

CLR's new cosmetic active 2019

## AnnonaSense CLR

Adaptogenic approach  
for skin health and well-being

### AnnonaSense CLR – Adaptogenic approach for skin health and well-being

#### THE EVOLVING SKINCARE CONSUMER

The average consumer is increasingly health-minded. Living a healthy lifestyle is a given for many consumers – “health is the new wealth.” However, we must keep in mind that they are “time-crunched” as well. On top of that, when thinking about skincare products, nowadays consumers are critical, want cosmetic brands to clearly communicate the advantages of their products and “want results now.” They want highly efficacious products that meet their personal needs. And their main need is to have healthy skin.

The need for healthy skin is reflected in an important trend in the skincare market. Dermocosmetics, cosmeceuticals and “Dr. brands” are showing and will continue to show strong growth, clearly much stronger than mainstream skincare and anti-skin aging products. Low-cost and mass market brands as well as conventional anti-aging skincare brands have recognized this trend, and these brands now also include efficacy claims based on skin health.

Where does this new consumer mentality come from? More than ever, consumers are recognizing their impact on the environment and the impact the environment has on them. The booming natural cosmetics market is a reflection of that. Global warming and environmental pollution, both caused by and a

burden to people, are good examples of topics which have catalyzed this movement. The consumer is clearly more aware than in the past and is much more involved in finding solutions for these problems.

The prevalence of skin ailments, such as acne, atopic dermatitis, allergies and psoriasis, is increasing in most parts of the world. Diabetes is a growing concern as well, where skin problems related to this disease are an increasingly common phenomenon. A growing number of people suffer from (chronic) itch and, especially in the industrialized world, about half of all people describe their skin to be sensitive. What is more, virtually everybody describes their skin as problematic, e.g. dry, oily, sensitive, etc. The fact that nobody seems to be satisfied with their skin health is another trigger for the health movement in the cosmetic market.

Healthy people feel better than unhealthy people. Inversely, acne, dry skin, itch, etc. have a large impact on people's quality of life, i.e., well-being. In the search of well-being, the health-minded consumer eats healthily, does a lot of sport regularly and takes good care of her or his skin. Improving skin health and well-being leads to an overall more positive feeling and better quality of life.

To provide the extremely demanding consumer with a highly effective and sustainable solution for increased skin health and well-being, CLR Berlin developed AnnonaSense CLR. It uses an innovative adaptogenic approach, stabilizing a healthy homeostasis in skin with immediate and long-lasting effects.

### Defining skin health and well-being

The pathophysiology of “unhealthy skin,” i.e., the biological processes taking place in the skin which lead to typical skin ailments like acne, dryness, atopic dermatitis, etc., is incredibly complex. Many different types of cells either reside in or can infiltrate the skin. Most importantly, skin resident cells are keratinocytes, sensory neurons, fibroblasts, melanocytes, Langerhans cells, adipocytes, and sebocytes. Classical immune cells like mast cells (filled with histamine), macrophages, neutrophils, T cells and B cells can also be found in the skin. Yet the above-mentioned resident skin cells also have an immunological function. The complexity of skin ailments lies in the fact that virtually all cells mentioned play a role in their pathophysiology. An inflammatory process can only occur when cell communication takes place. The production of inflammatory mediators is therefore a prerequisite for the onset and maintenance of inflammatory processes leading to skin ailments. Cell-cell communication is another such complex topic. Cells can communicate with each other through cytokines, chemokines, lipids, hormones, neuromediators, etc.

In view of the above, claiming that AnnonaSense CLR provides overall skin health and well-being would imply that it has an effect on all processes in their full complexity. This is obviously impossible. So, how can AnnonaSense CLR have a positive impact on overall skin health and well-being, and how can this be satisfactorily proven?

### The secret: influencing biological systems which are high in the hierarchy

There is a hierarchy within the different processes regulating health and disease. Some systems are only activated when other systems, higher in the hierarchy, “tell them to.” The goal would therefore have to be to find the biological system which is at the top of the hierarchy, which can regulate and even “overrule” all the other biological systems which can negatively impact health.

Our body is an endocannabinoid system (ECS). The ECS has more cellular receptor sites than any other receptor system in our body, illustrating its importance and potency for our body and its position in the hierarchy of our body’s biological systems. Activation of the ECS promotes homeostasis, a dynamic biological balance, in every cell, tissue and organ in our body. This includes our skin. Health and well-being are all about our body’s physiological ability to maintain homeostasis and the ECS is essential for this.

Our body is an endovanilloid system (EVS) as well. The EVS is, for the most part, the counterpart of the ECS. Where the ECS might be considered to be “the good system,” the EVS is “the bad system.” The EVS is a potent system, unfortunately high in the hierarchy as well. It plays a pivotal role in skin inflammation, for instance. The EVS is heavily involved in phenomena like redness, itch, and even pain in the skin.

The ECS and EVS are, luckily, strongly interwoven. This interaction between the two systems is so ubiquitous we can even speak of an ECS/EVS system. The ECS is virtually the only biological system in our body which can effectively overrule the EVS. ECS-induced promotion of homeostasis will lead to a reduction of EVS activity.

### The adaptogenic approach

AnnonaSense CLR was developed to act as a so-called adaptogen for the ECS/EVS system. Its mode of action focuses on two essential cellular receptors found on most skin and immune cells, one pivotal for the ECS and the other pivotal for the EVS. The delicate balance between the ECS and the EVS needs to be stabilized in order for a healthy homeostasis to prevail sustainably. Only a potent adaptogen stabilizes the balance in these physiological processes and can lead to a long-lasting homeostasis.

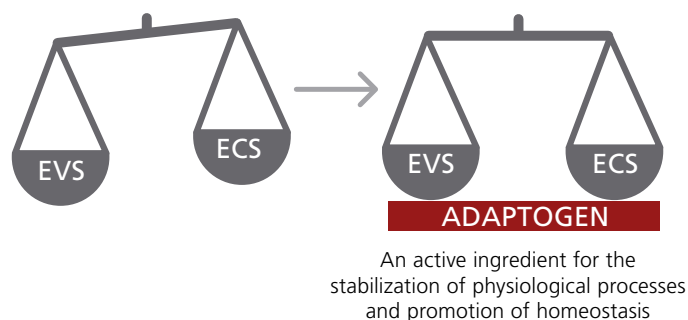


Fig. 1: The function of an adaptogen

Type-2 Cannabinoid Receptor (CB2), the most prevalent receptor in the ECS, is crucial in the ECS and able to modulate all levels of immunity. It is essential for maintaining or regaining a normal homeostasis. As skin is a tissue which is on the outside of our body, it is constantly challenged. CB2’s modulating, balancing properties are essential for skin’s ability to maintain or regain health and well-being.

On the other hand, the EVS is an important presence in the skin as well. The main cellular receptor for the EVS in the skin is the transient receptor potential channel, vanilloid subfamily member 1 (TRPV1). TRPV1 potently triggers inflammatory processes when triggered by heat, skin pH shift, UV light, inflammatory lipids (e.g. prostaglandins, leukotrienes), ROS, etc. TRPV1 is a strong regulator of inflammation in the skin.

As mentioned above, the ECS and EVS are strongly interwoven, with the anti-inflammatory ECS being able to overrule the inflammatory EVS. CB2 and TRPV1 respectively are essential components in this interaction. Activation of CB2 has a potent regulatory impact on the EVS. Activation of CB2 should, therefore, be the goal when the objective is to increase overall skin health and well-being.

AnnonaSense CLR is a product which improves overall skin health and well-being by acting on the ECS/EVS system in the skin. In order to prove this, different cell biological experiments need to be performed, answering questions like:

- Can AnnonaSense CLR positively influence the ECS, i.e., can it activate, be agonistic for CB2? If so, it should then rule down the EVS and the activity of TRPV1.
- How can this be proven? Activation of TRPV1 leads to inflammation. How to activate the TRPV1 specifically? Answer: Capsaicin is a molecule which can selectively interact with TRPV1 as an agonist. Capsazepine is an antagonist for TRPV1, it can block TRPV1 and avoid interaction of capsaicin with TRPV1.
- This allows for elegant cell biological studies, but how to quantify this reduction of TRPV1-induced inflammation? We can look at inflammatory mediators, but these need to be clearly linked with TRPV1 and relevant for loss of skin health and well-being. They should be playing an essential role in all known skin ailments.

#### The following markers were examined

- **Interleukin-1 $\beta$  (IL-1 $\beta$ ):** pro-inflammatory cytokine essential for the onset of inflammation
- **Interleukin-8 (IL-8):** chemokine, recruits immune cells, aggravates inflammation
- **Calcitonin Gene Related Peptide (CGRP):** neuromediator, promotes redness, itching, pain

Production of the above markers leads to increased production of other inflammatory mediators by virtually all cells present in the skin, underlining their importance.

## EFFICACY STUDIES – *in vitro* assays

### Agonistic effect of AnnonaSense CLR on CB2

293T-CB2 cells (CB2-expressing human epidermal keratinocytes) were incubated with different concentrations of AnnonaSense CLR, respectively a positive control for 10 minutes (WIN55, 212-2 [1  $\mu$ M]). The positive control is a specific agonist for CB2 and can, therefore, induce specific cellular processes induced by CB2. Subsequently the cells were treated with Forskolin (10  $\mu$ M) for 6 hours. Treatment with Forskolin led to an increase in the production of cyclic adenosine monophosphate (cAMP). Interaction with CB2 by the positive control substance and the different concentrations of AnnonaSense CLR had an impact on the Forskolin-induced cAMP production. CB2-induced reduction of Forskolin-induced cAMP production was determined, where the result obtained with the positive control was set at 100%.

### Results

At two different concentrations, treatment with AnnonaSense CLR showed a strong agonistic effect on the CB2 receptor, where the higher concentration of the two, 0.05%, was even able to outperform the positive control, a specific agonist for CB2. The potent CB2-agonistic properties of AnnonaSense CLR imply a potent effect on EVS-induced loss of skin health and well-being. TRPV1 plays a central role in inflammatory processes.

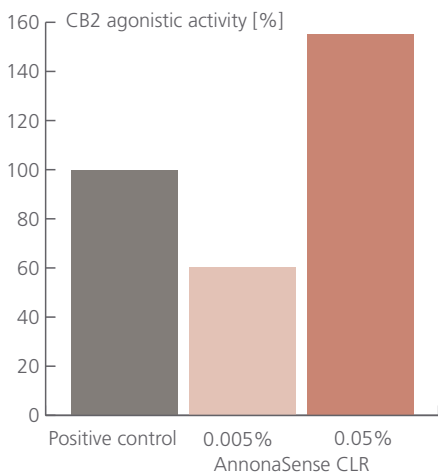


Fig. 2: Agonistic influence on CB2

### Reduction of TRPV1-induced inflammation, co-cultivation of keratinocytes – sensory neurons

Interaction between these keratinocytes and sensory neurons is essential for feelings of skin discomfort and irritation. To test if this product can improve skin well-being, the following experiment was performed (Fig. 3).

Primary human keratinocytes were co-cultivated with sensory neurons and incubated with AnnonaSense CLR at 0.1% and with capsazepine (10  $\mu$ M), respectively, as an antagonist for TRPV1 for 24 hours. Subsequently capsaicin (10  $\mu$ M) was applied for 60 minutes. Expression of TRPV1 induced IL-1 $\beta$ , IL-8 and CGRP was determined.

#### Results

Reduction of IL-1 $\beta$ , IL-8 and CGRP by positive control (capsazepine) was set at 100%. Expression of all three inflammatory mediators induced by AnnonaSense CLR was close to 80% of what was achieved with the positive control. This clearly illustrates the potency of AnnonaSense CLR to reduce the inflammation induced by TRPV1 as part of the detrimental EVS in the skin.

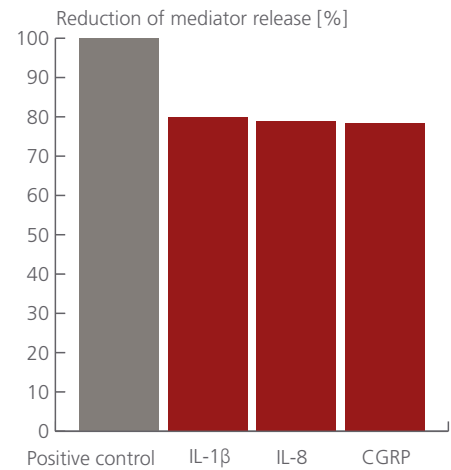


Fig. 3: Reduction of mediator release

### Reduction of TRPV1-induced inflammation, co-cultivation of keratinocytes

A similar experiment was performed only using primary human keratinocytes, with IL-8 as the marker (Fig. 4).

Primary human keratinocytes were cultivated with AnnonaSense CLR for 24 hours, after which capsaicin (0.1  $\mu$ M) was applied. IL-8 expression was measured at baseline and after treatment with capsaicin, with or without pre-incubation with AnnonaSense CLR.

#### Results

IL-8 production at baseline (untreated) was set at 100%. Treatment with capsaicin led to a 62.5% increase of the production of IL-8. Pre-incubation with AnnonaSense CLR, however, led to a clear decrease of IL-8 production, even lower than at baseline and even for the lowest concentration of AnnonaSense CLR used.

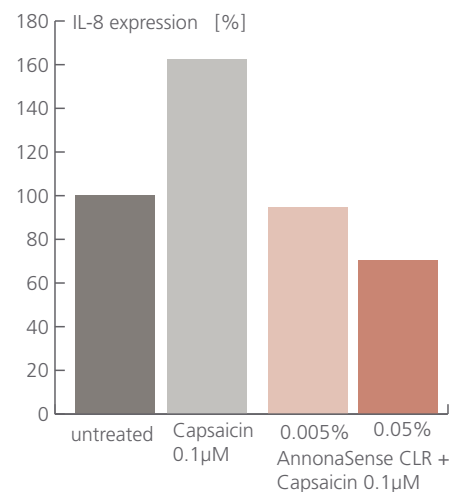


Fig. 4: Influence on IL-8 release

### Reduction of histamine-induced inflammation

Histamine is an important mediator in pruritus and plays a substantial role in skin irritation related to loss of skin health. In addition to its direct inflammatory effects, interaction of keratinocytes with histamine leads to increased production of further inflammatory mediators, especially neuromediators like CGRP (Fig. 5).

Primary human keratinocytes were cultivated with AnnonaSense CLR for 24 hours, after which histamine (100  $\mu$ M) was applied. CGRP expression was measured at baseline and after treatment with histamine, with or without pre-incubation with AnnonaSense CLR.

#### Results

CGRP production at baseline (untreated) was set at 100%. Treatment with histamine led to an almost 50% increase of the production of CGRP. Pre-incubation with AnnonaSense CLR, however, led to a clear decrease of CGRP production, even lower than at baseline.

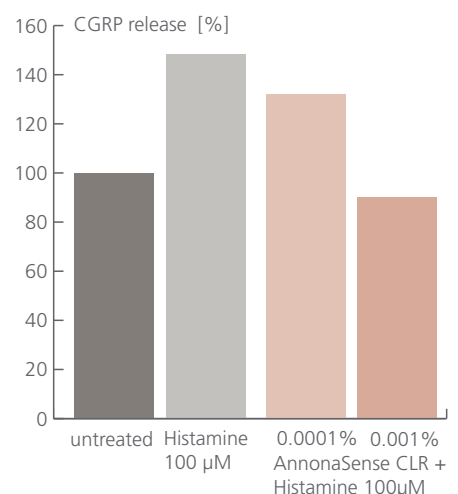


Fig. 5: Influence on CGRP release: Keratinocytes

### Conclusions from *in vitro* studies:

From our cell biological studies it could be clearly concluded that AnnonaSense CLR has potent *in vitro* activities. It strongly interacts with CB2 and can therefore effectively activate the endocannabinoid system (ECS) in our skin. A strong effect could moreover be proven in reducing TRPV1-induced inflammation. This action on TRPV1 – the main player in the endovanilloid system (EVS) in the skin – clearly illustrates the strength of AnnonaSense CLR as an adaptogenic agent, its ability to help gain and maintain a balance between the ECS and the EVS. This was further underlined by its activity on reducing histamine-induced inflammation.

## EFFICACY STUDIES – *in vivo* assays

*In vitro* studies showed the strong potency of AnnonaSense CLR in balancing two biological systems which stand high in the hierarchy of disturbing vs. maintaining a healthy immunological barrier. These two systems can essentially be considered to be one: the intertwined and highly significant ECS/EVS system. AnnonaSense CLR is a product promoting skin health and well-being. It is not just a soothing agent for the skin but provides the skin with the means to gain and maintain a healthy homeostasis, which improves overall well-being.

This brings us back to the description of health: the stronger our physiological ability to stay in homeostasis, the healthier we are. The healthier we are, the better our well-being. Skin with the ability to stay in homeostasis is not sensitive, not overly reactive. An objective evaluation of the effect of AnnonaSense CLR on the sensitivity/reactivity of skin is therefore one of the prerequisites for providing proof of AnnonaSense CLR providing better skin health and well-being.

As mentioned in the introduction of this article, a growing number of people suffer from skin ailments. Approximately 50% of people perceive their skin to be sensitive. At some point, virtually everybody suffers from skin irritations, redness and itch. In order to provide sufficient proof for AnnonaSense CLR to work effectively in daily life, the *in vivo* studies needed to be performed on volunteers who were representative of the general public, of people who want their skin to be healthier. For the subsequent *in vivo* studies more than 40 volunteers were included who reported their skin to be sensitive and dry. Twelve volunteers had atopic dermatitis, eight had type IV allergy, one had psoriasis, and one had diabetes type II. As the general consumer searching for better skin health and well-being, the volunteers showed a regular feeling of skin discomfort (e.g., itch). Both women and men were included, and the age range of the volunteers was 18–70 years.

### Assessment of skin sensitivity

One application of test product on 17 (for 250 Hz) and 18 (for 5 Hz) volunteers (including 5 with atopic dermatitis, 3 with type IV allergy, 6 with sensitive skin, 1 with diabetes type II). Skin sensitivity was determined by measuring current perception threshold (CPT) with Neurometer® CPT/C (Neurotron Inc., Baltimore, USA) at 250 Hz and 5 Hz before and after application of the test product. Baseline at  $t=0$  is set at 0%. Values are based on averages of individual results of all volunteers.

### Results

The 250 Hz experiment showed that the formulation with AnnonaSense CLR outperformed the corresponding placebo formulation by more than 35%. The 5 Hz experiment showed even clearer results: the formulation with AnnonaSense CLR performed more than 60% better than the placebo formulation. These results show unmistakably AnnonaSense CLR's potency of making the skin less sensitive. As irritation is a consequence of a loss of immunological balance, these results demonstrate that the skin's ability to stay in homeostasis is clearly increased. The skin is healthier, which should lead to increased well-being.

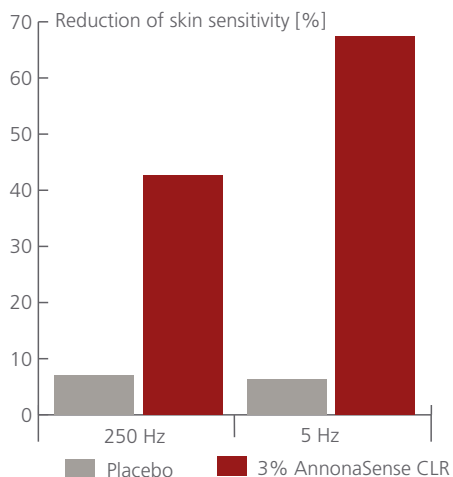


Fig. 6: Assessment of skin sensitivity

### Skin discomfort

One application of test product after occurrence of itch. Blinded, 22 volunteers tested formulation with AnnonaSense CLR, 20 tested placebo formulation. Itching was scored at t=0, 1 min, 5 min and 24 hrs. Baseline at t=0 is set at 0% (Fig. 7).

#### Results

Itch is a common phenomenon for the consumer. Instant, but especially long-lasting relief of itch is an important trait for a cosmetic formulation aimed at increasing the consumer's health and well-being. As itch is a phenomenon which is highly subjective, the placebo effect needs to be taken extremely seriously. Placebo formulation can relieve up to 40% of perceived itch without having any biological effect whatsoever on the biological processes involved in the itch. It is, therefore, important to prove with AnnonaSense CLR that it can lead to a relief of itch which is considerably greater than 40%. In this study this was clearly proven. Where the effects of the formulation containing AnnonaSense CLR were better than corresponding placebo after 1 and 5 minutes after applying the products, the effect after 24 hours was clearly larger than that of placebo. As could be expected, the placebo formulation was able to reduce itch by almost 37%. The same formulation additionally containing AnnonaSense CLR outperformed the placebo formulation by almost 18%, showing an overall reduction of itch by almost 55%.

### Skin appearance

In a double-blind test, 22 volunteers tested a formulation with AnnonaSense CLR and 20 tested a placebo formulation. Redness was scored at t=0 and after 17 days of twice daily application of test products. Baseline at t=0 is set at 0% (Fig. 8).

#### Results

When assessing skin health and well-being, skin appearance is as important as the feel of skin. An unhealthy appearance of skin can have important negative consequences for a person's well-being, i.e. quality of life. Reduction of skin redness in relation to skin health is therefore an important parameter to consider. In this study, 17 days of application of the test substance showed that the formulation with AnnonaSense CLR improved skin appearance by reducing redness due to scratching itchy skin. Where the placebo formulation reduced skin redness by 25%, the corresponding formulation containing AnnonaSense CLR clearly outperformed the placebo formulation, by reducing redness by more than 36%.

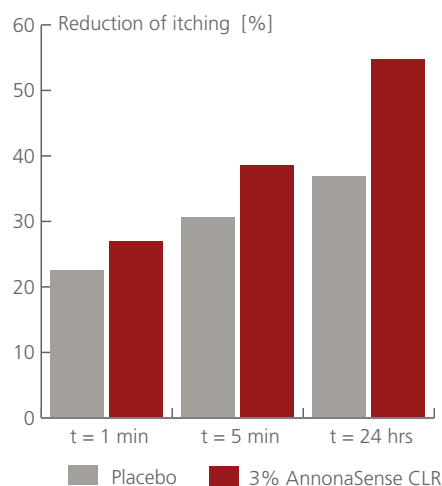


Fig. 7: Assessment of skin discomfort

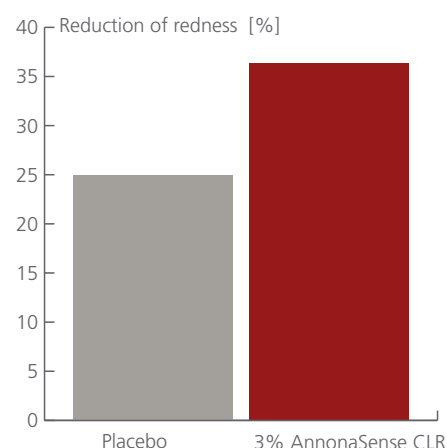


Fig. 8: Assessment of skin appearance

## Perception of skin health and well-being

Volunteers (n=22) who applied AnnonaSense CLR (3% in an aqueous gel) were given a questionnaire after 17 days of twice daily application (Fig. 9).

### Results

All previously performed *in vitro* and *in vivo* studies showed convincing results, clearly demonstrating that the use of AnnonaSense CLR was able to improve skin and reduce unpleasant sensations while also improving skin appearance. In this last study consumers were questioned on whether a simple aqueous gel containing 3% AnnonaSense CLR led to improvement of skin health and well-being. As the formulation was a gel, the biological effect of this vehicle could be considered to be minimal and the perceived effects of the use of this gel could be attributed to AnnonaSense CLR.

After 17 days of daily use of the product the consumers found that both the intensity and the frequency of itching were reduced by no less than almost 73%. A total of 54.5% of the users confirmed that the feeling of tension in their skin – a phenomenon often encountered by people with dry and unhealthy skin – was perceptibly reduced. The same number of users reported that the quality of their life was improved, and almost 64% of test persons stated that using this product had improved their well-being after only 17 days.

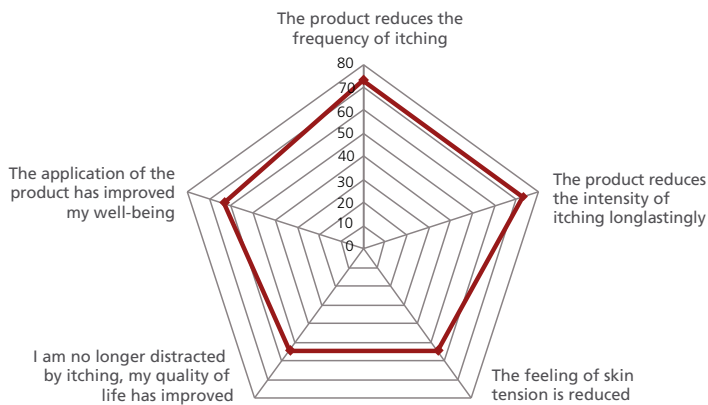


Fig. 9: Perception of skin health and well-being

## CONCLUSIONS

AnnonaSense CLR is a product based on the fruit of *Annona Cherimola*, common name *Cherimoya*. AnnonaSense CLR acts on two biological systems as an adaptogen. It establishes a stable homeostatic balance, which is the key to skin health. Through its adaptogenic action, AnnonaSense CLR activates the endocannabinoid receptor CB2. CB2 is pivotal for the endocannabinoid system (ECS) in the skin. With this action another receptor, TRPV1, is deactivated. CB2 can be considered to be an “on/off switch” for TRPV1. TRPV1 is the most important player in the detrimental endovanilloid system (EVS) of the skin. Deactivation of TRPV1 is essential for deactivation of inflammatory processes in the skin. A stable balance between the ECS and EVS is achieved. This leads to an improvement of skin health and well-being, which is both measurable and perceivable.

This was proven in a variety of *in vivo* studies. The skin becomes less sensitive and more balanced, and itch could even be perceptibly reduced. Skin appearance was improved, and a consumer study showed that perception of well-being and quality of life were improved.

AnnonaSense CLR is an all-natural active ingredient with potent action on one of the most important features for skin: **balance, health and well-being.**

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