Technical Report

Product

SILUSYNE®

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Revision

2

Lipotec

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Image and figure importance

Having the desired silhouette is nowadays a permanent goal for everyone. Our social image has personal and professional implications, so everyone is willing to improve it and correct the undesired physical features that can even influence our relationships.

Cellulite is an aesthetical problem which affects 90% of post-adolescent women and a small percentage of men, who consider it as an undesired skin disruption to solve. Cellulite is the final step of physiological changes that occur in the subcutaneous fat layer, which lead to an **increase in the volume of fatty tissue and the appearance of irregularities on the skin surface**, known as orange peel.

Moreover, **overweight** is an **increasing problem in modern society** due to the lack of exercise, extra caloric food intake and long working days. Having an elevated caloric food intake, results in an increase of fat storage. This accumulation causes the increase of the volume of the cells that keep the extra lipids and, consequently, of their surrounding tissue. As a result of this tissue growth, the volume of the area increases creating **visible and non attractive fat nodules (cellulite) and overweight.**

Many factors are involved in the storage of fat depots and the manifestation of cellulite including age, genetics, gender, extra caloric diet, lack of exercise, smoking, poor blood circulation and an unhealthy lifestyle in general. **Thighs, buttocks and abdomen** are the areas where cellulite mainly appears, but fat depots are also commonly found in the **stomach, breast** and face and neck in a smaller proportion. Although cellulite is clearly linked to these extra fat deposits, it is not exclusive of overweight individuals; it can also be found in lean women.

Overweight and cellulite are a problem for most of the worldwide population. People want to find an effective and preferably non invasive solution that allows them to reduce fat accumulation and visible nodules, improve their figure and feel better about themselves without surgery or complex treatments.

Adipose tissue and fat depots

Adipose tissue is a specialised connective tissue mainly located beneath the skin. There are two types of adipose tissue depending on its main function and their kind of adipocytes. In early stages of human life, brown adipose tissue (BAT) is necessary despite its low percentage (around 5%). However, **white adipose tissue (WAT) is the prevalent type and the small levels of brown tissue, present in youth, decrease with age**.

Human BAT is characterised by a high expression of mitochondrial genes and polygonal adipocytes, which contain a great number of mitochondria in the cytoplasm and several small lipid droplets. The main function of brown adipocytes is to **dissipate energy in the form of heat**.

On the contrary, **human WAT** functions as the **major storage site for the lipids incorporated by daily food intake.** Whenever the body requires energy for cells to use, lipids contained in its cells are burned. WAT contains **round adipocytes with a large single lipid droplet that are responsible for storing lipids.** It also has macrophages, fibroblasts, leukocytes and many collagen fibres that act as a support. **Preadipocyte** cells are present in the fat tissue as the precursors of adipocytes.

The main difference between white preadipocytes and adipocytes is the capacity of these last ones to store energy in the form of triglycerides (and cholesterol esters). **White adipocytes contain a large lipid droplet (80% of cell content),** forcing the nucleus and cytoplasm to remain in the periphery of the cell (Fig. 1).

Due to several factors such as genetics or high caloric intake, **fat deposits of human WAT can expand to such an extent that push and distort the connective tissue.** This local volume increase can also **induce irregularities in the junction line between dermis and hypodermis, increasing its length and making the skin surface appear uneven and with visible fat nodules** [1]. This extra lipid volume can obstruct the lymphatic drainage system making that waste materials like toxins or proteins cannot be removed properly.

Fig. 1. Preadipocyte and mature adipocyte.

These materials create an immobile network, together with the collagen fibres, where **fat cells are trapped**, leading to an inevitably **increase of local volume** and to the **appearance of unsightly irregularities** on the skin surface (cellulite).

Fat tissue volume and adipocyte differentiation

The volume of WAT is a function of both adipocyte number and size, so its enlargement can be caused either by an increase in the number of adipocytes or in lipid content.

Cellulite and overweight are related to a **rise in the adipose depots**, which is the result from an imbalance between food intake and energy expenditure. Cellulite can appear as a consequence of an extra local fat storage and it is known that overweight individuals produce more white adipocytes per year and have a greater number of mature adipocytes than thin individuals [2, 3]. For this reason, **acting on the cycle of the cells that have the capacity to store fat when they differentiate (white adipocytes) is one of the options to diminish fat deposits and their consequences in the skin appearance (cellulite and irregularities)**.

Total number of white adipocytes is constant in adult life. Therefore, adipocyte maturation rate must be the same as the adipocyte death rate to keep the equilibrium and maintain adipocyte number constant. For this reason, **diminishing the differentiation process rate** would alter this equilibrium resulting in a **lower number of mature white adipocytes**, which are the cells that store lipids in this tissue (Fig. 2). When the adipocyte differentiation rate is smaller than the adipocyte death rate, a **slimming effect** can be perceived because lipid accumulation decreases.

It is also important to mention that **white adipocyte precursors (preadipocytes) represent 15-50%** of the total adipose tissue cells, so acting on them would notably change WAT [4].

Considering the narrow relationship between cellulite and fat nodules, acting on adipocyte cycle will affect cellulite undesired manifestation.

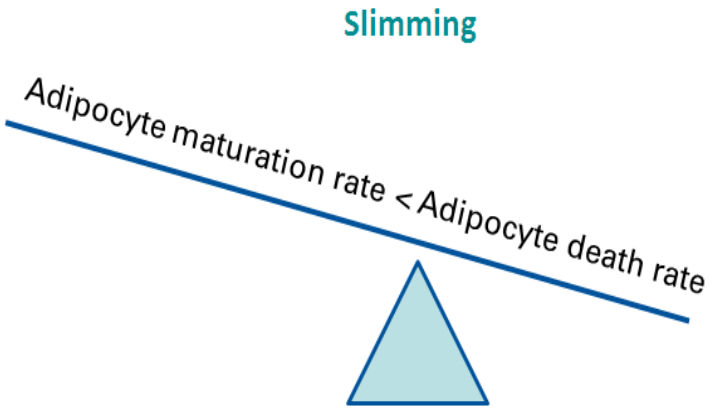

Fig. 2. Result of diminishing adipocyte differentiation.

Acting on the white preadipocyte differentiation is the key route to diminish lipid accumulation in WAT. Less fat tissue volume results in a slimmer figure.

PGC-1α effect on the slimming process

The differentiation process from preadipocytes to mature adipocytes is a complex process known as **adipogenesis** in which many factors and genes participate. Some genes need to be expressed as they are distinctive of mature adipocytes while the typical genes of preadipocytes need to be downregulated or almost inhibited in order to finally lead to the adipocyte phenotype [5]. For this differentiation process to happen, transcriptional factors are required. One of these key factors is Peroxisome proliferator-activated receptor-Gamma Coactivator 1 alpha (**PGC-1α**) due to its coactivation of a key receptor known as **PPARγ**.

PPARγ belongs to the Peroxisome Proliferator-Activated Receptors (PPARs) family, which is a group of **nuclear receptor proteins** that functions as transcriptional factors and regulates gene expression in cellular differentiation among other processes. This receptor forms heterodimers with Retinoid X Receptors which bind to specific regions on the DNA of target genes and regulate their expression. **PPARγ is predominantly expressed in the adipose tissue** and it is strictly necessary but not sufficient for preadipocytes to differentiate.

PGC-1α is a **transcriptional coactivator** that interacts with a broad range of transcriptional factors and nuclear receptors (including PPARγ) [6] (Fig. 3), **increasing the probability of certain genes related to adipocyte differentiation of being transcribed. In WAT, a robust induction of PGC-1α expression during *ex vivo* human subcutaneous preadipocyte differentiation was observed** [7], being its level as high as in mature adipocytes.

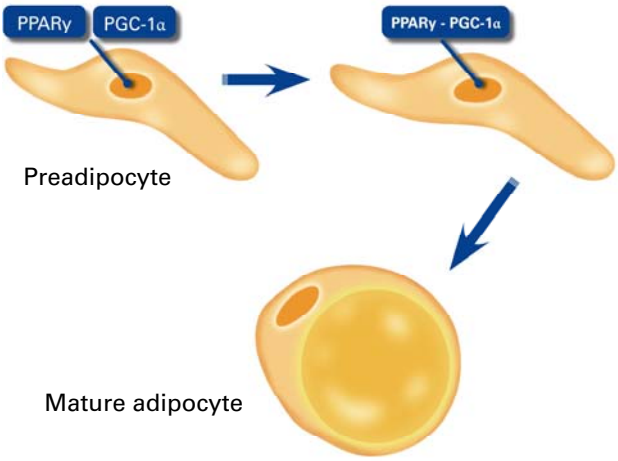

Fig. 3. PGC-1α effect on adipogenesis.

It was confirmed that in WAT, **PGC-1α interacts with PPARγ potentiating the expression of relevant genes related to adipocyte differentiation, thus stimulating adipogenesis**.

For this reason, diminishing PGC-1α would decrease adipocyte maturation and the number of adipocytes capable of storing lipids. As a result, storing fat in WAT would be more difficult and cellulite and overweight would be satisfactory reduced.

SILUSYNE®, new molecular mechanism for the persistent problem of cellulite

SILUSYNE® is a new ingredient for anti-cellulite and slimming products which contains a hexapeptide in a novel delivery system.

SILUSYNE® contains a hexapeptide with natural amino acids which was identified by a combinatorial chemistry approach from a library of 49,521,980 hexapeptides. The combinatorial peptide library was screened using the reporter gene assay in a stably transfected cell line where luciferase expression was controlled by PGC-1α promoter activity.

Lipotec developed a new specific delivery system in the submicron scale range with high encapsulation efficiency, suitable for a wide range of active ingredients. It combines techniques of microemulsion and microencapsulation and it allows improving the formulation of **SILUSYNE® peptide, in aqueous phases.**

SILUSYNE® showed to diminish adipocyte differentiation in white adipose tissue by decreasing PGC-1α levels *in vitro*.

***In vivo*, SILUSYNE®** proved to **significantly reduce the length of the dermo-hypodermal junction line**, which is related to the formation of cellulite and skin irregularities [1]. As a result, **skin becomes softer and flatter, making cellulite less visible.**

SILUSYNE® is the perfect ingredient for anti-cellulite and slimming cosmetic formulations.

In vitro efficacy

PGC-1α EXPRESSION IN HUMAN ADIPOCYTES

Efficacy of SILUSYNE® peptide was verified by measuring its effect in human subcutaneous preadipocytes in culture.

Human subcutaneous preadipocytes were incubated during 24 h in the Preadipocyte Growth Medium (PGM™-2), which was used as the basal control (non-treated non-differentiated cells). Differentiation was induced by changing this medium to the Preadipocyte Differentiation Medium (PDM-2), which was used as a control for non-treated differentiated cells. Afterwards, 25 or 100 µg/mL of SILUSYNE® peptide (Acetyl Hexapeptide-39) were added during the differentiation process and all samples (including controls) were incubated at 37ºC for 10 days.

Afterwards, cells were lysed, RNA was extracted and reverse transcription was carried out. The resulting cDNA was analysed by quantitative RT-PCR (Fig. 4).

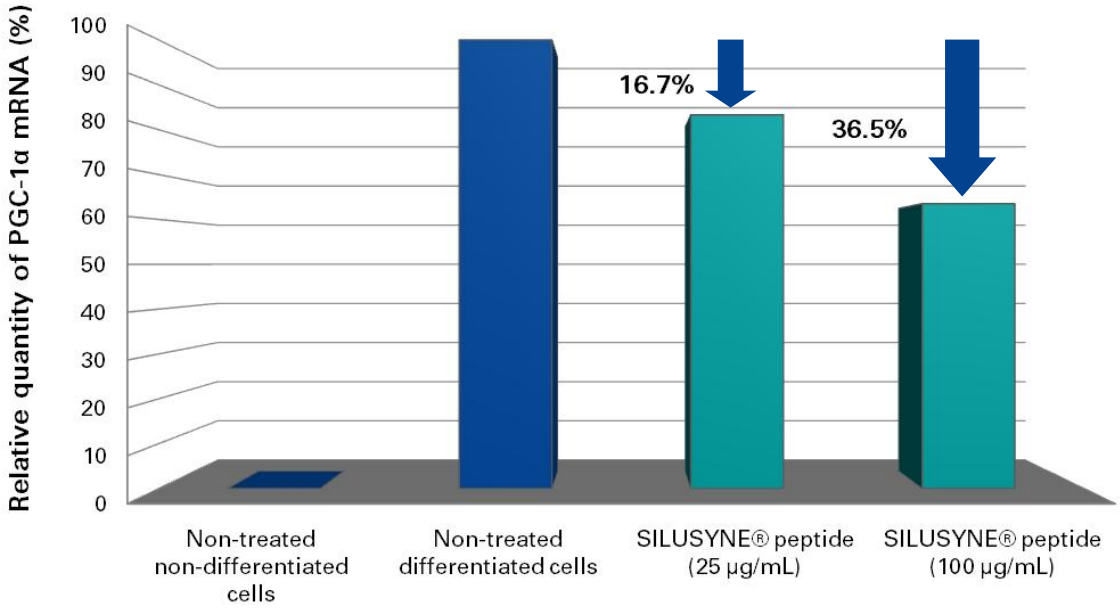

Fig. 4. PGC-1α mRNA expression relative quantity in human subcutaneous adipocytes after incubation with SILUSYNE® peptide.

Results showed that preadipocytes treated with **SILUSYNE® peptide had a lower expression of PGC-1α mRNA**. Compared to non-treated differentiated cells, Acetyl Hexapeptide-39 reduced PGC-1α transcription by 16.7% (at 25 µg/mL) and by 36.5% (at 100 µg/mL).

SILUSYNE® proved to reduce the expression of PGC-1α by 36.5% versus non-treated differentiated cells at 100 µg/mL.

EFFECT ON LIPID ACCUMULATION

Human subcutaneous preadipocytes were incubated during 24 h in the Preadipocyte Growth Medium (PGM™-2), which was used as the basal control (non-treated non-differentiated cells). Differentiation was induced by changing this medium to the Preadipocyte Differentiation Medium (PDM-2) and incubating the cells for 10 days in the presence of different treatments. SILUSYNE® peptide was tested at two different concentrations (25 and 100 µg/mL) and caffeine (200 µg/mL) was also included in the test. PDM-2 was used as a control for non-treated differentiated cells.

After 10 days, the supernatants were removed and wells were washed. Afterwards, 5 µL of AdipoRed™ reagent were added to each well and mixed.

The AdipoRed™ reagent is a hydrophilic solution that turns into fluorescent in hydrophobic environments. This facilitates the detection of the levels of intracellular lipid droplets accumulated during preadipocyte differentiation, which become stained. Fluorescence values were quantified at 535 nm (excitation at 485 nm), corrected with respect to basal fluorescence and normalised with respect to the fluorescence of the differentiation medium.

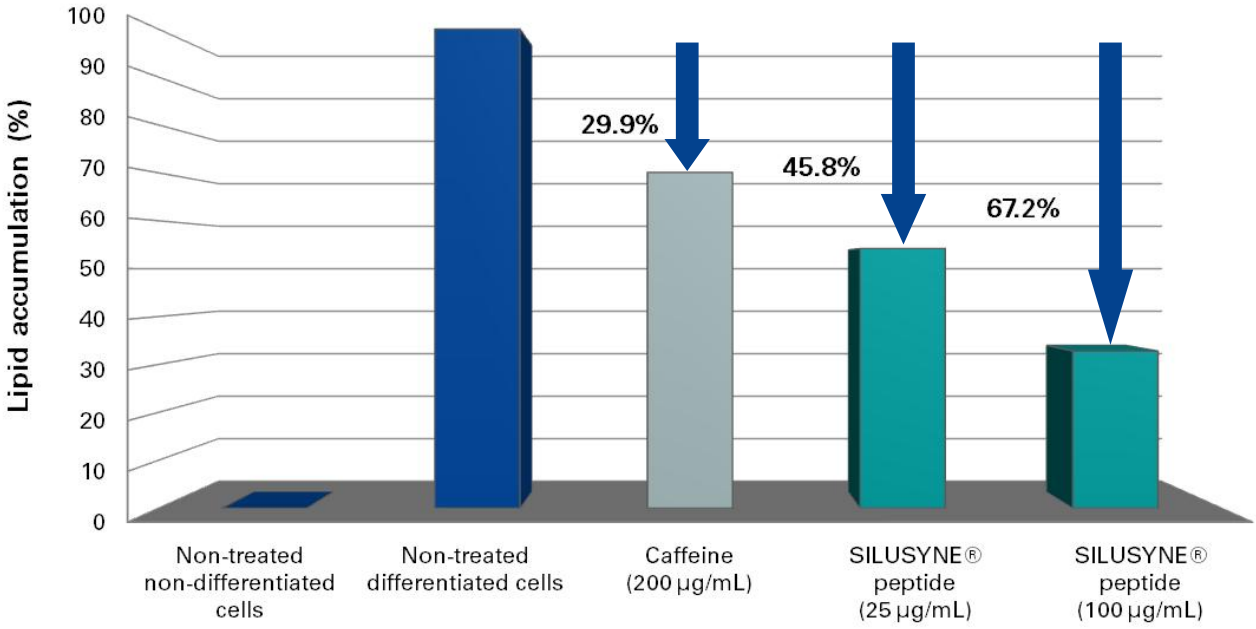

Fig. 5. Lipid accumulation in human adipocytes after different treatments.

The obtained values (Fig. 5) demonstrated that adipocytes coming from preadipocytes treated with **SILUSYNE® peptide had a lower lipid accumulation compared to non-treated differentiated cells or even to cells treated with caffeine**. Acetyl Hexapeptide-39 reduced lipid accumulation by 45.8% at 25 µg/mL and by 67.2% at 100 µg/mL.

SILUSYNE® showed to diminish lipid accumulation by 67.2% versus non-treated differentiated cells at 100 µg/mL.

In vivo efficacy

INSTRUMENTAL EVALUATION OF DERMO-HYPODERMAL JUNCTION

In order to determine the efficacy of SILUSYNE® as an anti-cellulite and slimming agent *in vivo*, its effects on the dermo-hypodermal junction were analysed by ultrasound ecography in B-scan mode (Ultrasound Scanner Dermascan C®). When cellulite is present, the inner disorder of the tissue increases and it makes this dermo-hypodermal junction line become more irregular and wavy, making it longer. For this reason, a decrease in the length of the dermo-hypodermal union means a more regular junction line and a better organisation of the tissue. This fact involves an improvement of the subcutaneous tissue uniformity and skin appearance.

For this study, 20 women between 25-45 years old with cellulite on thighs (Pinch test stage I-III) were selected and asked to apply the creams twice a day for 3 weeks. The placebo cream was applied on one thigh and the active cream with 2% SILUSYNE® was applied on the other.

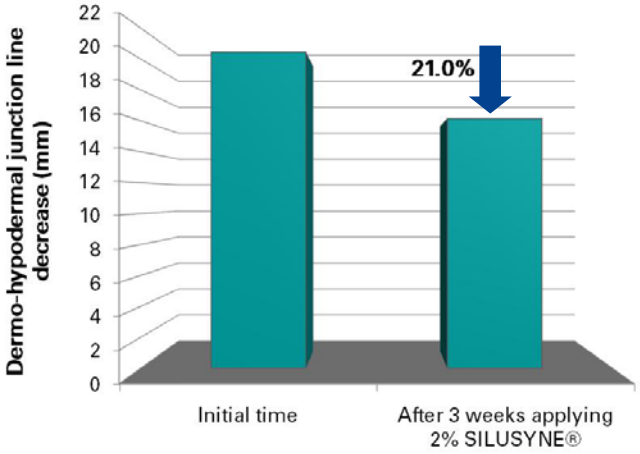

Fig. 6a. Dermo-hypodermal junction line decrease vs. initial time in presence of 2% Silusyne®.

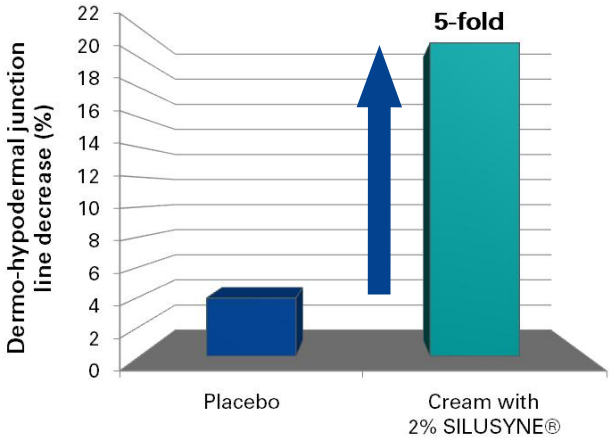

Fig. 6b. Dermo-hypodermal junction line decrease after three weeks.

After 3 weeks, areas treated with the cream containing **SILUSYNE® had notably reduced its dermo-hypodermal junction line by 21.0% versus initial time** (fewer irregularities in the junction line) contrary to placebo, which had no significant diminishing effect.

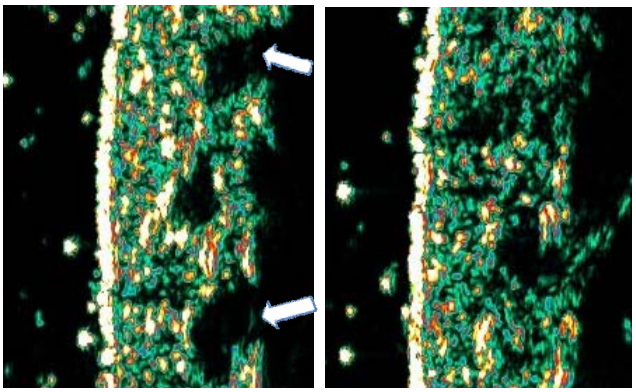

Fig. 7. Images from the dermo-hypodermal junction line of the tight of a volunteer at the initial time (left) and after 3 weeks applying a cream containing 2% Silusyne® (right).

SILUSYNE® reduced the dermo-hypodermal junction line improving the uniformity of the skin by 5-fold compared to placebo. This implies a more homogeneous and regular subcutaneous tissue, facilitating the skin surface to become flatter and with less cellulite.

Cosmetic properties

SILUSYNE®:

- **is a novel ingredient containing a hexapeptide included in a special delivery system ideal for anti-cellulite and slimming formulations.**
- **diminishes preadipocyte differentiation** in WAT by decreasing the expression of PGC-1α, which is closely linked to adipogenesis. Its effects were demonstrated *in vitro*, where it **reduced PGC-1α by 36.5%.**
- **reduces the lipid content in WAT**, as it can be seen *in vitro,* where it **diminished lipid accumulation by 67.2%,** obtaining even better reductions than caffeine.
- **improves skin uniformity** by reducing the length of the dermo-hypodermal junction line, related to cellulite and irregularities on the skin. Results of the *in vivo* study showed that this **junction was reduced by 21.0% versus initial time,** being **5 times more effective than the placebo**.

Cosmetic applications

SILUSYNE® can be incorporated in many formulations for **preventing and treating cellulite, slimming purposes** and as a **slimming complement** in hydrating, firming, remodelling, tanning and sun care products.

It can also be used as ingredient in formulations designed for usual sportsmen and sportswomen to enhance the slimming effect of the sport practise. Daily use products as body milks can also incorporate this ingredient to produce an extra slimming effect.

Technical data

INCI NAME OF THE ACTIVE INGREDIENT

Active ingredient	INCI name
SILUSYNE®	Isohexadecane, Sorbitan Sesquioleate, Acetyl Hexapeptide-39, Starch Hydroxypropyltrimonium Chloride, Sodium Hyaluronate, Potassium Cetyl Phosphate.

PRESENTATION AND PRESERVATIVES

Gel containing 0.05% of the peptide.

Code	Product presentation	Preservatives
PD205	SILUSYNE®	Phenoxyethanol, Sodium Benzoate

Application data

PROCESSING

SILUSYNE® can be added in the aqueous phase when formulating a gel. In case of preparing an emulsion, it should be added once the emulsion is formed. In both cases, it should be provided that the temperature is below 40ºC.

SILUSYNE® is stable at a pH range between 3.5 and 8.0.

INCOMPATIBILITIES

Not expected.

SOLUBILITY

Dispersible in water.

DOSAGE

A dosage of 2% of SILUSYNE® is recommended in final cosmetic formulations.

References

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Note: Graphs and photographs of efficacy tests are available for customer use provided that the final product contains the same concentration of active as the formulations in our tests. Customers must request written permission for use of the graphic material and/or ingredient tradenames to Lipotec. Customers are responsible for compliance with local and international advertising regulations.

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