Received: 18 August 2021 | Accepted: 24 September 2021

DOI: 10.1111/jocd.14527

JCD Journal of Cosmetic Dermatology WILEY

ORIGINAL ARTICLE

Effects of golden tomato extract on skin appearance—outlook into gene expression in cultured dermal fibroblasts and on trans-epidermal water loss and skin barrier in human subjects

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Funding information
This project was funded by Lycored Corp, USA (33 Industrial Parkway, Branchburg NJ 08876)

Abstract

Scope: Two experiments were performed to test the effects of rich tomato extract (Golden Tomato Extract, GTE) on human skin. In one experiment, the effects of this extract on gene expression in cultured human dermal fibroblasts were examined. In a second experiment, human subjects consumed the extract and trans-epidermal water loss (TEWL), and aspects of skin appearance were monitored.

Methods and results: Primary human dermal fibroblasts in culture were treated with the extract. After six hours, RNA was extracted, and gene expression was examined using Affymetrix Human Clariom D array processing. For the clinical study, 65 human subjects consumed a capsule once a day for 16 weeks, and various skin parameters were assessed at predetermined time intervals. Among the genes upregulated by GTE are genes that augment innate immunity, enhance DNA repair, and the ability to detoxify xenobiotics. GTE significantly reduced TEWL in subjects who had high TEWL at baseline, but it had no effect on TEWL in subjects who had lower TEWL at baseline.

Conclusions: Golden tomato extract may provide benefits to the skin by enhancing innate immunity and other defense mechanisms in the dermis and by providing antioxidants to the skin surface to optimize TEWL and the appearance of the skin.

KEYWORDS
antioxidant, fibroblasts, olfactory receptors, phytoene & phytofluene, transepidermal water loss (TEWL)

1 | INTRODUCTION

The tomato (*Solanum lycopersicum*) is a fruit that is eaten throughout the world. Tomatoes are rich in nutrients including vitamin C, potassium, essential amino acids, and various antioxidants. Tomatoes are the major dietary source of the potent antioxidant lycopene. Tomato consumption was demonstrated to provide protection against oxidative stress-related diseases including cancer and cardiovascular disease.[1,2]

The use of dietary supplements has been increasing worldwide. A survey of consumers in the United States covering 2015–2019 revealed upward trends in the use of various supplements.[3] Among the most used supplements are, in order of decreasing use: fish oil, glucosamine/chondroitin, probiotics/prebiotics, melatonin, coenzyme Q10, echinacea, cranberry pills, garlic extract, ginseng, and gingko biloba. Some of these supplements have demonstrated health benefits, while the effects of others are controversial.

[Correction added on November 8, 2021, after first online publication: The affiliation for Philip W. Wertz has been updated.]

Lumenato Supplement (Trade name: Lumenato); Oleoresin obtained from yellow tomato pulp. The supplement contains a mix of tomato carotenes predominantly phytoene and phytofluene, zeta carotene, and other naturally occurring tomato phytonutrients. In general, the carotenoids and tocopherols are antioxidants that provide numerous health benefits.[4-8]

Experiments conducted in the past two decades to study potential effects of oral intake of carotenoids on skin focused mainly on the effect of β-carotene and lycopene with scarce information on phytoene and phytofluene.[9,10] Specific benefits of lycopene and/or β-carotene include protection against UV-induced erythema and sun damage,[11-13] reduced oxidative stress,[14] and antioxidant/anti-inflammatory activities.[15] β-Carotene is, of course, the precursor of vitamin A and is known to supports collagen synthesis.[16,17] Lycopene has been shown to protect against UV damage to cultured human dermal fibroblasts.[4-6] In addition, it has been shown to suppress chemically induced skin cancer in an animal model.[7] This and its ability to ameliorate DNA damage induced by 4-hydroxyestradiol are thought to be attributable to its antioxidant properties.[8] In addition to antioxidant activity, phytoene and phytofluene are colorless carotenoids that absorb ultraviolet light.[18] This property could provide an additional level of protection to the skin.

The human skin is exposed to various sources of oxidative stress including ultraviolet light, ozone, and other air pollutants and metabolism of resident microorganisms.[19-21] These stressors accelerate skin aging resulting in wrinkles, reduced elasticity, and a rough texture.[22]

Fibroblasts are the most abundant cells in the dermis. They synthesize collagen and maintain the extracellular matrix.[23] During aging, collagen fibrils are progressively lost leading to wrinkling.[24] Fibroblasts play significant roles in innate immunity of the skin by producing cytokines to attract leukocytes to the dermis.[25] Fibroblasts also communicate with melanocytes in the basal layer of the epidermis to influence melanin synthesis.[26] Keratinocytes are the main cell type in the epidermis. They differentiate to produce a stratum corneum consisting of flattened, keratin-filled cells embedded in a lipid matrix.[27] The stratum corneum provides the permeability barrier of the skin. It serves to limit loss of water and electrolytes. The lipids of the stratum corneum consist mainly of ceramides, cholesterol, and fatty acids. One of the ceramides, the acylceramide, is quite unusual in that it contains 30- through 34-carbon long ω-hydroxyacids amide-linked to a long-chain base with linoleic acid ester-linked to the ω-hydroxyl group.[28] This linoleate-containing lipid is essential for the organization of the lipids and hence critical for barrier function.[29-31]

The purpose of the present study was to determine whether consumption of the GTE provides benefits to the skin. Toward this end, the effects of GTE on gene expression by dermal fibroblasts were examined using a microarray analysis. In a clinical study, human subjects consumed GTE over a sixteen-week period, TEWL and facial photography was monitored.

2 | MATERIALS AND METHODS

2.1 | Materials

The GTE extract, Trade name, Lumenato™ was provided by Lycored Corp (Branchburg NJ). Tetrahydrofuran (THF) was purchased from Sigma Chemical Company (St. Louis MO). Primary human dermal fibroblasts derived from the foreskin of a single donor, DMEM, fetal bovine serum, and the XTT cell viability kit were purchased from Thermo Fisher Scientific (Branchburg). The Qiagen RNeasy kit for RNA extraction was purchased from Qiagen (Germantown)S. A 0.1% (w/v) stock solution of dried tomato extract in THF was prepared for use in the gene expression experiment. GTE (110 mg) in soft gel capsules was used for the clinical study. The control capsules for this study contained paraffin oil instead of GTE.

2.2 | Gene expression

The effects of GTE extract at two concentrations: 0.001% and 0.0005% on gene expression in cultured human primary dermal fibroblasts were assessed using a microarray analysis.

The experiment included the following steps:

2.3 | Cytotoxicity assay

Cytotoxicity was evaluated using the XTT Cell Viability Assay (Thermo Fisher Scientific) according to manufacturer's instructions. Fibroblasts were seeded in 96-well plate and cultured overnight. The cells were incubated for 24 h in the presence of the compounds or controls and then re-incubated in the cell culture medium for additional 24 h. Untreated cells were used as a negative control. All treatments were performed in triplicate. Inhibition of viability of more than 20% of the control values was considered cytotoxic.

2.4 | Treatments of human dermal fibroblasts

Human dermal fibroblasts were cultured in 6-well plates for 24 h (Thermo Fisher Scientific/Nunc) and then treated with THF or GTE for 6 h. After incubation, cells were washed in PBS and lysates were prepared for total RNA extraction using a Qiagen RNeasy kit according to manufacturer's instructions. RNA concentration and purity were determined using a Nanodrop IMPLEN spectrophotometer (Westlake Village).

2.5 | Quality control of total RNA

RNAs isolated from dermal tissues were shipped to Advanced BioMedical Laboratories (Cinnaminson) on dry ice for processing.

The overall integrity of total RNA samples and RNA quality was confirmed. A proprietary algorithm that takes several QC parameters into account (eg, 28S/18S peak area ratios and unexpected peaks in the 5S region) was used to calculate the RNA integrity numbers (RIN). A RIN number of 10 indicates perfect RNA quality; a RIN number of 1 indicates degraded RNA. According to published data and our own experience, RNA with a RIN number >8 is of sufficient quality for gene expression profiling experiments. RIN number for all RNA samples was >8. Microarray analysis was performed using Affymetrix Human Clariom D array processing.

2.6 | Statistical analysis of gene expression data

The differential gene expression was obtained using a threshold of 0.05 for statistical significance (p value) and a log fold change of expression with absolute value of at least 0.6. The fold increase or decrease of the ratio gene expression in the treated cells to the control cells is asymmetric around zero. To avoid this problem, the up- or downregulation is usually expressed as the base 2 logarithm of the ratio. Therefore, an absolute value of 0.6 represents a 1.5-fold up- or downregulation. A value of 1 represents a twofold up- or downregulation.

2.7 | Human subjects

One hundred fourteen Japanese women aged 35 through 60 years old were screened to find 66 eligible subjects. The eligible subjects were all of Fitzpatrick skin type II or III and Glogau skin classification type II. Other inclusion criteria included ability to understand the study and willingness to comply with avoiding UV exposure and not taking supplements that could interfere with the study. Of the 63 subjects who completed the study, two had violated protocol, and so their data were not analyzed. This study was conducted in accord with the Declaration of Helsinki and was approved. All subjects gave written informed consent before the start of the study. Subjects were to make clinic visits at baseline, 4 weeks, 8 weeks, 12 weeks, and 16 weeks for photography, subjective assessments, and instrumental measurements. The 12-week clinical visit was canceled due to the COVID-19 pandemic. Subjects were to take one GTE capsule per day with food while maintaining their usual diet.

2.8 | Measurement of TEWL

Trans-epidermal water loss was measured under standard conditions at the clinic visits. A VapoMeter (Delfin Instruments, Tokyo, Japan) was used. The measurements were made on the left cheek on an area from the outer corner of the eye and on a plane with the small pointed eminence of the external ear (tragus). Prior to taking the measurement, subjects were acclimatized in an environmental room at 21°C and 50% relative humidity for 20 minutes. All skin measurements were taken by the same person. The skin measurement position was measured with a ruler in order to have the same position at each testing point.

2.9 | Photography and photography analysis

Subjects' photographs were obtained with the use of VISIA Evolution photography device.

Parameters of VISIA were obtained with using various VISIA modalities as elaborated below:

Subject positioning was critical and had to be repeated at each time point. Items such as stool height and careful placement of the subject's chin and forehead into the imaging device were maintained. Also, the subject's hair was off their face, jewelry removed, and a black drape used to standardize clothing. Subjects' front, left, and right views were captured with their eyes gently closed.

The images were further analyzed by Canfield Scientific (Canfield Scientific) to extract and report numerical values corresponding to various changes in the images as described below.

3 | RESULTS

The cytotoxicity assay revealed no toxicity of the tested compounds at the tested concentration. In the present study, 97 genes were significantly upregulated by treatment of human dermal fibroblasts with 0.001% GTE for 6 h. Of these, there was no readily available information relating the gene to skin for 55 genes. Many of these were pseudogenes, and seven were olfactory receptor genes. Fifty-eight genes were significantly downregulated, and there was no readily available information related to skin for 36 of these. With 0.0005% GTE, 121 genes were upregulated, and 44 genes were downregulated. There was some overlap with the findings with 0.001% GTE, but also some differences. Notably, twelve additional olfactory receptor genes were identified. The main genes to be discussed are listed in Table 1, and the olfactory receptor genes are listed in Table 2.

3.1 | Effect of GTE on TEWL

In the clinical study, 32 subjects had relatively high TEWL (value of ≥12.5 g/h/m^2) at baseline, while 29 subjects had lower TEWL values. The data from these two groups were analyzed separately. As shown in Figure 1, GTE supplementation had no effect on TEWL when analyzing for the entire study population. However, in the high TEWL cohort, GTE supplementation significantly reduced TEWL by 16 weeks.

The error bars represent one-half standard deviation with A and C going up and B and D going down.

TABLE 1 Gene of interest: Concentration a = 0.001% GTE. Concentration b = 0.0005% GTE

Gene	Level of up- or downregulation	*p* value	Concentration
IL6	0.860	0.002	a
CCL2	0.809	1.732e−4	a
CXCL1	0.682	8.31e−4	a
TNFPI3	0.689	0.002	a
DGAT2L6	0.658	0.026	a
ALOX5	0.788	0.014	a
BACH2	0.662	0.006	a
CYP4F11	0.736	8.498e−4	a
CYP4A22	0.689	0.015	b
B3GALT1	−0.756	0.003	a
B3GALT2	0.861	0.002	b

Note: IL6 and CCL2 were also significantly upregulated by 0.0005% GTE. The genes are listed from top to bottom in the order in which they will be discussed.

TABLE 2 Olfactory receptor genes up- or downregulated by GTE

Gene	Level of up- or downregulation	*p* value
OR52N4	0.984	0.002
OR5D15P	0.789	0.031
OR52B6	0.734	0.022
OR1F1	0.683	0.013
OR2L3	0.660	0.003
OR2V2	0.656	0.019
OR51AB1P	0.650	0.030
OR1B1	0.757	0.006
OR4C12	0.725	0.001
OR6C76	0.692	0.012
OR2AH1P	0.665	0.003
OR7A18P	0.660	0.001
OR1N2	0.626	0.030
OR10B1P	0.607	0.036
OR11L1	0.604	0.010
OR5BA1P	−0.615	0.005
OR5M11	−0.626	0.049
OR9A4	−0.654	0.002
OR4U1P	−0.727	0.002

Calculation of the individual relative change in TEWL for the group that exhibited the significant change ranged between 21 and 53% improvement from baseline. The VISIA photographs of these panelists that were further analyzed by Canfield Scientific pointed toward changes in skin appearance: redness, pores, and texture. Selected photographs are in a separate file of Supporting information for this manuscript.

4 | DISCUSSION

Topical products that address various skin ailments and disorders have been in use since ancient times. In recent decades, with the acknowledgment that the skin is nourished from the blood and with the belief that health equates beauty, the cosmetic and personal care industries are experiencing growth in nutritional supplements that claim improvement in skin appearance of various properties such as wrinkles, age spots, and glow. Taken together, the in vitro and in vivo studies for the evaluation of the effect of GTE on skin indicate that GTE affects the skin barrier and integrity in two complementary modalities: biological (innate immunity) and physical (the stratum corneum barrier). An illustration describing these two modalities is sketched in Figure 2 below.

A number of the upregulated genes appear to enhance dermal defensive mechanisms. These mechanisms include enhancement of innate immunity by recruitment of phagocytic cells and stimulated production of antimicrobial fatty acids, enhanced DNA repair, and enhanced detoxification of xenobiotics.

IL-17 expression was not upregulated after 6 h of GTE treatment. Presumably, it was upregulated at an earlier time, leading to upregulation of some of the genes in the IL-17 pathway at 6 hours. These include *IL-6*, *CCL2*, and *CXCL1*. *CCL2* and *CXCL1* are chemokine genes, which lead to attraction of neutrophiles and monocytes.[32,33] IL-17 and IL-6 act synergistically to increase production of cell survival molecules under conditions of stress.[34] Likewise, TNF-α is not upregulated at 6 h, but *TNFIP3* (TNF α-induced protein 3) is upregulated indicating the *TNF* gene was upregulated earlier. Although under some circumstance TNF-α has been shown to inhibit collagen synthesis,[35] under other circumstances TNF-alpha stimulates collagen synthesis by dermal fibroblasts,[36] which could inhibit formation of or possibly ameliorate wrinkles. TNFIP3 is a deubiquinating enzyme. It removes ubiquitin from cadherin to prevent degradation of cadherin.[37] Collectively, these observations indicate that GTE consumption activates both the IL-17 pathway and TNF-α pathway in dermal fibroblasts leading to enhanced antimicrobial defense. These two pathways act synergistically in activating antimicrobial defenses. It should be noted that when introduced to stressed cells (either by UVB or other oxidative stress) carotenoids are acting as anti-inflammatory agents by reducing the expression of anti-inflammatory cytokines.[38,39] This may be due to an adaptation to the environment. Carotenoids will protect from inflammation when stress occurs, but they may induce subclinical inflammation when the tissue is not stressed.

The gene product of *DGAT2L6* increases sebum production.[40] This could make lauric acid and sapienic acid more available at the skin surface. These fatty acids are antimicrobials.[41]

Upregulated *ALOX 5* protein acts upon arachidonic acid to produce 5-hydroperoxyeicosatetraenoic acid, which can then be converted to 5-hydroxyeicosatetraenoic (5-HETE) acid or to leukotriene A4 (LTA4). LTA4 is rapidly converted to leukotriene B4 (LTB4). 5-Hydroxyeicosatetraenoic acid can be oxidized to 5-oxoeicosatetraenoic acid. LTB4, 5-HETE, and 5-oxoeicosatetrenoic

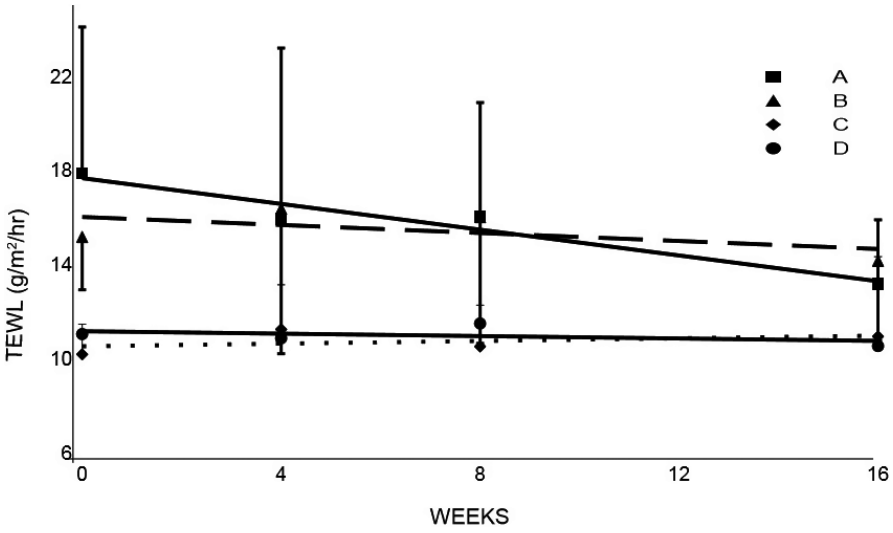

FIGURE 1 Effect of GTE on TEWL. (A) High TEWL-GTE (N = 18, One subject missed the 8-week clinic visit, 4 weeks $p = 0.428$, 8 weeks $p = 0.11$, 18 weeks $p < 0.001$). (B) High TEWL-Placebo (N = 14, One subject missed the 8-week clinic visit.4-week $p = 0.382$, 8-week $p = 0.506$, 16-week $p = 0.293$). (C) Low TEWL-GTE (N = 14, 4-week $p = 0.129$, 8-week $p = 0.49$, 16-week $p = 0.421$). (D) Low TEWL-Placebo (N = 15, One subject missed the 8- and 16-week clinic visits. 4-week $p = 0.099$, 8-week $p = 0.838$, 16-week $p = 0.231$

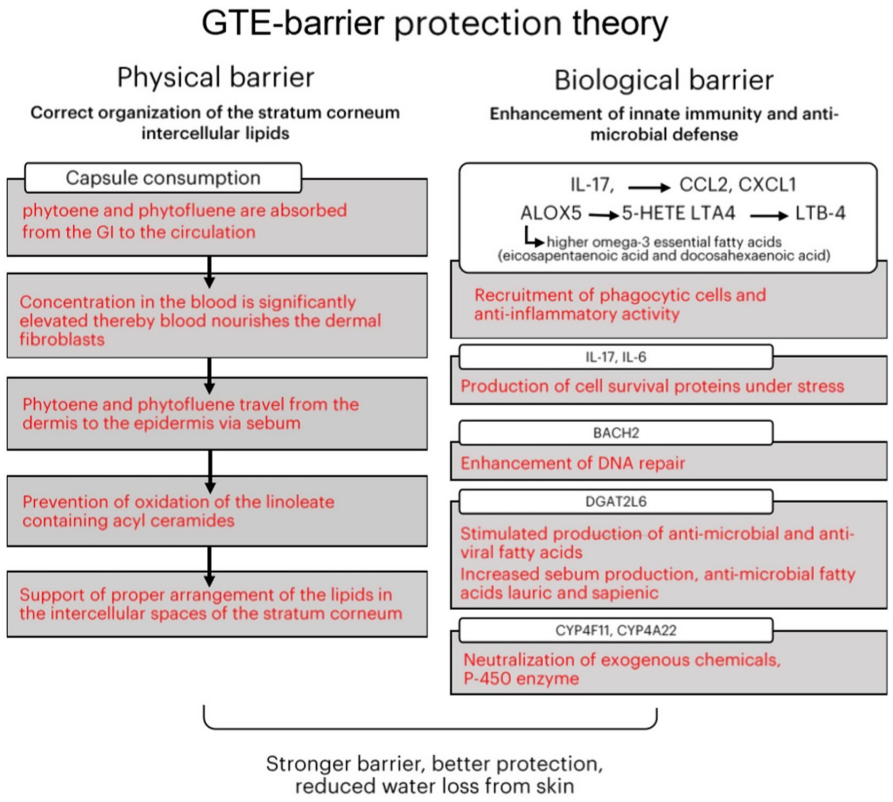

FIGURE 2 Illustration describing the theory of the two mechanisms by which GTE strengthens the physical and the biological barrier of the skin

acid act as chemotactic factors to attract neutrophils and monocytes, thereby enhancing innate immunity.[42-44] ALOX5 can also act on the higher omega-3 essential fatty acids (eicosapentaenoic acid and docosahexaenoic acid), and some of these products may be anti-inflammatory.[45]

BACH2 was significantly upregulated. The product of this gene is a marker of aging and DNA damage.[46] It has been implicated in B-cell differentiation and homeostasis of the immune system.

CYP4F11 codes for a P450 enzyme that could be protective against exogenous chemicals.[47] This P450 has been shown to hydroxylate a number of drugs.[48] Interestingly, while expression of *CYP4F11* was not significantly affected by treatment with 0.0005% GTE extract, a different P450 gene, *CYP4A22*, was upregulated at this lower concentration. This enzyme has been shown to ω-hydroxylate lauric and myristic acid and to be capable of hydroxylating a variety of aliphatic and aromatic substrates.[49]

A secondary but extraordinary finding of the present study was the upregulation of a series of genes coding for olfactory receptors. Olfactory receptors are G-protein coupled receptors. They have been found in a range of cell types other than olfactory neurons, including epidermal keratinocytes and melanocytes.[50] In one study, an olfactory receptor (OR2AT4) that responds to a synthetic

sandalwood odorant was identified in keratinocytes.[51] The interaction of the odorant with the receptor resulted in calcium uptake. A later study examined the role of this receptor in epithelial cells of the human hair shaft.[52] In this case, activation of the receptor resulted in prolonged hair growth *ex vivo*. In another study, two other olfactory receptors were identified in keratinocytes.[53] OR2A4/7 responded to cyclohexylsalicylate, and OR51B5 responded to isononyl alcohol. Activation of either of these olfactory receptors resulted in increased intracellular calcium. Olfactory receptors have also been detected in melanocytes.[54] This receptor, OR51E2, responds to β-ionone resulting in increased intracellular calcium and stimulated melanin synthesis. β-ionone is the odorous compound from violets. It is formed from carotenoids such as β-carotene by eccentric cleavage with the BCO2 enzyme (beta-carotene 9′,10′-oxygenase). Although olfactory receptors have been reported in 3T3 cells, an embryonic fibroblast-derived cell line, the present study may be the first to identify numerous olfactory receptor genes in human dermal fibroblasts indicating a role of such genes in skin. The study suggests that GTE regulates the expression of these genes.

It was also noteworthy that two galactosyltransferase genes were affected by GTE. One of these, *B3GALT1*, was significantly downregulated, while the other, *B3GALT2*, was upregulated.

Both β-1,3-galactosyltransferases transfer a galactosyl residue from UDP-galactose to a terminal N-acetylglucosamine of a glycoprotein or ganglioside.[55,56] The product of *B3GALT2* can also transfer galactose to a terminal galactose, but the affinity for this substrate is much lower.[56] These alterations in galactosyl transferase activities will alter the carbohydrate presentation at the cell surface. Consequences of this modification are currently unknown.

As noted previously, the lipids of the stratum corneum consist mainly of ceramides, cholesterol, and fatty acids. The fatty acids are mostly 20- through 28-carbons long saturated species. Most of the ceramides also have few readily oxidizable functional groups. These lipids are ideal for resisting oxidative damage. The one exception is the linoleate-containing acylceramide.[28] This lipid is readily oxidized on exposure to air if not protected by an antioxidant. Linoleic acid is essential for the barrier function of the skin.[57,58] Linoleic acid from the circulation is taken up by the epidermis and is initially incorporated into a small pool of triglycerides.[59] It is rapidly transferred to phosphoglycerides and then to an acylglucosylceramide.[60-62] This is the glucosylated precursor of the linoleate-containing acylceramide.[28] The glucose β-glycosidically is attached to the primary hydroxyl group of the long-chain base. This linoleate-containing acylglucosylceramide is associated with lamellar granules.[63] In the late stages of the keratinization process, the contents of the lamellar granules are extruded into the intercellular space, and glucosylceramides are deglycosylated to produce ceramides, including the linoleate-containing acylceramide.[28] The linoleate-containing acylceramide is essential for barrier function.[29-31] Isolated linoleate-containing acylceramide is rapidly oxidized on exposure to air. Under optimal conditions, alpha-tocopherol is delivered to the skin surface via sebaceous secretion, and this protects lipids and proteins from oxidation.[64,65] Other antioxidants are also delivered in sebum; however, alpha-tocopherol is the major one under most circumstances. Under conditions of oxidative stress, such as UV light exposure, squalene, cholesterol, and keratins have been shown to become oxidized, and the oxidized keratins were shown to be in the outer stratum corneum.[19,66] It seems likely that under such compromised conditions, the linoleate-containing acylceramide would also become oxidized. This would compromise the barrier function of the skin and lead to elevated TEWL. This could account for the subjects with higher TEWL at baseline. Although some carotenoids diffuse from the dermis into the epidermis, a major portion of the carotenoids in the dermis are delivered to the skin surface via sebum secretion and/or sweating.[19,66] This could provide additional protection to the acylceramide leading to restoration of barrier function with reduced TEWL. The present results from Figure 1 show that after 16 weeks of GTE supplementation the TEWL subjects have TEWL values lower than the placebo controls. If the linear trend was to continue, an additional 10 weeks of supplementation would have reduced TEWL to the same range as the low-TEWL group.

The stratum corneum lipids are organized into multilamellar structures in the intercellular spaces of the stratum corneum with a major periodicity of 13 nm that is evident in transmission electron micrographs[67] and from X-ray diffraction data.[68] This 13 nm periodicity is observed with reconstituted stratum corneum lipids; however, if the acylceramide is omitted this periodicity is not seen by either electron microscopy or X-ray scattering.[29,69] Mutations of enzymes that are uniquely involved in acylceramide synthesis result in various autosomal recessive congenital ichthyoses with impaired barrier function.[70] These genes include *ELOV4*, *CYP4F22*, *PNPLA1*, and *ABHD5*. The product of *ELOV4* is a fatty acid elongase, which elongates from C26-CoA to C30-CoA and beyond.[71] *CYP4F22* codes for the P450 that ω-hydroxylates the very long fatty acids.[72] The product of *PNPLA1* transfers linoleate to the ω-hydroxyl group,[73] and the product of *ABHD5* is a coactivator of PNPLA1.[74] In addition, in essential fatty acid deficiency, oleate replaces linoleate in the acylceramide and TEWL increases to at least fivefold.[75] In Gaucher disease, the β-glucocerebrosidase that deglycosylates acylglucosylceramide to produce acylceramide is defective. In this situation, the ultrastructure of the intercellular spaces of the stratum is altered, and the TEWL is markedly elevated.[76] These observations reinforce the concept that the linoleate-containing acylceramide is essential for normal barrier function.

Astaxanthin is a carotenoid found in salmon, salmon roe, and some shrimp,[77] and it has been reported to have benefits for skin health and homeostasis.[77] In one randomized placebo-controlled study involving 36 male subjects, after 6 weeks of consuming 6 mg of astaxanthin per day TEWL was significantly reduced in the astaxanthin group compared to controls.[78] Astaxanthin was also reported to lead to improvements in minor wrinkles, age spot size, hydration, and elasticity. In another study, astaxanthin was shown to increase the minimal erythema dose of UV after 9 weeks of supplementation at 4 mg/day.[79] When TEWL was measured at the site of irradiation, it was increased at both the control and astaxanthin group, but the increase was significantly less in the astaxanthin group.

A number of studies have shown that when TEWL is reduced by various interventions hydration[80-84] and skin surface smoothness[80] increased, and scaliness[84] and pore area[80] decreased. Likewise, in studies in which the skin was irritated by topical application of chemicals[85] or by using harsh soap[84] TEWL and erythema both increased. Thus, TEWL correlates with a number of skin appearance parameters. In the present study, correlation was found between reduction of TEWL and various attributes in skin's appearance that were captured by VISIA photography and qualitatively and quantitatively analyzed (see Supporting information file attached to this manuscript). Specifically, reduction in skin redness and skin pores and overall improvement in skin texture (reduction in roughness) and appearance has been demonstrated to accompany a reduction in TEWL. Skin redness is a sign of inflammation that may be subclinical levels of an underlying disease, a manifestation of primary irritation or an allergic reaction. Since carotenoids are known anti-inflammatory and antioxidant agents, the attenuation of skin redness may be explained by such activity, but the fact that the reduction in skin redness was strongly associated with reduction in TEWL points toward a potentially more complex multi-functional path. Moreover, the skin barrier is known to maintain a relatively stubborn homeostasis, even if such is abnormal. This means, for example, that when challenged by a detergent, abnormal pH, or other irritating conditions, in most cases it will bounce back to its baseline within hours to days.

Subjects from the high TEWL cohort who received the GTE supplement for 16 weeks showed various improvements in facial features including a smoother appearance with fewer pores and reduced redness (Figures S1-S5).

In summary, the studies presented in this publication, reveal a unique effect of GTE on skin at the dermal and epidermal levels. These carotenoids and tocopherols strengthen the skin barrier and equip it with tools to cope with environmental insult. Moreover, we demonstrated a correlation between barrier strength to specific skin appearance-related benefits such as reduction in redness and in pores and skin smoothening effect. We are also excited to report, for the first time, the existence of olfactory receptors in human dermal fibroblasts and their significant up regulation by GTE. While additional studies are required to further substantiate the barrier improvement claims as well as the role and function of the olfactory receptors, we believe that these studies are key in paving a path to biological modalities that are novel.

CONFLICT OF INTEREST

Elizabeth Tarshish served as a employee of Lycored Ltd. Karin Hermoni acted as a employee of Lycored CORP. Yoav Sharoni provided consulting fee that was paid by Lycored. Philip W. Wertz provided consulting fee that was paid by Lycored. Nava Dayan provided consulting fee that was paid by Lycored.

AUTHOR CONTRIBUTIONS

Elizabeth Tarshish and Karin Hermoni involved in conception and design of the work and final approval of the version to be published. Yoav Sharoni involved in final approval of the version to be published. Philip W. Wertz involved in data analysis and interpretation, and critical revision of the article. Nava Dayan involved in data analysis and interpretation, drafting the article, and critical revision of the article.

ETHICAL STATEMENT AND PATIENT CONSENT

This study was conducted in accord with the Declaration of Helsinki and was approved by Aisei Hospital Ueno Clinical Research Ethics Committee of Japan (IRB # 12000071). All participants have read and signed informed consent.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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How to cite this article: Tarshish E, Hermoni K, Sharoni Y, Wertz PW, Dayan N. Effects of golden tomato extract on skin appearance—outlook into gene expression in cultured dermal fibroblasts and on trans-epidermal water loss and skin barrier in human subjects. *J Cosmet Dermatol*. 2022;21:3022–3030. https://doi.org/10.1111/jocd.14527