Plumping the hypodermis – A new approach to joyfully embrace yourself

Klein, Marco Jan[1]*; Ferreira Melo, Elaine[1]; Carulla, Patricia[1]; Grau-Campistany, Ariadna[1]; Mateu, Miriam[1]; Pastor, Silvia[1]

[1] Lipotrue S.L., Barcelona, Spain

* Marco Jan Klein, C/Imaginació 12, Pol Ind Les Marines, 08850 Barcelona, Spain, +34 935477609, mklein@lipotrue.com

Abstract

In the past years there has been increasing evidence that the facial subcutaneous white adipose tissue (sWAT) could play an important role in skin aging and should therefore be included in developments for facial skin rejuvenation. The age-related change in facial subcutaneous white adipose tissue is associated with a reduction in its volume, a change in communication between dermal and adipose layers and alteration of collagen structure and content. The sum of these factors can significantly affect the mechanical stability of the skin and cause the development of signs of aging such as wrinkles. In this study, we present an innovative approach to prevent major factors in age-related facial white adipose tissue degradation by activating adipogenesis, lipogenesis, boosting the anti-oxidant and anti-inflammatory response and improving the communication between the dermis and hypodermis. In order to demonstrate improvements in all relevant areas concerning the loss of facial white adipose tissue we used amongst others a gene expression approach, *in vitro* and *in tubo* experiments, a fibroblast and adipocyte co-culture assay, and a clinical study with Caucasian female volunteers. In addition, improvement in skin appearance seems to have a satisfying emotional effect on the volunteers, which could be demonstrated in an independent clinical study performed by neuroscience experts.

Keywords:

facial aging, adipose tissue, adipocytes, inflammation, well-being

Background

Adipose tissue consists mainly of adipocytes, its precursor cells and vascular endothelial cells, which are embedded in connecting tissue consisting of collagens and other extracellular matrix components that serve as a scaffold for these cells [1,2]. A major cause of change in the mechanical stability of the skin and the appearance of signs of aging such as wrinkles are changes in the facial subcutaneous white adipose tissue (sWAT). Similar to damage caused by extrinsic and intrinsic factors in the epidermis and dermis, also the hypodermis suffers from these factors [3]. These events can lead to a replacement of healthy dermal white adipose tissue (dWAT) by inflamed fibrotic structures that macroscopically presents itself as a sunken, saggy face partly due to unfavorable relocation of facial fat and weakened structures [3,4]. Among other things, UV light can negatively influence the hypodermis by penetrating the skin deep enough to reach the tips of dWAT columns. However, since the hypodermis can communicate with the dermis via these aforementioned dWAT columns, the production of collagen can be stimulated that same way as well, and volume, firmness and inflammation can be restored or even improved [2,5,6].

In white adipose tissue (WAT) intrinsic (e.g., mitochondrial oxidative) or extrinsic (e.g., UV irradiation) stress and thus the generation of reactive oxygen species (ROS) impact the metabolic function of facial fat cells [7]. Increased adipocyte oxidative stress decreases adipogenesis and secretion of adipokines. In general, chronic prolonged oxidative stress negatively influences the overall homeostasis of white adipose tissue, including inadequate cellular antioxidant defenses and impaired mitochondrial function [7].

Various regulating enzymes are involved in the formation of mature adipocytes from preadipocytes. In addition to the proper functioning of adipogenesis, also lipogenesis and avoidance of lipolysis are aspects of healthy facial white fat tissue. In the transcriptional cascade regulating the terminal differentiation of adipocytes, the key adipogenic transcription factors are CAAT/enhancer-binding protein α (C/EBPα), peroxisomal proliferator-activated receptor g (PPARγ) and the regulator of lipogenic genes sterol-regulatory-element-binding protein 1 (SREBP1) [8,9,10].

PPARγ activates the promoter of the gene encoding C/EBPα and vice versa, creating a positive-feedback loop. In addition, PPARγ and C/EBPα induce the expression of genes that are involved amongst others in lipogenesis, including those encoding glucose transporter GLUT4 (also known as SLC2A4), fatty-acid-binding protein (FABP4)), lipoprotein lipase (LPL), perilipin and the secreted factor adiponectin, an adipokine. Recent genome-wide binding analyses have revealed that PPARγ and C/EBPα cooperate on multiple binding sites in promoter regions, together regulating a wide range of genes expressed in developing and mature adipocytes [11,12].

Lipid droplets (LDs) are the major cellular organelles for the storage of neutral lipids, and besides a proper formed collagen scaffold, they can be considered the main aspect of sWAT volume. Therefore, reduction of the facial sWAT volume in aging can be mainly connected with three possible mechanisms: reduction of the adipocytes' number (impaired adipogenesis), reduction of the single cells' volumes (lipolysis), and with "beiging" of white adipocytes, which is generally connected with volume reduction in these phenotypically modified cells [1].

There are enzymes that promote lipogenesis, such as fatty acid synthase (FASN), but also enzymes that favor lipolysis e.g., lipase E (LIPE), Enoyl coenzyme A hydratase 1 (ECH1) or Growth differentiation factor 15 (GDF15) [13]. FASN, an enzyme encoded by this gene, is a multifunctional protein with the main function of catalyzing the synthesis of palmitate from acetyl-CoA and malonyl-CoA, in the presence of nicotinamide adenine dinucleotide phosphate (NADPH), into long-chain saturated fatty acids [14]. LIPE is a protein whose short form is expressed in adipose tissue, among others, where it hydrolyzes stored triglycerides to free fatty acids [15]. ECH1 is a widely known enzyme involved in lipid metabolism, and is implicated in browning of WAT. ECH1 regulates the thermogenic program by inhibiting rapamycin signaling in mammals, which may explain the potential mechanism for regulation of fat browning by ECH1 [16]. GDF15 is a brown adipokine released by brown and beige cells in response to thermogenic activity and is able to promote fat lipolysis, thermogenesis, and oxidative metabolism in human adipose tissue [17].

Of further importance for the maintenance of lipid droplets in mature adipocytes is the lipid droplet-associated protein perilipin. It coats the surface of lipid droplets and thus protects them from the body's own lipases. It controls the lipid metabolism of adipocytes, performing essential functions in the regulation of basal and hormonally stimulated lipolysis, and may also be involved in increasing the synthesis of triglycerides, and therefore the accumulation of lipid content [18].

Adipocytes are imbedded in a special extracellular matrix (ECM) structure containing different types of collagen. This structure and the volume of the sWAT are significantly modified during aging. The modification is connected with the loss of the sWAT volume and its collagen network, which leads to a corresponding stiffness of the sWAT. Such alterations can directly or indirectly influence the mechanical properties of the adjacent skin [19,20]. As mentioned earlier, adipocytes from sWAT can physiologically interact with skin fibroblasts influencing their synthetic properties, which can indirectly modify the mechanical properties of the composite skin [21,22,23]. Exposure of facial preadipocytes to conditioned medium from UVA-irradiated epidermal-dermal cells strongly inhibited the differentiation of these cells into mature adipocytes as both chronic and single UVR exposure significantly reduces master adipogenic factors such as peroxisome proliferator-activated receptor γ (PPARγ) [24].

Although sWAT and dermis appear anatomically as absolutely different tissues with a clearly defined boundary, it seems obvious that these tissues strongly interact with each other. An important process that involves communication between the two tissues with different cell types, which can significantly influence the sWAT volume and be directly involved in facial aging, is the recently discovered AMT (adipocyte-myofibroblast transition). Reduction in the dWAT in a chronically UV-irradiated skin was shown to correlate with replacement of this volume by fibrotic structures indicating the involvement of AMT. This substitution of sWAT by fibrosis was observed also after application of different physical stressors and thus can be effectively involved in the volume loss connected with aging processes [3,25,26]. In order to maintain a healthy sWAT, in addition to homeostasis of adipogenesis and lipogenesis, therefore, another important factor is to avoid inflammation and previous oxidative stress. sWAT structure and its mechanical properties are known to be strongly

modified by inflammation, suggesting that inflammation is involved in facial sWAT loss and hence in facial aging. Exposure of preadipocytes to conditioned medium from sun-irradiated epidermal dermal equivalents containing inflammatory cytokines such as IL-1α, IL-6, IL-11 and TNF-α inhibited the differentiation of these cells into mature adipocytes and resulted in a reduction in triglyceride levels [3].

In order to protect adipose tissue and our skin from such negative modifications, an attempt can be made to improve the general anti-oxidant abilities of the skin, but adipocytes themselves can also react to such insults. They can release adipokines which are cytokines secreted by adipose tissue. It has been shown that adipokines such as adiponectin attenuates inflammatory responses to multiple stimuli by modulating signaling pathways in a variety of cell types [27]. Studies indicate that adiponectin overexpression significantly increased preadipocyte differentiation and cell viability, inhibited MCP-1, IL-6, IL-8 and TNF-α expression, and upregulated PPARγ and C/EBPα expression [28].

Another aspect of adiponectin besides its anti-inflammatory properties and thus preventive maintenance of the well-organized sWAT, is the active up-regulation of the production of ECM components (i.a., collagen) by dermal fibroblasts that supports a healthy hypodermis. This communication is feasible as fibroblasts possess adipokine receptors that upon activation lead to the expression of type I collagen [29].

Methods

Origin of the active (INCI: Cimicifuga Racemosa Root Extract, Vitis Vinifera (Grape) Fruit Cell Extract)

The active ingredient is a blend of two botanical extracts, Vitis Vinifera and Cimicifuga Racemosa. Vitis vinifera is the Latin name of the common grape vine (Monastrell grape variety from Spain), and its extract is known for the abundance of polyphenols, anthocyanins a subtype of flavonoids, and other antioxidants, which can even be enriched due to the origin of the extract, a so-called callus culture. The root extract of Black Cohosh (Cimicifuga Racemosa), which originally could be found in North America, is very rich in polyphenols and isoflavones.

Total antioxidant capacity

The antioxidant capacity of the compound was measured by means of the Trolox equivalent antioxidant capacity (TEAC) assay. Briefly, different concentrations of BL4BTE0044_0013 were incubated with the stable radical cation, $ABTS^{\bullet-}$, which is a blue chromophore, in order to assess if they are able to neutralize it into the colourless $ABTS^{2-}$. The degree of decolorization, measured at 405 nm, induced by the compound is related to that induced by Trolox, a soluble analogue of vitamin E with a high antioxidant capacity, giving the TEAC value, which can be used to rank antioxidants.

Modulation of genes by qPCR

Preadipocytes were seeded and differentiated into mature adipocytes. During differentiation, 2% BL4BTE0044_0013 were added during the whole process (9 days) or once preadipocytes were terminally differentiated into mature adipocytes, during the last 48 h. Untreated cells were used as negative control.

Then, cells were lysed for RNA extraction using Qiagen RNeasy Mini kit. cDNAs were obtained by reverse transcription using a commercial kit (Thermofisher). Taqman assay probes were used for RT-qPCR using StepOne plus instrument. ΔΔCt method was used for data analysis. Samples were normalized with the relative expression of a housekeeping gene GAPDH.

Lipid content quantification

Preadipocytes were seeded and differentiated into mature adipocytes (9 days). In order to evaluate the effect of BL4BTE0044_0013 on adipogenesis, different concentrations of the ingredient were added to differentiation medium during the whole process. Finally, Adipored assay was performed as follows:

Cells were washed twice with PBS. Then, Adipored™ Assay Reagent (Lonza) was added to each well and cells were incubated at room temperature for 15 minutes, under horizontal shacking. Finally, fluorescence of each well was measured with a FLUOstar fluorimeter at 485 nm of excitation and 530 nm of emission.

Lipid content values were normalized using the relative fluorescence units (RFU) of non-treated mature adipocytes (basal, considered a 100% of lipid content), obtaining the % of lipid content.

Perilipin quantification by immunofluorescence

Fully differentiated adipocytes were treated for 5 days with 3% BL4BTE0044_0013 diluted in AM-1 medium. Basal samples were also incubated with AM-1 medium but without the ingredient. After treatment, samples were washed twice with PBS, fixed with 4% formaldehyde and permeabilized with Triton 0.2%. Next, samples were blocked for 1 hour with PBS/BSA 5%, in order to avoid nonspecific antibody binding.

For perilipin staining, samples were incubated overnight with 1:100 anti-perilipin primary antibody in PBS/BSA 1%, at 4ºC. After proper washing with PBS, cells were stained with 50 µg/mL Phalloidin (against actin filaments) and 5 µg/mL of the secondary antibody Goat anti-Rabbit IgG (against primary antibody, Thermo), during 1 hour at room temperature and dark conditions. Finally, samples were washed three times with PBS and coverslips were mounted using Prolong-Gold with DAPI, that stains nuclei, and samples were kept protected from light at 4ºC until microscopic images were acquired.

Microscopic images were acquired using 10x objective. Three replicates were stained for each condition and images of at least five different fields of each coverslip were acquired using the same settings.

Dermal Fibroblasts and Subcutaneous Adipocytes co-culture

Preadipocytes were differentiated into mature adipocytes for 7 days. Separately, fibroblasts (HDF) were seeded on transwell inserts. When adipocytes were completely differentiated, transwell inserts with fibroblasts were transferred to the plate with adipocytes, and both cell lines were co-cultured for 24 h in the presence of different concentrations of BL4BTE0044_0013 (1, 2 or 3%).

After treatment, culture medium was collected. Part of this conditioned medium was used for quantifying both interleukin-8 (IL-8) and adiponectin through ELISA (Enzyme-Linked ImmunoSorbent Assay), whereas the remaining medium was transferred to another cell culture plate with HDF cells already seeded on coverslips. After 4 days of treatment, HDF cells were fixed and collagen I was stained for immunofluorescence. Images were acquired using a fluorescence microscope and Image J software was used to analyse and compare the mean fluorescence per cell.

Clinical efficacy

The clinical efficacy of the ingredient was assessed in a clinical assay on 20 Caucasian female volunteers (50-60 years old). The study was conducted versus placebo: volunteers applied the active product, a cream containing the blend at a concentration of 3% in half of the face and the placebo formulation on the other half, according to a previously randomization list. Product efficacy was evaluated after 7 (T7), 14 (T14) and 28 (T28) days of daily use.

Skin profilometry was assessed by means of Primos 3D (GFMesstechnik GmbH). Primos 3D is a non-contact in vivo skin measurement device based on structured light projection. In conjunction with a comprehensive 3D measurement and evaluation software, the sensor allows to evaluate skin surface properties (i.e. wrinkle depth, volume, roughness etc.). In this study wrinkle volume in the crow's feet area and the nasolabial fold was evaluated. Moreover, the plumping effect on cheekbones was assessed using PrimosCR high resolution high field. Furthermore, radiance and erythrema were evaluated in this study.

Finally, the effect on an emotional level of the use of the botanical blend was assessed on 10 volunteers (50 to 60 years old) by means of a vocal marker assay as well as a projective test.

Briefly, for the vocals markers, loudness (vocal intensity, dB) and pitch (tone, Hz), which can be assigned to an emotional stress level, were measured before and after 28 days of daily use of a cream containing 3% of BL4BTE0044_0013. Moreover, in the projective test, a trio of different images, with tress emotional dimensions (positive active-dynamism, positive active – serenity/balance or negative) were presented to the volunteers 15 times and they must choose one of the three images at each time. The percentage is calculated by the number of images related to the specific mood. The analysis and the quality control of all data was performed by the neuroscience expert Arnaud Aubert.

Statistical analysis

For all experiments, each condition was performed at least in triplicates (n=3) in three independent assays (N=3). For all data, mean values of the different experiments and SEM values are shown. All data was normalized versus basal. Statistical data analysis was performed using Student t-test by comparison of treated cells vs non-treated cells (Basal) where ***$p<0.001$, **$p<0.01$ and *$p<0.5$. All graphics shows the mean ± SEM. For the in vivo assay, statistical data analysis was performed using Student t-test.

Results.

Total antioxidant capacity

The active ingredient presents at all concentrations tested an antioxidant capacity equivalent to more than 255 µM of Trolox (Fig.-1), reaching an antioxidant activity equivalent to 604 µM Trolox at 0.2 mg/mL and 689 µM Trolox at 0.3 mg/mL.

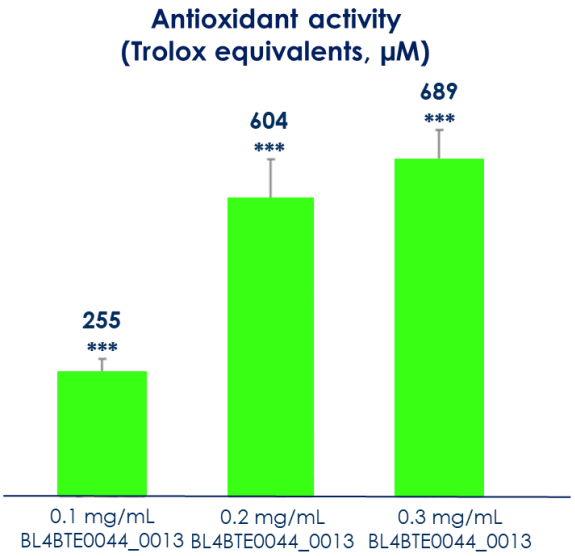

Fig.-1: Dermal and adipose tissue protection by antioxidant activity. Trolox Equivalent Antioxidant Capacity (TEAC) tested *in tubo* at 0.1, 0.2 and 0.3 mg/mL active ingredient.

Modulation of genes by qPCR

Positive effects on lipogenesis, lipolysis and adipogenesis were evaluated by modulation of relevant genes. For early differentiating preadipocytes, incubation with 0.2 mg/mL of active for 48 h results in an upregulation of genes PPARG, CEBPA, FATP/SLC27A1 and LPL by 1.2-fold, 1.64-fold, 1.16-fold, 2.69-fold, respectively, and downregulation of genes LIPE, ECH1 and GDF15 by -1.8-fold, -1.17-fold and -1.19-fold, respectively. Moreover, when late terminal differentiated preadipocytes are treated for 7+2 days with the active at 0.2 mg/mL, genes PPARG, CEBPA, SREBF1, FABP4, FATP/SLC27A1, LPL, SCD and FASN are upregulated by 1.98-fold, 2.21-fold, 1.36-fold, 1.58-fold, 1.68-fold, 1.75-fold, 1.82-fold and 2.66-fold, respectively and GDF15 is downregulated by -1.70-fold. Positive effects are observed on inflammation- and ECM-related gene expression when late terminal

differentiated preadipocytes are treated for 7+2 days with the active at 0.2 mg/mL. Genes COL1A1, COL4A1, TIMP2 are upregulated by 1.23-fold, 1.46-fold and 1.42-fold, respectively, and genes IL6, MMP1, MMP3 and TNFa by -1.56-fold, -8.68-fold, -2.57-fold and -1.19-fold, respectively, are downregulated (Table-1).

Table-1 Gene expression modulation in Human preadipocytes (fold change vs. basal)

Modulation of gene expression (fold change vs. basal)					
0.2 mg/mL, 48h, early differentiated preadipocytes		0.2 mg/mL, 7+2d, late differentiated preadipocytes			
LIPE	-1.8	**CEBPA**	2.21	**IL6**	-1.56
GDF15	-1.19	**LPL**	1.75	**MMP1**	-8.68
ECH1	-1.17	**FABP4**	1.58	**MMP3**	-2.57
FATP/SLC27A1	1.16	**FAS**	2.66	**TNFa**	-1.19
PPARG	1.2	**FATP/SLC27A1**	1.68	**COL1A1**	1.23
CEBPA	1.64	**GDF15**	-1.70	**COL4A1**	1.47
LPL	2.69	**SREBF1**	1.36	**TIMP2**	1.42
		PPARG	1.98		
		SCD	1.82		

Lipid content quantification

Results show that when differentiating preadipocytes are incubated with different concentrations of active (0.1, 0.15 and 0.2 mg/mL) for 7+2 days a positive effect on differentiation and significant increase of lipid content by 4%, 7% and 17%, respectively, can be observed in a concentration dependent manner, when compared with untreated cells (Fig.-2).

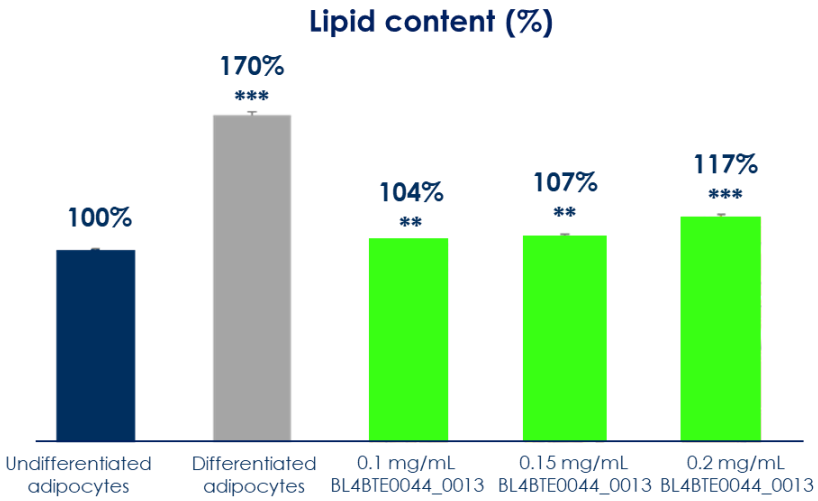

Fig.-2: Lipid content [%]. Effect on differentiation and accumulation of lipid content in differentiating preadipocytes after treatment with 0.1, 0.15 and 0.2 mg/mL active ingredient for 7+2 days. After exposure, lipid content was measured using AdipoRed™ fluorescence assay (λ=520 nm).

Perilipin quantification by immunofluorescence

As seen in Fig.-3, when human adipocytes are incubated with 0.3 mg/mL of active ingredient an increase in accumulation of lipid droplet protecting coating protein perilipin on the cell membrane surface by 33% is observed, when compared with untreated cells.

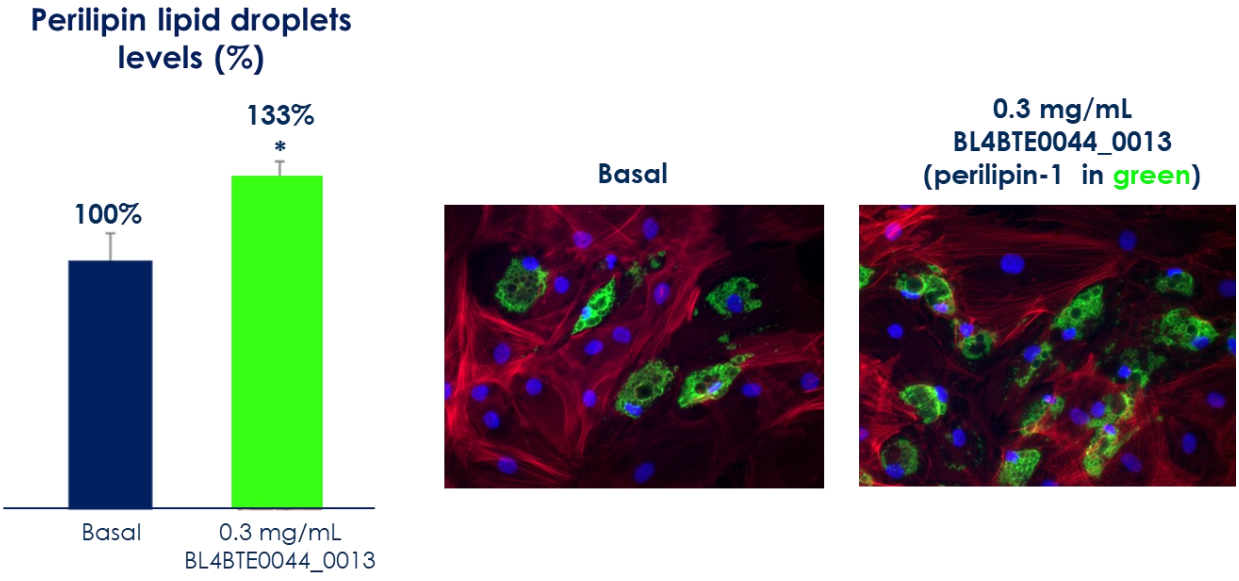

Fig.-3: Perilipin quantification [%]. Effect on accumulation on perilipin on lipid droplets after treatment of human adipocytes for 48 h with 0.3 mg/mL of active ingredient. Immunofluorescence measurement, Alexa 488.

Dermal Fibroblasts and Subcutaneous Adipocytes co-culture

The active ingredient can improve inflammaging in dWAT by lowering pro-inflammatory cytokines (IL-8 by -66%) and increasing anti-inflammatory factors (adiponectin by 22%). Furthermore, a novelty of the active ingredient's action is the improvement in communication between the hypodermis, mainly adipocytes, and the dermis/adjacent fibroblasts. We found that adiponectin released mainly by adipocytes after treatment with the active ingredient triggers the production of collagen (collagen I by 43%) in adjacent fibroblasts (Fig.-4)

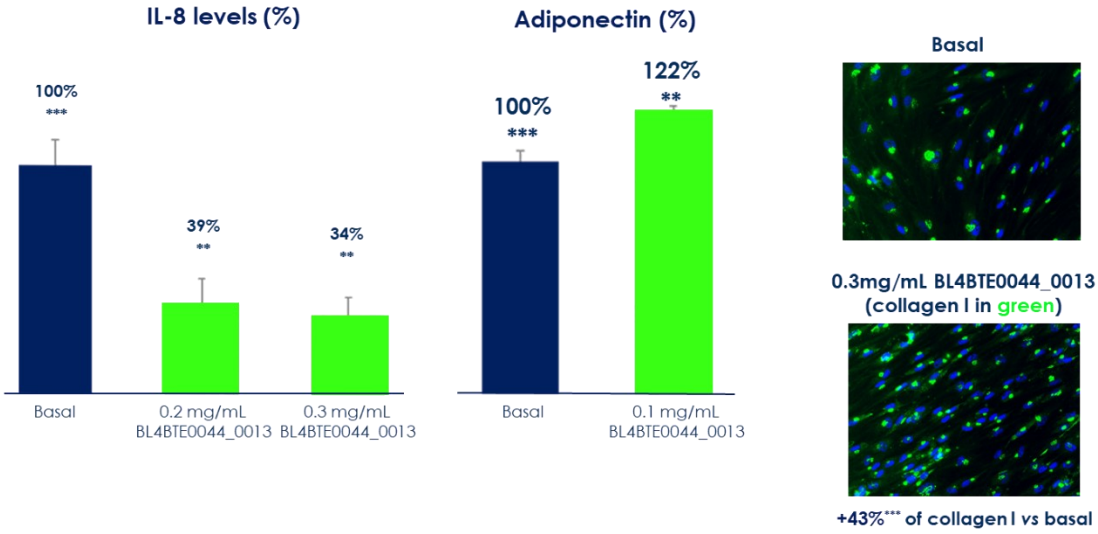

Fig.-4: Positive modification related to inflammatory cytokine IL-8 (-66%) and anti-inflammatory adipokine adiponectin (+22%) after treatment of a co-culture of human dermal fibroblast (HDFa) and differentiated adipocytes for 24 h with 0.1, 0.2 and 0.3 mg/mL active ingredient. Human dermal fibroblast (HDFa) treated with supernatant co-culture (HDFa + hAD) for 4 days, immunofluorescence, Alexa 488.

Clinical efficacy

In a clinical study, brightness (significant increase in radiance by 25.2%, Fig.-5), erythema (significant decrease of -7.8%, Fig.-6), wrinkles in the crow's feet (significant reduction by -10.7%, Fig.-7) and nasolabial fold (significant reduction by -6.3%, Fig.-8), and a plumping

effect on the cheekbones (significant increase of 0.52 mm in the measurement along the curvature of the cheekbones, Fig.-9) were assessed.

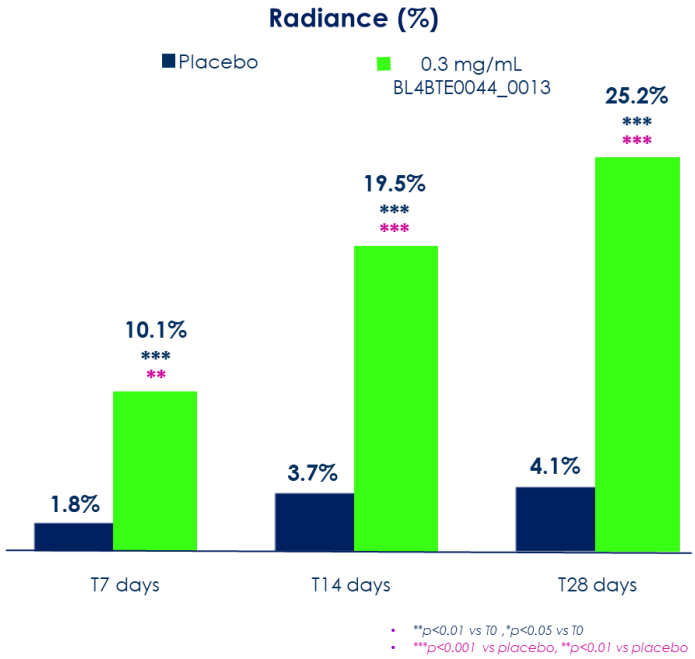

Fig.-5: Significant increase in brightness/radiance after treatment with the active ingredient at 0.3 mg/mL vs placebo by 10.1%, 19.5% and 25.2% after application of 7 days, 14 days and 28 days, respectively.

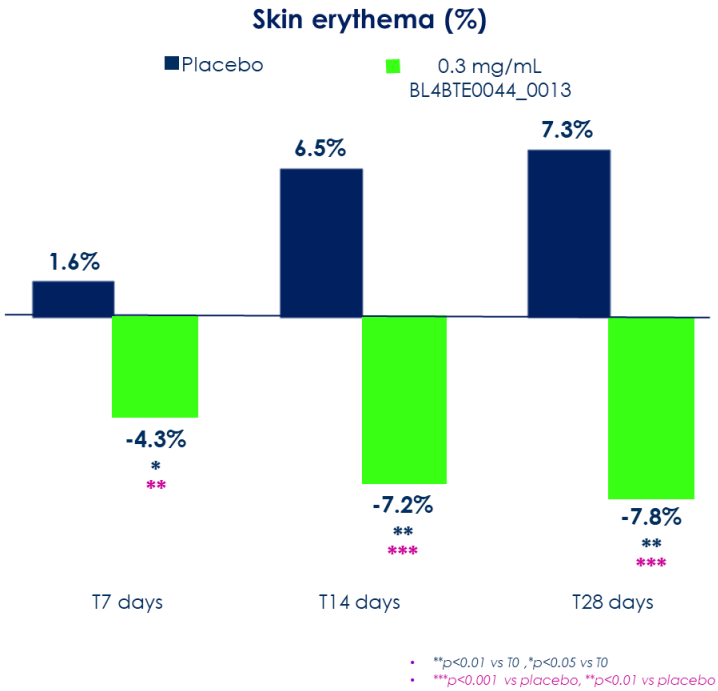

Fig.-6: Significant decrease in skin erythrema after treatment with the active ingredient at 0.3 mg/mL vs placebo by -4.3%, -7.2% and -7.8% after application of 7 days, 14 days and 28 days, respectively.

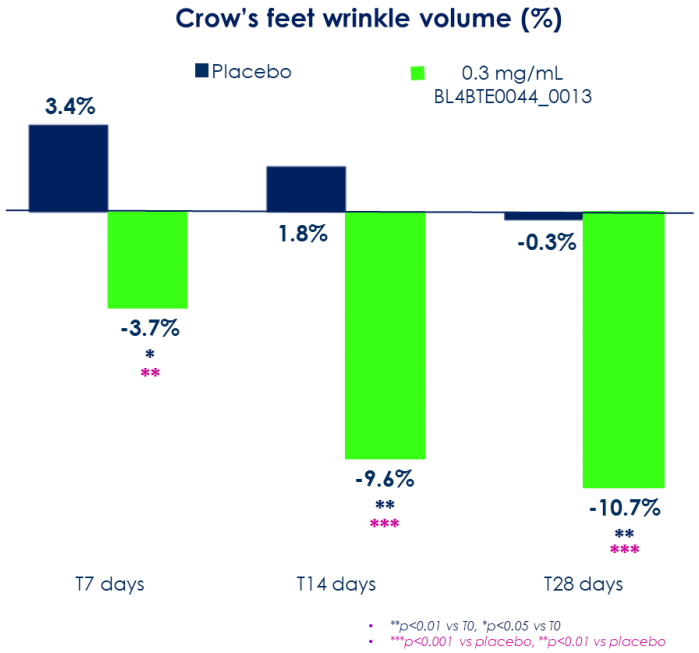

Fig.-7: Significant reduction in the crow's feet wrinkle volume after treatment with the active ingredient at 0.3 mg/mL vs placebo by -3.7%, -9.6% and -10.7% after application of 7 days, 14 days and 28 days, respectively.

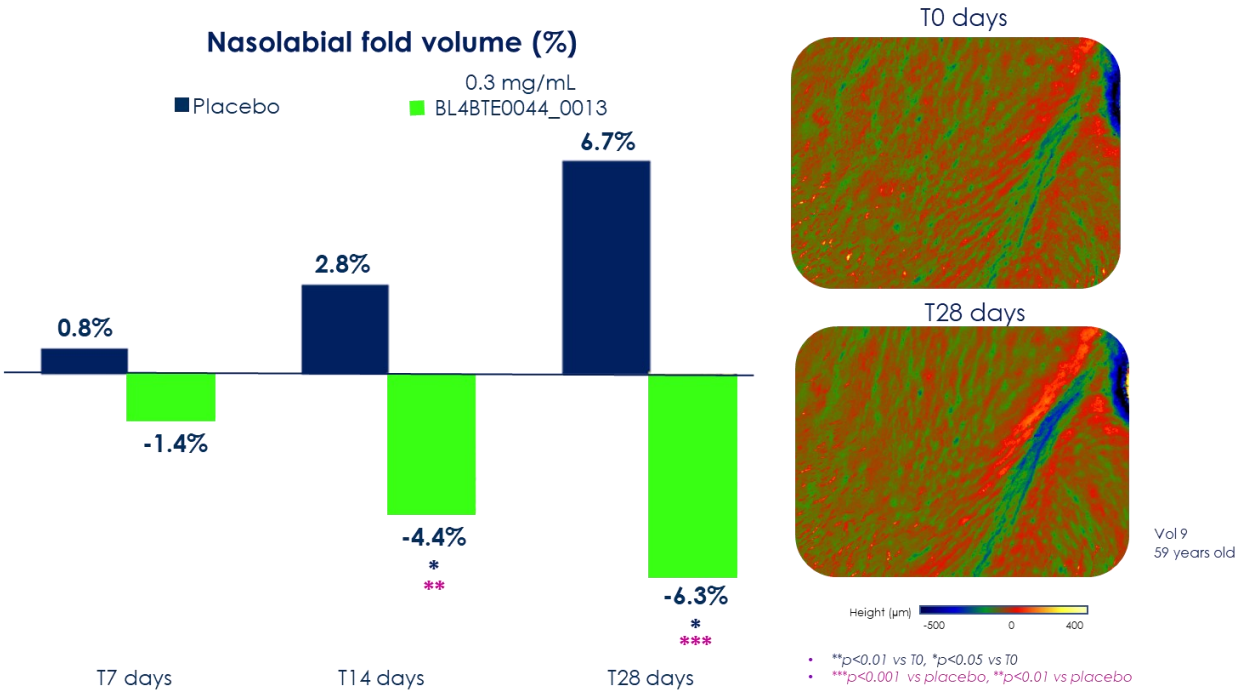

Fig.-8: Significant reduction in the nasolabial fold volume after treatment with the active ingredient at 0.3 mg/mL vs placebo by -1.4%, -4.4% and -6.3% after application of 7 days, 14 days and 28 days, respectively (left). PRIMOS 3D image of the nasolabial fold of volunteer 9 (59 years old) at day 0 and after 28 days of treatment with 0.3 mg/mL active ingredient (right).

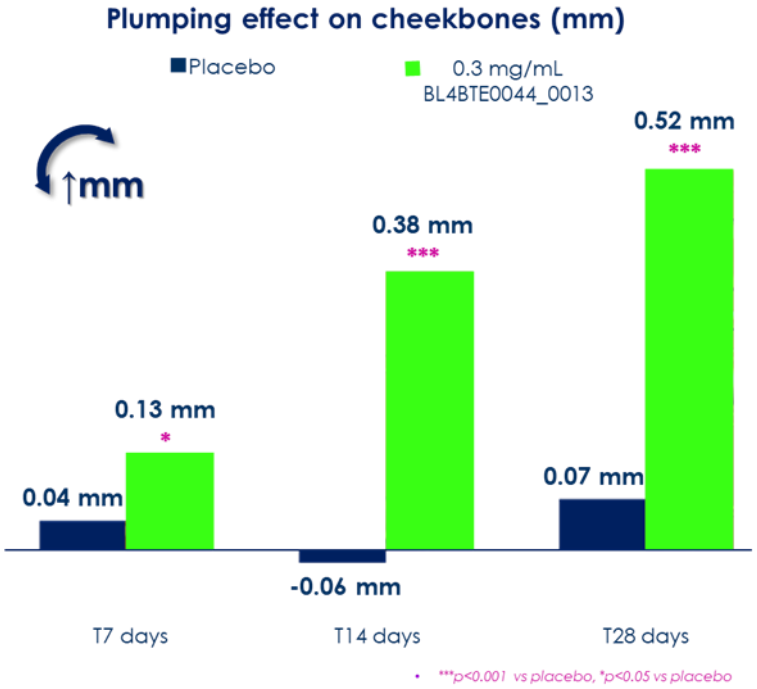

Fig.-9: Significant plumping effect on cheekbones after treatment with the active ingredient at 0.3 mg/mL vs placebo by 0.13 mm, 0.38 mm and 0.52 mm after application of 7 days, 14 days and 28 days, respectively.

In an additional clinical study, mood improvement by application of the active ingredient for 28 days vs day 0 was evaluated. A projective test revealed that balance (positive passive) could be improved by 7.1%, dynamism (positive active) by 6.5%, and negative emotions could be decreased by 50% (data not shown). Experts can interpret the change in vocal markers of emotional stress that are usually only receivable on a subconscious level. Loudness could be reduced by -4.1 dB and the vocal pitch could be lowered by -5.1 Hz which supports the idea of mood improvement (Fig.-10).

Fig.-10: Vocal markers of emotional stress could be improved after treatment of the whole face with 0.3 mg/mL of active ingredient for 28 days. Loudness could be reduced by -4.1 dB and the vocal pitch could be lowered by -5.1 Hz which can be interpreted as mood and well-being improvement of the volunteers.

Discussion.

Anti-aging products are the most common type of products on the cosmetic market, along with other skin application. However, almost all cosmetics are aimed solely at improving the epidermis and dermis, their ECM components and hydration. Our data indicate that our active ingredient BL4BTE0044_0013 (INCI: Cimicifuga Racemosa Root Extract, Vitis Vinifera (Grape) Fruit Cell Extract) can activate adipogenesis, lipogenesis and reduce lipolysis in the facial hypodermis that constitutes of adipocytes, its precursor cells and vascular endothelial cells. These cells are embedded in connecting tissue consisting of collagens and other extracellular matrix components that serve as a scaffold for these cells, and is mainly provided by fibroblast of the adjacent dermis. Furthermore, the anti-oxidant and anti-inflammatory response could be boosted and communication between the dermis and hypodermis could be improved to deal with the general loss of facial white adipose tissue when aging.

We found that the active ingredient can improve inflammaging in dWAT by lowering pro-inflammatory cytokines (IL-8 by -66%) and increasing anti-inflammatory factors (adiponectin by 22%). Due to its high antioxidant molecule content, we were also able to confirm the antioxidant activity for the active ingredient (689 µM Trolox equivalents) as expected. A novelty of its action is the improvement in communication between the hypodermis, mainly adipocytes, and the dermis/adjacent fibroblasts. We found that adiponectin released mainly by adipocytes after treatment with our active ingredient triggered the production of collagen (collagen I by 43%) in adjacent fibroblasts. Furthermore, the active is not only improving the surrounding of adipose tissue, but has also positive effects on adipogenesis, the formation of new adipocytes from stem cells (e.g., upregulation of genes: SLC27A1 1.68-fold, PPARG 1.2-fold, CEBPA 2.21-fold, SREBF1 1.36-fold, FABP4 1.58-fold, SCD 1.82-fold, PPARG 1.98-fold, FASN 2.66-fold and LPL 2.69-fold, and downregulation of genes: LIPE -1.8-fold, ECH1 -1.17-fold and GDF15 -1.70-fold). We could find an effect on differentiation, but also an accumulation of lipid content (lipid content by 17% and perilipin by 33%) in the adipocytes themselves, that is essential for a macroscopic plumping effect.

A clinical study was then carried out with Caucasian female volunteers between the ages of 50-60 years with menopausal symptoms. The cream was applied twice a day against placebo

using a split-face approach. The results were analyzed after 7, 14 and 28 days of application. We assessed brightness (significant increase in radiance by 25.2%), erythema (significant decrease of -7.8%), wrinkles in the crow's feet (significant reduction by - 10.7%) and nasolabial fold (significant reduction by -6.3%), and a plumping effect on the cheekbones (significant increase of 0.52 mm in the measurement along the curvature of the cheekbones).

In an independent clinical study, we evaluated the mood enhancing effects on 10 Caucasian women, which applied a product containing our active at 0.3 mg/mL, twice a day on the whole face for a period of 28 days. A projective test revealed that balance (positive passive) could be improved by 7.1%, dynamism (positive active) by 6.5%, and negative emotions could be decreased by 50%. For a normal human being changes of vocal markers of emotional stress are usually only receivable on a subconscious level, but experts can interpret the change in loudness and pitch, and assign them to an emotional stress level. After 28 days loudness could be reduced by -4.1 dB and the vocal pitch could be lowered by -5.1 Hz. Improvement in skin appearance seems to have a satisfying emotional effect on the consumer, which can be evaluated by the two parameters measured.

Conclusion.

The active ingredient BL4BTE0044_0013, a unique botanical blend of Vitis vinifera and black cohosh extracts, induces adipogenesis, lipid accumulation and prevents the adipose tissue aging by modulating its inflammation and increasing the collagen I content in the hypodermis due to the communication between adipocytes and fibroblasts. The active is able to reduce crow’s feet and nasolabial wrinkles as a plumper ingredient, soothes erythema and brings radiance to the skin. Also, it increases the positive mood and decreases the vocal markers of stress. These last results suggest that there is an indirect effect of the active ingredient's benefits on improving facial skin quality, which may translate into an improvement in people's mood. In other words, BL4BTE0044_0013 appears to enhance our overall well-being in addition to and because of its general anti-aging benefits achieved by improving the hypodermis.

Conflict of Interest Statement.

None.

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