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Extracts from *Glycine max* (soybean) induce elastin synthesis and inhibit elastase activity

Renbin Zhao[1], Elizabeth Bruning[1], Dianne Rossetti[1], Barry Starcher[2], Miri Seiberg[1] and Violetta Iotsova-Stone[1]

[1]The Johnson and Johnson Skin Research Center, CPPW, A division of Johnson and Johnson Consumer Product Companies, Skillman, NJ, USA; [2]Health Center at Tyler, University of Texas, TX, USA

Correspondence: Miri Seiberg, The Johnson and Johnson Skin Research Center, CPPW, A division of Johnson and Johnson Consumer Product Companies, Skillman, NJ, USA, Tel.: 1-908-874-2325, Fax: 1-908-874-1254, e-mail: mseiber@its.jnj.com

Abstract: Elastic fibres are essential extracellular matrix components of the skin, contributing to its resilience and elasticity. In the course of skin ageing, elastin synthesis is reduced, and elastase activity is accelerated, resulting in skin sagging and reduced skin elasticity. Our studies show that non-denatured *Glycine max* (soybean) extracts induced elastin promoter activity, inhibited elastase activity and protected elastic fibres from degradation by exogenous elastases *in vitro*. Mouse and swine skins topically treated with soybean extracts showed enhanced elastic fibre network and increased desmosine content. Elastin expression was also augmented in human skin transplanted onto SCID mice in response to soy treatment. These data suggest that non-denatured soybean extracts may be used as skin care agents to reduce the signs of skin ageing.

Keywords: elastase – elastin – *Glycine max* – non-denatured – skin ageing – soy – soybean

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The resilience of the skin depends on the functionality of its elastic fibres. Elastic fibre formation is a multi-step process engaging a variety of factors. It involves tropoelastin synthesis and secretion, and its deposition on microfibrils followed by cross-linking into mature fibres (1–4). In addition, growth factors, such as TGF-β, IGF-1 and IL-1β induce elastin expression (5–9), while TNF-α and EGF inhibit elastin synthesis (10,11). Loss of elastic fibres is a major contributing factor of skin ageing (12,13). Chronological ageing, UV exposure and inflammation, all increase the release of elastases, and in particular of human neutrophil and human macrophage elastases (HLE and HME) (14–16). These elastases degrade elastic fibres, resulting in skin sagging and wrinkling (14,16–21). Indeed, the inhibition of fibroblast-derived elastase protects skin's elastic fibres and reduces wrinkle formation in rodents (19,22,23).

Our previous studies showed that treatments with non-denatured soybean extracts and with the soybean derived protease inhibitors (STI and BBI) result in skin lightening (24–26) and in delayed hair growth (26). During the clinical studies, we noticed an improvement in skin elasticity of soy-treated subjects (27). Here we report that soybean extracts enhance the elastic fibres in skin by inducing elastin promoter activity and by inhibiting elastases.

To understand the mechanism of action of the soybean extracts, we generated two elastin promoter-reporter constructs, and their activities were examined in transiently transfected H9c2 cells (rat cardiac myoblasts). A 2.2 kb promoter-reporter construct (Elp2.2, Fig. S1a) showed lower basal promoter activity than a shorter, 0.5 kb construct (Elp0.5, Fig. S1a,b), because of negative cis-acting elements between −2260 and −495 (28). Treatment of Elp2.2 transfected cells with soybean extracts resulted in a dose-dependent increase in elastin promoter activity (Fig. 1a). No induction was observed with the Elp0.5 promoter (not shown), suggesting a possible de-repression, via regulatory elements located between −2260 and −495 bp.

HME and HLE activities were measured *in vitro* using fluorescent-labelled peptide substrates (Appendix. S1). Soybean extracts inhibited both HME (Fig. 1b) and HLE (Fig. 1c) in a dose-dependent manner, yet with different degrees of inhibition. HLE was inhibited by 70% with 0.1% soy extracts, while HME was only inhibited by 40% (Fig. 1b,c). The soy proteins STI and BBI showed only 30% and 40% inhibition of HLE respectively, at concentrations ten times higher than those present in the tested soybean extracts, while soy isoflavones and BSA did not

Abbreviations: HLE, human leukocyte elastase; HME, human macrophage elastase; STI, soybean trypsin inhibitor; BBI, Bowman-Birk inhibitor; SCID, severe-combined immunodeficient; IHC, immuno-histochemistry; ECM, Extracelluar matrix; DE, dermal–epidermal.

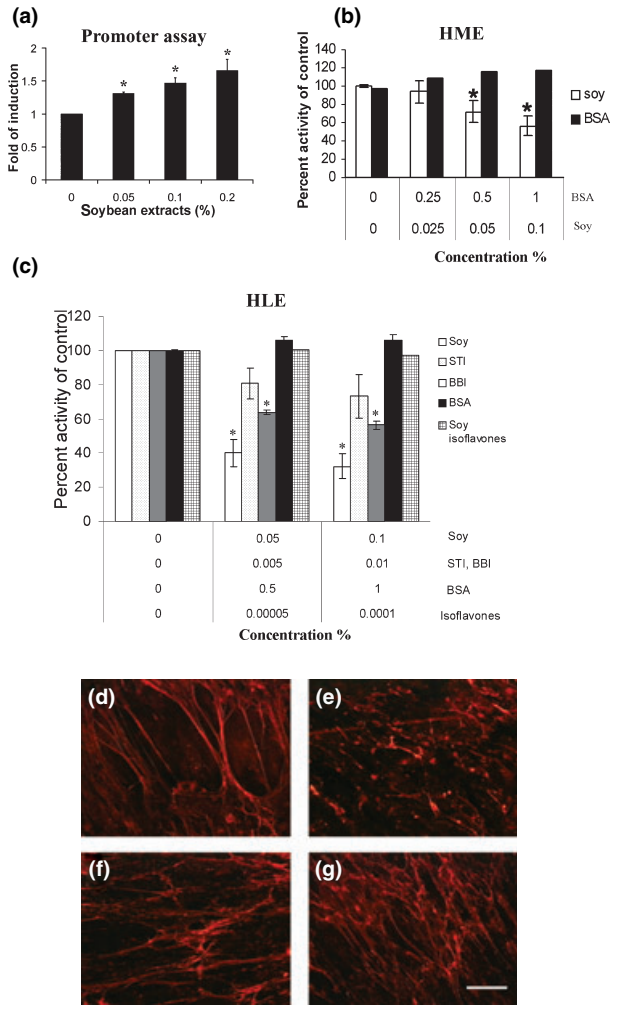

Figure 1. Soybean extracts induce elastin promoter and inhibit elastases. (a) Soybean extracts induce elastin promoter activity. Data were presented as fold of induction. Results from 5 experiments are summarized. Each bar represents mean ± SD * significant with $P \leq 0.001$. (b, c) HME (b) and HLE (c) activities were measured in the presence of soybean extracts, STI, BBI, soy isoflavones and BSA, at different concentrations as indicated. The extract contains approximately 0.001% STI and BBI and 0.0001% isoflavones. Results are presented as percent activity of control. Each bar represents mean ± SD * significant with $P < 0.01$ ($N = 5$ for soy and $N = 3$ for STI, BBI). (d–g) Elastic fibres produced by primary fibroblast cultures were visualized by IHC with polyclonal anti-elastin antibody. (d) Untreated control culture; (e) Fibroblasts treated with 0.1 U/ml HLE for 1 h; (f, g) Fibroblasts treated with 0.1 U/ml HLE in the presence of 0.02% (f) and 0.05% (g) soybean extracts. Bar = 50 μM.

inhibit HLE (Fig. 1c). Heat-denatured soybean extracts showed similar elastase inhibitory activity to that of the non-denatured soy, (Fig. S1c), suggesting that STI, BBI and isoflavones are not the main elastase inhibitors in soy.

Further studies were carried out *in vitro*, to evaluate the protective effect of soybean extracts against elastases. Soybean extracts protected elastic fibres, produced by primary dermal fibroblasts (Fig. 1d), from degradation by exogenous HLE. Elastic fibres appeared fragmented within one hour of exposure to 0.1 U/ml HLE (Fig. 1e). When incubated with soybean extracts during the HLE treatment, the elastic fibres were protected from degradation, partially at 0.02% (Fig. 1f) and completely at 0.05% (Fig. 1g). Ellagic and tannic acid can protect newly synthesized elastic fibres from elastase degradation, and therefore enhance elastogenesis in fibroblast cultures (29). The protective capacities of soybean extracts against elastases suggest that they may also contribute to elastogenesis.

To test the *in vivo* activity of the soybean extracts, SKH-1 hairless mice (Fig. 2a,b) and Yucatan swine (Fig. 2c,d) were treated for 8 and 12 weeks, respectively, with either soybean extracts or vehicle (Appendix S1). Luna elastin staining of the soy-treated mouse and swine skins demonstrated an increase in elastic fibre staining in the upper dermis, adjacent to the dermal–epidermal (DE) junction (Fig. 2b,d). Soy-treated swine skins revealed an increase in branched, fine elastic fibre that were perpendicular to the DE junction (Fig. 2d). Skins treated with soybean extracts showed no abnormal elastin accumulation or any other histological abnormalities. Next, we showed the induction of tropoelastin expression in mouse skins. A 2.4-fold increase in tropoelastin mRNA was demonstrated in soy-treated mouse skins relative to vehicle (QPCR, Fig. 2e). To measure elastin content in skin, we quantified desmosine crosslinks, which are unique to elastic fibres (30). Desmosine analysis of swine skins showed an increase from 104 ± 15 pmole/mg protein (vehicle-treated) to 163 ± 17 pmole/mg protein in soy-treated skins, with an average increase of about 57% (Fig. 2f), suggesting that soy not only induces elastin synthesis, but also increases the amount of mature elastic fibres.

To relate the findings to human skin, two facial skins (facelifts, obtained with informed consent), transplanted onto SCID mice, were treated for 7 weeks with soy extracts or vehicle. Treatment with soy extracts significantly induced tropoelastin expression, compared with respective controls (3.4- and 1.8-fold, QPCR) (Fig. 2g). Different basal levels of tropoelastin expression were documented with the two individuals; however, soy treatment induced tropoelastin expression in both donors, relative to their baselines (Fig. 2g). The levels of tropoelastin induction in these human skin grafts were in the range of those observed in the soy-treated mouse skins. Confirmation of elastin induction by histology was not feasible due to massive solar elastosis in these skins both pre and post-transplantation. Treatment of these human skins with soy extracts not only induced tropoelastin expression, but also increased the expression of fibulin-5, a major microfibril component (Fig. 2g), suggesting the concerted regulation of elastic fibre assembly. Soy extracts also induced the

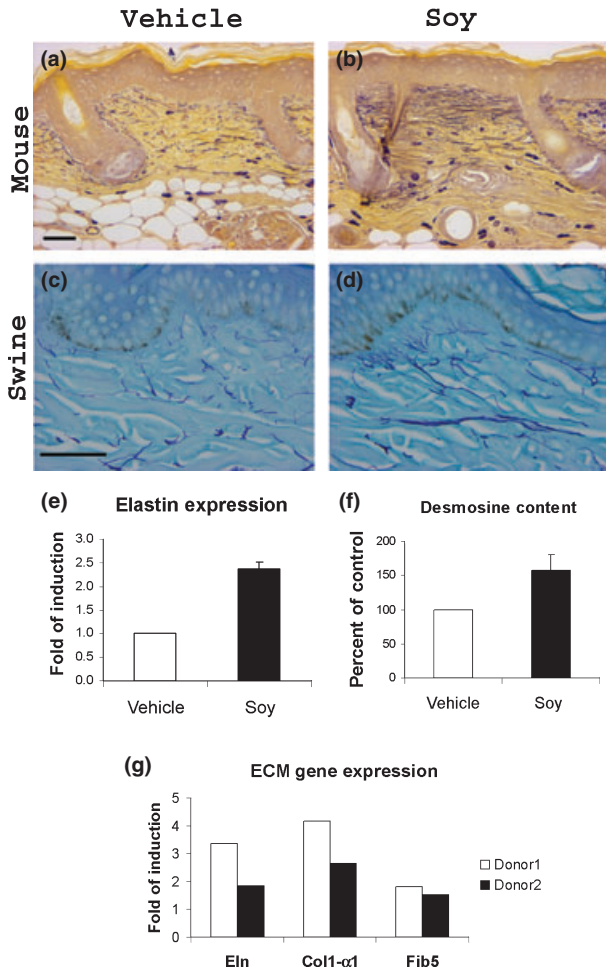

Figure 2. Soybean extracts enhance the elastic fibre network *in vivo*. (a, b) SKH-1 mice were treated with soy extracts (b) and vehicle control (a), for 8 weeks, and elastic fibres were visualized histologically by Luna elastin staining. Elastic fibres stained purple in a yellow background. (c, d) Swine skins were topically treated with soybean extract (d) or vehicle (c), for12 weeks and stained for elastin fibres (stained purple in a blue/green background). Bar = 50 μM (e) RT-PCR analysis of RNAs isolated from SKH-1 mouse skins treated with soy extracts and control for 1 week. Tropoelastin expression was normalized to GAPDH and results were averaged from three mice per group. Fold of induction was calculated as ratio of treatment to control. (f) Desmosine analysis of swine skins topically treated with soybean extracts or vehicle for 12 weeks. Desmosine results were averaged from both left and right side of the treated swine. (g) Soy extracts induced elastin, collagen-1 alpha-1 and fibulin-5 expression in human skins transplanted onto SCID mice. Fold of induction was calculated as the level of expression in soy treated skin, relative to control from the same donor.

expression of collagen1-α1, a major component of the dermal extracellular matrix, suggesting that soy may have effects on additional ECM genes (Fig. 2g).

We present *in vitro* and *in vivo* data for soybean extracts to induce the expression of tropoelastin and fibulin-5, which are both essential for the de novo formation of elastic fibres. We also show the inhibition of elastases by soybean extracts, which prevents elastic fibre degradation. These two activities of the soybean extracts suggest their usefulness in skin care, particularly in the prevention and reduction of the loss of elastic fibres associated with skin ageing.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1. (a) Two promoter fragments of 2.2 kb (Elp2.2) and 0.5 kb (Elp0.5) in length upstream of the elastin coding region, were cloned into luciferase reporter constructs. A promoterless vector was used as negative control. An internal control carrying Thymidine kinase promoter with the Renila luciferase (pRL-TK) was also included. □ Elastin promoter sequence. ⚂ Elastin coding sequence; ■ Firefly luciferase; ▨ Thymidine kinase promoter; ▥ Renila luciferase. (b) Relative promoter activities of the two elastin- promoter fragments were measured by transient transfection into rat cardiac myoblast H9c2 cells. The results are expressed as relative promoter activities of the ratio between Firefly and Renila luciferase measurements. Each bar represents mean ± SD. (c) HLE activity was measured in the presence of non-denatured and heat-inactivated soybean extracts, at different concentrations as indicated. Results are presented as percent activity of control and averaged from triplicate of readings. Each bar represents mean ± S.D.

Appendix S1. Materials and methods.

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Letter to the Editor

A method for the analysis of milk and egg allergens for the atopy patch test

Cinzia Ballabio[1], Alessandro Fiocchi[2], Alberto Martelli[2], Silvia Pecora[3], Samuele E. Burastero[4] and Patrizia Restani[1]

[1]Department of Pharmacological Sciences, University of Milan, Milan, Italy;
[2]Melloni Paediatria, University of Milan Medical School at the Melloni Hospital, Milan, Italy;
[3]ALK Abellò Italy, Lainate, Italy;
[4]San Raffaele Scientific Institute, Milan, Italy
Correspondence: Samuele Burastero, MD, San Raffaele Scientific Institute, 58, via Olgettina, 20132 Milan, Italy, Tel.: +39 2 2643 4730, Fax: +39 2 2643 4723, e-mail: s.burastero@hsr.it

Abstract: The patch test with food antigens (atopy patch test, APT) has been reported as a more specific method than prick or RAST for the early detection of cow's milk and/or egg sensitizations in children. Standardization of APT extracts is a major issue on the road towards full clinical exploitation of this assay. Here, we used sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) to characterize sensitivity and specificity of commercial preparations of APT for milk and egg allergies, which are expected to improve the reliability of this test, when compared with fresh food allergen sources. We found that: (i) SDS-PAGE is an appropriate technique for quality control of APT and (ii) commercial milk and egg APT are equivalent to fresh food preparations in terms of allergen content. Clinical trials aimed at characterizing sensitivity and specificity of APT in the diagnosis of food allergy in children will benefit from this technique.

Key words: allergy – atopy patch test – egg allergens – milk allergens – SDS-PAGE

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Abbreviation: APT, atopy patch test.

