# Use of Soy Isoflavones for Stimulation of Skin Collagen Synthesis

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**Abstract**

As known tyrosine kinase inhibitor and slightly estrogenic active, genistein, the bioactive form of the predominant soy isoflavones, has a big potential to stimulate collagen in the skin. A clinical skin penetration study showed that there is no in situ activation of biologically inactive isoflavone glycosides. The study showed further that only genistein encapsulated into lecithin liposomes penetrates into deeper stratum corneum layers. In another clinical study, a cream with liposomal genistein was found to significantly increase skin thickness, firmness and hydration.

## Introduction

Flavonoids form a large group of plant polyphenols. They are subdivided into anthocyanins, which are responsible for the colors from blue to red in flowers and fruits, and the colorless or white-to-yellow flavones, flavonols and isoflavones.

Many of these flavonoids are physiologically active compounds that may have disease-preventing properties. Studies have been carried out mainly on cancer, cardiovascular disease and postmenopausal problems. In addition to their potent antioxidant properties, their mechanisms of action may include specific inhibition of enzymes or binding to receptors.

Soy isoflavones became well known as natural alternative to estrogens used in hormone replacement therapies. The decline in estrogen production at menopause increases the risk for osteoporosis and causes accelerated skin aging expressed in thinning of the skin and the corresponding loss in elasticity and wrinkle formation. Several studies show that estrogen therapy preserves collagen content and so elastic properties and thickness of the skin. Hormone replacement therapies are widely used to relieve postmenopausal symptoms but estrogens are also debated because they seem to induce an increased risk of breast and uterus cancer as long-term side effect.

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 to the principal circulating estrogens in humans, isoflavones are bound at a much lower rate ($10^3$-fold weaker). However, they do compete with estrogens for receptor sites and they have been shown to protect against osteoporosis without affecting the uterus and breast.

Genistein, the predominant isoflavone in soy, is a well-known inhibitor of protein tyrosine kinases. Several reports describe a regulatory effect of genistein on collagen metabolism by the inhibition of protein tyrosine kinases. Figure 1 describes the role of this kinase in the breakdown signalling pathway.

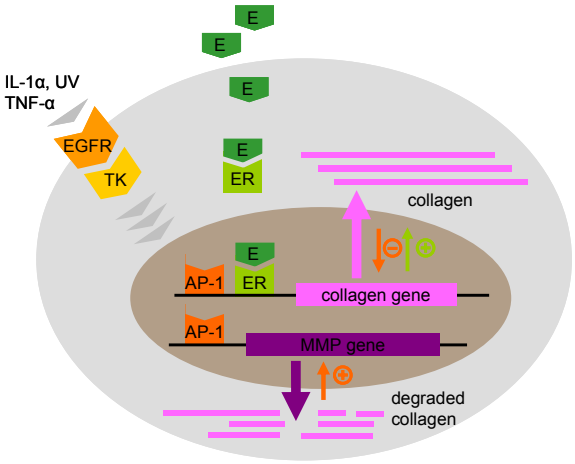


Figure 1: Cellular regulation of collagen formation and breakdown. The signalling for breakdown is shown in red and that one for formation in green. E, estrogen; ER, estrogen receptor; EGFR, epidermal growth factor receptor; TK, tyrosinase kinase.

The activator protein-1 (AP-1) is the principal transcription factor involved in downregulation of collagen (1). Activation of AP-1 suppresses the expression of collagen and up-regulates the expression of matrix metalloproteinase (MMP) genes. MMPs are endopeptidases that are specific for collagen degradation. Activation of AP-1 is induced by pro-inflammatory cytokines (IL-1alpha, TNF-alpha) and

UV light. The epidermal growth factor (EGF) receptor on the surface of fibroblast cells is the cellular gate in the signalling pathway. The intrinsic part of the EGF receptor is a tyrosine kinase (TK), which is activated in response to receptor binding. UV light and the inflammatory cytokine IL-1 have also been found to activate the kinase (2). Following activation, the kinase phosphorylates other kinases in the signal transduction cascade, leading finally to the activation of AP-1.

By inhibiting the tyrosine kinase, genistein disrupts the signalling cascade and prevents so the degradation of collagen and the decline in the formation of new collagen. As estrogen receptor agonist, genistein stimulates the production of new collagen. As shown in Figure 1, estrogen or estrogen agonists such as genistein can easily pass through the cell membrane of fibroblasts and bind to the estrogen receptor in the cytoplasm. After activation, the receptor moves to the nucleus where it induces the expression of collagen. Genistein is the perfect plant active to stimulate collagen formation and thus to increase skin thickness.

Most of the flavonoids in plants are found in the glycoside form, which means they are attached to a sugar residue (see figure 2). As such, they are water-soluble and can be kept in the aqueous plant vacuoles. The glycosides are usually biologically inactive in humans because of low cellular uptake and because they do not fit into the binding sites of enzymes and receptors. After oral application, transformation into active molecules occurs through the action of hydrolytic enzymes in the intestine. But what happens with the glycosides in the stratum corneum?

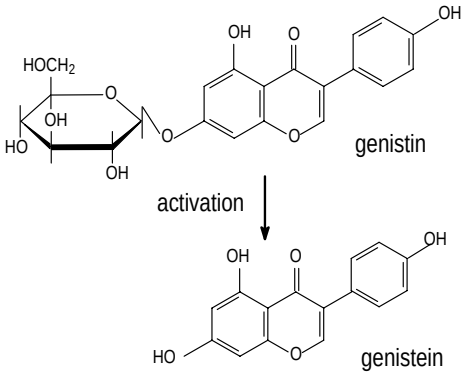


Figure 2: Transformation of the glycoside genistein to the bioactive aglycone genistein

This article describes a clinical study on skin metabolism of genistin, the glycoside of genistein, and on the effect of encapsulation of genistein into lecithin liposomes on skin penetration. In a second part, a clinical study on the efficacy of liposomal genistein in an anti-aging formulation is shown.

**Study of Percutaneous Absorption and Skin Metabolism of Soy Isoflavones**

In order to analyze penetration of the soy isoflavone genistein and to verify whether its glycoside (genistin) is hydrolyzed in skin, we conducted a study with ten human volunteers and three different test preparations:

- Formula a - Isoflavone aglycone genistein incorporated into lecithin liposomes (0.3% genistein)
- Formula b – Same as Formula a but without liposomes to address their influence on penetration
- Formula c - Isoflavone glycoside genistin incorporated into lecithin liposomes to determine the rate of hydrolysis (0.55% genistin which corresponds to the same molar concentrations as in the genistein preparations)

The different preparations were applied to test areas on the inside of the forearm. After 4 hours, skin samples were taken by stripping the test areas with adhesive tapes. The dose of genistein applied on the stripped area was 750 µg. This method gently removes the stratum corneum. After solubilisation of the material from the tapes, the concentration of genistein was analyzed by HPLC.

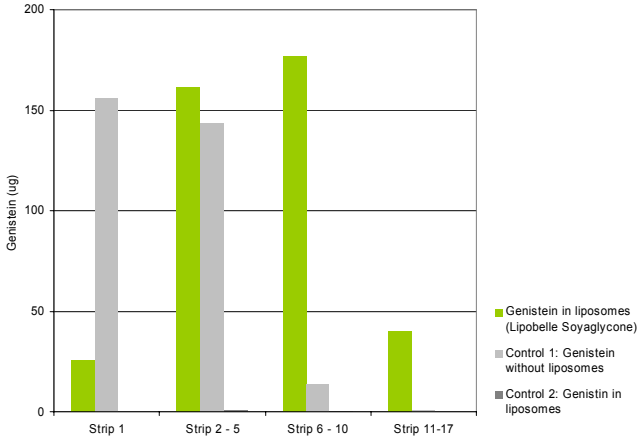


Figure 3: Percutaneous absorption and metabolism of soy isoflavones. Genistein in liposomes (green); Genistein without liposomes (light grey); genistin in liposomes (dark grey).

The study results are shown in Figure 3. The genistein in Formula b remained mainly in the outermost stratum corneum (strips 1 to 5). Only 4% of it was found in strips 6 to 10 and nothing in deeper layers. The distribution profile of the genistein in Formula a compared to that of the genistein in Formula b was clearly shifted to deeper stratum corneum layers. The largest portion of genistein was detected in strips 6 to 10. In the skin areas where formula c was applied, no significant amounts of genistein could be detected in the stratum corneum.

The study clearly shows that isoflavone glycosides are not hydrolyzed in significant amounts four hours after application. Therefore, topically applied isoflavone glycosides cannot exert significant physiological effects in the skin. The tape stripping results show that

liposomes can strongly enhance the penetration of genistein. Instead of remaining in the outermost layers, genistein in liposomes penetrates into deeper layers of the stratum corneum (strips 2 to 10).

**Study on the Efficacy of Liposomal Genistein in an Anti-Aging Formulation**

A cream containing 90 mg/kg genistein, encapsulated into lecithin liposomes, was tested in a study with 20 women between age 55 and 64. The product was applied twice daily on the inner side of the forearm. The same cream without genistein served as a control. Several skin parameters were measured after 2 and 3 months of product application, including skin thickness by ultrasonic measurement, skin firmness by cutometry, skin roughness by a digital micromirror device and skin hydration by corneometry. All skin parameters were determined 8 to 12 hours after the last product application.

After 3 months, skin thickness in subjects using the genistein cream had increased by 11 %. In comparison, skin thickness in subjects using the control cream did not change significantly (Figure 4).

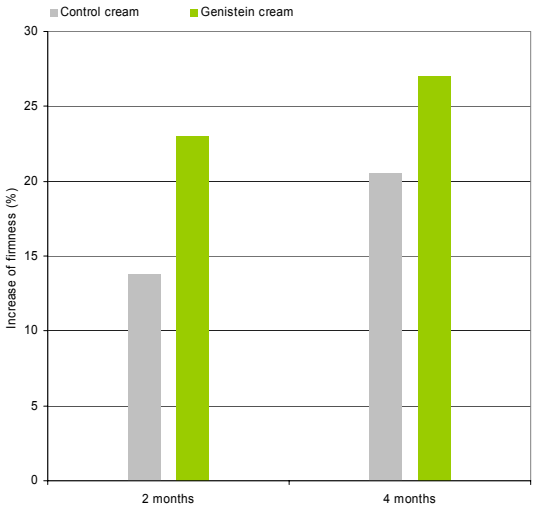


Figure 4: Comparison of skin thickness when treated with cream formulated with and without genistein.

This improvement was statistically significant by the Wilcoxon matched pairs signed rank test ($p < 0.05$). The application of genistein cream also improved other skin parameters slightly, such as firmness (Figure 5), roughness and hydration (Figure 6).

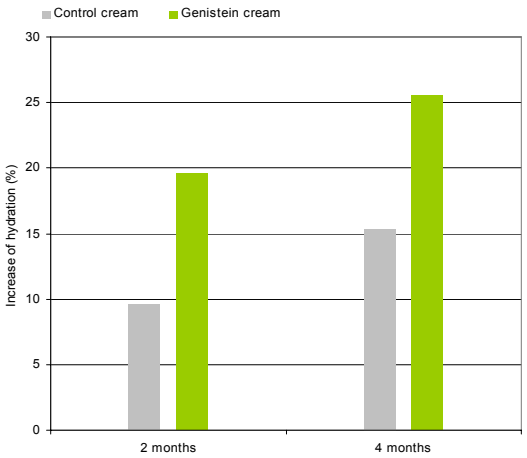


Figure 5: Comparison of skin firmness.

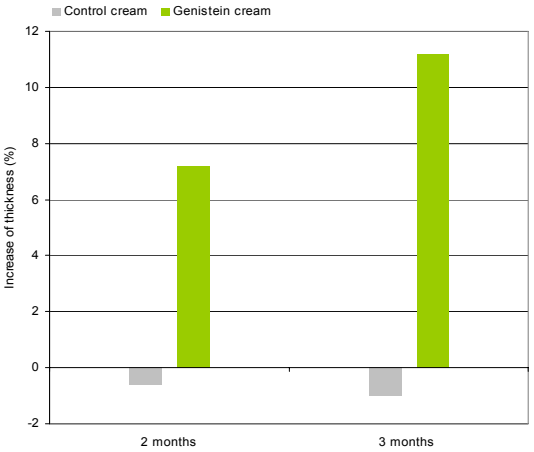


Figure 6: Comparison of skin hydration.

As skin thickness is principally determined by the concentration of collagen in the dermis, this study clearly suggests a positive effect of genistein on collagen content. To determine whether this result was based on genistein interacting with the estrogen receptors in the skin or with the receptor protein tyrosine kinase on skin cells or with both needs further study.

**References**

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