Slimming and Tissue Strengthening: Soy Isoflavone for a Perfect Body

Dr. Daniel Schmid, Esther Belser, Sandra Meister

Mibelle Biochemistry, Switzerland

February 2009

Abstract

An ingredient comprising the soy isoflavone genistein, caffeine, carnitine and an extract of the Spirulina platensis algae was found in a clinical trial to be very effective against cellulite. In vitro studies in a 3D-full skin model, showed the capacity of genistein to inhibit differentiation of preadipocytes into adipocytes and to stimulate production of collagen IV. Thus, genistein counteracts cellulite on two levels; it reduces storage of lipids and it strengthens the skin epithelium.

Introduction

Dimpling of the skin of the buttocks and thighs is known as cellulite, a typical problem for women. They have a characteristic depot of subcutaneous adipose tissue in the gluteal-femoral region (pear shape). The adipose tissue is a type of connective tissue that is specialized for the storage of fat (triglycerides). There is also a gender-related difference in the anatomy of the adipose tissue. In women, the organization of the subcutaneous fat cell chambers and of the connective tissue septa that divide them, allows vertical stretching of the fat chambers. After stretching, the chambers protrude into the reticular dermis, leading to the visible dimples of the skin.

Cosmetic ingredients to treat cellulite can be categorized into 3 groups:

A Compounds that reduce the volume of the adipose tissue
B Compounds that strengthen the skin
C Compounds that stimulate microcirculation

This article describes an anti-cellulite ingredient comprising the soy isoflavone genistein, caffeine, carnitine and an extract of the Spirulina platensis algae.

Caffeine is the best known representative of group A. Like theobromine (chocolate) and theophylline (tea) it belongs to the group of xanthine derivatives. They stimulate mobilisation of lipids by increasing the release of catecholamines and by inhibiting the activity of the enzyme phosphodiesterase. This enzyme normally degrades cyclic AMP (cAMP), an important signaling molecule in the lipolysis process. Inhibition guarantees a high pool of cAMP. This in turn activates the enzyme lipase which is responsible for the hydrolysis of triglycerides. The long chain fatty acids are then oxidized to CO2 and H2O to produce energy. Caffeine also stimulates microcirculation because the increased release of catecholamines will induce vasodilation. The improved blood circulation in the cellulite area helps in the breakdown of fat.

Carnitine, a quaternary ammonium compound, is another group A active. Carnitine's role is to carry long chain fatty acids into the mitochondria. These are the cellular organelles where fatty acids are oxidized.

Spirulina platensis is a filamentous blue-green algae species that naturally lives in tropical and subtropical lakes of high alkalinity. The extract of Spirulina cells is a rich source of carotenoids, unsaturated fatty acids, essential amino acids and minerals. The carotenoids protect the skin against oxidative stress. The algae extract is known to stimulate elasticity and firmness of the skin and thus contributes to skin strengthening (group B active). A firmer skin resists better the vertical stretching of the fat chambers into the reticular dermis in the cellulite areas.

The use of the soy isoflavone genistein as an anti-cellulite active seems at first glance not to be logical because soy isoflavones are known as phytoestrogens. Cellulite is a typical female phenomenon and thus primarily regulated by estrogen. The structures of soy isoflavones, heterocyclic phenols, are very similar to the steroidal estrogen. Thus they can bind to some extent to estrogen receptors. Compared with the principal circulating estrogens in humans, isoflavones are bound at a much lower rate (103-fold less). Genistein, the predominant isoflavone in soy, is also a well-known inhibitor of protein tyrosine kinases (1).

Kinases are involved in the signaling process inside cells. In the skin, they are essential components of the signal transduction pathway that is activated by radicals and inflammatory cytokines and leads to the expression of collagen- and elastin-degrading enzymes (2;3). These enzymes, called matrix metalloproteinases, are key players in skin aging. Genistein can block this signaling pathway and as a consequence, radicals and inflammatory cytokines can no longer induce the breakdown of collagen and elastin. In this way, genistein helps to strengthen the skin (group B active).

Several scientific reports show a further physiological activity of genistein. It is an effect that is specifically directed at adipocytes. Genistein was shown to act as a negative regulator of adipogenesis (4;5). Thus, genistein seems to be an efficient anti-cellulite active, belonging to the categories A and B. In the next two chapters, in vitro studies are described that demonstrate the efficacy of genistein to reduce the formation of adipose tissue and to enhance the concentration of collagen in a 3D-full skin model.

Inhibtion of the Differentiation of Preadipocytes into Adipocystes

The mass of the adipose tissue can be controlled either by the recruitment of new adipocytes (adipogenesis) or by the volume of the existing adipocytes (Figure 1).

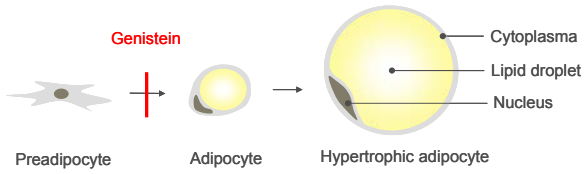
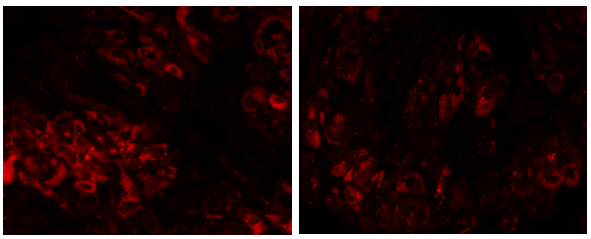


Figure 1: Differentiation of preadipocytes leads to the formation of new adipocytes

Adipogenesis is the differentiation process of preadipocytes (fibroblast-like) into adipocytes. The conversion is normally regulated by various hormones and is associated with the coordinate induction of key enzymes of the lipid metabolism. In this study, the effect of genistein on adipogenesis was investigated. Human preadipocytes were cultured until the cells reached confluence. Then the medium was replaced by a specific differentiation medium with genistein. The controls were preadipocytes kept in the normal medium (not differentiated control) and preadipocytes cultured in the differentiation medium without genistein. The conversion of preadipocytes into mature adipocytes was analyzed using two methods: 1. Demonstration of accumulation of lipids in large fat droplets (adipocyte-specific phenotype). The lipid droplets were labeled with a fluorescent dye (AdipoRed®). The number of total cells was controlled by Hoechst staining of the cell nuclei. 2. Analysis of adipocyte-specific metabolism by gene array. A chip containing 96 genes related to adipogenesis was used.

Compared to the cells in the differentiation medium without genistein, labeling of the lipid droplets showed that genistein at 25 µM reduced adipogenesis by 31% after 3 days and by 42% after 5 days incubation in the differentiation medium. This strong inhibitory effect is clearly visible in the fluorescence pictures (figure 2).

Figure 2: Fluorescence labelling of lipid droplets (right: differentiation medium with genistein; left: control without genistein)



Also the results of the gene array analysis reflected the efficacy of genistein to inhibit adipogenesis (figure 3).

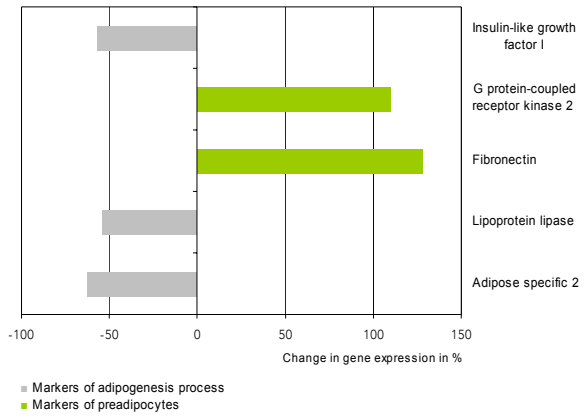


Figure 3: Effect of genistein on gene expression of preadipocytes in the differentiation medium

Compared to the cells in the differentiation medium without genistein, typical markers for mature adipocytes were reduced whereas markers for preadipocytes were found at a much higher level.

Stimulation of Collagen IV in the Basement Membrane

Most important for the strength of the skin tissue are the collagen proteins. There are at least 14 types of collagens that differ in their structural organization. Collagen IV forms sheet-like networks and is specific for basement membranes. The skin basement membrane is a thin matrix between the epidermis and the dermis and serves as an adherent connection between the two skin layers. The basement membrane is very important for the attachment of the basal keratinocytes and for their proliferation. A natural basement membrane is also formed in 3D-full skin models. This model was used to analyze the effect of genistein on the synthesis of collagen IV. The in vitro skin model was incubated for several days with a medium containing 5 µM genistein. The concentration of collagen IV was analyzed with immunofluorescence microscopy using anti-collagen IV

antibodies. The number of total keratinocyte and fibroblast cells was controlled by Hoechst staining of the cell nuclei.

The immunofluorescence pictures (figure 4) show the localization of collagen IV in the basement membrane. Incubation of the skin model in a medium with genistein clearly stimulated the synthesis of collagen IV (+ 53% after 6 days). The analysis of cell nuclei showed that this stimulation was specific and not only due to an increased cell proliferation.

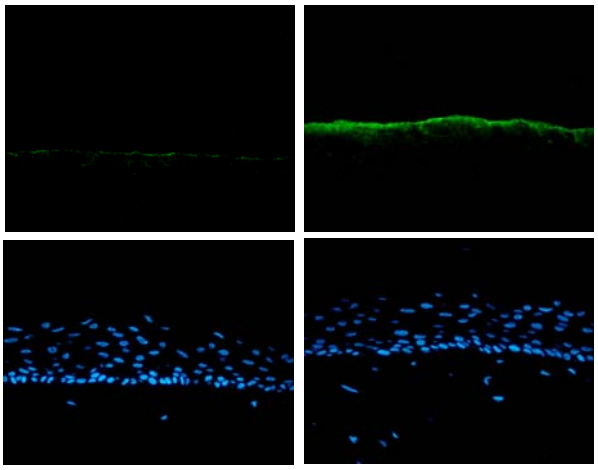


Figure 4: Immunofluorescence labelling of collagen IV (upper row) and cell nuclei (lower row). The pictures on the right are from 3D-full skin models incubated with genistein. On the left side are pictures from skin models incubated without genistein

Clinical Study with an Anti-Cellulite Formulation

A cosmetic ingredient to treat cellulite (Iso-SlimComplex), comprising the soy isoflavone genistein, caffeine, carnitine and an extract of the Spirulina platensis algae, was tested in a clinical study over 6 weeks with 20 women aged from 39 to 58 with light to heavy cellulite. A cream containing 4% IsoSlimComplex was applied once daily on the right thigh. The other thigh stayed untreated and served as a control. The following skin parameters were measured: smoothness (PRIMOS system), elasticity (cutometer), thigh circumference and cellulite degree (clinical scoring).

The results clearly demonstrated the efficacy of Iso-SlimComplex. Compared to the untreated areas, elasticity and smoothness improved after 6 weeks' application by 57% and 41% respectively (figure 5). The circumference of the right thigh was reduced by 3.8 cm after 6 weeks whereas the circumference of the untreated thigh was only reduced by 0.8 cm. There was a visible improvement of the cellulite appearance. The cellulite degree dropped from 3.7 to 2.5 after 6 weeks treatment (Figure 6). There was no visible change in cellulite on the untreated thigh.

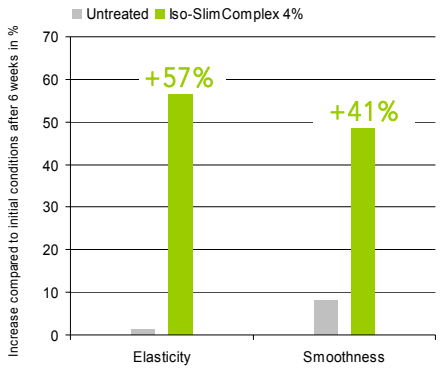


Figure 5: Improvement of skin elasticity and smoothness after 6 weeks application of a cream with 4% Iso-SlimComplex

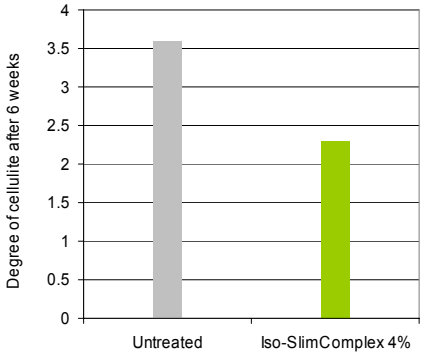


Figure 6: Reduction of cellulite appearance after 6 weeks application of a cream with 4% Iso-SlimComplex

References

(1) Akiyama T., Ishida J., Nakagawa S., Ogawara H., Watanabe S., Itoh N., Shibuya M. and Fukami Y. (1987) Genistein, a specific inhibitor of tyrosine-specific protein kinases. J. Biol. Chem. 262: 5592-5595

(2) Kajanne R., Miettinen P., Mehlem A., Leivonen S.-K., Birrer M., Foschi M., Kähäri V.-M. and Leppä S. (2007) EGF-R Regulates MMP Function in Fibroblasts Through MAPK and AP-1 Pathways. J. Cell. Physiol. 212: 489 – 497

(3) Xu Y., Shao Y., Voorhees J.J. and Fisher G.J. (2006) Oxidative inhibition of receptor-type protein-tyrosine phosphatase kappa by ultraviolet irradiation activates epidermal growth factor receptor in human keratinocytes. J. Biol. Chem. 281: 27389 – 27397

(4) Park H.J., Yang J.Y., Ambati S., Della-Fera M.A., Hausman D.B., Rayalam S. and Baile C.A. (2008) Combined effects of genistein, quercetin, and resveratrol in human and 3T3-L1 adipocytes. J. Med. Food 11: 773-783

(5) Liao Q.C., Li Y.L., Qin Y.F., Quarles L.D., Xu K.K., Li R., Zhou H.H. and Xiao Z.S. (2008) Inhibition of adipocyte differentiation by phytoestrogen genistein through a potential downregulation of extracellular signal-regulated kinases 1/2 activity. J. Cell. Biochem. 104: 1853-1864

Bolimattstrasse 1, 5033 Buchs / Switzerland
Phone: +41 62 836 17 31 Fax: +41 62 836 14 05
info@mibellebiochemistry.com, www.mibellebiochemistry.com