

Topically Applied Soy Isoflavones Increase Skin Thickness

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Epidemiological studies indicating an association between diet and disease led to the investigation of a series of bioactive plant compounds. In Japan, for example, the incidences of cardiovascular disease, hormone-dependent cancers (breast, uterus and prostate) and menopausal symptoms (osteoporosis and hot flashes) are all substantially lower than in western countries. The traditional Japanese diet includes a significant amount of soy-based foods such as tofu and tempeh.

The physiologically-relevant compounds in soy are the isoflavones, a subgroup of polyphenolic plant compounds. Isoflavones, or phytoestrogens, adopt a chemical structure very similar to that of the human hormone estrogen after hydrolysis of the sugar moiety (Figure 1). The isoflavones

contained in soybeans appear predominantly in the form of polar, water-soluble glycosides, such as genistin.

Many dietary supplements with soy isoflavones already exist. In most cases, supplements contain only isoflavone glycosides, the molecular form that is not biologically active because there is no cellular uptake of glycosides. However, after ingestion, intestinal glucosidases and intestinal bacterial metabolism transform the glycosides into the physiologically active form.

There is an interest in isoflavones in the cosmetic industry because these compounds may have potential in the treatment of skin aging. We assume that two different activities of isoflavones could affect the skin by binding estrogen receptors and inhibiting the protein tyrosine kinase-signaling pathway.

Since the skin does not have the hydrolytic activity of the intestine, the isoflavones must be in the active aglycone form for skin care applications. In this rather apolar form, isoflavones penetrate easily into deeper skin layers and into the skin cells. Unfortunately, these aglycones have a poor solubility in water and in oil. We have therefore developed a process to produce a water-soluble isoflavone aglycone preparation based on liposomes that is suitable for cosmetic applications, which we have reported previously.¹ This article summarizes the first study conducted on the efficacy of isoflavones in the prevention of skin aging.

Key words

Soybeans, isoflavones, genistein, estrogen, collagen, matrix metalloproteinase

Abstract

This placebo-controlled study in humans has shown that soy isoflavones have a potent effect on the improvement of skin thickness in aged skin. The authors discuss the possible mechanisms of action.

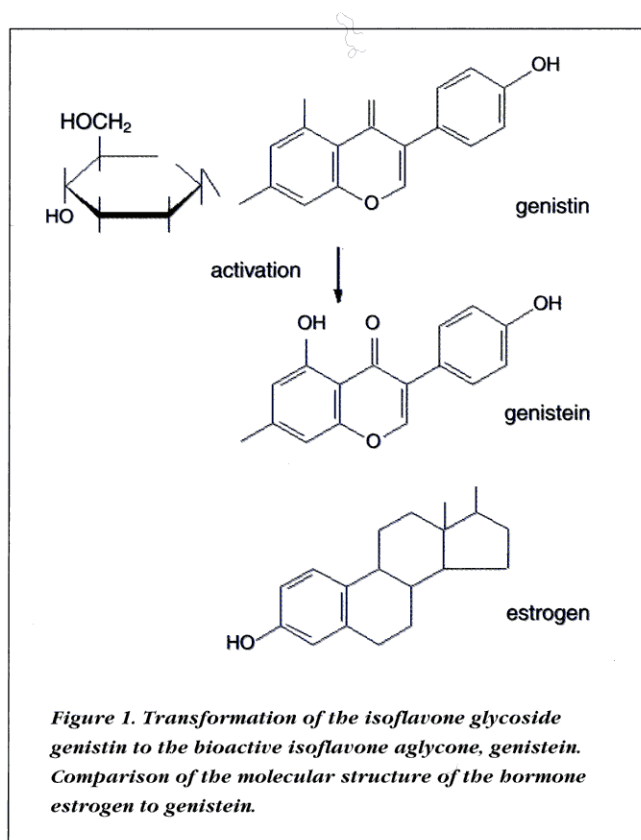
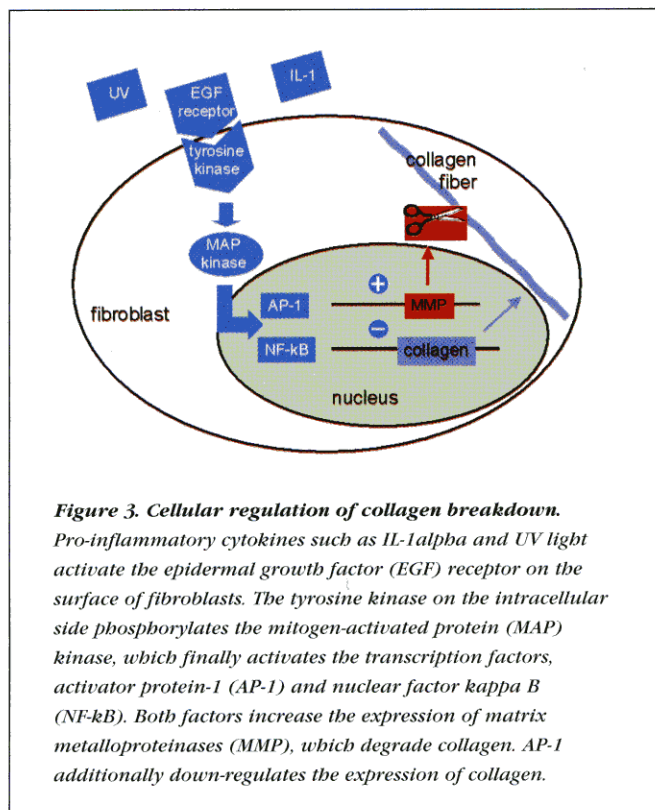
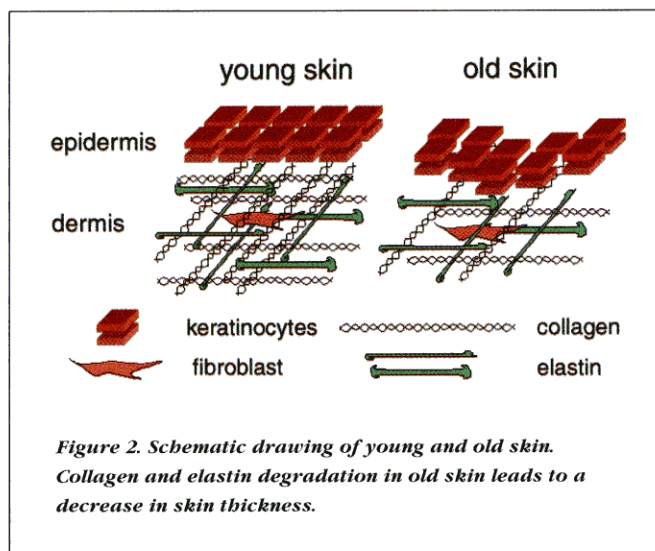


Figure 1. Transformation of the isoflavone glycoside genistin to the bioactive isoflavone aglycone, genistein. Comparison of the molecular structure of the hormone estrogen to genistein.



Skin Aging

The skin is composed of two cell layers, the epidermis and the dermis, with the corresponding major cell types being keratinocytes and fibroblasts, respectively. The function of the outer epidermis is to provide a barrier against water loss and external chemical injury. The underlying dermis is rich in an extracellular matrix (ECM) that is essential for providing strength and elasticity to the skin. The ECM is composed of fibrillar collagen bundles and elastic fibers in a complex array of proteoglycans and other matrix com-

ponents. During aging, the skin becomes thinner and less elastic (Figure 2). As collagen accounts for the biggest component of the matrix, regulation of collagen biosynthesis and degradation is central to the skin aging process.

Several factors ultimately lead to cutaneous aging. The Hayflick Phenomenon predicts that fibroblasts undergo cellular senescence with advanced age.² This results in a switch from a matrix-producing to a matrix-degrading phenotype. Another mechanism in skin aging is based on reactive oxygen species (ROS) as by-products of the oxidative cell metabolism (Free Radical Theory). A general decline in hormone production is a further important aging mechanism; the effect of declining estrogen production on skin has been well documented. At menopause, when the production of estrogens drops drastically, skin aging is more pronounced than before. The principal external factor that causes premature skin aging is UV light.

Cellular Regulation of Collagen Breakdown

A basic knowledge of collagen formation and breakdown is necessary to fully understand the effect of genistein in the treatment of skin aging. The fibroblasts synthesize and secrete individual polypeptide chains of types I and III collagens as procollagen precursors. Regulation of procollagen expression is controlled at the transcriptional level, with two transcription factors involved in down-regulation of collagen: the activator protein-1 (AP-1) and nuclear factor kappa B (NF-kB) (Figure 3). Both factors are induced by pro-inflammatory cytokines (IL-1alpha and TNF-alpha) or UV light. AP-1 can directly suppress collagen expression. But both AP-1 and NF-kappaB reduce collagen by up-regulation of the expression of matrix metalloproteinase (MMP) genes. MMPs comprise a large family of zinc-dependent endopeptidases that are specific for collagen degradation.

The epidermal growth factor (EGF) receptor on the surface of fibroblast cells is the cellular gate in the signaling pathway. The intrinsic part of the EGF receptor is a tyrosine kinase activity, which is activated in response to receptor binding. UV light and the inflammatory cytokine IL-1 have been found to induce tyrosine phosphorylation in a manner similar to EGF.³ Following activation, the kinase phosphorylates other kinases in the signal transduction cascade, like those of the mitogen-activated protein (MAP) kinase pathway. The signaling pathway ends in the cellular nucleus with the activation of AP-1 and NF-kappaB. It has been suggested that H₂O₂ as well as other ROS are produced upon UVA radiation. ROS, produced either by UVA or as by-products of the oxidative cell metabolism, may account for the expression of matrix metalloproteinases through induction of inflammatory cytokines.

Biochemical Properties of Isoflavones in Skin

Isoflavones as selective estrogen receptor modulators: Postmenopausal skin aging has been found to be the result of lower collagen production.⁴ Studies show that

topical estrogen replacement therapies can increase the skin collagen content.⁵ Although estrogen receptors have been identified in skin cells, it is not known how estrogen receptor binding ultimately results in increased skin collagen.

Classical hormone replacement therapies to fight postmenopausal symptoms, including hormone-related skin aging, are highly disputed because estrogen appears to increase the risk of breast and uterine cancers. Thus pharmaceutical companies have recently developed selective estrogen receptor modulators (SERMs), synthetic estrogen-like compounds, that are safer because they exert an estrogenic effect only in selected tissues, such as in bones to prevent osteoporosis (Table 1).

Although isoflavones are not steroids, they fit estrogen receptors very well. In humans, two different estrogen receptors exist, ERalpha and ERbeta. Compared with estrogen, the soy isoflavone genistein (the active form of genistin) has a lower affinity for ERalpha but about the same affinity for ERbeta. Unlike estrogen, genistein has been shown to even reduce the risk of breast and uterine cancers. In this sense, genistein can be regarded as a natural SERM with a high potential in prevention of hormone deficiency-related disorders such as skin aging.

Isoflavones as protein tyrosine kinase inhibitors: Genistein is a well-known inhibitor of protein tyrosine kinases as it competes at the ATP side. Several reports describe a regulatory effect of genistein on collagen metabolism by the inhibition of protein tyrosin kinases.

This is the case for certain cancerous cells, where genistein has been found to down-regulate the expression of MMPs and up-regulate the tissue inhibitor of metalloproteinase (TIMP).^{6,7} Inhibition of MMPs (collagenases) is considered an important step in prevention of growth and spread of a metastatic tumor.

But also in normal cells genistein stimulates collagen production by interacting with kinases. This has been shown in bone cells by Yoon et al.⁸ and in skin fibroblasts by Ravanti et al.⁹

Table 1. Selective tissue effects of the synthetic estrogen-like compound raloxifene and soy isoflavones compared to estrogen¹²

Target tissue	Estrogen	Raloxifene	Isoflavones
Brain	++	-	+
Uterus	++	-	-
Breast	++	-	-
Bone	++	++	+
Cardiovascular	++	++	+

++ = strong
+ = medium
- = no effect

Wang et al.¹⁰ found that genistein blocks the signaling pathway from UVB to activation of AP-1 by specific inhibition of the kinase at the growth factor receptor site.

All these publications indicate a high potential for genistein in up-regulation of collagen production by inhibition of the protein tyrosine kinase.

Soy Isoflavones in Anti-Aging Cosmetics

The scientific literature on the physiological effects of genistein shows interesting possibilities for its application in cosmetics, particularly in anti-aging cosmetics. The decrease in skin collagen caused by chronological aging is even more pronounced after menopause as collagen decrease accelerates, due to hypoestrogenism.

A consequence of this skin aging is reduced skin thickness. In our study, we therefore used skin thickness as a major parameter to measure the theoretical skin benefits of topical application of genistein. Only two substances, estrogen and retinoic acid, have previously been shown to significantly increase skin thickness upon topical application. However, neither agent is allowed for use in cosmetics in most countries for safety reasons.

Clinical Study of Genistein

The safety of genistein as a cosmetic ingredient was first confirmed by a repeated patch test in human subjects for the determination of the irritation and photo-irritation potential.

A cream containing 90 mg/kg genistein in a suitable carrier, as described by Schmid et al.,¹ was tested in a study with 20 women between age 55 and 64. The product was applied twice daily on the inside 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 micro-mirror 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). This improvement was statistically

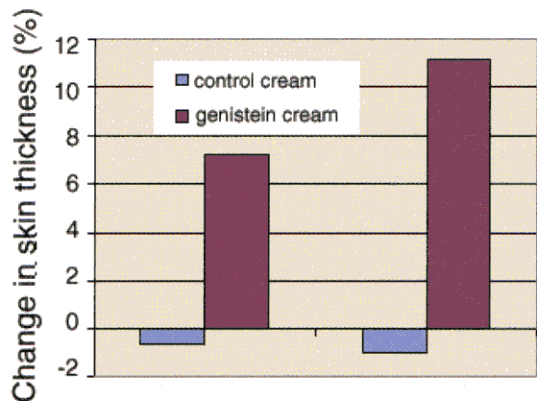


Figure 4. Comparison of skin thickness when treated with cream formulated with and without genistein

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 elasticity, roughness and hydration.

Genistein therefore appears to have a specific effect on skin thickness that is not based on a swelling of the stratum corneum. No side effects were reported.

Furthermore, the effect of genistein cream on skin thickness as determined in our study was better than the 7.7% increase after one year found in a study¹¹ with oral estrogens.

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.

Conclusion

We conclude that isoflavone aglycones from soy, such as genistein, are very interesting molecules for cosmetic products. We therefore are studying new applications of our genistein preparation such as the treatment of UV-stressed skin and adipose tissue in cellulite.

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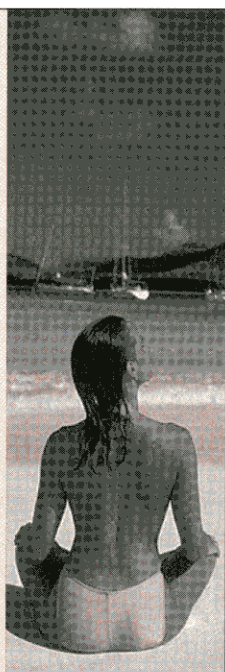
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