

An evaluation of the effect of a topical product containing C-xyloside and blueberry extract on the appearance of type II diabetic skin

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Summary

Background Diabetes is a multisystem disease caused by the presence of chronic hyperglycemia, which leads to increased oxidative stress. Many of the changes observed in type II diabetic patients can be traced to the increased production of advanced glycation end products, also known as AGEs. AGEs are produced as a result of a nonenzymatic reaction with glucose interacting with proteins, lipids, and nucleic acids. AGEs are also present in normal skin with advancing age and contribute to the senescence of many body organs, including the skin.

Aims This research evaluated the effect of a topical product formulation containing blueberry extract, an AGE inhibitor, and C-xyloside, a GAG synthesis stimulator, applied twice daily on the hand, arm, and facial skin of 20 type II diabetic females. Diabetic skin was chosen for evaluation because AGEs are found in increased concentration in diabetic skin, representing a model for accelerated aging.

Materials and Methods This single-center study enrolled 20 female type II diabetics aged 55+ years with mild to moderate fine lines, wrinkles, and hyperpigmentation on the face and hands. Subjects used the study product on their face, hand, and inner forearm twice daily for 12 weeks. Ordinal grading on a 4-point scale (0 = none, 1 = mild, 2 = moderate, 3 = severe) of facial fine lines, wrinkles, firmness, radiance, skin tone, skin smoothness, hyperpigmentation, creping, density, sagging, and overall appearance was performed by the investigator at baseline, week 4, week 8, and week 12. Tolerability, subject assessments, digital photography, AGE measurements, skin caliper measurements, and corneometry were also performed at each time point.

Results 19/20 subjects successfully completed the study. The presence of AGEs was documented by skin autofluorescence. The 12-week duration of the study was insufficient to measure a change in skin AGEs, but longer application of the study product might produce different results. No tolerability issues were noted. There was a statistically significant increase in skin caliper measurements on the face ($P = 0.004$) and arm ($P = 0.014$) as well as corneometry measurements ($P < 0.001$) consistent with enhanced moisturization at week 12. The dermatologist investigator also found statistically significant improvement in fine lines ($P = 0.01$), firmness ($P = 0.011$), radiance ($P < 0.001$), skin tone ($P = 0.014$), skin smoothness ($P < 0.001$), creping ($P < 0.004$), and overall appearance ($P < 0.001$).

Conclusion This study examined a topical product containing an AGE inhibitor and a GAG synthesis stimulator designed for the unique needs of diabetic skin.

Keywords: protein glycation, diabetic skin, C-xyloside

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Introduction

Diabetes is a multisystem disease caused by the presence of chronic hyperglycemia, which leads to increased oxidative stress.¹ Many of the changes observed in diabetic patients can be traced to the increased production of advanced glycation end products (AGEs). AGEs are produced as a result of a nonenzymatic reaction with glucose interacting with proteins, lipids, and nucleic acids.² Specifically, an aldose interacts with amino groups of proteins and the subsequent Amadori rearrangement leads to complex molecules that cross-link collagen leading to vascular stiffness, the postulated cause of diabetic vascular changes and skin stiffness associated with poor wound healing and ulcer formation on the lower extremities.³ Wound healing may also be impaired by intracellular AGE formation leading to the quenching of nitric acid and reduced growth factor function. AGEs also induce intracellular signaling leading to enhanced oxidative stress manifesting in the release of prosclerotic cytokines.⁴ Further, circulating AGEs can bind to the receptor for AGEs (RAGE) on different cell types enabling modulation of gene expression.^{5,6} RAGE expression is postulated to be upregulated in glomerular epithelial cells, vascular smooth muscle cells, and inflammatory mononuclear phagocytes and lymphocytes in diabetes, accounting for the observation of multisystem disease in diabetic patients.⁷

AGEs are found in increased amounts not only in type I and type II diabetic patients, but also in aging individuals.⁸ It appears that their formation is accelerated in diabetes leading to premature chronic disease from the conversion of short-lasting cellular activation to sustained cellular dysfunction.^{9,10} However, with aging, AGEs are found in increasing quantities affecting every cell type in the body. The pathologic effects of AGEs include increased vascular permeability, inhibition of vascular dilation by interfering with nitric oxide, oxidation of LDL and induction of cytokine secretion.¹¹ All of these factors increase oxidative stress, which promotes aging.

The intermediate products formed from protein glycation are known as Amadori, Schiff base and Maillard products, named after the researchers that identified the reaction. The concept of protein glycation is not new to the dermatologist. It is glycated proteins formed via the Maillard reaction that create the formation of the brown skin stain produced by self-tanning preparations. Self-tanning creams contain a sugar that glyicates the keratin protein to form a brown-colored substance, known as melanotoids.¹² This reaction is also used in foodstuffs to brown the tops of hamburger rolls. Monnier and Cerami

demonstrated that the same glycation reaction can also occur in the human body, resulting in AGEs.

Current anti-aging research has focused on methods of inhibiting and reversing AGEs. Our interest was in investigating a topical blueberry extract that had previously demonstrated antiglycation effects *in vitro*, due to the presence of antioxidant flavonoids. The blueberry extract was studied in a model where the protein albumin and the sugar ribose produced nonenzymatic glycation. Aminoguanidine, a potent inhibitor of glycation, was used as a positive control. The blueberry extract demonstrated a dose-response inhibition of glycation, when compared with the aminoguanidine control.¹³

The formulation that we studied in this research combined the blueberry extract with beta-C-xyloside (Pro-xylane™ L'Oréal, Paris, France). This environmentally sustainable ingredient was obtained as xylose, a monosaccharide, from the beechwood tree, native to western Europe. The C-xyloside is synthesized via Knoevenagel condensation of unprotected xylose in water followed by a catalytical reduction to yield C-beta-D-xylo-pyranoside-2-hydroxy-propane. C-xyloside increases the synthesis of glycosaminoglycans (GAGs), as demonstrated in human dermal fibroblast cultures.¹⁴ In addition, C-xyloside may have a direct action on keratinocytes renewal through functional interactions with growth factors, thus mediating epidermal homeostasis.¹⁵ These effects result in younger appearing skin, as the synthesis of GAGs increases the water binding capacity of the dermis and growth factors stimulate cell turnover.

This research evaluated the effect of a topical product formulation containing blueberry extract, an AGE inhibitor, and C-xyloside, a GAG synthesis stimulator, on the hand, arm, and facial skin of diabetic female subjects. Diabetic skin was chosen for evaluation as AGEs are found in increased concentrations in diabetic skin, representing a model for accelerated aging. The objectives of this study were to demonstrate the tolerability and efficacy of a topical product in diabetic female subjects with mild to moderate photoaging.

Method

This single-center IRB-approved (Concordia Clinical Research, Beach Haven, NJ, USA) study enrolled 20 female type II diabetic subjects aged >55 years with mild-to-moderate fine lines, wrinkles and hyperpigmentation on the face and hands. Subjects who had hypersensitivity to any of the product ingredients were excluded. Subjects were not allowed to use any other

topical cosmeceuticals, topical retinoids, or moisturizers during the 12-week study.

Subjects used the study product on their face, hand, and inner forearm twice daily for 12 weeks. In addition, they were provided with a sunscreen and cleanser. Ordinal grading on a four-point scale (0 = none, 1 = mild, 2 = moderate, and 3 = severe) of facial fine lines, wrinkles, firmness, radiance, skin tone, skin smoothness, hyperpigmentation, creping, density, sagging, and overall appearance was performed by the investigator at baseline and weeks 4, 8, and 12. Additional assessments were made by the investigator on the same four-point scale regarding product performance on the hand evaluating skin tone, smoothness, hyperpigmentation and overall appearance. The investigator and subjects also performed skin tolerability assessments on the same four-point ordinal scale evaluating erythema, edema, dryness/scaling, burning, stinging, itching, tingling and tightness on the face.

Digital high-resolution photography was performed on the frontal face, 45° to the right and 45° to the left for documentation purposes at baseline and weeks 4, 8, and 12. Bioinstrumentation of facial skin was performed to assess hydration and elasticity at baseline and weeks 4, 8, and 12. Hydration measurements were obtained with pin probe corneometry (DermaLab; Cortex Technologies, Hadsund, Denmark) and skin elasticity measurements were obtained with a suction device (DermaLab). Skin caliper measurements for skin thickness were performed on the face over the right malar eminence and the left inner mid-forearm at baseline and weeks 4, 8, and 12.

Advanced glycation end products were measured on the left forearm to validate the presence of increased glycated proteins in the diabetic subjects at baseline and weeks 4, 8, and 12. The AGEs were measured with a prototype device (AGE Reader; DiagnOptics Technologies, Groningen, the Netherlands). The non-invasive device measurement is based on the fact that some glycated proteins, such as pentosidine, *N*-carboxymethyl-lysine, and *N*-carboxyethyl-lysine, autofluoresce when exposed to 300- to 420-nm radiation.¹⁶ Other studies have demonstrated that autofluorescence is increased in diabetic patients, especially in those with neuropathy, vasculopathy, nephropathy and cardiovascular disease.¹⁷

The skin autofluorescence device illuminated a 1-cm² area of the left forearm with 300- to 420-nm radiation. The forearm skin was firmly pressed against the device to prevent light from entering the optic aperture. The radiation reflected from the skin was measured using the device spectrophotometer in the 300- to 600-nm range.

The autofluorescence was calculated as the average radiation intensity per nanometer in the 420- to 600-nm range divided by the average light intensity per nanometer in the 300- to 420-nm range. This calculation resulted in an AGE score. Based on a database of normal and diabetic subjects, the device helped determine the relationship between the reported subject's autofluorescence and the normal age-corrected population mean. A value of $P \leq 0.05$ was considered statistically significant. A two-tailed unpaired Mann–Whitney test was used to analyze the nonparametric ordinal data.

Results

Nineteen of 20 subjects successfully completed the study with one subject unable to complete due to an unrelated hospitalization. All subjects who entered the study possessed well-controlled diabetes type II on oral hypoglycemics. The presence of AGEs was documented by skin autofluorescence. The 12-week duration of the study was insufficient to measure a change in skin AGEs, but longer application of the study product might produce different results.

Skin caliper measurements demonstrated a gradual increase in skin thickness from baseline to week 12. The facial measurements demonstrated a statistically significant increase in skin thickness at weeks 8 ($P = 0.031$) and 12 ($P = 0.004$). A statistically significant increase in caliper reading was also observed on the arm at week 12 ($P = 0.014$). A statistically significant increase in the average of two corneometry measurements consistent with increased skin hydration was observed at weeks 8 ($P < 0.001$) and 12 ($P < 0.001$).

The skin elasticity measurements were conducted with five suction and relaxation cycles. The three final cycle readings were averaged. While the data did not reach statistical significance, there was a positive trend. The amount of force required to stretch the skin to a predetermined distance decreased from 27.01 at baseline to 24.46 at week 4, to 23.22 at week 8 and to 22.25 at week 12. This progressively lower number means that the skin became more elastic and less rigid. Skin rigidity is a common finding in diabetic patients perhaps related, in part, to the presence of glycated dermal matrix proteins. The positive trend might translate into statistical significance if the sample size of the study were increased.

The investigator facial assessments demonstrated a statistically significant improvement in facial radiance ($P < 0.001$), skin smoothness ($P < 0.001$), and overall appearance ($P = 0.007$) at week 4. These benefits continued into week 8. By week 12, there was also statistically

significant improvement in fine lines ($P = 0.01$), firmness ($P = 0.011$), radiance ($P < 0.001$), skin tone ($P = 0.014$), skin smoothness ($P < 0.001$), creping ($P < 0.004$), and overall appearance ($P < 0.001$).

The investigator also assessed the efficacy of the product on the hand. A statistically significant improvement in hand skin smoothness ($P < 0.001$) and overall appearance ($P = 0.002$) was observed at week 4. This improvement continued into weeks 8 and 12 with statistically significant improvement in smoothness ($P < 0.001$) and overall appearance ($P < 0.001$).

No tolerability issues related to erythema, edema and scaling were observed by the investigator. Subjects reported that stinging of the skin decreased at weeks 4 ($P = 0.022$), 8 ($P = 0.005$), and 12 ($P = 0.026$). There was also a decrease in burning at weeks 8 ($P = 0.012$) and 12 ($P = 0.050$). Finally, itching ($P = 0.040$) and tingling ($P = 0.024$) were decreased at week 12 consistent with an excellent tolerability profile.

Discussion

This study examined skin attributes in a population of type II diabetic female subjects and their response to a topical product designed to meet the unique needs of diabetic skin. Diabetes is an interesting disease model to study as abnormal glucose homeostasis triggers abnormalities in virtually every organ, in part due to protein glycation. One of the more commonly used tests to monitor the severity of diabetes is the hemoglobin A1c level, which is an early glycation end product that is reversible. However, further molecular rearrangements resulting from oxidation produce AGEs, which are not reversible.¹⁸ Irreversible AGEs include carboxymethyl-lysine and pentosidine linked to polypeptides.¹⁹ Pentosidine produces cross-link-induced increased tissue rigidity affecting the mechanical properties of skin.^{20,21} This altered extracellular matrix is less susceptible to hydrolytic turnover resulting in the accumulation of structurally inadequate matrix molecules.²² In addition, intracellular glycooxidation occurs in the presence of elevated serum glucose levels, which alter the properties of critical growth factors, such as fibroblast growth factor.²³ AGEs diminish vascular barrier function, enhance vascular cell adhesion molecule-1 (VCAM-1), quench nitric oxide and alter the balance of cellular coagulant properties.²⁴ These changes account for the accelerated aging and poor wound healing that occur in diabetic patients.

The presence of AGEs in the skin of the type II diabetic study population was verified by measuring the auto-fluorescence of the skin when excited by UVA radiation.

The collagen cross-links were measured by fluorescence at 440 nm upon excitation by 370 nm.²⁵ The subjects that were selected possessed above-average AGE scores due to the presence of type II diabetes.

These diabetic subjects were evaluated for their skin attributes following 12 weeks of applying the study product containing blueberry extract and C-xyloside. The blueberry extract functioned as an AGE inhibitor and C-xyloside functioned to stimulate GAG synthesis. Efficacy was evaluated using several parameters to more fully understand the effect of the topical product on diabetic skin.

A trend toward increased skin flexibility/elasticity was demonstrated, but the 12-week study needed to be extended with a larger sample size to determine if statistical significance could be achieved. This could be attributed to increased GAG synthesis and increased skin water content as demonstrated by a statistically significant increase in skin caliper measurements and corneometry by week 12. These findings were visually verified by the investigator who noted improvement in skin roughness, radiance, fine lines, creping, firmness and overall appearance.

The findings of this study were aimed at more fully understanding the anti-aging benefits of a topical product targeted for a diabetic population. Prior to this time, it had been assumed that products could be developed for general skin use, making designations for skin needs by classifying individuals as dry, normal or oily skin. With the increasing prevalence of diabetes type II in the US population, more research should be aimed at maximizing the skin performance and appearance of skin in these individuals characterized by premature protein glycation.

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