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Triple Strength against Cellulite

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abstract

Cosmetic strategies against cellulite generally have the following starting points: fat decomposition, lymphatic drainage and skin firming. The aim of all the strategies, which interact with the lipid metabolism, is to reduce the volume of adipose tissue. This can be achieved by preventing the formation of adipocytes, suppressing lipogenesis or stimulating lipolysis. Activation of the microcirculation encourages drainage of the tissue by means of the lymphatic system and accelerates the removal of fatty acids by the vascular system. The cosmetics market has shown an increased demand for natural and plant-based slimming substances. Using RAHN's knowledge and expertise, some of the candidates that showed the best prospects of success were combined, further developed and tested in terms of their potential. This has resulted in the creation of SLIMEXIR®, an innovative and sophisticated cosmetic active ingredient to combat cellulite.

Introduction

Many women struggle with areas of fat on their abdomen, legs, bottom and arms. Things become especially problematic when the excess pounds on buttocks and thighs are coupled with cellulite. Young women have fewer problems with dimples because cellulite usually only develops with age. Excess storage of fat, weak connective tissue and resultant fluid retention, as well as delayed lymphatic circulation, are responsible for cellulite. The adipose tissue pushes into the upper layers of the skin causing an unwanted orange peel skin surface (**Fig. 1**).

Enlargement of adipose tissue can be explained by two possible mechanisms: hypertrophy (cell size increase) and hyperplasia (cell number increase). An excessive supply of energy primarily increases the size of the existing adipocytes. Adipocytes are able to increase their volume up to 60-fold. However, the mass of fat can also be increased by the formation of additional adipocytes, especially when the fat absorption capacity of the existing cells is already exhausted.

There are a variety of factors that have to be addressed in combat against cellulites. The first is an excess of fat. The major targets as mentioned above would be prevention of new adipocytes formation, suppression of lipogenesis and stimulation of lipolysis. Second, activation of the microcirculation in the tissue encourages drainage and accelerates the removal of fatty acids. Third, the skin surface needs to be firmed and strengthened. Our idea was to develop a sophisticated product, which refine the silhouette and address the cellulite. We wanted to approach the problem from more than one angle and therefore we have searched for inspiration in areas close to cosmetics, such as the food industry. RAHN created SLIMEXIR® a natural and plant-based cosmetic active targeting exactly the main culprits responsible for cellulite.

Active Ingredients

Xanthines have the best documented effect against cellulite. They stimulate the decomposition of fat by increasing the lev-

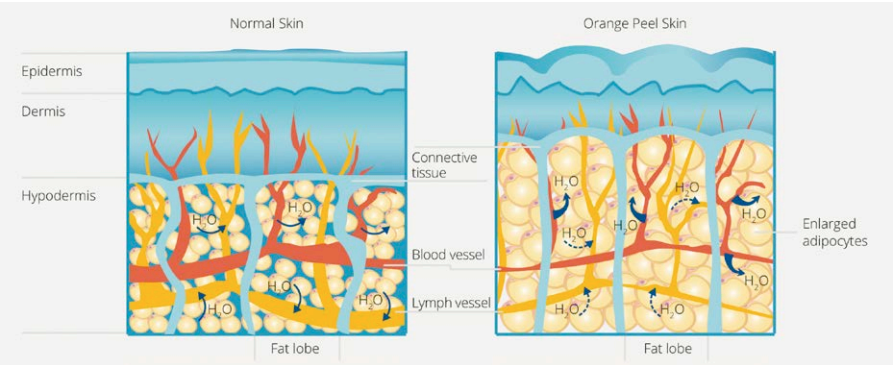

Fig. 1 Subcutaneous adipose tissue. Subcutaneous adipocytes are localised in the hypodermis. They are surrounded by fibrous septae and separated in lobes. Cellulite formation is a result of weak connective tissue and the enlargement of fat cells. Due to the elasticity of the connective tissue, the fat lobes bulge upwards (right hand picture). In addition, interstitial fluid collects in the upper layers of the skin, accentuating skin irregularities.

el of cAMP in the adipocytes and increase the cutaneous microcirculation. Thus caffeine is very frequently found as an active ingredient in anti-cellulite products. The effectiveness of caffeine can be improved by adding other xanthines. Therefore, different xanthines have been combined in X-Melt® (INCI: Xanthine) in the best possible manner to achieve the optimal synergistic effect. We have shown that effectiveness of caffeine was improved by adding other xanthines such as theobromine (3,7-dimethyl xanthine), 1,7-dimethyl-xanthine and 7-methyl xanthine.

Artichoke extract (INCI: Cynara Scolymus Leaf Extract) has a choleretic effect. In other words, it leads to an increased production of bile and it has been used since ancient times against dyspeptic disorders. The main bioactive substances are different caffeoylquinic acids and glycosides such as cynarin and luteolin. From a cosmetic perspective artichokes are a perfect fit as an active ingredient to fight problem areas as they stimulate the circulation and have a draining effect.

Levan's (INCI: Fructan) positive effects on health have been known for centuries. It helps to detoxify the body and has a calming effect on the stomach. Levan is an unusual non-structural polysaccharide. It is one of the principal fructan variants composed of fructose molecules with a terminal glucose residue. Levan has exceptional cosmetic properties: It provides moisture (comparable to that of hyaluronic acid) and soothes skin irritations. The large chain length with many branches ensures strong cohesion amongst the molecules as well as strong cohesion with the adjoining boundary surfaces. This leads to firming and strengthening of the skin surface.

Materials and Methods

In vitro Studies

Reduction of fat storage: The *in vitro* experiments were carried out with the pre-adipocyte cell line (3T3-L1). As a result of their capacity to divide, pre-adipocytes can be grown in cell cultures and matured into adipocytes within 6 days using a differentiation medium. During the differentiation phase the cells were incubated with X-Melt®. The morphology was determined by microscopy and the lipid content was determined by staining with Sudan-II.

Stimulation of fat decomposition: Primary subcutaneous adipocytes of a slightly overweight 46-year-old woman (BMI 27.4) were incubated for three hours with ingredients of SLIMEXIR® and caffeine as a reference compound. Subsequently, the quantity of released free fatty acids was determined in the cell supernatant by means of spectrophotometry at 540 nm.

Fragmentation of lipid droplets: Human adipocytes were treated for 6 hours with SLIMEXIR® or with 3-Isobutyl-1-methylxanthine (IBMX)/isoproterenol mix as a positive control or were left untreated. After incubation the cells were stained with

fluorescent probes (Bodipy and Hoechst dye) and an antibody against perilipin (anti-perilipin A). Pictures were taken using a confocal microscope with 60x magnification.

In vivo Study

Reduction of fat nodes, improvement of skin firmness/elasticity and fat melting: 20 female subjects with healthy, Caucasian skin with visible cellulite (18-65 years) were tested in a double-blind, placebo controlled, randomised, hemi-body study. Test formulations (emulsion with 0 % and 3 % SLIMEXIR®) were applied twice daily. Objective assessment of visible signs of cellulite was done by a dermatologist using a clinical score of the fat node stage on each hemi-body on D0, D28, D56 and D84 (without pinching). Firmness in the abdomen and the neck area and elasticity in the hips, gluteus, arms and neck area was determined using conventional cutometry (2 or 6 mm probe, depending on the zone measured, MPA 580). FLIR E50bx camera (forward looking infrared) was used to produce a thermal image. The visualisation is mapped in the false colour image (from black to white). Namely, the brighter the colour, the warmer the measured area.

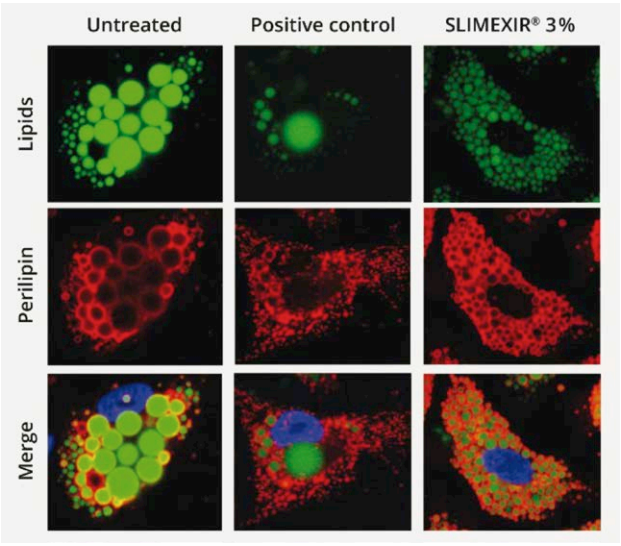

Fig. 2 SLIMEXIR® induces a restructuring of the lipid droplets. Primary adipocytes were incubated for 6 hours with SLIMEXIR® or a control medium and subsequently visualized by means of confocal microscopy. Untreated adipocytes (untreated) show large lipid droplets (top panels, green), which are protectively enclosed by perilipin (middle panels, red). This is more clearly apparent in the merged pictures shown in the bottom panels (cell nuclei in blue). The control experiment (middle column) with IBMX and isoproterenol (positive control) show a strong effect on the lipid droplet fragmentation and fat decomposition. The treatment effect with SLIMEXIR® (SLIMEXIR® 3 % column) closely resembles the situation of the positive control.

Results

The *in vitro* study results were already published in SÖFW 2011. To give an overview, we provide a brief summary:

Reduction of fat storage: X-Melt® successfully prevents the maturation of the adipocytes and the storage of fat. In concentrations ≥ 0.005 % X- Melt® reduced the accumulation of lipids by up to 90 %. The effect of caffeine requires much higher concentrations (≥ 0.010 %). This clearly shows synergistic effect of X-Melt® over caffeine alone.

Stimulation of fat decomposition: The level of free fatty acids in the supernatant is a measure of the lipolytic activity of the cells. The incubation with two different ingredients of SLIMEXIR®, X-Melt® or artichoke extract increased the release of free fatty acids more than 3-fold, even at concentrations of 0.001–0.005 %. This demonstrates that the components of SLIMEXIR® markedly stimulate the decomposition of fat.

Fragmentation of lipid droplets: Treatment with a positive control or SLIMEXIR® leads to a restructuring of the lipid droplets in adipocytes (**Fig. 2**). The lipid droplets are disintegrated and gradually dissolved. This experiment supports the hypothesised mechanism of perilipin phosphorylation (see **Fig. 8**). SLIMEXIR® activates the breakdown of fat via the cAMP-dependent protein kinase A (PKA)

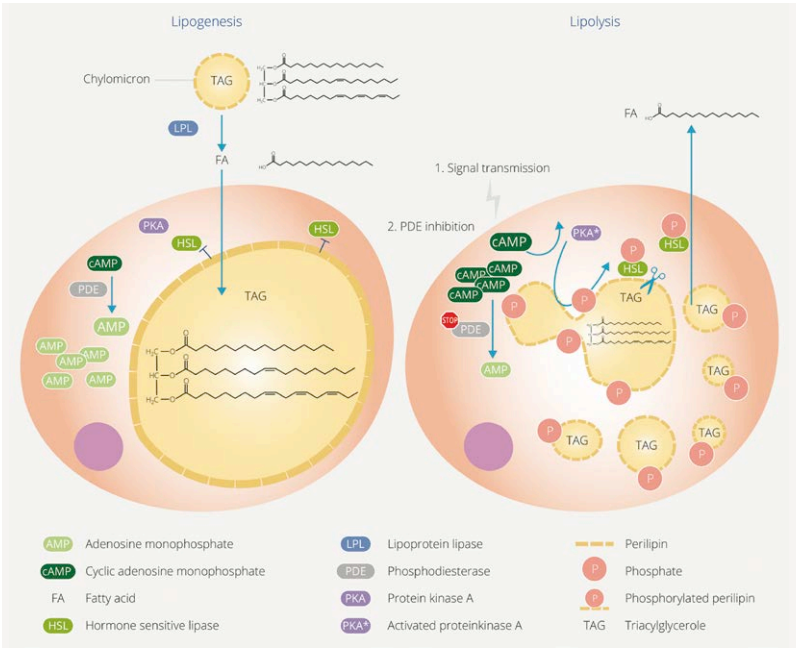

Fig. 8 Lipid metabolism in adipocytes. Lipogenesis: In its normal state, perilipin prevents the decomposition of fat by preventing the lipidmetabolising enzyme HSL (hormone sensitive lipase) from accessing fat deposits. The decomposition of fat is controlled via the content of the messenger substance, cyclic adenosine monophosphate (cAMP). Under normal conditions, the level of cAMP is kept low as the enzyme phosphodiesterase (PDE) breaks down the cAMP into AMP, which prevents the activation of this process. Lipolysis: An increased cAMP level either through the transmission of a signal (1) or by the inhibition of PDE (2), leads to an activation of the cAMP-dependent protein kinase A (PKA). PKA phosphorylates both perilipin and HSL, which induce two simultaneous effects. Firstly, perilipin changes its spatial structure and becomes a docking station for the activated HSL, leading to fat degradation. Secondly, the fat vacuole fragments into thousands of tiny lipid droplets. The enlarged surface area of the lipid droplets additionally facilitates the ability of HSL to gain access [3-5].

pathway (for more detailed information see **Fig. 8**). In brief, PKA is activated either by signal transmission or by the PDA inhibition. Activated PKA phosphorylates perilipin and HSL. This in turn leads to fragmentation of the large lipid droplets. The enlarged surface area of the lipid droplets facilitates access by HSL leading to the desired fat decomposition.

In vivo Study

SLIMEXIR® visually reduces fat nodes, improves skin firmness/elasticity and melts fat away (*in vivo* study)

Clinical evaluation and photographic documentation: Cellulite appearance was evaluated dermatologically without pinching. The application of 3 % SLIMEXIR® significantly improved skin appearance (fat nodes/cellulite) over baseline and placebo (**Fig. 3**). After 56 and 84 days of treatment with 3 % SLIMEXIR® an improvement of 10 % and 18 % respectively was observed. The treatment with 3 % SLIMEXIR® after 84 days was 100 % more effective than placebo.

Firmness: Application of 3 % SLIMEXIR® significantly increased firmness in the neck and abdomen region by 22 % and 29 % after only 28 days of treatment. Effects were statistically significant over baseline and placebo (**Fig. 4**).

Elasticity: Improvement of skin elasticity in the region of hips, gluteus, arm and neck was linearly better over the treatment time. It is noteworthy that the treatment after 84 days revealed an improvement of 10.9 % for the neck region, 7.5 % for the hips, 5.2 % for the arm region and 3.3 % for

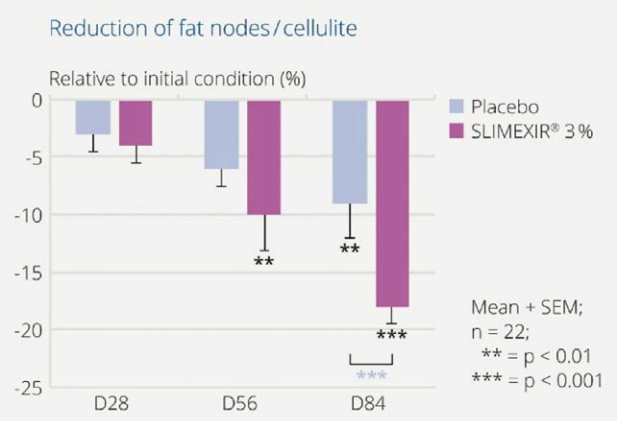

Fig. 3 SLIMEXIR® reduces fat nodes/cellulite. The treatment with 3 % SLIMEXIR® revealed reduction of fat nodes after 56 and 84 days of 10 % and 18 %, respectively. After 84 days, the treatment with 3 % SLIMEXIR® was twice as effective as for the placebo. The statistical values in blue relate to the comparison of SLIMEXIR® with the placebo, whereas the black values relate to the comparison with the initial condition. Wilcoxon signed-rank test.

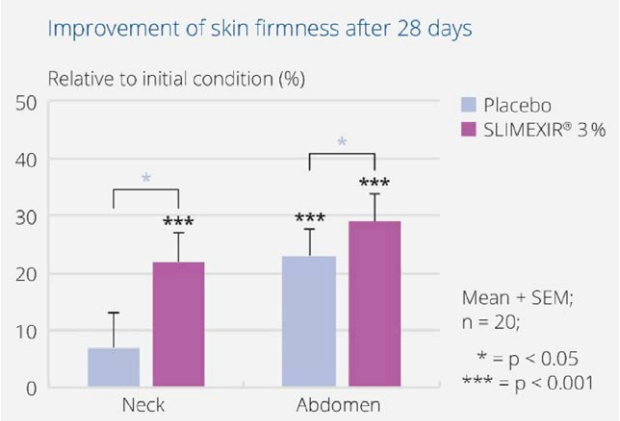

Fig. 4 SLIMEXIR® significantly improves skin firmness. A 28-day treatment with SLIMEXIR® 3 % improved skin firmness significantly by 22 % in the neck region and by 29 % in the abdomen region. The statistical values in blue relate to the comparison of SLIMEXIR® with the placebo, whereas the black values relate to the comparison with the initial condition. Wilcoxon signed-rank test.

the gluteus. The effects were significant over baseline and placebo. The Wilcoxon signed-rank test was used for the statistical evaluation.

Photographic documentation: Visual images of lower body part supported the analytical data. **Fig. 5** shows a representative example of test subject number 6. The semi-body approach clearly shows the efficacy of SLIMEXIR® (right) over placebo (left) after 84 days.

Thermographic images: Skin temperature was measured by infrared thermography (visualised in false colour image) before and after the treatment to assess changes in temperature and its homogeneity. The temperature increased continuously during the treatment (**Fig. 6**). After 28 days, it increased by 2.5 %, after 56 days by 3.5 % and after 84 days by 4.8 %. The application of placebo reached a saturation plateau after 56 days, while the SLIMEXIR® effect was significant over baseline and placebo. The data indicates an improvement in the microcirculation and in fat burning (**Fig. 7**).

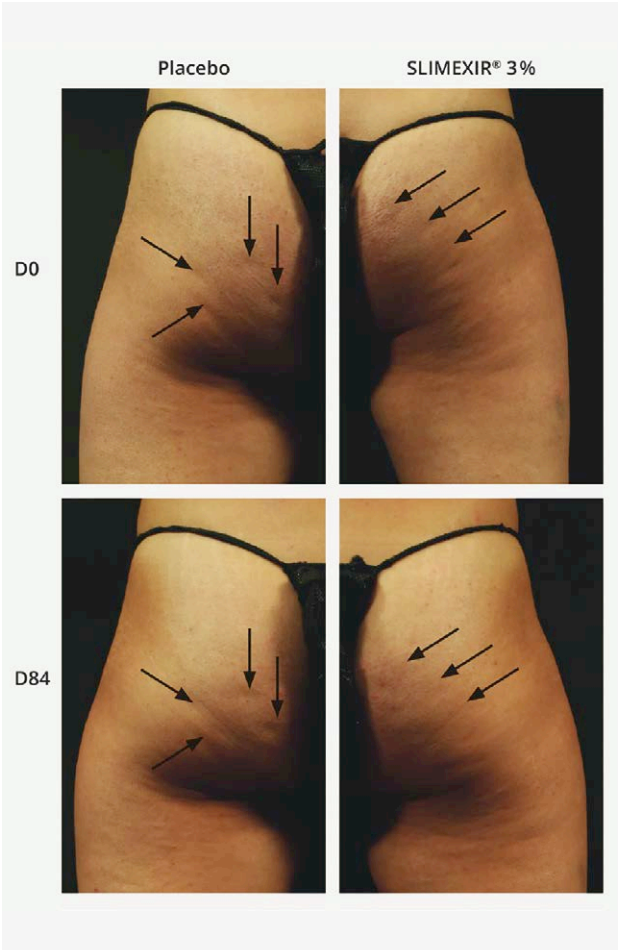

Fig. 5 SLIMEXIR® visibly reduces cellulite. Representative image of test subject number 6 at the beginning of the study (D0) and after 84 days of placebo and 3 % SLIMEXIR® application, respectively. The black arrows indicate prominent skin irregularities. Treatment with the placebo did not visibly improve the cellulite after 84 days of treatment, while treatment with 3 % SLIMEXIR® visibly improved the skin condition.

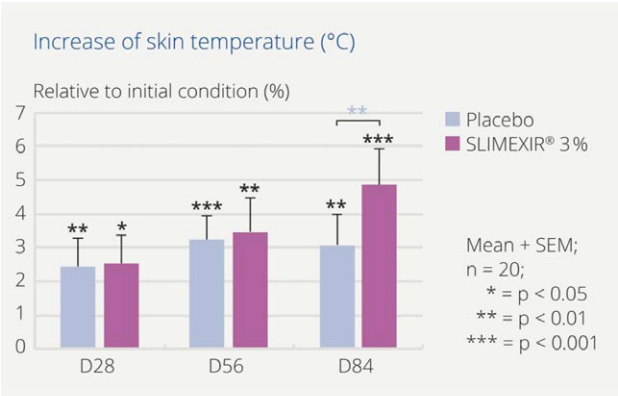

Fig. 6 SLIMEXIR® significantly increases the skin temperature. The increase of the skin temperature (°C) in the hips region after 28, 56 and 84 days of treatment is shown. While the placebo effect reached plateau saturation after 56 days, a 3 % SLIMEXIR® application revealed a continuous temperature rise during the study (2.5 % after 28 days, 3.5 % after 56 days and 4.8 % after 84 days). The effects were significant over baseline and over placebo after 84 days. The statistical values in blue relate to the comparison of SLIMEXIR® with the placebo, whereas the black values relate to the comparison with the initial condition. Wilcoxon signed-rank test.

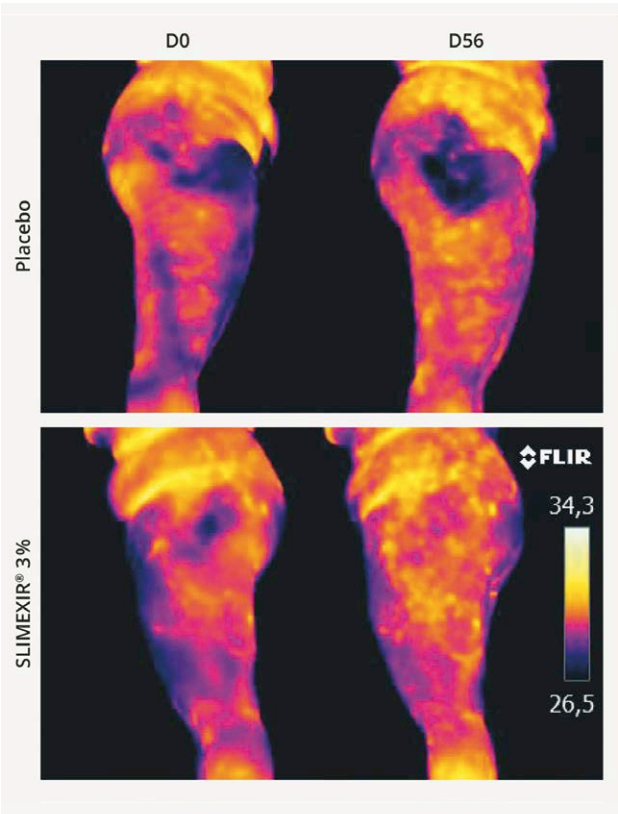

Fig. 7 SLIMEXIR® visibly improves microcirculation and fat burning. False colour image of test subject number 1. The temperature is expressed in °C. The placebo was applied on one side of the body and on the other 3 % SLIMEXIR®. Each subject was its own control. The top panel shows placebo effects and the lower panel 3 % SLIMEXIR® on day 0 and day 56. The colour palette ranges from black (cold) to white (warm). Pictures show clear enhancement and homogenising of the temperature in the lower panel after 56 days, indicating enhanced microcirculation and fat burning after application of 3 % SLIMEXIR®.

Discussion

The sophisticated mix of xanthines in X-Melt® inhibits the maturation of pre-adipocytes into adipocytes probably via inhibition of the CCAAT/enhancer binding protein and peroxisome proliferator activated receptor PPARγ, two main adipogenic transcription factors [1]. Additionally, it may be supported by inhibition of the AMPK/MAPK signalling pathway during the early stage of adipogenesis [2]. In this way, lipogenesis is reduced and less fat is stored in the adipocytes.

In addition, lipolysis is activated via a cAMP dependent pathway: X-Melt® and artichoke extract have a positive effect on the breakdown of already existing fat, by inhibiting the enzyme phosphodiesterase (PDE) and reducing phosphatide phosphohydrolase (PAP) activity (**Fig. 8**). Owing to its special composition, X-Melt® outperforms the current reference substance, caffeine, inhibiting lipogenesis and activating lipolysis [3-5].

This is supported as SLIMEXIR® triggers the fragmentation and decomposition of the lipid droplets in the adipocytes. The postulated mechanism goes via phosphodiesterase inhibition and the perilipin phosphorylation it induces. The effects obtained are similar to those seen for the positive control with 3-Isobutyl-1-ethylcantine/isoproterenol, an effective phosphodiesterase inhibitor.

Furthermore, the artichoke extract with its high cynarin content promotes drainage via the lymphatic system and the removal of the fats via the vascular system. X-Melt® and artichoke extract act synergistically on this.

The large levan molecules with many lateral chains form a film on the skin ensuring surface firmness and providing a moisturising effect nearly identical to that provided by hyaluronic acid. It was reported that it may function as an anti-irritant and that it counteracts inflammation [6]. In general, it produces a velvety skin effect.

SLIMEXIR®'s triple mode of activity (**Fig. 9**), namely fat decomposition, drainage effect and skin hydration/smoothness ensures high effectiveness on problem areas *in vivo*. SLIMEXIR® changes the whole body sensation: from head to toe.

Conclusion

SLIMEXIR® is a very efficient anti-cellulite agent. It improves all conditions relevant to cellulite like skin firmness, dermal structure and water content, microcirculation and the outer appearance of the skin. The postulated mechanisms proven by our *in vitro* experiment are perfectly matched by the *in vivo* studies.

References

[1] *Kim, A.R., et al.*, Caffeine inhibits adipogenesis through modulation of mitotic clonal expansion and the AKT/GSK3 pathway in 3T3-L1 adipocytes. BMB Rep, 2016. 49(2): p. 111-5.

[2] *Jang, Y.J., et al.*, Theobromine inhibits differentiation of 3T3-L1 cells during the early stage of adipogenesis via AMPK and MAPK signaling pathways. Food Funct, 2015. 6(7): p. 2365-74.

[3] *Lafontan, M.*, Adipose tissue – What's new? Kosmetische Medizin, 2008. 5: p. 246-253.

[4] *Brasaemle, D.L.*, Thematic review series: adipocyte biology. The perilipin family of structural lipid droplet proteins: stabilization of lipid droplets and control of lipolysis. J Lipid Res, 2007. 48(12): p. 2547-59.

[5] *Marcinkiewicz, A., et al.*, The phosphorylation of serine 492 of perilipin a directs lipid droplet fragmentation and dispersion. J Biol Chem, 2006. 281(17): p. 11901-9.

[6] *Kang, S.A.*, Levan: Applications and Perspectives. MICROBIAL PRODUCTION of BIOPOLYMERS and POLYMER PRECURSORS, ed. M.U. Bernd H. A. Rehm Institute of Molecular BioSciences, New Zealand. 2009: Caister Academic Press.

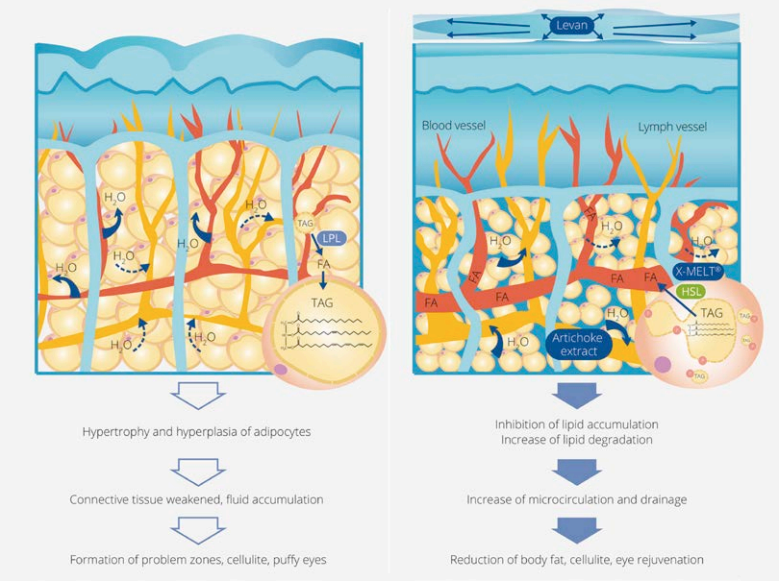

Fig. 9 SLIMEXIR®'s triple mode of activity. X-Melt® reduces lipogenesis by inhibiting the maturation of the pre-adipocytes into adipocytes and activates lipolysis. Stored fat is fragmented into smaller lipid droplets, degraded by HSL and excreted. The artichoke extract stimulates the cutaneous microcirculation and improves removal of the decomposed fat. The high molecular weight levan provides a firming film on the skin, also providing it with moisture to give a velvety skin feeling.

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