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Juvenile look and desired silhouette

Nowadays, age related consequences on human body are not considered attractive. However, time does not obey to canons of beauty and its effects can be seen in all organs, including skin which is the most important for personal appearance. As we age, skin gets thinner, loses firmness and gravity also acts pulling the skin downwards, leading to a loose, lax and saggy skin [1]. These facts, together with a loss of the adipose tissue, which can act as a supporting tissue of the skin, alter facial morphology and provoke undesired and visible changes in people when aging.

Considering the whole body, the beauty stereotypes established by society are not always in accordance to real women and men silhouettes. Women need to have a perfect and feminine figure for being considered attractive and men are supposed to have an athletic but also well-developed shape as well. In most cases, people do not have these specific silhouettes.

As a result of these imposed canons of beauty, most people are not fully satisfied with their personal image and would like to change specific areas of their anatomy to feel themselves more attractive. As the most extensive organ of the body, the skin and the fatty tissue beneath can perform these changes easily and without invasive treatments.

Without any doubt, the face is the most exposed and visible body area of a person, so the aging process produces important modifications on it. If the adipose tissue volume lost when aging is regained, the unpleasant consequences of aging would be in part diminished. The result would be a younger and good-looking appearance.

This increase of adipose tissue can be stimulated in certain areas of the whole body (breast, buttocks, lips or hands) in order to change silhouette and personal image according to personal needs and desires.

People want to find an efficient and non aggressive way to increase tissue volume where they specifically want, including facial and body areas.
Aging and adipose tissue

Adipose tissue is mostly localised beneath the skin and it is a specialised connective tissue with two variants, depending on the main function of the tissue and the kind of adipocytes. Brown Adipose Tissue (BAT) is present in a really small percentage in humans but it is found to be necessary in newborns and early stages of human life. The aging process diminishes even more these already low levels of brown adipose tissue so White Adipose Tissue (WAT) is the most abundant type in adult life.

The main features of human BAT include an elevated expression of mitochondrial genes and polygonal adipocytes, which present a high number of mitochondria in the cytoplasm and numerous lipid droplets. The principal purpose of the brown adipocytes is to generate and dissipate heat from stored energy.

On the contrary, the major human storage site for the lipids incorporated by food intake is the WAT. This tissue stores lipids until the body requires energy, moment in which they are burned. Round adipocytes with a unique lipid droplet are the typical cells of WAT together with its precursor cells, known as preadipocytes. Apart from these two cell types, WAT also contains macrophages, fibroblasts, leukocytes and many collagen fibres, which act as the supporting structure for all these cells.

White adipocytes contain a wide lipid droplet (80% of cell content), forcing the nucleus and cytoplasm to remain in the periphery of the cell. This ability to store triglycerides (and cholesterol esters) is one of the most important differences between adipocytes and its precursors, as preadipocytes cannot accumulate lipids.

Young and old people have a very different adipose tissue distribution and quantity. With age, fat tissue decreases and gets redistributed. During this natural process, fat has a tendency to go from the subcutaneous stores to visceral depots, muscle, liver and other ectopic sites [1]. Therefore, several body regions see their volume decreased with age (cheeks, facial oval, hands or breast), depending also on the personal genetics. As a result, silhouette and facial morphology suffer remarkable changes, which are not always expected or happily accepted.

If the adipose tissue and adipocyte fat deposits can be increased, the global volume of the selected area will also augment. This situation will produce a development of local volume with the consequent improvement on facial appearance and curvy silhouette.
Breast importance and morphology

The relevance of breast in the silhouette of women is clear in the contemporary cultures. It seems one of the most important areas to define either a woman is considered attractive or not. For this reason, since its early development in girls (normally between 9-14 years old) it becomes an aesthetical concern for all women.

Breast can be described as a modified sudoriferous gland situated at both sides of the sternum and positioned between the skin and the pectoral muscle. These semispherical glands are basically formed by lobules, connective tissue, adipose tissue and Cooper’s ligament, although they also have blood vessels, nerves and lymphatic vessels, which make it sensitive.

The lobules of the breast are separated by connective tissue but also connected and joined together in a final channel which leads to the nipple.

As a function of some personal circumstances or physiological global changes, breast volume can vary a bit as it happens during pregnancy, for example.

The adipose tissue represents a high percentage of the total gland composition as it is placed around the lobules and also between the gland and the muscle behind. For this reason, its size variation has a great effect on global breast size.

The adipose tissue is basic to determine breast shape and size.
Adipocyte maturation

The volume of WAT varies as a result of its adipocyte number and size. For this reason, the increase of volume can be produced by an increment of the number of adipocytes or by an increase of lipid content.

In adult life, the equilibrium is maintained between adipocyte maturation rate and death rate, so the total number of adipocytes remains constant in number.

Having this into consideration, increasing the differentiation process rate would modify this equilibrium resulting in a higher number of mature white adipocytes, which are the cells capable of storing lipids in WAT (Fig. 1). When adipocyte differentiation rate is superior to adipocyte death rate, a replenishing effect can be perceived because lipid storage increases.

White adipocyte precursors represent 15-50% of the total adipose tissue cells [2], so changing them would highly modify adipose tissue.

Fig. 1. Result of increasing adipocyte differentiation.

Preadipocyte differentiation is the key route to locally increase lipid storage in WAT. A local augment of the adipogenesis process leads to an extra volume in the desired areas; face, breast or buttocks among them.
PGC-1α and volume increase

Adipogenesis is the process by which preadipocytes convert into mature adipocytes. It is a complex process where the typical genes of mature adipocytes must be expressed while the distinctive genes of preadipocytes need to be downregulated or almost inhibited [3]. As a result of this gene regulation and many interacting factors, the adipocyte phenotype is obtained. There are some key factors without which the differentiation would not be successful. One of the key factors is the Peroxisome proliferator-activated receptor-Gamma Coactivator 1 alpha (PGC-1α) because of 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. These receptors act as transcriptional factors and regulate gene expression in cellular differentiation processes. PPARγ is essential in the adipose tissue and it forms heterodimers with Retinoid X Receptors which bind to specific regions on the DNA of target genes and regulate their expression. This receptor is strictly necessary but not sufficient for preadipocytes to differentiate.

PGC-1α is a transcriptional coactivator that increases the probability of certain genes of being transcribed by interacting with a broad range of transcriptional factors and nuclear receptors (including PPARγ) [4]. In WAT cells, a high induction of PGC-1α expression during ex vivo human subcutaneous preadipocyte differentiation has been seen, rising to the levels found in mature adipocytes [5].

Considering this increase, it is confirmed that PGC-1α coactivates PPARγ potentiating the expression of genes linked to adipocyte differentiation, finishing in a stimulation of adipogenesis (Fig. 2).

If there was an increase of PGC-1α expression, the adipocyte maturation rate would raise, resulting in a growth of the number of adipocytes capable of storing lipids. As a consequence, fat depots would be more easily formed and the desired area would see its fat tissue volume augmented.
ADIFYLINE® is a new hexapeptide containing natural amino acids ideal for formulations to increase fat tissue volume in specific and local areas. It 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.

ADIFYLINE® showed to increase adipocyte differentiation in WAT by raising PGC-1α in vitro. The increase of mature adipocytes capable of storing lipids in the white adipose tissue was confirmed in vivo. ADIFYLINE® produced an augment of fat tissue volume in the tested areas (face and breast), which had an evident increase of volume.

ADIFYLINE® is the perfect ingredient to diminish the effects that the aging process causes in the skin.

In facial formulations, it can increase the supporting tissue beneath the skin improving facial appearance in mature individuals as well as increasing direct volume. In body products, it can be used to enhance the volume of certain areas like breast or buttocks.
In vitro efficacy

PGC-1α EXPRESSION IN HUMAN ADIPOCYTES

Efficacy of ADIFYLINE® 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 (PGMTM-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 also used as a control for non-treated differentiated cells. During the differentiation process, 0.5 or 0.1 mg/mL ADIFYLINE® were added and all samples (including controls) were incubated at 37 ºC 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. 3).

![Fig. 3. PGC-1α mRNA expression relative quantity in human subcutaneous adipocytes after incubation with ADIFYLINE®.](image)

These values showed that preadipocytes treated with ADIFYLINE® had a higher expression of PGC-1α mRNA compared to non-treated differentiated cells. ADIFYLINE® increased the expression of PGC-1α by 25.6% at 0.1 mg/mL and by 61.1% at 0.5 mg/mL.

ADIFYLINE® increased the expression of PGC-1α by 61.1% versus non-treated differentiated cells at 0.5 mg/mL.
EFFECT ON LIPID ACCUMULATION

Human subcutaneous preadipocytes were incubated during 24 h in PGM™-2, which was also used as the basal control (non-treated non-differentiated cells). Differentiation was induced by changing this medium to PDM-2 and incubating the cells for 10 days in the presence of the different treatments. ADIFYLINE® was tested at two different concentrations (0.5 and 0.1 mg/mL) and 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, which facilitates the detection of the levels of intracellular lipid droplets accumulated during preadipocyte differentiation (they become stained).

Fluorescence values were read at 535 nm (excitation at 485 nm), normalised with respect to basal fluorescence and to the fluorescence of the differentiation medium.

Fig. 4. Lipid accumulation in human adipocytes after different treatments, including ADIFYLINE®.

Results demonstrated that adipocytes coming from preadipocytes treated with ADIFYLINE® had an increase in lipid accumulation compared to non-treated differentiated cells. ADIFYLINE® enlarged lipid accumulation in white adipocytes by 27.9% at 0.1 mg/mL and by 32.4% at 0.5 mg/mL.
In vivo efficacy

INSTRUMENTAL EVALUATION OF FACIAL VOLUME INCREASE

In order to evaluate the efficacy of ADIFYLINE® in increasing skin volume, a group of 22 female volunteers between 50-60 years old was selected. In this in vivo study, the tested areas were the cheeks and volunteers were asked to apply the cream containing 2% ADIFYLINE® SOLUTION twice a day for 14 days. The volume of the cheeks was analysed by fringe projection at the beginning and at day 14. This technique allows the direct acquisition of the morphology of the studied area in 3D, being able to obtain the volume in mm³. The volumes obtained at the end of the treatment were compared to the initial values (Fig. 5).

After 14 days, areas treated with the cream containing 2% ADIFYLINE® SOLUTION had a significant 11.9% volume growth versus initial time. Results showed as well that with ADIFYLINE®, 79% of volunteers had a volume expansion after 14 days.

ADIFYLINE® produced a visible cheek volume increase of almost 12% after 14 days, versus initial time.

Fig. 5. Measurement of cheek volume of volunteers.

Fig. 6. Real 3D images from a volunteer at the initial time (left) and after 14 days applying a cream with 2% ADIFYLINE® SOLUTION (right).
INSTRUMENTAL EVALUATION OF BREAST VOLUME INCREASE

For the evaluation of the in vivo efficacy of ADIFYLINE® in increasing local volume (breast), a panel of 22 females between 25 and 40 years old was selected. As specific criteria, volunteers needed to have an 80-90 European bra cup size and a stable weight. They were asked to apply twice a day the placebo cream in a bust and the cream containing 2% ADIFYLINE® SOLUTION in the other, for 56 days. Measurements of breast volume were taken at the beginning and at day 14, 28 and 56 using the Fast Optical In vivo Topometry Technique (FOITS), which allows reconstructing the surface and volume of the breast based on the principle of optical interferometry. The relative volume of the area and 3D images were obtained. The differences versus initial time were measured (mm³) as well as the evolution of the breast volume of the volunteers versus initial time normalised with respect to placebo results.

Results at the end of the study showed that ADIFYLINE® generated a clear positive growing tendency in breast volume, while placebo did not. Moreover, the volume increase in the area where the cream containing 2% ADIFYLINE® SOLUTION was applied was 30 times higher than the placebo at day 56.

ADIFYLINE® improved breast volume increase by 30-fold compared to placebo results, at day 56.
Cosmetic properties

ADIFYLINE®:

- **enhances adipocyte maturation by boosting PGC-1α expression in WAT** (closely linked to adipogenesis). *In vitro*, it showed to raise PGC-1α levels by 61.1%.

- **induces a higher lipid accumulation in WAT**, which was confirmed *in vitro* as it increased lipid storage by 32.4%.

- **augments local volume by raising subcutaneous adipose tissue**, which was confirmed *in vivo* when cheek volume grew by almost 12%.

- **increases body volume by growing adipose tissue volume**; *in vivo* breast volume raised by 30 times more than placebo.

Cosmetic applications

ADIFYLINE® can be incorporated in many formulations and products to **increase local volume**. It includes **facial redefining products, breast firming formulations**, facial anti-aging products, make up for mature women, lip care products and make up, specific formulations for the cleavage area (mature women), and anti-aging products for hands, which lose adipose tissue with age.

Moreover, it can also be incorporated as a complementary ingredient in hydrating, firming and sun care products for specific body care areas (buttocks and breast) with a replenishing effect.
Technical data

**INCI NAME OF THE ACTIVE INGREDIENT**

<table>
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<th>Active ingredient</th>
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<tr>
<td>ADIFYLINE®</td>
<td>Acetyl Hexapeptide-38</td>
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**PRESENTATION AND PRESERVATIVE**

Solution containing 0.05% of active ingredient.

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<th>Code</th>
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<th>Preservative</th>
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<tr>
<td>PD210</td>
<td>ADIFYLINE® SOLUTION</td>
<td>Preservative free</td>
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**Application data**

**PROCESSING**

ADIFYLINE® SOLUTION needs to be added in the aqueous phase. In case of emulsions, it should be added once the emulsion is formed and at temperatures below 40 °C.

ADIFYLINE® is stable at a pH range between 3.0 and 8.0.

**INCOMPATIBILITIES**

Not expected.

**SOLUBILITY**

Soluble in water, ethanol and glycols (glycerin, butylene glycol).

**DOSAGE**

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


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.

The specific situation of the trademark in each country may vary and we recommend that you contact us for updated information.

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