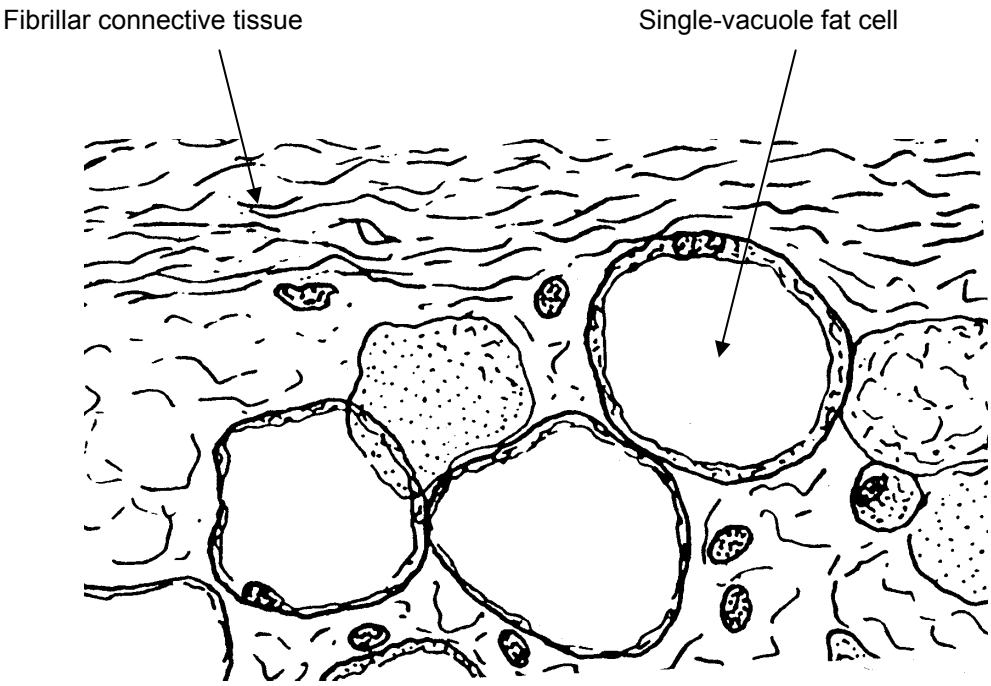
Fatty acid

⇒ is cleaved through β-oxidation into C_2-units which leave the mitochondria as citrate after additional biochemical steps.
Citrate subsequently enters the citric acid cycle and is completely degraded to water and CO_2.

→ **Citrate**

3. Ruscogenin in Butcher's Broom extract activates and increases the lymph flow leading to a reduction of accumulated tissue fluids. Swollen cells are drained and cleansed of deposits and reduced to their original size. Pressure within the tissue is diminished and the tissue structure is tightened due to the build-up of cross-linked collagen and elastin.

2.1 Fat Cells within Subcutaneous Tissue



Ruscogenin drains the cells of harmful deposits, inhibits inflammation, and **tightens and fortifies the tissue by means of its anti-elastase activity.**

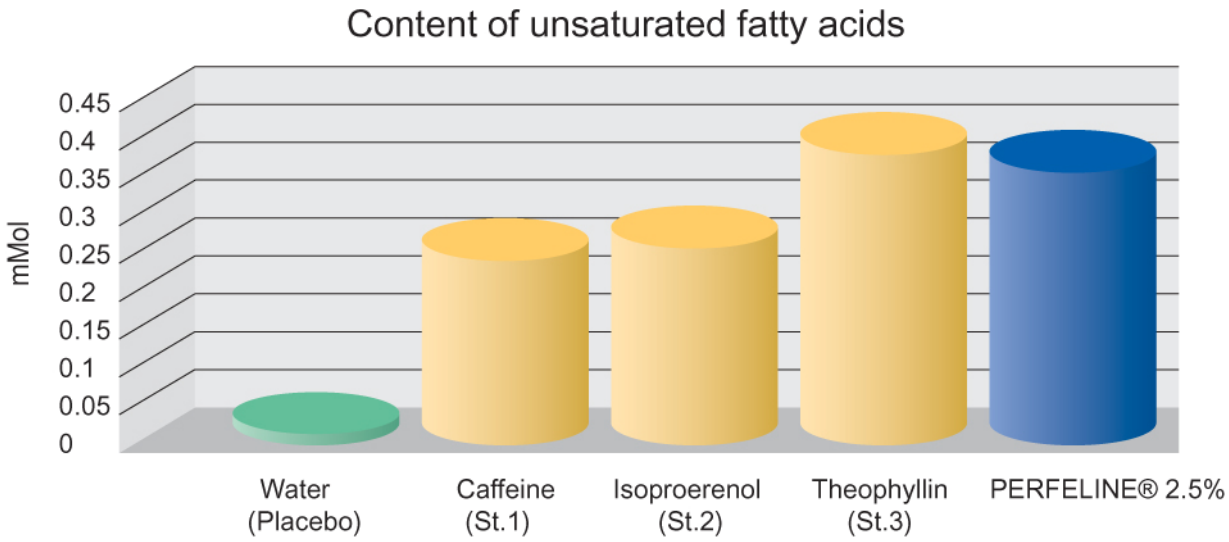
The active substances contained in PERFELINE® are synergistically balanced and succeed in affecting the symptoms of cellulite, leading to an attractive appearence.

3 Efficacy Tests

3.1 Lipolysis (in-vitro)

The optimization of fat degradation (lipolysis) should take place due to the inhibition of phosphodiesterase by both caffeine and Butcher's Broom extract. An in-vitro test was carried out to validate this assumption.

In a test, PERFELINE® and human fat cells were mixed and stirred for 2 hours at 37°C. The same procedure was used with water as a placebo instead of PERFELINE®. In order to verify the assay procedure, 3 analogous standards, whose expected results were known to the test institute, were used in parallel. Thereafter, the amounts of unsaturated fatty acids were measured in all samples using the NEFA-C Kit (manufactured by WACO). The results obtained using standard tests showed that the test procedure was correct, thus indicating that the results from test samples were significant.



The test indicated that at a concentration of 2.5% PERFELINE®, the content of unsaturated fatty acids compared to the placebo increased by a factor of 21.

In fatty acid determination assays, the values of PERFELINE® correlated with those of the standard with the highest lipolysis activity.

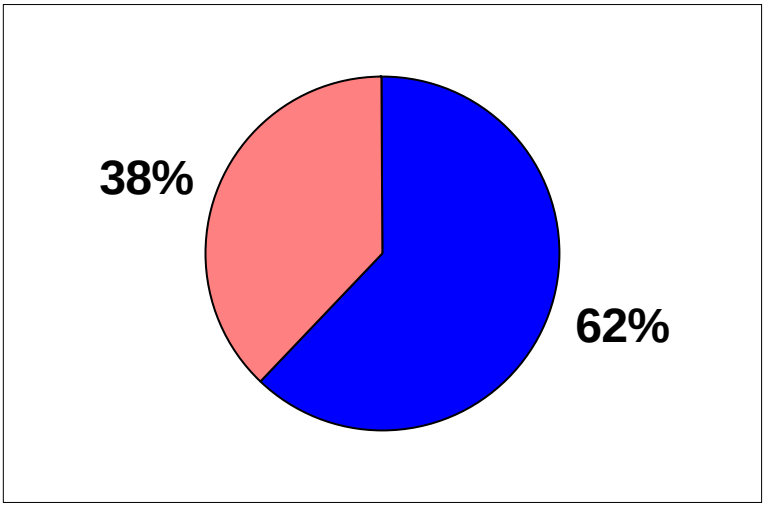
Based on these findings, the independent test institute concluded that PERFELINE® can be considered to be a product leading to intensive lipolysis activity.

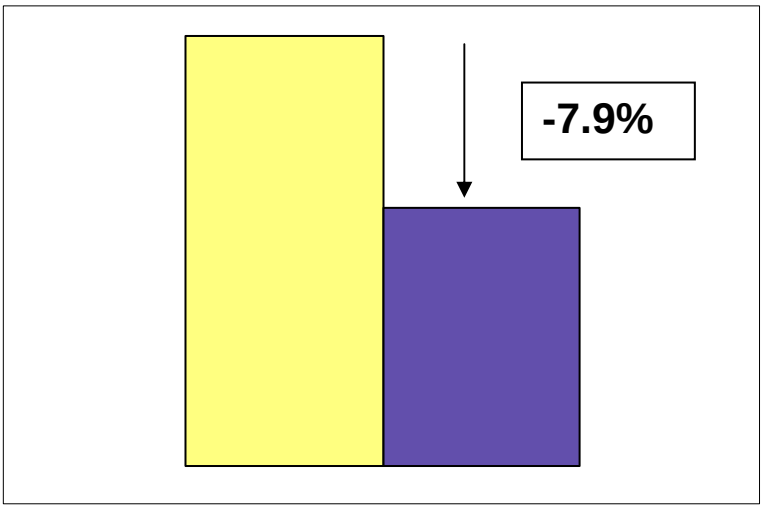
3.2 Reduction of Fat Layer (in-vivo)

An independent test institute determined the efficacy of PERFELINE® in a test conducted with 15 women over a 4 week period.

Measurements were carried out with a 20 MHz ultrasound device. The values of the thickness of the dermis (at constant thickness) enabled determination of proportional changes in the fat layer.

An emulsion of 5% PERFELINE® was applied daily on one leg, while the other leg was treated with the same emulsion without PERFELINE® (placebo). Defined locations were measured by means of ultrasound on day 0 and after 4 weeks from inception of the treatment. In order to obtain results attributable to PERFELINE®, the values obtained with the placebo were deducted from the values obtained with the cream containing the active substance.





A positive effect due to PERFELINE® was observed in 62% of the persons tested. The reduction of the fat layer reached values of up to 25%.

The average reduction of the fat layer was 7.9%.

3.3 Ultrasound Measurements of in-vivo Studies

Method of Measurement
The in-vivo studies have been carried out by conducting measurements with a 20 MHz ultrasound device. The density of the dermis was measured before and after treatment. The complex set of data obtained were processed with a special computer program which enabled to determine the average reduction of fat infiltration into the dermis.

Visualization
Fat infiltration into the dermis is showed in black on the computer screen. The less fat infiltration into the dermis, the bigger is the density of the dermis leading to an increase of the dermis color intensity on the screen.

Conclusions
By measurement of dermis density, the decrease of fat infiltration into the dermis can be evaluated. After a treatment time of four weeks with BODY 0102, the reduced fat infiltration is clearly visible even without instrumentation (see picture on following page).
It is clearly visible that the amount of fat infiltration is reduced and the color intensity of the dermis is increased while the measurement indicates a higher density level of the dermis.

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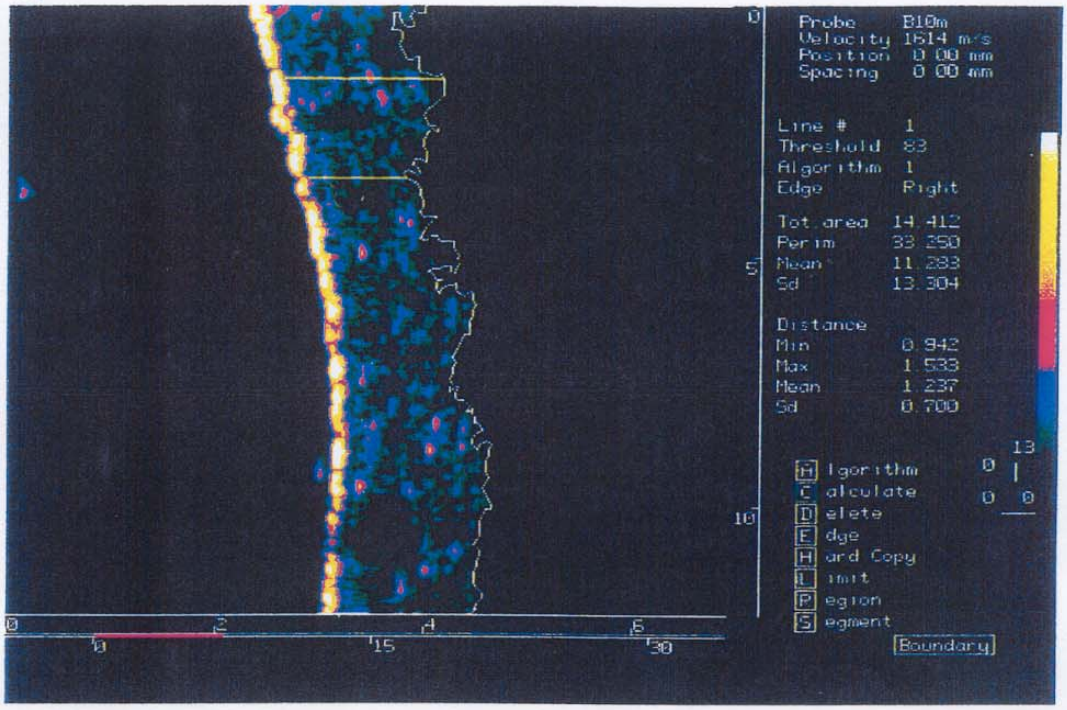
Cream/C06
Cream/C07

Volunteer 28 : Treated thigh with product « Cream/C06 »

D0



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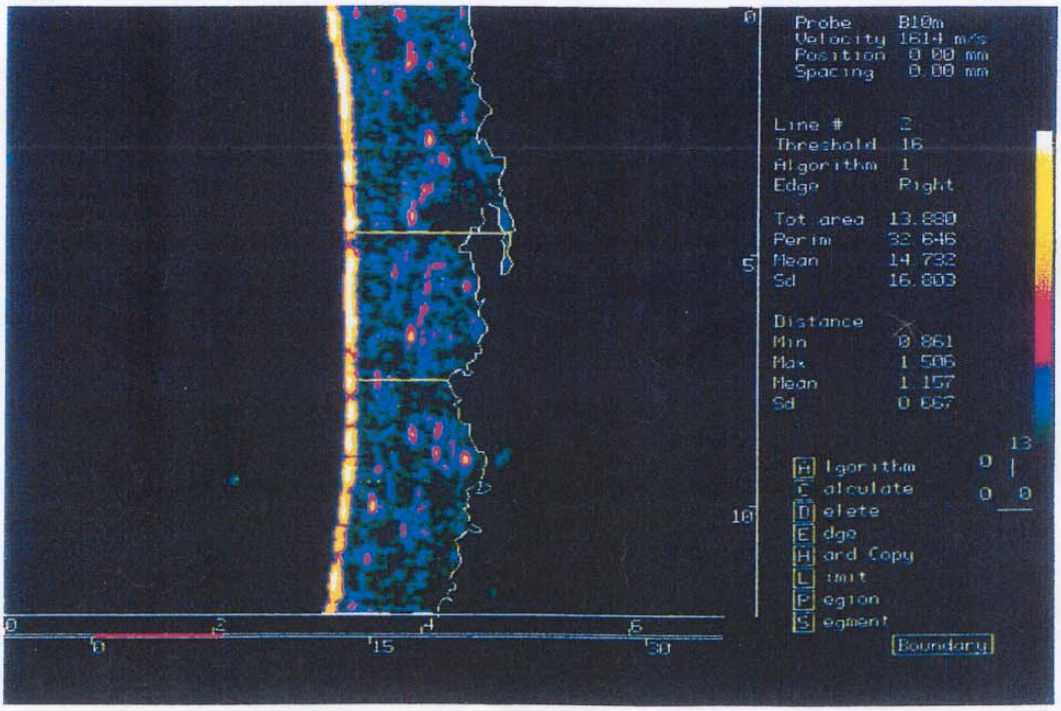


4 Use and Storage

4.1 Summary

The key characteristics of an active substance against cellulite are fat degradation, increase of micro-circulation, as well as anti-inflammatory properties. The above sections described PERFELINE® as an effective substance against cellulite.

4.2 Areas of Application

- Cellulite products
- Slimming products

4.3 Origin of Ingredients

PERFELINE® is of botanical, biotechnological and synthetic origin. It does not contain any animal-derived substances or any substance derived from mineral oils.

4.4 Suggestions for Storage

PERFELINE® should be stored at cool temperatures (15 - 20°C) and away from light. Shelf life is 24 month after production. PERFELINE® is a product with natural ingredients. Slight fallout may occur over time.

4.5 Suggestion for Use

No particular procedures are required.

4.6 Suggested Usage Concentrations

3% - 8%

5 Product Specification

Test Parameter	Test Equipment	Specification Value
Appearance	TP 063	Brown, clear solution
Odor	TP 063	Characteristic
Consistency	TP 063	Fluidity of water
pH-value, direct	TP 006	4.80 - 5.35
Density 20°C	TP 007	1.021 – 1.027 g/ml
Refraction index 20°C	TP 044	1.348 – 1.354
Dry contente	TP 020	> 8.0 %
Total plate count	TP 032	< 50 cfu/g

6 Registration

6.1 INCI Name

EU: Aqua, Carnitine, Caffeine, Ruscus Aculeatus Root Extract

USA: Water, Carnitine, Caffeine, Ruscus Aculeatus Root Extract

Japan: Water, Carnitine, Caffeine, Ruscus Aculeatus Root Extract

6.2 Registration and Contents

INCI EU	Content in %	CAS-Nr.	EINECS-Nr.
Aqua	> 50	7732-18-5	231-791-2
Carnitine	5-10	541-15-1	208-768-0
Caffeine	1-5	58-08-2	200-362-1
Ruscus Aculeatus Root Extract	1-5	84012-38-4	281-682-9

6.3 Other Substances

0.20% potassium sorbate and 0.10% sodium benzoate are added to preserve PERFELINE®.

7 Toxicology and Safety Assessment

7.1 Toxicology

Test Parameter	Test Method	Assessment
Oral toxicity	Inform. from literature	Not toxic *
Skin irritation	Rep. human patch test with 30 people using 20% aqueous solution	Not irritating
Eye Irritation	BECAM test (in-vitro), undiluted	Slightly irritating
Sensitization	Rep. human patch test with 30 people using 20% aqueous solution	Not sensitizing
Mutagenicity	AMES test (in-vitro), undiluted	Not mutagenic

*Oral toxicity is assessed as non-toxic based on literature sources that refer to the LD50 for carnitin of >5 g/kg and for caffeine of >0.20 g/kg.
Literature sources describe Butcher's Broom extract as non-toxic. In view of individual usage concentrations, an assessment of non-toxicity is considered appropriate. However, an exact oral toxicity assessment has not been carried out.

7.2 Environmental Toxicology

Test Parameter	Test Method	Assessment
Biol. Degradability	OECD 301 F	Readily biodegradable
Daphnia limit test	100 mg/liter, 48 hours	Not toxic

In all likelihood PERFELINE® will not have any environmental toxicological impact. Its Bioaccumulation can be ruled out as PERFELINE® is soluble in water and biodegradable.

7.3 Water Hazard Class (WHC)

Substance	Information from Literature	Assessment
Water	none	WHC 0
L-carnitin	Merck Index	WHC 0
Caffeine	Merck Index	WHC 1
Butcher's Broom extract	none	unknown

As Butcher's Broom extract is of botanical origin and is used in traditional medicine as an infusion, the addition of this extract is not considered devaluating. The good biological degradability and the non-toxicity as determined with the Daphnia limit test confirmed these assumptions.
For these reasons, PERFELINE® is assessed as a product with a water hazard class value of 1.

7.4 Safety Assessment

PERFELINE® can be classified as harmless based on comprehensive toxicological data and environmental toxicological information. During testing only slight eye irritation was detected. No other toxic characteristics of any kind emerged. In efficacy tests no incompatibilities were detected.

8 Test Emulsion and its Effects

Efficacy tests were conducted using a particularly light non-fatty and rapidly absorbed base, as is required in modern slimming products.

Stability data and physical characteristics of the test cream BODY 0102 are reported below.

8.1 Product Description

White cream-gel, pH value 6.5, viscosity of approximately 15,000 mPas, centrifugation at 6,000 rev/min (20 min.): No separation, droplet size: 2-10 µm

8.2 Recipe: BODY 0102

Phase	Substance	INCI name (EU)	% [w/w]
A	Water demin.	Aqua	78.00
	Glycerol 86%	Glycerin	4.00
	Carbopol ETD 2020	Acrylates/C10-30 Alkyl Acrylate Crosspolymer	0.40
	NaOH solution 10%	Aqua, Sodium Hydroxide	1.20
B	Cetiol OE	Dicaprylyl Ether	5.00
	Cetiol SN	Cetearyl Isononanoate	2.00
	Parfum Vert de Bambou 21401	Parfum	0.20
	Uniphen	Phenoxyethanol, Methyl-paraben, Ethylparaben, Propylparaben, Butylparaben	1.00
C	Keltrol F	Xanthan Gum	0.20
D	Ethanol 96%	Alcohol	3.00
E	PERFELINE®	Aqua, Carnitin, Caffeine, Ruscus Aculeatus Root Extract	**5.00**

			100.00

8.3 Production of Emulsion

1. Production does not require heating.
2. Mix water and glycerol. Add Carbopol on top of water surface and let moisten for 10-15 minutes. Then mix briefly.
3. Neutralize mixture using the NaOH solution. A gel builds up.
4. Premix Phase B and shortly before adding to the above mixture, disperse Phase C into Phase B.
5. Add Phase B/C to Phase A and homogenize.
6. Add Phase D to the mixture obtained so far. Stir until homogenous.
7. Depending on size of oil droplets, homogenize shortly.
8. Add Phase E to the mixture obtained so far. Mix until homogenous.

8.4 Stability Data

The emulsion BODY 0102 should feature droplet sizes of approximately 2-10 micro meters.
The following results were obtained with heat stability tests conducted with the emulsion:

	After 1 month	After 2 months	After 3 months
at 3°C	OK	OK	OK
at 20°C	OK	OK	OK
at 40°C	OK	OK	OK
at 50°C	OK	OK	OK

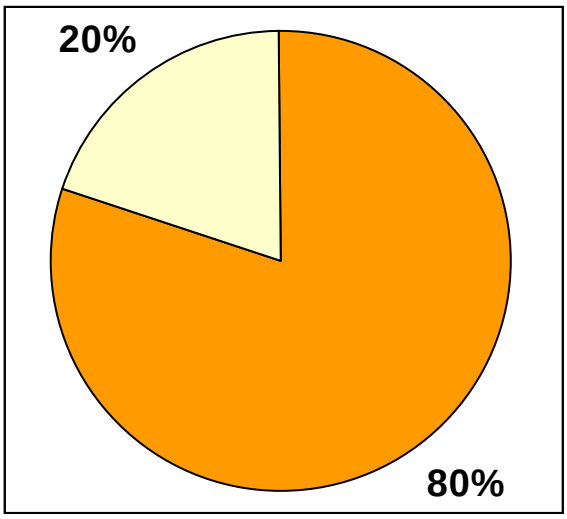
Temperature stability tests resulted in no changes of droplet size, of viscosity or of pH levels at any temperature measured.

8.5 Efficacy of Cream BODY 0102

The slimming activity of test cream BODY 0102 was determined by an independent institute based on a sample of 14 women.

In each case, the cream was applied to one leg for a period of 4 weeks. The other leg of each person was left untreated. Prior to testing and after 28 days of testing, predetermined locations of the skin were examined with a 20 MHz ultrasound device.

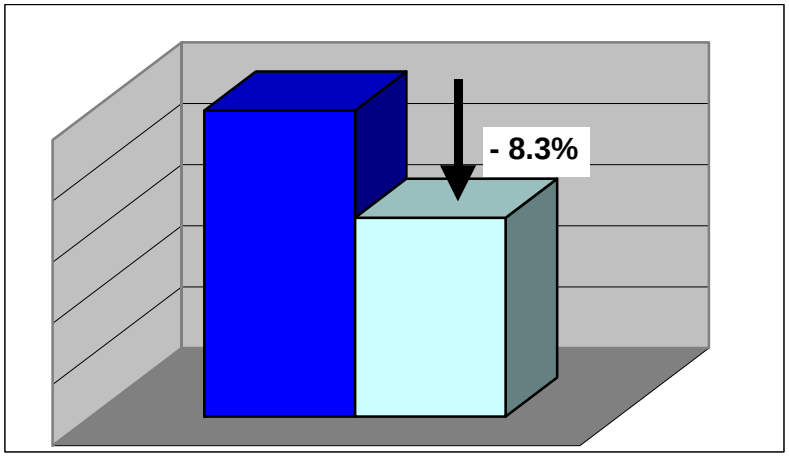
The thickness of dermis that did not change was used as the basis for calculating percentage changes in fat layer decrease. In order to determine real effects caused by the test emulsion, changes on the untreated leg were deducted from the results obtained from the sections treated with cream BODY 0102.



Cream BODY 0102 resulted in a positive effect with 4 out of 5 people tested: The fat layer reduction was up to 30%.

The average decrease of the fat layer was 8.3%.

A reduction of leg circumference was achieved on 12 of the 14 women (86%) tested



8.6 Summary

The residue drainage effectiveness of Butcher's Broom extract and the lipolytic activity of PERFELINE® make this product well suited for slimming / anti-cellulite products.
As shown above, the efficacy of PERFELINE® has been confirmed with both in-vivo and in-vitro studies. The efficacy was demonstrated based on human tests in two ways, in comparison to a placebo and also in comparison to untreated skin.

The correlation of efficacy values obtained with the two in-vivo studies are significant. In fact, a comparable decrease of the fat layer by about 8% was observed, and in both cases about 60% of the people tested which responded to PERFELINE® showed a decrease of fat layer in the range of 5-25% and 5-30%, respectively.

	PERFELINE® Results	
	against placebo	against untreated skin
Persons with positive effect in the in-vivo test	62%	80%
Average reduction of fat layer of these persons	7.9%	8.3%
Distribution of percentage reduction of these persons	60% in the range of 5% to 25%	64% in the range of 5% to 30%

8.7 Conclusions

As the comparative values of these studies demonstrate, the slimming effect is attributable to PERFELINE® and is not a result of the base used or of the application procedure.

Due to the complex biochemical processes involved, not all persons tested can be expected to respond to the application of a slimming product. In addition, in-vivo studies of this type always vary depending on the individuals involved. Hence deviations of measured values occur. Such statistical deviations seldom lead to identical results in double testing assays. Despite this consideration, the above in-vivo and in-vitro studies are good indicators as to whether an effect can be expected and to what extent the effect occurs.

The results and their correlation obtained with the above measurements demonstrate that PERFELINE® leads to a significant slimming effect.
This effect is based on the lipolytic activity of PERFELINE® as demonstrated with in-vitro tests and is based on the effectiveness of Ruscogenin contained in Butcher's Broom extract.

9 Bibliography

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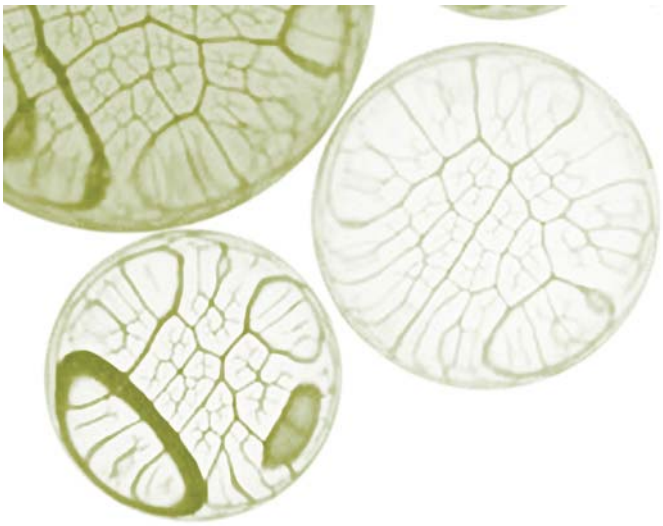

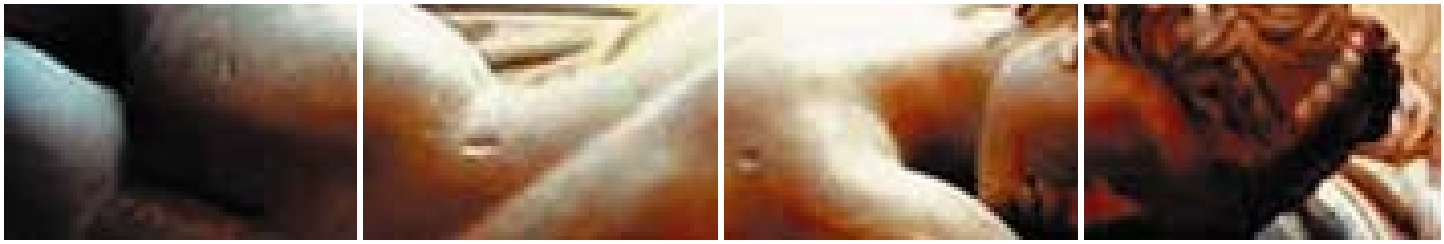

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