LUCASMEYER COSMETICS

ACTIVE INGREDIENTS

TECHNICAL FILE

ADIPOFILL™

BIO-CONTROLLED LIPOFILLING

L-ornithine Ionosome™

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Lipohormesis concept

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Decreases the appearance of nasogenian fold for a more plumper and youthful look

SUMMARY

INCI NAME	Water (1°) (and) Propanediol (2°) (and) Ornithine (3°) (and) Phospholipids (4°) (and) Glycolipids (5°)
CAS	(1°) 7732-18-5, (2°) 504-63-2, (3°) 70-26-8, (4°) 8002-43-5, (5°) /
EINECS	(1°) 231-791-2, (2°) 207-997-3, (3°) 200-731-7, (4°)232-307-2, (5°) /
ORIGIN	L-ornithine, amino acid obtained by biotechnology from vegetable starch, encapsulated in an Ionosome™.
COSMETIC PROPERTIES	• Activation of HIF-1α • Adipogenesis activation • Lipogenesis activation • Lipolysis inhibition
SKIN BENEFITS / POTENTIAL CLAIMS	• Reduces the signs of aging: nasogenian folds • Has a lipofilling-like effect • The skin is smoother and appears plumper and more lifted
APPLICATIONS	• Anti-aging care • Anti-wrinkle skin care • Skin care for mature skin • Alternative to surgery • Breast and buttock volume enhancer • Anti-aging treatment for hands • Eye care • Lip care • Men's care
RECOMMENDED DOSAGE	0.5-2%
USAGE PH RANGE	4.5 - 6.0
INCORPORATION	At the end of the formulation (< 40˚C)
INCOMPATIBILITIES	None known

INTRODUCTION

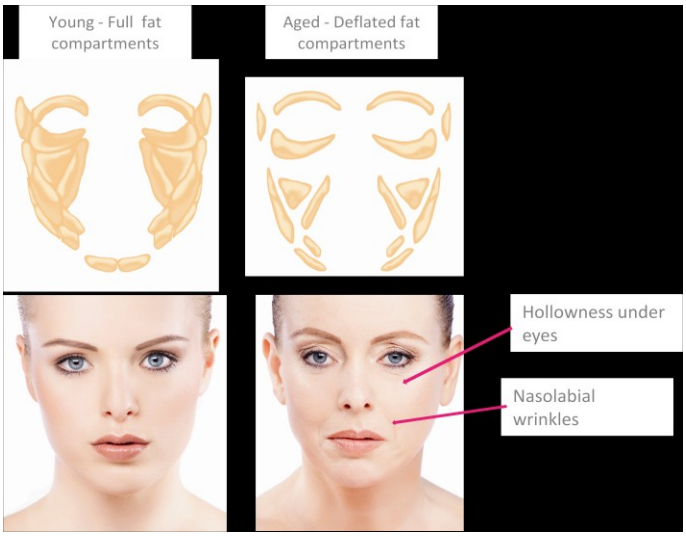
With population growth and more information available on how unhealthy lifestyles increase aging, products that can help consumers look younger are more popular than ever.

A wide choice of anti-aging ingredients and finished products can be found on the cosmetic market, all acting on different biological targets, with different mechanisms and for different applications.

A global aging involves different aging processes:

- Cellular aging with a dysfunction of the cell metabolism: decrease in cellular respiration, decrease in synthesis of hyaluronic acid, collagen, elastin, etc.
- Cutaneous aging characterized by the visible signs of aging such as loss of elasticity and firmness, appearance of wrinkles, sagginess, dark spots, rosacea, etc.
- Structural aging due to a lipoatrophy which is a loss of adipose tissue located on face and hands.

The subcutaneous adipose tissue is responsible for the plump aspect of the face. With aging, this fat pad progressively melts due to a disturbance of adipocyte metabolism comprising as well a reduction of the adipocyte number as the adipocyte size due to a decrease of the lipid content. This fat loss is especially localized at the temples (sagging of the eyebrow by lacking of lateral support), under eyes (hollow eye look, dark rings) and in the malar area (increase of the nasogenian fold, loss of high cheekbone). This atrophy of the facial soft tissues makes the facial bones appear more prominent.



To reduce the appearance of the signs of aging due to lipoatrophy, plastic surgery is a well-known method with the use of lipofilling technology. Lipofilling comprises a harvest of fat from area such as buttock followed by its injection on the volume-deficient parts of the face. This fat autograft allows the restoration of the initial volume of a face.

Lipofilling-like cosmetic products are already present on the market and act according to one of the 3 possible following ways of action:

-Increase in adipocyte number: adipogenesis
-Increase in the lipid synthesis: lipogenesis
-Reduction of the loss of adipocyte volume: inhibition of lipolysis

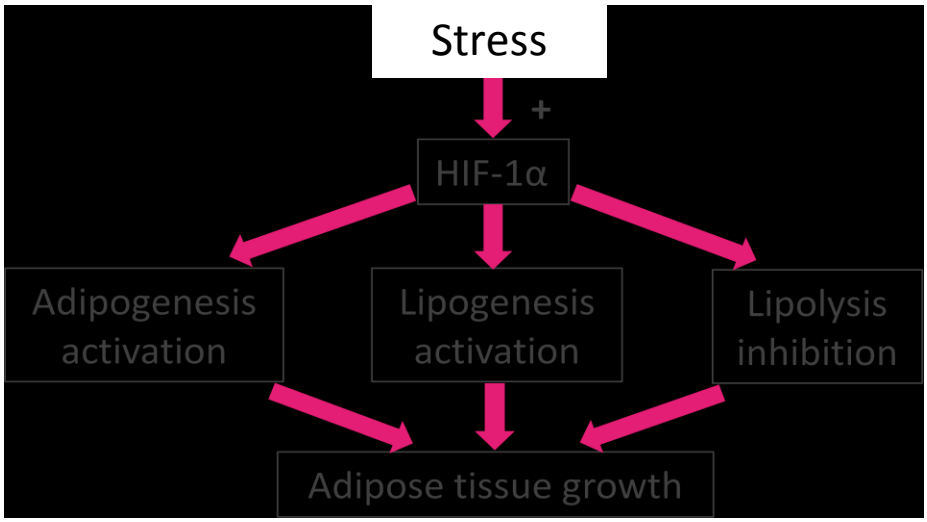
The last scientific data have inspired the development of a unique anti-aging skin ingredient acting with a lipofilling-like effect with a global and non-invasive patented mechanism.

THE CONCEPT OF LIPOHORMESIS

A hot trend emerged in 2010 in the cosmetic industry is the hormesis concept.
The word “hormesis” comes from the greek root “hormeticos”, which means ”stimulation”. It can be defined as a homeostatic and adaptative beneficial response of cells to microstress exposure in order to correct a physiological imbalance. This concept is applied to cosmetic products by the presence of active ingredients which induce micro-stimuli able to improve skin defense system and to better prepare and arm skin to fight further stress.

A next step in cosmetic innovation is the lipohormesis. Lipohormesis is a hormesis phenomenon applied to adipose tissue.

It has been recently described that various stresses in the adipose tissue are able to stimulate its expansion through the activation of Hypoxia Inducible Factor (HIF-1α), a transcription factor.
For example, it has been recently described that the induction of a hypoxia in the adipose tissue is able to activate angiogenesis and fatty tissue growth [Kalinina NI *et al.*, 2009]. This phenomenon explains the obesity vicious circle. As the fat tissue increases, some areas are in hypoxic conditions (lack of oxygen). To correct the situation, cells activate the HIF-1α that induces fat tissue expansion to compensate lack of space and oxygen.

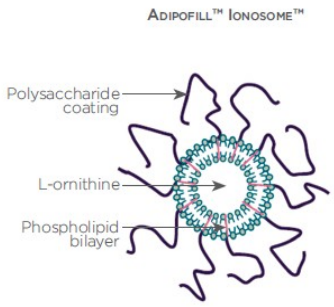


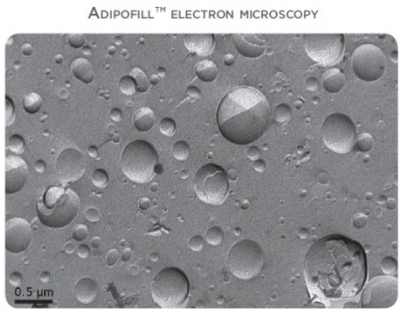
Lipohormesis is therefore an innovative pathway to induce fatty tissue growth in order to compensate lipoatrophy and have a lipofilling-like effect.

Adipofill™: Bio-controlled lipofilling

Adipofill™ is L-ornithine, an amino acid obtained by biotechnology from vegetable starch, encapsulated in an Ionosome™.

Ionosome™ is a proprietary resistant delivery system developed to encapsulate charged molecules. The phospholipid-bilayer membrane of the vesicle is stabilized by a protective polysaccharide-coating and makes the encapsulation more stable face to destabilizing molecules of the formula. The encapsulation allows a deeper penetration to target adipocytes and a higher efficacy.





Adipofill™ reproduces lipohormesis phenomenon by activating HIF-1α gene expression and the related genes acting on the global adipocyte mechanism:
-The activation of adipogenesis (transformation of pre-adipocytes into adipocytes).
-The activation of lipogenesis (triglycerides synthesis).
-The inhibition of lipolysis (breakdown of lipids into free fatty acids).
It has been demonstrated that Adipofill™ decreases free fatty acid release from adipocytes. This triglyceride storage increase inflates adipocytes leading a bigger size.

With this global action on the adipocyte anabolism, Adipofill™ induces cell fat refilling and increases fatty tissue volume to mimic lipofilling results.

By acting with a non-invasive and bio-controlled mechanism, Adipofill™ clinically improves the signs associated with structural aging, such as nasogenian fold depth, skin smoothness and roughness for a plumper and youthful look.
Adipofill™ is thus a sophisticated and high-tech product that fights the aging process for a facial rejunevation.

Adipofill™ is able to reverses age-related lipoatrophy to reduce the appearance of structural aging and plump up the skin.

Reverse effect of Adipofill™

EFFICACY STUDIES

ADIPOCYTE GENIC EXPRESSION

ACTIVATION OF HIF-1α

INTRODUCTION

High-throughput technologies and platforms are facing a new challenge because of the growing number of data engendered by microarrays. The growing mass of information is requiring specific tools to structure and organize the relation between molecules, genes and proteins. Thanks to PredictSearch™ software, genomic data permit to identify new functions or pathway through emerging keywords, discovered activities triggered by new molecules and so to determine contextual biological processes and activities within functional networks. By determining the main signaling pathway induced by a molecule, this software can predict the global physiological impact and biochemical cascades as the result of the modulation of gene expression.

PROTOCOL

Principle

The objective of the study was to determine the transcriptional effects of the L-Ornithine compound in human differentiated subcutaneous adipocytes.

Biological material

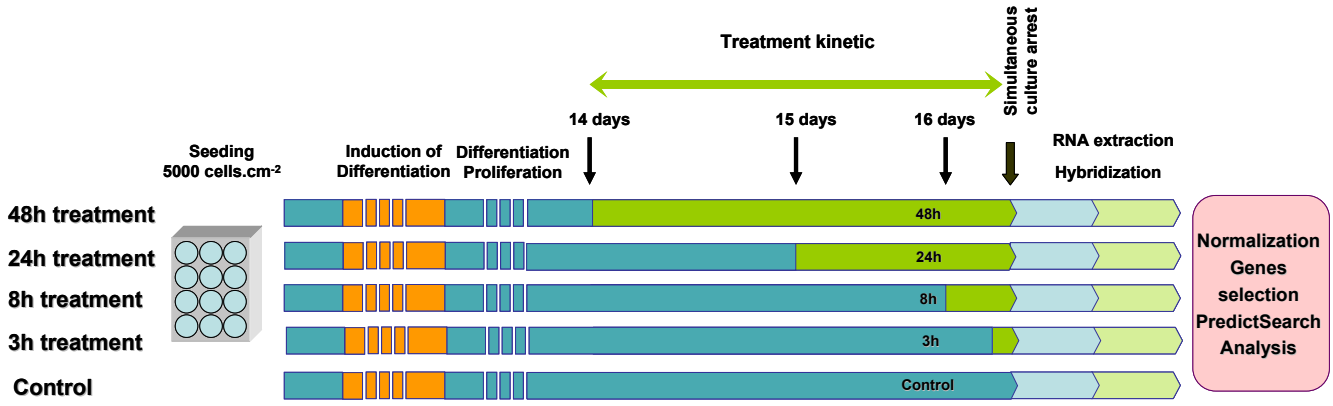
Human subcutaneous preadipocytes (Ref C-12731) were isolated from skin biopsies processed during surgical intervention of healthy non-diabetic 18-60 years old patients with normal Body Mass Index (20 to 25). Cells were amplified in appropriate culture medium (Ref C-27417), and then seeded in 9.6 cm^2 wells (COSTAR cell culture plates 12 wells, Corning, Ref. CLS3513, Sigma-Aldrich) at a density of 5,000 cells per cm^2. Three days after seeding, adipogenic differentiation was induced with specific medium (Ref C-27437), maintained for 72 hours and replaced with adipocyte-specific medium (Ref C-27439). These conditions were maintained until full differentiation, i.e. 14 days.

Each culture was duplicated in order to perform both RNA and protein extractions.

Tested molecules

Stock solution 1%: 10 mg of L-Ornithine were resuspended in 1 mL of H_2O.
0.02% L-Ornithine (equivalent to 0.2% of Adipofill™): The stock solution was directly diluted in culture medium (1:50).

RNA extraction



Total RNAs were extracted using the RNEasy Mini kit (QiaGen), according to the manufacturer's recommendations. A DNAse I treatment has been performed to avoid genomic DNA contamination.

Purified RNAs were quantified using the Nanodrop 1000 (ThermoFisher) spectrometer and qualitatively evaluated by capillary electrophoresis on Agilent BioAnalyzer.

- **Sample processing and microarrays hybridization**

500 ng RNA of each sample have been processed for retro-transcription, amplification and Cy3 labeling using the Quick Amp Labeling Kit, one-color (Agilent Technologies).

Three Whole Human Genome Arrays (4x44K, Agilent Technologies) were hybridized as recommended by the manufacturer.

- **Data processing and normalization**

The microarray data were quantified according to the protocol of the quantification software (GeneExtractionFeature V10.5.5.1). The dataset was normalized by Quantile Method with the R statistical software (inter- and intra-array normalization).

- **Genes selection and main results**

Gene modulation was evaluated by determining a ratio calculated as follow: intensity value of treated condition versus intensity value of the corresponding control. Only genes expressed with an intensity value ≥ 60 have been taken into account. Genes submitted to PredictSearch™ analysis were selected when the ratio was superior or equal to 1.45 or activated and maintained at three consecutive time points.

RESULTS

The overall induction of specific L-Ornithine-responding genes suggests that distinct activities appear in the course of the kinetic.
Furthermore, most of the genes that are found modulated, are up-regulated by L-Ornithine.

Overall effect
L-Ornithine treatment induces a slight hypoxic shock associated to transitory up-regulation of inflammation-associated genes, especially encoding cytokines. Then the inflammation cascade returns to normal state after 8 hours. This phenomenon would enhance adipocyte differentiation (adipogenesis) associated to lipogenesis.

Hypoxic shock and oxidative stress
Therefore, several lines of evidence suggest that L-Ornithine activates hypoxia and subsequent oxidative stress. However, it should be emphasized that these effects triggered by L-Ornithine seem to be transient since the induced expression of the genes involved decreases over time.

Inflammation
The inflammatory process activated by L-Ornithine seems tightly controlled over time, as shown by decreased induction of inflammatory genes after 8h, probably explained by up-regulation of all those genes involved in retro-control of inflammation.

Adipogenesis
An association of both LFNG-mediated NOTCH signalling activation and the maintenance of HES6 up-regulation through the 48 hours of treatment suggest that L-Ornithine impacts on adipogenesis.

Angiogenesis
Vascular endothelial cells express ERBB-receptors and can respond to NRG1/Neuregulin 1 by proliferation and angiogenesis [Kalinowski, A. *et al.*, 2010].

Fatty acids storage
Induction of GK/Glycerol Kinase stimulates glycerol incorporation into triglyceride, inducing FA re-esterification for storage in human subcutaneous adipose tissue. GK also reduces FA secretion from adipocytes [Guan HP. *et al.*, 2002].

Oxidative stress triggers glucose oxidation
L-Ornithine-induced hypoxia and inflammation trigger an intracellular oxidative stress that is illustrated by an up-regulation of Reactive Oxygen Species (ROS)-induced genes such as SOD2, PPP1R1A, PPP1R1B/DARPP-32, the phosphodiesterase PDE3B as well as the monocarboxylate co-transporters SLC16A7/MCT-2, SLC16A12 and SLC16A14. Such transcriptional effects represent an adaptive response to reduced O_2 tension [Di Virgilio F., 2004]. Hence, L-Ornithine exerts metabolic effects through Acetyl-CoA production in all aspects of lipid biogenesis.

Lipogenesis

Surprisingly, these results suggest that L-Ornithine induces concomitant FA uptake (as indicated by the up-regulation of genes such as APOB, LPL and LDLR), *de novo* FA synthesis and FA storage.

CONCLUSION

Altogether, our study suggests that L-Ornithine triggers the activation of the adipocyte-specific anabolism by concomitant fatty acids uptake, lipogenesis, lipolysis inhibition and lipid storage in differentiated subcutaneous adipocytes. In addition, it is possible that L-Ornithine would accentuate adipogenesis as several known adipocyte differentiation induced genes are up-regulated (Leptin/LEP, Adiponectin/ADIPOQ, ACOT2, CORIN), GLUL, PDE3B). Thus, the effects induced by L-Ornithine would mimic a process related to hormesis that is the term for favourable biological responses to low exposure to stressors. This repair process not only fixes the damage caused by the stressor but also other low-levels damages that might have accumulated before without triggering the repair mechanism.

INCREASE IN LIPID STORAGE IN HUMAN ADIPOCYTES BY ADIPOFILL™

OBJECTIVE

The aim of this study was to confirm the data obtained by the genic expression study by the evaluation of the effect of Adipofill™ on the adipocyte specific anabolism. In this study, the fatty acids release from human normal adipocytes and the average diameter of adipocytes before and after treatment have been measured.

PROTOCOL

Biological materials

Human normal adipocytes were obtained from a piece of surgical resection. The subject was 34 years old.

Tested product

0.05 and 0.2 % Adipofill™ batch 1130801C120 without preservative system.

Reference product

Theophylline at 10^{-3} M was used as a reference product.

Incubation protocols

Suspensions of adipocytes were incubated at 37°C under gentle agitation for a 2, 15 and 24 hours periods in the absence (untreated), or in the presence of the reference product or in the presence of increasing concentrations of Adipofill™.

Adipofill™ was directly dispersed in the reactional medium.

Evaluation protocols

- Non-esterified fatty acid assay

At the end of the incubation periods, non-esterified fatty acids (NEFA) were quantified in the reactional media by using a sensitive and specific spectrophotometric method.

- Oil red O staining and adipocyte diameter measurements procedure

At the end of the 2h incubation period, adipocytes were rinsed two times with a phosphate buffered solution (PBS) and were fixed with formaldehyde 10 % (v/v). Adipocytes were then incubated for a 2h period in a solution of isopropanol containing 0.5 % (w/v) of oil red O. At the end of the incubation period, cells were rinsed two times and were resuspended in PBS. Optical microscopy photographs of the cells were then taken and measurements of adipocyte diameter were achieved by using dedicated software.

Statistics

- NEFA measurements

Results are expressed as mmol/l of NEFA.

Statistical significance of the difference observed between the "Untreated" and the "Reference product" groups, was assessed by a student t-test (**: $p<0.01$).

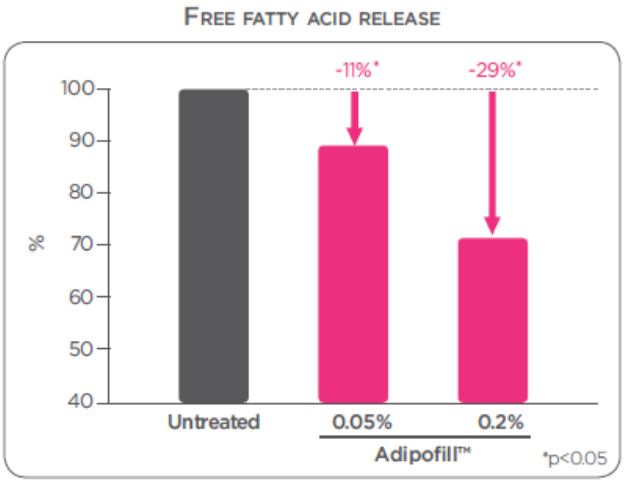
Statistical significance of the differences observed between the "Untreated" and the Adipofill™ groups, was assessed by a one way ANOVA analysis, followed by a Holm-Sidak test (*: $p<0.05$).

- Adipocyte diameter measurements

Results are presented to show the proportion of adipocyte diameters < and > to 190 pixels.

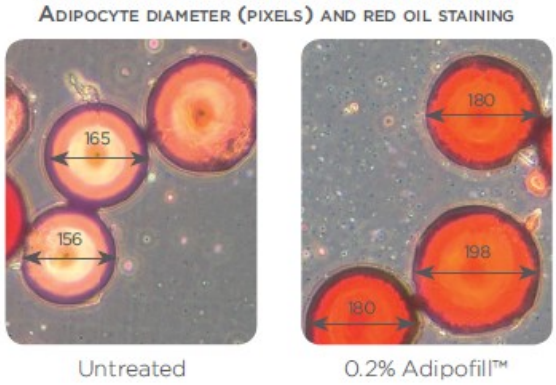
RESULTS

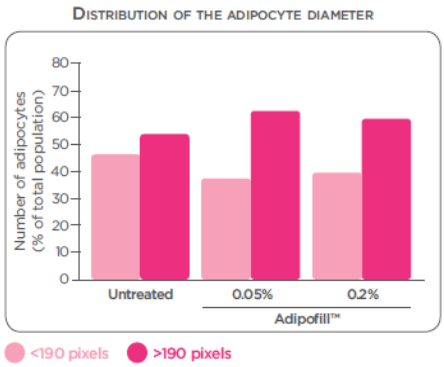
Effect of Adipofill™ on free fatty acids release from adipocytes :



0.05% and 0.2% Adipofill™ significantly reduces the fatty acid release from human normal adipocytes: - 11 % ($p<0.05$) and - 29 % ($p<0.05$) reductions, respectively.

Adipocytes size:





The increase in red intensity is correlated to a higher lipid content in adipocytes.
Adipofill™ induces a slight increase in the adipocyte diameter.

CONCLUSION

The results confirm the data obtained by genic expression study about the capacity of Adipofill™ to inhibit lipolysis and decrease the release of fatty acids from adipocytes.

The triglyceride storage increase inflates adipocytes leading to a bigger volume and diameter.

CLINICAL STUDIES

EVALUATION OF THE LIPOFILLING EFFECT

In vivo profilometry and evaluation by a dermatologist

OBJECTIVE

The aim of the study is to evaluate the lipofilling effect of Adipofill™ at 2% in a finish product formula during a clinical trial of **2 months**. The study has been carried out by a dermatologist.

PROTOCOL

Subjects

20 healthy female volunteers aged between 40 and 60 years with wrinkles, especially nasogenian folds.

Test conditions

For 60 days the volunteers applied an emulsion cream containing either 2% of Adipofill™ or a placebo. The trial cream and the placebo cream were applied randomized split-face twice a day in the morning and evening.

Tested creams

Placebo (batch 11.347.01/02-C122):

Phase A:	83.5%	Water
	0.5%	Emulmetik™ 930
	4%	Biophilic™ S
	0.5%	Xanthan gum
Phase B:	10%	Prunus amygdalus dulcis oil
	1%	Dekaben C
	0.3%	Tocopherols
Phase C:	0.2%	Fragrance

Active cream (batch 11.348.01/02-C122):

Phase A:	81.5%	Water
	0.5%	Emulmetik™ 930
	4%	Biophilic™ S
	0.5%	Xanthan gum
Phase B:	10%	Prunus amygdalus dulcis oil
	1%	Dekaben C
	0.3%	Tocopherols
Phase C:	0.2%	Fragrance
Phase D:	2%	Adipofill™

Clinical evaluation

At days 0 and 30 or 60, the evaluated parameters on both half-faces are:

-Skin profilometry by means of instrumental analysis.
Nasogenian fold depth and skin roughness of each volunteer were measured using Primos 3D profilometry.
This technique allows to:
a) take a high resolution image of the skin,
b) take a 3 dimensional image,
c) analyze the profilometrical information of the 3D images.

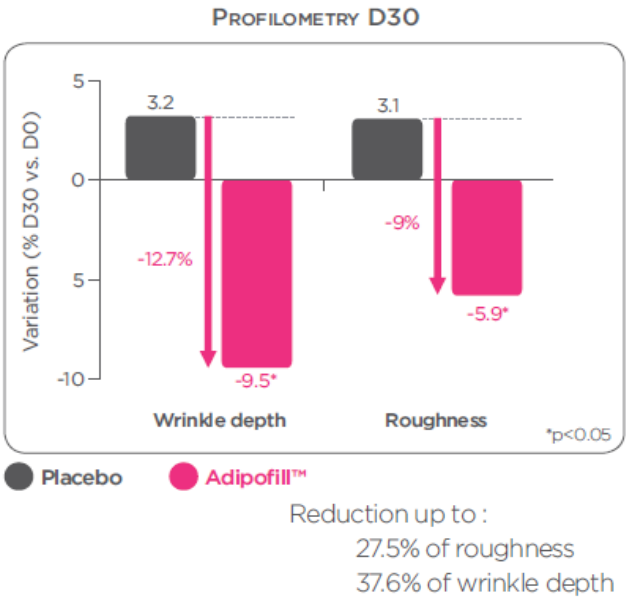
-Skin smoothness by means of dermatological, clinical evaluation. The dermatologist evaluated smoothness according to a clinical score scale.

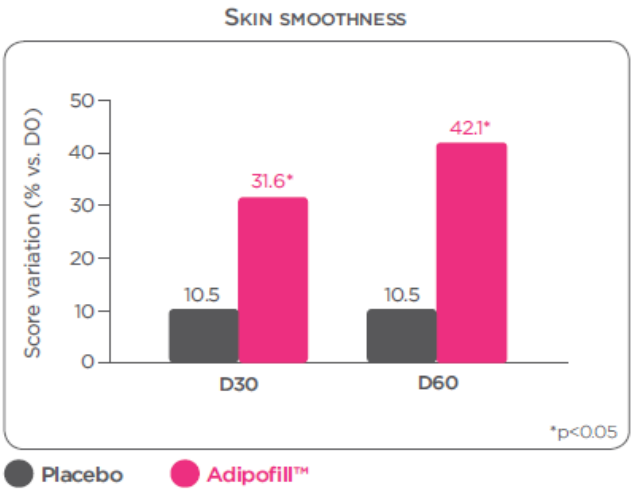
-Self-assessment by means of a questionnaire answered at the end of the study.

RESULTS

Profilometry Primos 3D & dermatological evaluation

Results are expressed in terms of variation of the evolution of wrinkle depth and roughness after 30 days of the placebo- and Adipofill™-treated sides.





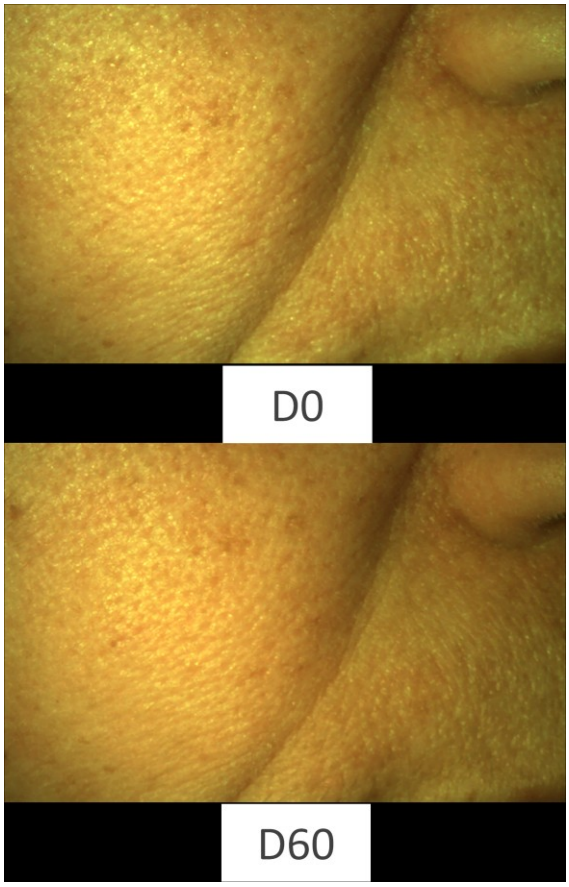
Reduction up to :
27.5% of roughness
37.6% of wrinkle depth

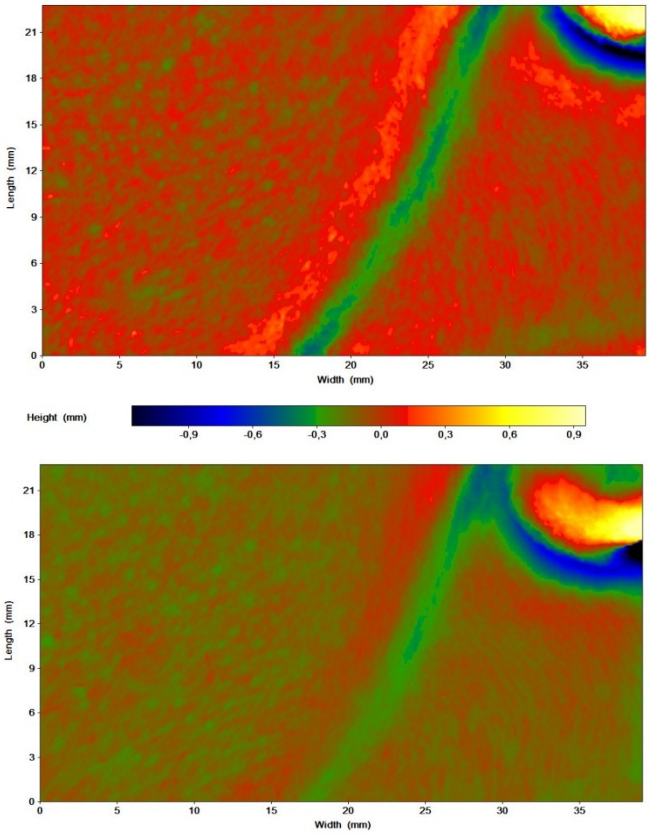
As shown, Adipofill™ significantly reduces the wrinkle depth and roughness after 30 days of treatment: -9.5% and -5.9%, respectively; whereas the placebo-treated half of the face shows no significant variation.

The dermatologist has evaluated a significant improvement of skin smoothness after 30 days and even more after 60 days; whereas the placebo-treated half of the face shows no significant variation.

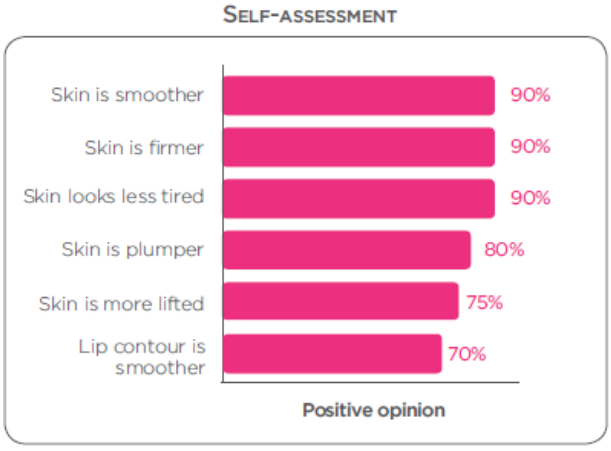
The appearance of nasogenian fold is reduced after only 30 days of application.

Illustrative photographs





Self-assessment



According the answers, the use of a finished product containing Adipofill™ leads to a skin:

- smoother
- firmer
- less tired
- plumper

CONCLUSION

By reducing nasogenian fold depth and skin roughness, Adipofill™ plays a major role in the reduction of the signs of aging, especially those relating to the face lipoatrophy.

Adipofill™ significantly reduces the appearance of nasogenian fold after only one month.

CONCLUSION

Adipofill™ is a patented L-ornithine amino acid encapsulated in an Ionosome™. Adipofill™ reproduces lipohormesis phenomenon by activating HIF-1α gene expression and related genes involved in the global adipocyte anabolism. Adipofill™ therefore mimics lipofilling result in a non-surgical way. By acting on adipose tissue growth with a bio-contolled and non-invasive mechanism, Adipofill™ clinically reverses the age-related signs of lipoatrophy.

Adipofill™ is thus an innovative, sophisticated and high-tech ingredient that fights the aging process for a plump and youthful look.

COSMETIC APPLICATIONS

- Anti-aging care
- Anti-wrinkle skin care
- Skin care for mature skin
- Alternative to surgery
- Breast and buttock volume enhancer
- Anti-aging treatment for hands
- Eye care
- Lip care
- Men's care

RECOMMENDATION FOR USE

Adipofill™ is easy to use and has excellent stability.

Adipofill™ should be incorporated at the end of the manufacturing process, at a temperature below 40°C.

RECOMMENDED DOSAGE: 0.5–2%

LA SPIRIT CREAM

	INGREDIENTS	INCI NAME	%
A	Deionised Water	Water	77.10
	Dermofeel™ PA-12	Sodium Phytate	0.10
	Chlorphenesin	Chlorphenesin	0.30
	Phenoxyethanol	Phenoxyethanol	0.80
	Granlux® AOX-GL	Glycerin (and) Picea Abies Extract (and) Alcohol	1.00
	Biophilic™ H	Hydrogenated Lecithin (and) C12-16 Alcohols (and) Palmitic Acid	4.00
B	Dermorganics GMS-SE	Glyceryl Stearate SE	1.00
	Mirasil DM350	Dimethicone	4.00
	Dekanex 2006 FG	Hydrogenated Polydecene	3.00
	Phytosqualan	Squalane	2.00
	Lecigel™	Sodium Acrylates Copolymer (and) Lecithin	1.50
C	Adipofill™	Water (and) Propanediol (and) Ornithine (and) Phospholipids (and) Glycolipids	2.00
	Progeline™	Glycerin (and) Water (and) Dextran (and) Trifluoroacetyl Tripeptide-2	2.00
	Exo-H™	Butylene Glycol (and) Alteromonas Ferment Filtrate	1.00
D	Douceur 3246	Fragrance	0.20

GENIC EXPRESSION ANNEX

Hypoxic shock and oxidative stress

Among genes modulated by L-Ornithine at all time points, the highest-induced gene encodes for NR4A3/NOR1$^{(48.2;\ 1.6;\ 2.9;\ 1.8)}$, an orphan nuclear receptor of the NR4A family [Pei L. et al., 2005]. Thus, the over-expression of these genes may indicate an early induction of an inflammatory process upon L-Ornithine treatment. This process is illustrated by the up-regulation of RELB$^{(5.1;\ 2.5;\ 1.4;\ 1.2)}$, a subunit of the NF-kappaB complex, as well as the induction of its targets PTGS2/COX2$^{(11.7;\ 2.4;\ 0.7;\ 0.9),}$ encoding Cyclo-oxygenase 2, and SOD2$^{(2.1;\ 1.7;\ 1.7;\ 1.2)}$, encoding Superoxyde Dismutase, whose role is to remove superoxide radicals from hypoxic cells. Moreover, it has been shown that NR4A3/NOR1 is a downstream effector of HIF1A$^{(1.4;\ 1.8;\ 1.1;\ 1.1)}$ (Hypoxia Inducible Factor 1) signaling involved in the survival response of endothelial cells to hypoxia [Martorell L. *et al.*, 2009].

Even though HIF1A is modestly induced at 8h at the transcriptional level, post-transcriptional activation of this factor has been reported. Indeed, accumulation of intracellular pyruvate increases the DNA-binding affinity of HIF1A to its target promoters. In hypoxic conditions, lactate acid, pyruvate and ketone bodies accumulate within adipocytes, and specific monocarboxylate co-transporters, such as SLC16A12$^{(1.8;\ 1.1;\ 1.5;\ 0.9)}$ and SLC16A14$^{(2.0;\ 1.6;\ 1.9;\ 1.4)}$, translocate to the membrane to release these extra metabolites. [Halestrap AP. and Meredith D., 2004]; [Pérez de Heredia F. *et al*., 2010].

Hypoxia, that induces apoptosis but also triggers adaptive mechanism to ensure cell survival, is known to increases the expression of genes such as IL6$^{(10.8;\ 2.2;\ 1.7;\ 0.9),}$ NOX4/NADPH Oxidase 4$^{(2.9;\ 5.3;\ 1.5;\ 1.4),}$ TGM2$^{(4.5;\ 4.2;\ 1.4;\ 1.5)}$ and ANGPTL4$^{(7.3;\ 6.9;\ 1.0;\ 0.8)}$.

NOX4/NADPH Oxidase 4 encodes an oxygen sensor protein which catalyzes the reduction of molecular oxygen to various reactive oxygen species (ROS), resulting in oxidative stress. These ROS trigger TGM2 activation, and are involved in cell differentiation.

Therefore, several lines of evidence suggest that L-Ornithine activates hypoxia and subsequent oxidative stress associated to an inflammatory process. However, it should be emphasized that these effects triggered by L-Ornithine seem to be transient since the induced expression of the genes involved decreases over time.

Inflammation

Two major inflammatory cytokines, IL6$^{(10.8;\ 2.2;\ 1.7;\ 1.2)}$ and IL33$^{(2.5;\ 8.9;\ 0.9;\ 0.6)}$ (a ligand of IL1RL1$^{(2.1;\ 1.9;\ 2.0;\ 1.1)}$), show an increased expression in preadipocytes following exposure to hypoxia [Wang B. et al., 2007], [Wood IS. et al., 2009] and can be released by hypoxic adipocytes to trigger inflammation, angiogenesis and to regulate insulin sensitivity. IL1RL1, a member of the interleukin 1 receptor family, is induced by pro-inflammatory stimuli such as IL6.

The inflammatory process activated by L-Ornithine seems tightly controlled over time, as shown by decreased induction of inflammatory genes after 8h, probably explained by up-regulation of all those genes involved in retro-control of inflammation.

Adipogenesis

Although the master gene of adipogenesis PPARG was not modulated following L-Ornithine treatment, its target genes such as GK/Glycerol Kinase$^{(1.8;\ 2.7;\ 1.3;\ 1.1)}$, FABP3$^{(1.9;\ 1.3;\ 1.5;\ 0.8)}$) and FABP4/aP2$^{(1.4;\ 1.0;\ 1.5;\ 1.4)}$, also involved in adipogenesis are found to be induced.

An association of both LFNG$^{(13.6;\ 4.1;\ 2.0;\ 2.2)}$-mediated NOTCH signalling activation and the maintenance of HES6$^{(3.6;\ 1.5;\ 2.0;\ 1.7)}$ up-regulation through the 48 hours of treatment suggest that L-Ornithine impacts on adipogenesis.

ST3GAL5/GM3-synthase$^{(1.9;\ 1.9;\ 1.8;\ 1.2)}$ participates in the induction of differentiation as a direct target of PPARB and PPARD [Sanderson LM. *et al*., 2009].

Among genes induced by L-Ornithine, FAM65B(1.6; 3.4; 1.8; 1.1), RET/CDHF12(3.2; 1.9; 2.7; 2.6) and IL1RL1 encode proteins known to be induced during the differentiation process [Yoon S. *et al*., 2007], [Iwashita T. *et al*., 1996], [Wade KC. *et al*., 2006].
This network highlights a potential effect of L-Ornithine on the activation of adipogenesis through at least in part a control of the NOTCH signalling pathway that can be illustrated by the overexpression of LFNG. As mentioned, L-Ornithine triggers the up-regulation of NR4A1 and NR4A3. These nuclear receptors are involved in the regulation of glucose and lipid metabolisms in insulin-sensitive tissues, and seem to be independent from PPARG activation. NR4A1(9.2; 1.4; 0.8; 0.7), NR4A3 [Chao LC. *et al*., 2008] and SOD2 [Lechpammer S. et al., 2005] are negative regulators of adipogenesis suggesting that they may participate to regulate negatively adipogenesis induced by L-Ornithine.

Angiogenesis
Vascular endothelial cells express ERBB-receptors and can respond to NRG1/Neuregulin 1(1.7; 4.3; 1.7; 1.0) by proliferation and angiogenesis [Kalinowski, A. *et al*., 2010].
Fatty acids storage
Induction of GK/Glycerol Kinase(1.8; 2.7; 1.3; 1.1) stimulates glycerol incorporation into triglyceride, inducing FA re-esterification for storage in human subcutaneous adipose tissue. GK also reduces FA secretion from adipocytes [Guan HP. *et al*., 2002], [Leroyer SN. *et al*., 2006]. ANGPTL4(7.3; 6.9; 1.0; 0.8) encoded protein is directly involved in triglyceride homeostasis through LPL(1.8; 1.3; 1.7; 1.7), suggesting a tight regulation of fatty acids storage after L-Ornithine treatment [Sukonina V. et al., 2006]. Long chain FA metabolism is also important in L-Ornithine-treated adipocytes as shown by the up-regulation of the peroxysomal enzyme FAR2/Fatty AcylCoA Reductase(2.0; 1.8; 2.1; 1.3).

Oxidative stress triggers glucose oxidation
L-Ornithine-induced hypoxia and inflammation trigger an intracellular oxidative stress that is illustrated by an up-regulation of Reactive Oxygen Species (ROS)-induced genes such as SOD2(2.1, 1.7, 1.7,1.2), PPP1R1A(1.7; 1.0; 1.5; 1.4), PPP1R1B/DARPP-32(2, 1.1, 1.8, 1.6), the phosphodiesterase PDE3B(1.7, 1.1, 1.6, 1.5) as well as the monocarboxylate co-transporters SLC16A7/MCT-2(2.0; 1.3; 1.5; 1.6), SLC16A12(1.8; 1.1; 1.5; 0.9) and SLC16A14(2.0; 1.6; 1.9; 1.4). Such transcriptional effects represent an adaptive response to reduced O2 tension [Di Virgilio F., 2004], [Pereyra-Munoz N. et al., 2006], [Jansen S. *et al*., 2009], [Pérez de Heredia F. *et al*., 2010], [Abdollahi M. *et al*., 2003].
Such moderate oxidative stress conditions have been shown to synergize with insulin signalling to increase glucose uptake through the insulin-responsive glucose transporter SLC2A4/GLUT4(2.3; 0.; 2.2; 72.2). SLC2A4/GLUT4 expression can also be induced by secreted adiponectin, encoded by ADIPOQ(1.6; 1.0; 1.4; 1.3) and specifically expressed in differentiated adipocytes [May JM and de Haën C., 1979].
SREBF1/SREBP1(1.7; 0.9; 1.5; 1.5) expression can be induced both by ADIPOQ and by moderate oxidative stress. Activation of SREBF1/SREBP1 transcription factor is denoted by the substantial albeit moderate expression of its reported target genes LDLR(1.8; 0.7; 1.3; 1.3), LPL, FASN(1.5; 0.9; 1.5; 1.3) or ACSS2(3.1; 1.8; 2.4; 1.7), all involved in lipogenesis.
Up-regulation of key metabolic enzymes is a significant feature of L-Ornithine-induced effects, which seems to trigger glycolysis associated to pyruvate production, necessary for Acetyl-CoA synthesis. Especially, Pyruvate Carboxylase/PC(1.8; 1.2; 1.7; 1.7) catalyzes the carboxylation of pyruvate to oxaloacetate and ACSS2-encoded cytosolic enzyme catalyzes the activation of resulting acetate to produce Acetyl-CoA, the major substrate of lipid synthesis.
Hence, L-Ornithine exerts metabolic effects through Acetyl-CoA production in all aspects of lipid biogenesis.

Lipogenesis

L-Ornithine exerts metabolic effects through a general albeit moderate up-regulation of many genes involved in all aspects of lipid biogenesis.

Fatty acids transport and uptake are increased after L-Ornithine treatment as indicated by the up-regulation of FABP3, FABP4/aP2, LPL, ACSL1$^{(1.5;\ 1.1;\ 1.4;\ 1.4)}$, ACSL3$^{(1.4;\ 1.2;\ 1.2;\ 1.2)}$, ACSL4$^{(2.1;\ 2.1;\ 1.1;\ 1.1)}$, ACSL5$^{(1.9;\ 1.5;\ 1.6;\ 1.4)}$, OLR1 $^{(1.4;\ 1.6;\ 1.1;\ 1.1)}$.

Early expressed Fatty acid transport protein 4/SLC27A4$^{(1.3;\ 1.7;\ 1.4;\ 1.1)}$ is able to activate PPARD signalling pathway in adipocytes, mediating the transcription of genes involved in FA transport (FABP3, LPL, ACSL1, ACSL3, ACSL4, ACSL5, OLR1), long chain FA synthesis (ACSL1, ACADL$^{(1.4;\ 1.0;\ 1.5;\ 1.5)}$), adipocyte differentiation (ANGPTL4, FABP4*, SORBS1$^{(2;\ 1.5;\ 1.7;\ 1.4)}$, PLIN1$^{(1.7;\ 1.2;\ 1.5;\ 1.5)}$, MMP1$^{(1.4;\ 2.1;\ 0.8;\ 0.8)}$) and in gluconeogenesis (GK, AQP7$^{(1.6;\ 1.2;\ 1.5;\ 1.4)}$). Up-regulation by L-Ornithine of ACACA$^{(1.8;\ 1.4;\ 1.5;\ 1.5)}$, ACACB$^{(1.7;\ 1.1;\ 1.7;\ 1.6)}$ and FASN also suggests the activation of FA biosynthesis as these genes encode enzymes that are involved in multiple steps of this process.

Glycerol kinase GK leads to triglycerides synthesis, allowing the storage of both neo-synthezised and taken-up FA into L-Ornithine-treated adipocytes. Concomitant to this FA storage, up-regulation of PLIN1 and Leptin/LEP$^{(1.8;\ 1.4;\ 1.5;\ 1.4)}$, both markers of adipocytes maturation, lead to an inhibition of triglyceride lipolysis [Kakuma T. *et al*., 2000].

Surprisingly, these results suggest that L-Ornithine induces concomitant FA uptake (as indicated by the up-regulation of genes such as APOB$^{(2.1;\ 1.1;\ 1.9;\ 1.6)}$, LPL and LDLR), de novo FA synthesis and FA storage.

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