Inhibition of adipogenesis for anti-cellulite activity

By Irene Montaño*, Daniel Schmid* and Fred Zülli*

Introduction

Etymologically, cellulite is defined as localized metabolic disorder of the subcutaneous tissue which provokes an alteration of the body shape. Every person knows how cellulite looks like, 80 to 90 percent of all women have some cellulite on their thighs or buttocks but only a few women know what it really is. Several factors cause this problem including heredity, lack of the appropriate type of exercise and a diet high in fat. However, no single factor can be blamed 100 percent.

Cellulite is not an illness from a medical point of view; it is a unique and distinctive layer of subcutaneous body fat that becomes more prominent in women than in men because of different fat structures. In women, subcutaneous fat cell chambers and the connective tissue septa that divide them, allow vertical elongation of the fat deposits. After stretching, they protrude into the reticular dermis, leading to visible dimples. If the connective tissue which separates the dermal and the adipose tissue layers is inherently weaker or becomes progressively thinner and looser, the adipose tissue starts to extrude outwards into the dermis, a process manifested as cellulite (fig. 1).

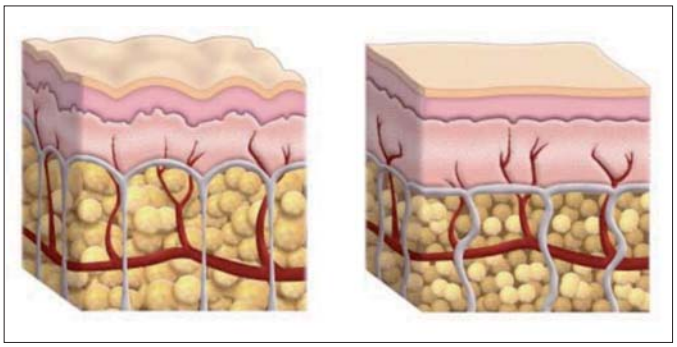

Figure 1: Fat distribution in subcutaneous tissue. Left, skin section with cellulite. Right, normal skin. When the uppermost layer of fat remains smooth in subcutaneous tissue, the skin remains relatively even.

Flavonoids are compounds that significantly affect all human organs, including the skin [1]. They occur naturally and are widely distributed in vegetables, fruits, seeds, nuts and beverages such as tea and red wine. Flavonoids are polyphenolic molecules, diverse in chemical structure and biochemical properties. Normally they occur in nature as glycosides and are physiologically inactive. To make them biologically active, the glycosides have to be hydrolyzed (fig. 2).

The predominant isoflavone in soy is genistein, a heterocyclic phenol with a structure very similar to the steroidal estrogen (fig. 2). Thus,

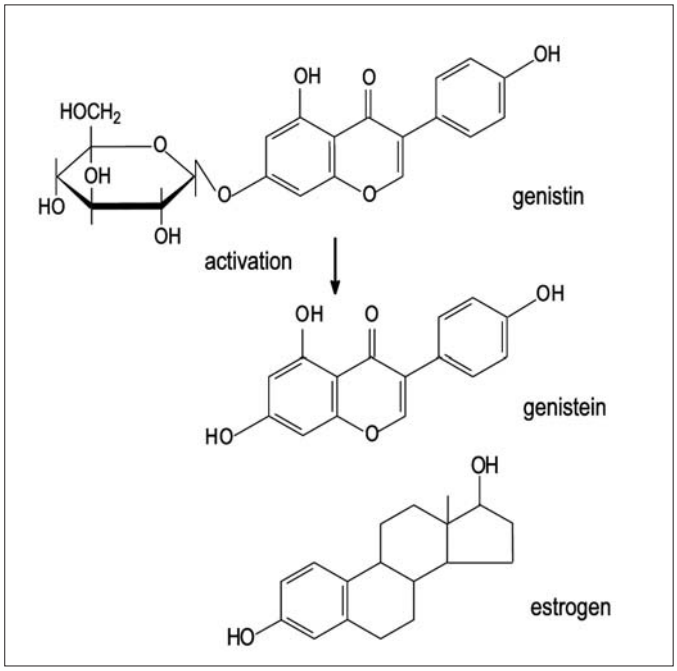

Figure 2: Transformation of the isoflavone glycoside genistin to the bioactive isoflavone aglycone genistein. Comparison of the molecular structure of the hormone estrogen to genistein.

it can bind to some extent to estrogen receptors. Genistein is a well-known inhibitor of protein tyrosine kinases [2]. 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 [3,4]. These enzymes, called matrix metalloproteinases, are major factors 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 reinforce the skin.

A number of scientific reports show an additional physiological activity of genistein; it was shown to reduce adipogenesis [5,6]. Thus, genistein seems to be an efficient anti-cellulite active. This article describes the capability of genistein to reduce the formation of adipose tissue in an in vitro study. A 3D skin model established the efficacy of genistein to increase the concentration of collagen. Finally, a clinical study showed the reduction of cellulite and the improvement of the elasticity and smoothness of the skin by using a cosmetic ingredient based on genistein.

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Genistein Stimulates Collagen IV Production in the Basement Membrane

The first step in the genesis of cellulite is the initial breakdown of the collagen fibers that separate the fatty deposits (trabeculae). There are at least 14 types of collagens in the skin with different structures. In the basement membrane collagen IV forms sheet-like networks.The skin basement membrane is a thin matrix between the epidermis and the dermis and serves as connective tissue between the two skin layers. The basement membrane is very important for anchoring the basal keratinocytes and for their proliferation. A 3D skin model was used to analyze the effect of genistein on the synthesis of collagen IV. For several days, the in vitro skin model was incubated with a medium containing 5 µM genistein. The concentration of collagen IV was analyzed with immunofluorescence microscopy using anti-collagen IV antibodies.The total number of keratinocyte and fibroblast cells was assessed by Hoechst staining of the cell nuclei.

The immunofluorescent stainings (fig. 3) shows the localization of collagen IV in the basement membrane.The 3D skin model treated with genistein for 6 days showed an increase of 53% in the synthesis of collagen IV; the number of cell nuclei was not increased indicating that genistein specifically stimulated collagen production.

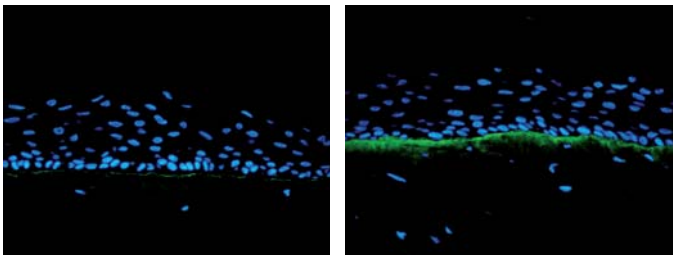

Figure 3: Immunofluorescence labeling of collagen IV (green) and cell nuclei (blue) with Hoechst. Cross section of 3D skin incubated without genistein (right) and with genistein (left).

Inhibition of the Adipogenesis Process

With age, cellulite becomes more visible and one reason is that the number of adipocytes increases.The mass of the adipose tissue can be controlled either by the creation of new adipocytes (adipogenesis) or by the volume of the existing adipocytes. In adipogenesis the preadipocytes, which are fibroblast-like cells, differentiate 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 the following study, we investigated the effect of genistein on adipogenesis. Human preadipocytes

were cultured till the cells reached confluence and then incubated for 7 days in a differentiation medium containing genistein. Two different culture controls were run in parallel: preadipocytes cultured in the differentiation medium without genistein and preadipocytes cultivated in non differentiating medium. The formation of mature adipocytes was followed by two different methods:

1. lipids accumulation in large fat droplets (adipocyte-specific phenotype) through the labeling with a fluorescent dye (AdipoRed®); in parallel the total number of cells was measured by Hoechst staining.
2. Analysis of adipocyte-specific metabolism by gene array. A chip containing 96 genes related to adipogenesis was used.

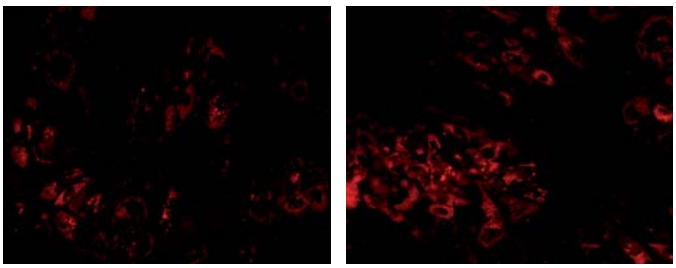

Figure 4: Lipid droplets labeled with a fluorescent dye. Culture of adipocytes in differentiating medium without genistein (left) and with genistein (right).

The labeling of the lipid droplets in the cells cultivated in the differentiation medium showed that genistein at 25 µM reduced adipogenesis by 31% after 3 days and by 42% after 5 days in comparison to the control. Figure 4 clearly shows the difference in fluorescence. These results correlate with those obtained with the gene array analysis. The DNA microarray technology showed that genistein reduced markers of the differentiation of preadipocytes into adipocytes and increased genes expressed predominantly in preadiposites (table 1).

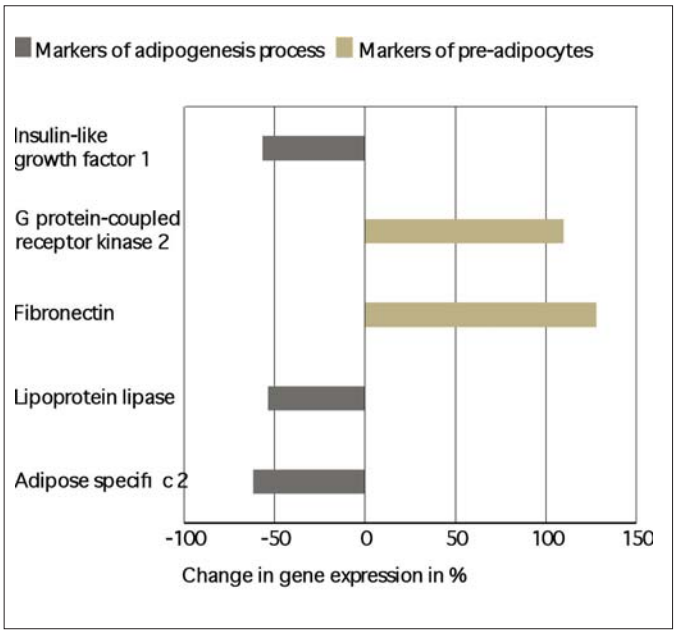

Table 1: Gene Expression was compared to that of control culture kept in differentiating media without genistein.

Clinical Study: Visible Anti-Cellulite effect

In a clinical study a cosmetic ingredient (Iso-SlimComplex) comprising genistein, caffeine, carnitine and an extract of the Spirulina platensis algae was used to treat cellulite. Over 6 weeks the gel-based formulation containing 4% IsoSlimComplex, was applied once daily on one thigh of 20 women aged from 39 to 58 with light to heavy cellulite. The other thigh was not treated and served as a control. The following skin parameters were measured: cellulite degree (clinical scoring), thigh circumference, smoothness (PRIMOS system) and elasticity (cutometer).

The results showed that Iso-SlimComplex visibly reduced cellulite. The cellulite degree dropped from 3.7 to 2.5 after 6 weeks' treatment (fig. 5) while there was no visible change on the untreated thigh. The circumference of the right thigh was reduced by 3.8 cm on average after 6 weeks whereas the circumference of the untreated thigh was

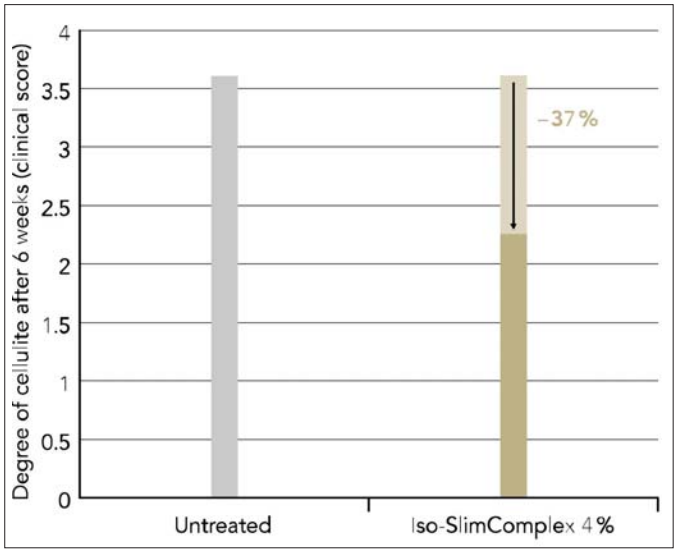

Figure 5: Reduction of cellulite appearance after 6 weeks' application of a cream with 4% Iso-SlimComplex.

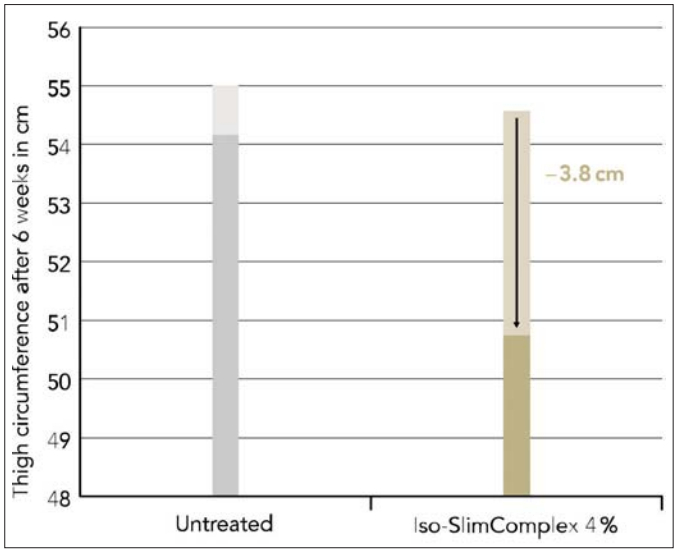

Figure 6: Slimming effect on the tight circumference after 6 weeks' application of a cream with 4% Iso-SlimComplex.

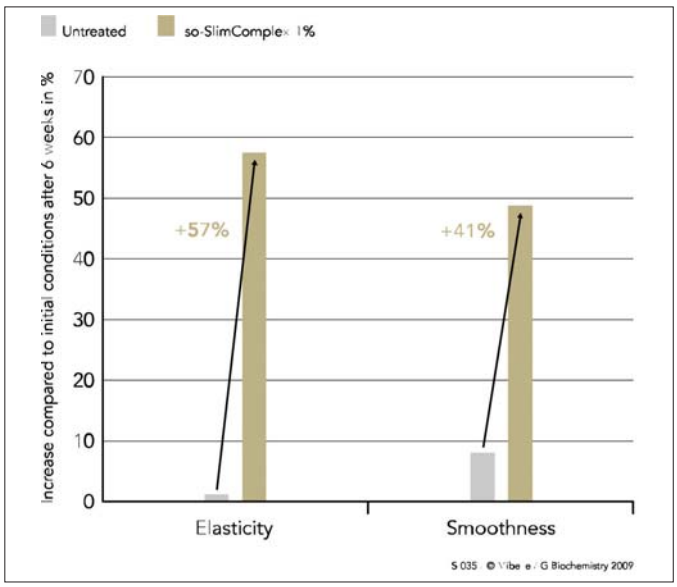

Figure 7: Improvement of skin quality after 6 weeks' application of a cream with 4% Iso-SlimComplex.

only reduced by 0.8 cm (fig. 6). Smoothness and elasticity improved after 6 weeks' application by 57% and 41% respectively compared to the untreated areas (fig. 7).

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