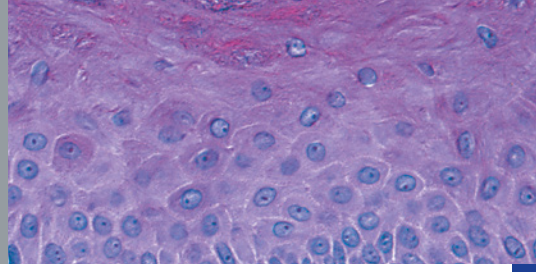
# The Science of Melanogenesis



## A Review of Novel Substances That Can Restrict Excess Melanin Production and Accelerate Cell Turnover

## Summary


Hyperpigmentation disorders may occur when skin cells produce excessive amounts of melanin. Additionally, abnormal skin surface cell turnover rates can cause skin to retain excess melanin for prolonged time periods. Several substances have been discovered that have the ability to restrict excess melanin production as well as accelerate cell turnover in patients with pigmentation disorders.

When combined together, these agents can both interrupt melanogenesis at multiple points in the biochemical pathway, as well as help mitigate the appearance of accumulated melanin at the skin surface, leading to lighter and brighter skin.

***Stachys officinalis***, a botanical extract, has antioxidant and anti-inflammatory properties both pre-clinically and clinically. In particular, *Stachys* resolves papules in acne patients by 30% and pustules by 50% after 15 days of treatment.

**N-acetyl glucosamine** (NAG) lightens skin by inhibiting the glycosylation of tyrosinase. NAG (3%) applied topically to skin equivalent cell cultures significantly inhibits melanin content in a dose-dependent manner. Clinically, treatment with a 4% niacinamide plus 2% NAG combination for 8 weeks was significantly more effective at reducing hyperpigmentation than niacinamide alone.

**Glutathione** has antioxidant properties that eliminate free radicals and inactivates tyrosinase. In a split-face trial, glutathione treatment inhibited melanin production significantly more than control.

**Soybean extracts** act as antioxidants and inhibit tyrosine kinase. In a 12-week clinical study, mottled hyperpigmentation improved at least 1 grade in 90% of the soy treated group, compared to 53% in the vehicle group ($P \leq 0.05$). Soy extracts have been proven in vivo to modulate the protease activated receptor pathway (PAR-2) to reduce melanosome pigment transfer for skin-lightening effects.

**Retinol** initiates accelerated epidermal renewal and cell turnover rate to help reverse the sign of accumulated melanin at the skin surface. Retinol also stimulates the skin's natural rebuilding process by supporting the expression of glycosaminoglycans, chemicals that bind to water molecules, leading to firmer skin with fewer lines and wrinkles, even in the elderly.

**Ascorbic acid** is primarily known for its antioxidant ability, but it has also been shown to significantly lighten skin in patients with melasma. Ascorbic acid acts as a keratolytic agent that exfoliates surface skin cells to further reverse the signs of accumulated melanin at the skin surface.



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# Introduction

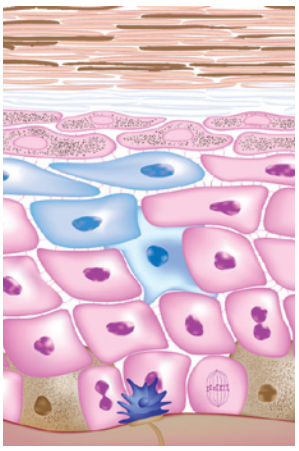
*Hydroquinone (HQ), which has been used for more than 50 years, is often described as the gold standard of skin lightening.[1-3] Despite its general effectiveness, however, some patients never achieve adequate reductions in pigmentation.[4-6]*

*HQ inhibits tyrosinase by binding histidines or copper at the active site of the enzyme.[7] The clinical effectiveness of HQ 4% in the treatment of melasma and other pigment disorders has been shown in multiple studies, both as monotherapy[4-6] and in combination with other ingredients.[8,9]*

*Based on observation of a loss of effectiveness after long-term treatment with HQ, researchers hypothesize that after constant exposure to HQ, melanocytes eventually become resistant to it, which leads to increased production of melanin (rebound pigmentation) and potential development of ochronosis.[10] However, with a pulse treatment protocol, the patient is initially treated with HQ for 4 to 6 weeks to establish a baseline level of skin lightening, followed by a 4 to 6 week interval to allow the skin to stabilize by treatment with retinol, antioxidants, and anti-inflammatory agents. With this protocol, the clinician can use HQ in rotation with other products as needed without losing efficacy and can extend the period between HQ treatments.[11]*

# Molecular Mechanisms of Normal Pigmentation

To recognize the value of products that can lighten and brighten the skin for patients with pigmentation disorders due to aging or medical conditions, an understanding of the pigmentation process at the molecular level in healthy skin is essential.



Melanocytes produce melanosomes, which are intracellular organelles that produce melanin starting from the amino acid L-tyrosine.[12,13] Tyrosine catalyzes the next two steps in melanin synthesis: 1) the hydroxylation of tyrosine to 3-(3,4-dihydroxyphenyl)-alanine (DOPA)[11]; 2) the oxidation of DOPA to dopaquinone.[14,15] The first step is crucial because the subsequent reactions that proceed automatically at physiological pH[11] include tyrosinase-related protein 1 (TYRP1) and dopachrome tautomerase (DCT), and other enzymes involved in the melanogenic pathway leading to eumelanins (that lead to brown-black skin) and pheomelanin (that lead to red-yellow pigmentation).[12]

# Molecular Mechanisms of Hyperpigmentation

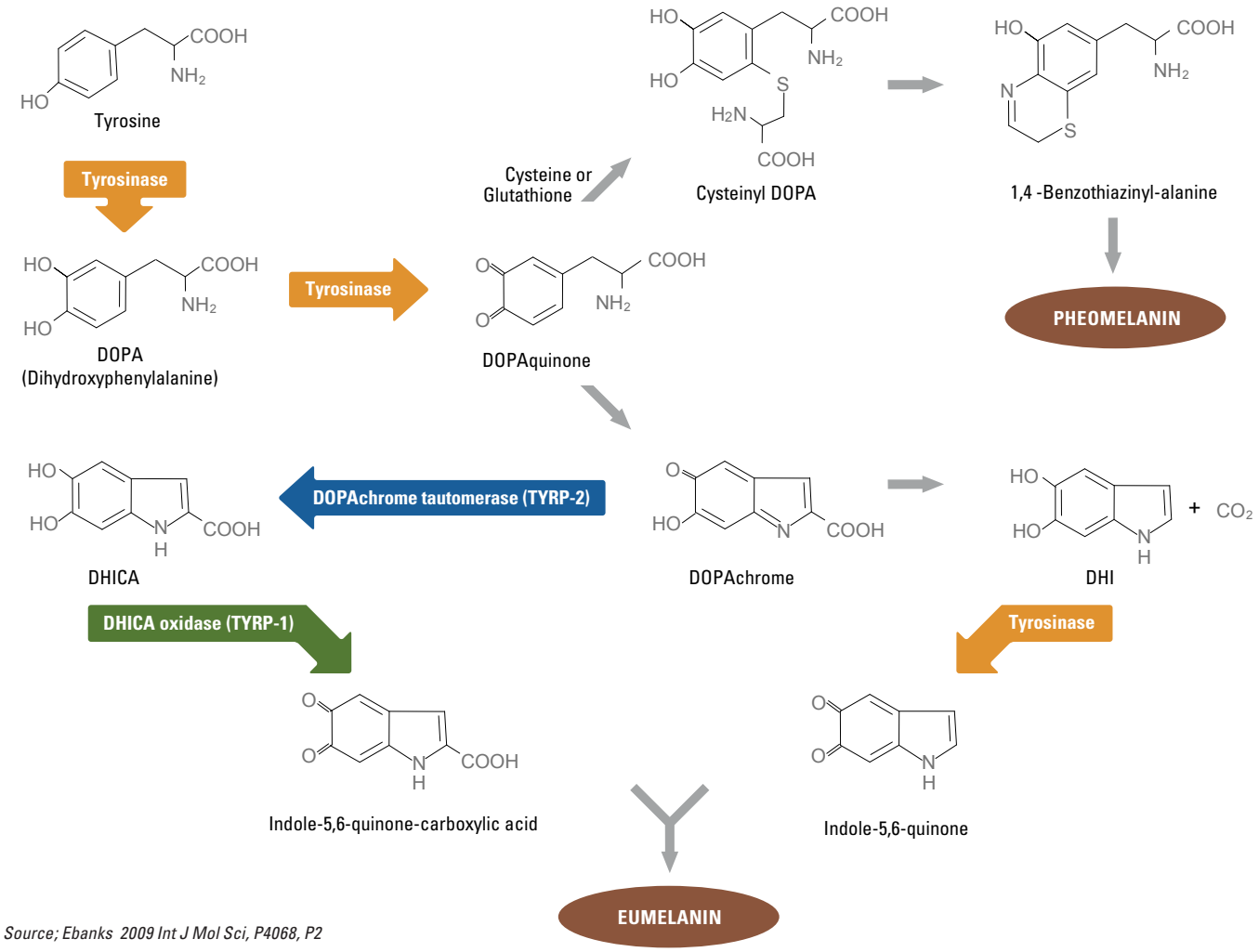
Abnormal pigmentation may develop at a number of points in the biochemical cascades that regulate healthy pigmentation. Hyperpigmentation disorders may occur when melanocytes produce too much melanin, or when epidermal cells retain too much melanin if cell turnover is abnormal.[2]

Theoretically, any substance that inhibits the catalytic function of tyrosinase could be used to cosmetically brighten the skin. However, the clinician must balance the patient's desire for lighter skin with the need to protect the skin from ultraviolet radiation. Ideally, the brightening agent should slow melanin synthesis, but not stop it entirely.[16]

Depigmenting agents can have potent effects by acting on one or more steps in the melanogenic pathway (starting with

PROCESS OF MELANOGENESIS WITHIN EPIDERMAL MELANOSOMES

*(Interference and inhibition of tyrosinase in the melanogenic pathway could help lighten the skin)*




*Source; Ebanks 2009 Int J Mol Sci, P4068, P2*

inflammation), melanosome transfer, or post-transfer pigment processing and degradation.[7]

At the start of the melanogenesis, inflammatory processes may lead to hyperpigmentation. Prostaglandins, leukotrienes, and thromboxanes all upregulate tyrosinase activity, and therefore upregulate pigmentation in response to inflammation.

By the same token, a substance that interferes with inflammatory processes, like *Stachys officinalis* (see additional details in a later section), would likely minimize pigmentation.

At the next step in the melanogenic pathway, the melanocyte begins producing tyrosine which is then further biologically converted by the tyrosinase enzyme. Any substance which upregulates this step in the cascade would increase pigmentation levels. In contrast, any substance which inhibits glycosylation of tyrosinase, such as n-acetyl glucosamine, could help lighten the skin.

Continuing in the cascade, the tyrosinase enzyme converts tyrosine into L-dihydroxyphenylalanine (L-DOPA). Any substance which inactivates tyrosinase by blocking its maturation and transfer to the premelanosomes, such as glutathione, could help lighten skin.

Next in the pathway, dopachrome is converted to 5,6 ihydroxyindole-2 carboxylic acid (DHICA). Any substance which interferes with this or subsequent steps in the pathway, like soy isoflavones, could help lighten skin.

Once melanin synthesis is complete, other substances can minimize the appearance of melanin, by dispersing the melanin granules, to appear less dense.

# Skin Lightening Agents that Capitalize on Mechanisms of Hyperpigmentation

There have been dramatic advancements in the discovery of plant enzymes' and novel agents' ability to restrict excess melanin production and accelerate cell turnover for patients with pigment disorders.

## *STACHYS OFFICINALIS*

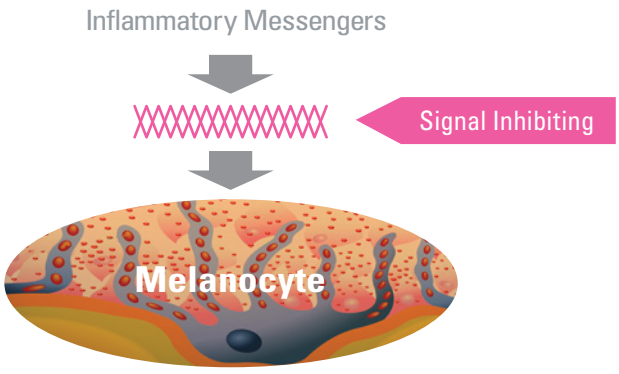
*Stachys officinalis* is the botanical name for the herb known as wood betony.[17] According to folklore dating back to antiquity, this plant has properties that make it useful when applied externally to treat wounds, and to treat parasites, digestive problems, and hangovers when taken orally.[17,18]

### Chemical Components of *Stachys Officinalis*

Many different substances can be isolated from *Stachys officinalis*, including phenylethanoid, iridoid, and diterpenoid glycosides; flavonoids; and stachydrine (a pyrrholidine alkaloid).[17,19]

### Mechanism of Action of *Stachys Officinalis*

*Stachys officinalis* acts as an inflammatory mediator that inhibits signal processing in the melanocyte in the presence of inflammatory messengers. Extracts of *S. officinalis* also have antioxidant properties.[17]




### Efficacy of *Stachys Officinalis*

In laboratory studies, extracts of *Stachys chrysanthemum* and *Stachys candida* have been shown to inhibit leukotriene C4, selectively inhibit TX-synthase enzyme, inhibit histamine release, and inhibit the secretion of tumor necrosis factor alpha and interleukin 6.[18,20,21]

Investigators evaluated the anti-inflammatory activities of aqueous extracts of the aerial parts of ten Hungarian *Stachys* species using a standard model of inflammation – carrageenan-induced edema in the rat's paw.[18] In this model, carrageenan injection causes acute inflammation, leading to a peak increase in paw thickness within 4 hours.[20] Aqueous extracts of *S. officinalis*, when administered orally, inhibited edema by 26.6%, compared to the 41.0% inhibition shown in the positive control, diclofenac sodium.[18]

Other investigators evaluated the anti-inflammatory activities of an acetone and methanol extract of the aerial parts of *Stachys byzanthina C. Koch* using the same standard model of inflammation.[20] In particular, the acetone extract inhibited carrageenan-induced edema in the rat paw in a dose-dependent fashion over the dose range 50-200 mg/kg ($P<0.0015$).[20]

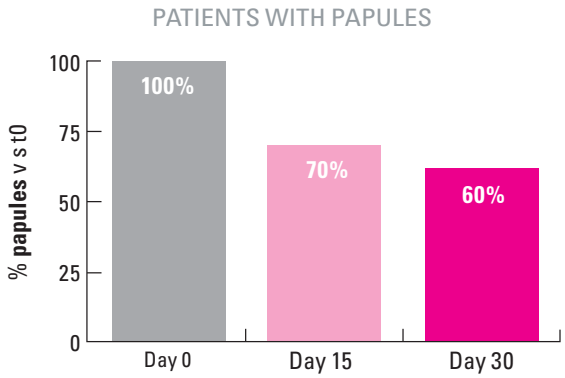
Methanol extracts of two related types of *Stachys* (*S. chrysantha and S. candida*) significantly inhibited the release of the prostanoid thromboxane B2 (TXB2) in human platelets that had been stimulated by calcium ionophore.[21] Thromboxane inhibition with the *Stachys* extracts (89.34%) was only slightly lower than that seen with the reference drug ibuprofen (96.34%).[21]

Although all of these studies involved other extracts from *Stachys* genera that were not of the species *officinalis*, and none of them involved topical administration, it is likely that *S. officinalis* extracts also exhibit anti-inflammatory characteristics. All species of *Stachys*, including *Stachys officinalis*, produce iridoids, chemicals that have shown to be at least partially responsible for the anti-inflammatory effect of *Stachys* species.[19]

#### Clinical Studies Using *Stachys*

A clinical study with 29 subjects (15 to 25 years old) with acne, subjects applied a cream with 1% of *Stachys officinalis* twice a day for 30 days. Efficacy outcome variables measured at days 15 and 30 included absolute number of acne lesions, colorimetry, skin hydration, and transepidermal water loss.[22]

Subjects' skin improved visibly, with resolution of rash and inflammation, and colorimetry demonstrating reduction of pigmentation/redness. The outcomes relevant to evaluating the anti-inflammatory mechanism of action for *Stachys* are as follows. All subjects' skin had papules at baseline. At day 15, 70% of subjects had papules, which represents a 30% reduction from baseline. The anti-papular effect increased further by day 30, at which time approximately 60% of patients had papules. All subjects' skin had pustules at baseline.




By day 15, only 50% of subjects had pustules remaining. By day 30, effectiveness had waned, with approximately 60% of subjects having pustules.[22]

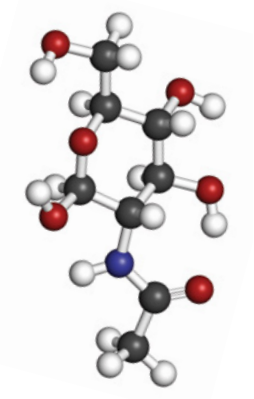
### Safety Issues With *Stachys*

Clinical safety of *Stachys* was rated as high in study cited above, with no further details supplied.

## N-ACETYL GLUCOSAMINE (NAG)

### Mechanism of Action of Glucosamine

N-acetyl glucosamine (NAG), is an amino-sugar that inhibits the glycosylation of tyrosinase, which accounts for its depigmenting effect. In addition, NAG acts as a substrate for the production of humectants that maintain hydration of the dermis, such as hyaluronic acid, heparan sulfate, and proteoglycans.[1] Investigators hypothesize that NAG and its metabolites (which include glucosamine and glucuronic acid) act as additional substrates for in situ production of hydroquinone.[23]



### Efficacy of N-Acetyl Glucosamine

#### Preclinical Studies Using Glucosamine

Glucosamine inhibits lipid carrier-dependent glycosylation of protein, which in turn inhibits pigmentation and changes the structure and function of melanogenic compartments of B16 melanoma cells.[7]

NAG in concentrations ranging from 1% to 5% applied topically to skin equivalent cell cultures induced declines in melanin content in a dose-dependent manner. In particular, the changes compared to vehicle control compared to the 3% NAG concentration were statistically significant ($P$=0.0350).[23]

In vitro experiments showed that NAG on skin cell equivalents induced upregulation of gene expression relating to protein and epidermal turnover that could contribute to the pigmentation reduction observed in the skin equivalent cell cultures.[23]

#### Clinical Studies Using N-Acetyl Glucosamine

Kimball et al conducted a randomized, double-blind, vehicle-controlled trial with 202 Caucasian female subjects (ages 40–60) had moderate to moderately severe irregular hyperpigmentation, 188 (93%) of whom completed the study.[24] Subjects in the niacinamide plus NAG group received a topical night-time cream formulation containing 4% niacinamide plus 2% NAG and a topical daytime lotion formulation which also contained 4% niacinamide plus 2% NAG, for 8 weeks.[24] Investigators calculated the spot area fraction as the ratio of total hyperpigmented area to total skin measurement area, multiplied by 100.[24] Although hyperpigmentation worsened from baseline during the study in both groups, the increases in spot area fraction were significantly smaller in the niacinamide plus NAG group at 6 and 8 weeks.[24]

In an 8-week, double-blind, split-face study among 50 Japanese female subjects (ages 25–55), topical 2% n-acetyl glucosamine was effective in improving the appearance of facial hyperpigmentation based on computer images.[23] This study reported an overall directional spot area fraction change at a combined 4 and 8 weeks ($P$= 0.089).[23] Investigators also conducted an 8-week, double-blind, placebo controlled, split-face study among 35 Caucasian female subjects (ages 35–65) with solar lentigines on both sides of the face.[23] There were 3 test formulations: a placebo control, 4% niacinamide, and 4% niacinamide plus 2% n-acetyl glucosamine.

At week 8, the combination formulation was significantly more effective at reducing hyperpigmentation spot area than was niacinamide alone ($P$= 0.017), which in turn was more effective than the control formulation.[23]

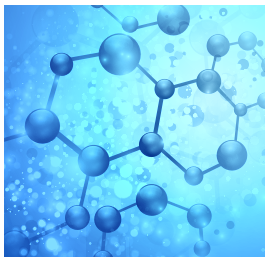
### Safety of N-Acetyl Glucosamine

N-acetyl glucosamine was well-tolerated clinically by participants in the clinical studies mentioned above, with fewer than 3.5% developing such adverse reactions as mild-to-moderate skin irritation in both studies.[23,24]

## GLUTATHIONE

### Mechanism of Action of Glutathione

Glutathione has several roles in inhibiting the pigmentation process. At the start of the cascade, it has antioxidant properties that eliminate free radicals and peroxides that initiate melanogenesis. In addition, glutathione inactivates tyrosinase in two ways: by chelating copper from the enzyme and by inhibiting glycosylation, another step that is critical for melanogenesis to progress.[7] Glutathione is also involved in the conversion of dopaquinone to pheomelanin.[7]



### Efficacy of Glutathione

#### Preclinical Studies Using Glutathione

Alena et al evaluated the ability of injected glutathione to counter depigmentation induced by N-acetyl-4-S-cysteaminylphenol (N-acetyl-4-S-CAP) in black and yellow mice after multiple intraperitoneal injections in black and yellow mice.[25]

#### Clinical Studies Using Glutathione

A randomized, double-blind, two-arm, placebo-controlled trial investigated the skin lightening effects of orally administered glutathione in 60 healthy subjects. Melanin indices decreased significantly more among glutathione-treated subjects than controls on the right side of the face ($P = 0.021$) and the sun-exposed left forearm ($P = 0.036$).[26]

### Safety of Glutathione

Glutathione was well-tolerated over 4 weeks in the study cited above, but long-term safety needs to be researched in a larger sample.[26]

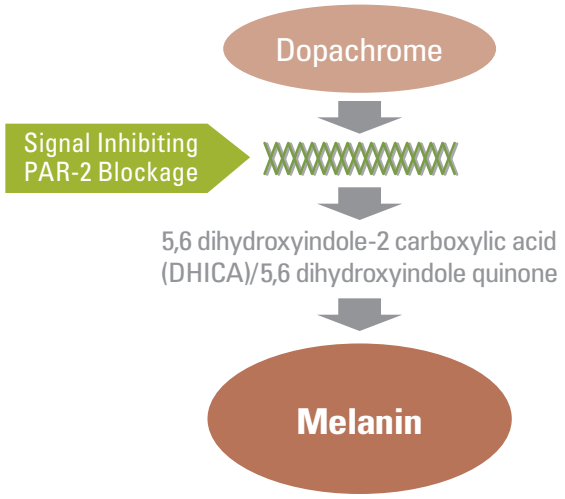
## SOY ISOFLAVONES

### Mechanism of Action of Soy Isoflavones

Soybeans contain many components that are biologically active in the skin, such as antioxidants (isoflavones like genistein) and small proteinase inhibitors including soybean trypsin inhibitor (STI) and Bowman-Birk protease inhibitor (BBI). Topically applied genistein is readily absorbed into the viable human epidermis.[27] Its main role in skin pigmentation is to inhibit tyrosine kinase. In its chemically reduced form it also acts as an antioxidant by scavenging reactive oxygen species.[27]

Protease-activated receptor 2 (PAR-2) cell receptors are found on the surface of keratinocytes but not on melanocytes.[28] PAR-2 mediates the transfer of melanosomes from melanocytes to the surrounding keratinocytes making up the epidermal melanin unit.[29] Soybean trypsin inhibitor (which affects proteolysis) and Bowman-Birk inhibitor (which interferes with chymotrypsin), inhibit the activation of PAR-2 cell receptors. This in turn reduces keratinocytes ability to phagocytize melanosomes thus inhibiting melanosome transfer, which leads to a reversible depigmentation.[7,30] PAR-2 receptors are involved in the development and regulation of inflammation, so they could play a role in conditions such as post-inflammatory hyperpigmentation.[28] PAR-2 expression increases in human skin after exposure to UV radiation.[28]

SOY ISOFLAVONES INHIBIT MELANOSOME TRANSFER



## Efficacy of Soy Isoflavones

### Preclinical Studies Using Soy

Soymilk and soybean extract reduces pigmentation in dark-skinned Yucatan pigs treated for 8 weeks. The data suggest that STI and BBI inhibit PAR-2 activation, reduce keratinocyte phagocytosis, thus inducing a reduction in skin pigmentation. Soymilk also contains trace amounts of free fatty acids and their acyl CoA esters that can inhibit trypsin and may participate in PAR-2 inhibition, thus inducing skin lightening. In addition, soybeans contain isoflavones, which are antioxidants that may downregulate tyrosinase's DOPA oxidase activity.[7]

### Clinical Studies Using Soy

Kang et al conducted a clinical study of the effects of the soy isoflavone, genistein, in healthy subjects. After pretreatment with genistein and exposure to UV radiation, investigators keratomed or biopsied the skin for analysis. Pretreatment with genistein inhibited both ERK activity by 60% ($P<0.05$) and reduced UV induction of cJun protein by 70% ($P<0.05$).[27]

Johnson & Johnson conducted a 12-week, randomized, double-blind, vehicle-controlled study of 68 subjects, 63 of whom completed the study.[29] At week 12, mottled hyperpigmentation improved at least 1 grade in 28 of 31 (90%) of the soy-treated group, compared to 17 out of 32 subjects (53%) in the vehicle group (based on clinical evaluation, colorimetry, and digital photography) ($P\leq0.05$).[29]

## Safety of Soy Isoflavones

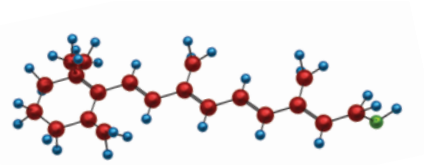
Topically applied soy isoflavones are well-tolerated. Because the inhibition of melanosome transfer by soybean trypsin inhibitor is reversible, side effects are minimal.[1] During the trial described above, erythema, dryness, itching, and burning/stinging did not change from baseline to final follow-up, although one subject in the control group developed mild contact dermatitis.[29]

# RETINOL

## Mechanism of Action of Retinol

Retinol disassociates into retinoic acid, which is the biologically active form of vitamin A, by enzymes beneath the surface of the skin. Retinol stimulates the skin's natural rebuilding process by supporting the expression of glycosaminoglycans, chemicals that bind to water molecules.[31] This binding of water results in desirable, visible changes, such as firmer skin with a more even tone and texture, and fewer lines and wrinkles.

In addition, retinol enhances the proliferation of epidermal cells, thus thickening the epidermis, and upregulates the expression of cellular retinoic acid binding protein (CRABP) II and CRABP mRNAs and proteins.[31] Retinol also inhibits induction of collagen-degrading matrix metalloproteinases by exposure to ultraviolet light, and associated declines in collagen production.[31]



Retinol Molecular Structure

## Efficacy of Retinol

### Preclinical Studies Using Retinol

Retinol inhibited melanin synthesis in melanoma cells stimulated by alpha-melanocyte stimulating hormone (alpha-MSH) or 3-isobutyl-1-methylxanthine by inhibiting the expression of tyrosinase.[32] When applied to black guinea pigs for 10 days, retinol (0.025%) in combination with monobenzylether of hydroquinone lightened the skin of black guinea pigs, used as a model for vitiligo.[33]

### Clinical Studies Using Retinol

Clinical studies have demonstrated retinol's effects on the skin. Visibly, fine lines and wrinkles significantly improved after 12 weeks of retinol treatment among participants in a controlled clinical trial.[34]

Although initial clinical studies of retinol involved participants of middle age, retinol has also been effective and safe in the elderly. Kafi et al conducted a controlled clinical trial in 18 human volunteers (mean age 87 years) exposed to 0.4% retinol lotion on one arm compared to 18 who received vehicle 3 times a week for 24 weeks.[35] This trial showed a statistically significant induction of type I procollagen protein from baseline to week 24 ($P = 0.049$). The study also showed that retinol stimulated a statistically significant difference in glycosaminoglycan expression compared to control ($P = .02$).[35]

## Safety of Retinol

Importantly, retinol is also less irritating than retinaldehyde, leading to less erythema and scaling than occurs with retinoic acid.[31,34] Individuals treated with topical retinol may experience photosensitivity, so patients are advised to avoid excessive sun exposure and to apply a broad-spectrum sunscreen.[31]

Retinols are generally safe, but there is a slight potential for a retinoid reaction, although it is more common with the stronger retinoids like tretinoin and tazarotene. This reaction generally occurs within a few weeks of beginning treatment, and is characterized by itching, a burning sensation where the product was applied, erythema, and peeling.[31]

Adding natural agents like sitosterol, a recognized component of the *Buddleja* plant family, concomitantly with retinol may counteract some of these irritant effects.[31] Importantly, during 30 years of retinoid availability, young adults treated with topical retinoids over the long term have not experienced systemic side effects.[31]

## ASCORBIC ACID-2 GLUCOSIDE

### Mechanism of Action of Ascorbic Acid-2 Glucoside

Ascorbic acid is primarily known for its antioxidant ability to scavenge and neutralize superoxide radicals.[36] Ascorbic acid interferes with melanin synthesis by chemically reducing oxidized dopaquinone back to DOPA, reversing the melanogenic process.[37] Unfortunately, native ascorbic acid is highly unstable and oxidizes and decomposes in aqueous solutions. A more stable form of ascorbic acid is ascorbic acid glucoside, also known as 2-O-alpha-D-glucopyranosyl-L-ascorbic acid (AA-2G). AA-2G is hydrolyzed into ascorbic acid by skin cells.[38]

### Efficacy of Ascorbic Acid-2 Glucoside

#### Preclinical Studies Using Ascorbic Acid-2 Glucoside

Native ascorbic acid has been shown to have many properties that benefit the skin, including antioxidant, anti-inflammatory, and sunscreening properties.[39]

Ascorbic acid 2-glucoside shares many of these properties. Investigators evaluated the ability of L-ascorbic acid glucoside, also known as 2-O-alpha-D-glucopyranosyl-L-ascorbic acid (AA-2G), to inhibit damage induced by UVB exposure to human keratinocytes derived from squamous cell carcinoma.[40,41] Study results demonstrated that AA-2G is protective effect against UVB-induced damage in human epithelial cells. AA-2G also has demonstrated antioxidant properties,[42] and has also been shown to promote collagen synthesis in human skin fibroblasts.[43]

# Conclusions

*Researchers have determined the chemical changes that occur at every step of the melanogenic pathway, both in healthy individuals and in those with pigmentation disorders. The data derived from these studies drive the scientific determination of agents that can interfere with specific points in that pathway. By using a product that combines these agents, each of which has different mechanisms of action, to disrupt hyperpigmentation at multiple points, clinicians can help their patients with melasma and post-inflammatory hyperpigmentation.*

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