Quetzalcoatl University of Veracruz

Tyrosinase inhibitors – mechanisms of action and their effect on the treatment of hyperpigmentation

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In
Biology

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1. Introduction

Hyperpigmentation is a common skin condition that profoundly impacts a patient's emotional and psychological well-being, significantly diminishing their quality of life. Often overshadowed by other dermatological issues, it is regrettably dismissed as a mere cosmetic concern, leading to underdiagnosis and improper treatment. Patients frequently express feelings of embarrassment, frustration, and uncertainty (Amin M.T. et al, 2023).

Pigmentary disorders are very common among individuals with darker skin tones. A survey conducted in the United States involving over 1,400 patients identified dyschromia as the second most frequent dermatological diagnosis in Black and Hispanic populations. This contrasts with light-skinned patients, where dyschromia did not even rank among the top ten most common diagnoses. This finding, regarding the prevalence of pigmentary disorders in those with darker skin tones, has been demonstrated in additional studies, such as (Amin M.T. et al.,2023; Susruthi R., Mayra B d. C.M, Neelam A , N.,2019; Chi Z. et al.,2023; Ana C. H, Luciane D. B. M., Hélio A. M.,2014; Erica C. D., Valerie D. C., 2010; and more).

One form of hyperpigmentation is melasma, also known as chloasma. It is a persistent disorder characterized by brown or gray pigmentation, usually involving the face and its characteristic distribution is symmetrical. Although melasma can appear in both sexes, it is more common in women, especially during pregnancy. Post inflammatory hyperpigmentation (PIH) is another common hyperpigmentation disorder that occurs due to hyper melanosis after skin trauma or inflammation. Unlike melasma, this condition has no gender or age bias. (J. M. Gillbro, M. J. Olsson, 2011).

Human skin tone is derived from the outermost layer of the skin, the epidermis, where the pigment-producing cells, the melanocytes, are located. Melanocytes are found in the basal cell layer, at the bottom of the epidermis, above the basement membrane, or the dermo-epidermal junction. Melanin pigment granules are transferred from the melanocytes to the keratinocytes through extensions (dendrites), where they are packed inside special vesicle-like organelles called melanosomes. Each melanocyte can interact with 40 neighboring keratinocytes via its dendrites, forming an epidermal melanin unit (Chi Z. et al.,2023).

Upon exposure of the skin to UV radiation (as well as other activating factors such as hormonal influences, inflammation, and stress), melanogenesis is enhanced by the activation of the key enzyme in melanogenesis, tyrosinase. Tyrosinase is a glycoprotein located in the membrane of the melanosome. Melanogenesis occurs in the melanosomes while they are inside the melanocyte. Two types of melanin are synthesized within the melanosomes: eumelanin and pheomelanin. Eumelanin is an insoluble polymer that is dark brown to black in color, whereas pheomelanin is a soluble polymer containing sulfur that is light red to yellow (J. M. Gillbro, M. J. Olsson,2011).

The enzyme tyrosinase is the target of most melanin production inhibitors designed to treat hyperpigmentation. It catalyzes the first two steps of melanin production: the hydroxylation of L-tyrosine to L-dihydroxyphenylalanine (L-DOPA) and the subsequent oxidation of this o-diphenol to its corresponding quinone, L-dopaquinone (figure 1).

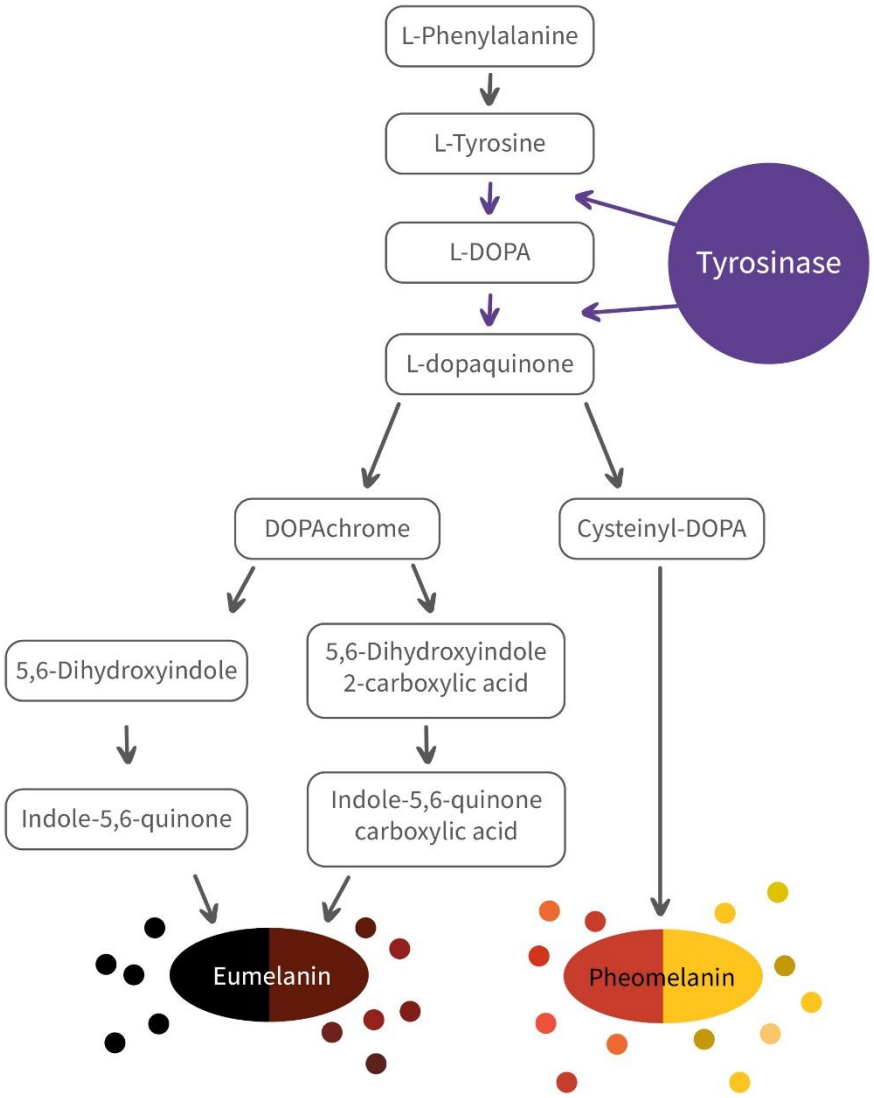

Figure 1: Melanin synthetic pathway. Melanin synthesis begins with catalyzation of the substrates L-phenylalanine and l-tyrosine to produce L-DOPA via phenylalanine hydroxylase (PAH), tyrosinase and partly tyrosinase hydroxylase 1 (TH-1). The pathways are then divided into eumelanogenesis or pheomelanogenesis (J. M. Gillbro, M. J. Olsson,2011).

Baseline skin tone has a crucial impact on its response to factors that cause hyperpigmentation, factors that exacerbate pigmentation, as well as the effective treatment of this condition. Skin tone is one of two parameters that determine skin phototypes, which are classified according to the Fitzpatrick scale. The second parameter is the skin's response to sun

exposure. The Fitzpatrick scale was originally designed to provide clinicians with a diagnostic and therapeutic tool regarding the risk of sunburn and the development of skin cancer with prolonged UV exposure. It is now known that skin phototype is a good predictor of its tendency toward hyperpigmentation (Pathak M.,2004 and others).

The classification method was developed by Thomas B. Fitzpatrick in 1975. In its early stages, back in 1972, Fitzpatrick outlined only 3 skin types (1, 2, 3) which he described based on their response to mid-day exposure, in a preliminary study conducted in Australia. These classifications were recognized even then and became popular as an aid in the field of dermatology. In that year, 1972, the FDA also adopted this classification and used it for the determination of SPF values (Pathak M.,2004).

Today, the Fitzpatrick classification outlines 6 skin phototypes (Table No. 1) according to the basic skin tone and reaction to sun exposure, ranging from 1 (very fair) to 6 (very dark) (Sachdeva S, Indian J,2013).

	Sunburn and tanning history (defines the phototype)	Constitutitve color (unexposed buttock skin)
1	Burns easily, never tans	Very fair
2	Burns easily, tans minimally with difficulty	Fair
3	Burns moderately, tans moderately and uniformly	Fair to medium
4	Burns minimally, tans moderately and easily	Beige-olive, lightly tanned
5	Rarely burns, tans profusely	Moderate brown or tanned
6	Never burns, tans profusely	Dark brown or black

Table no. 1: Fitzpatrick phototypes. Adopted from (Sachdeva S, Indian J,2013).

All active ingredients, treatments, and methods for addressing hyperpigmentation are based on two main mechanisms:

1. Inhibiting or reducing the overproduction of melanin pigment.
2. Removing dead skin cells or exfoliation in its various forms.

Inhibiting, balancing, or reducing melanin production primarily helps stop the formation of excess pigmentation in areas where melanin production is unbalanced. This can be referred to as 'treating future pigmentation.' This is one part of the treatment, while the second part should address the hyperpigmentation that has already occurred.

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Exfoliation or the removal of dead skin cells addresses visible pigmentation. Exfoliation can be done through active ingredients applied in a medical or aesthetic clinic and by using special equipment like lasers or IPL technology, and can also be performed simultaneously through daily-use products by the patient

Using category 1 ingredients alone (balancing melanin production), without the involvement of exfoliating ingredients or ingredients that accelerate skin cell turnover, will give slower results, because it relies solely on the natural skin cells turnover, while the clearing of the spots can be accelerated with the help of exfoliants. Using ingredients and methods from category 2 alone can help on some level, but it does not address the continued excessive production of melanin, and hyperpigmentation may continue to occur for the same reasons that caused it to occur in the first place.

In this review, i will refer to the treatment of hyperpigmentation using ingredients that reduce or balance pigment production. However, it must always be remembered that while this component of the treatment is essential and critical to its success, it is only a part of it. Where it is relevant, i will also mention the issue of peeling or accelerating skin cell turnover as part of the complete hyperpigmentation treatment.

2. Hyperpigmentation Types

The two types of hyper pigmentation that result from excessive melanin production are melasma and PIH. There are other types of discoloration that result from melanocyte proliferation, such as age spots, birthmarks, flat and raised moles, and more. In this context, only the spots caused by overproduction of melanin will be discussed. Although melasma and PIH are considered different types of pigmentation, there are many commonalities between them in terms of causes and mechanisms of formation. In fact, some studies such as Ana C. H, Luciane D. B. M., Hélio A. M. (2014) suggest that individuals prone to melasma are more likely to develop PIH. It's important to remember that the definition of melasma is based on the distribution of the spots, not on their cause. Melasma has a variety of triggers that ultimately lead to hyperpigmentation with a characteristic visual appearance: irregular skin spots on the face, symmetrically distributed on the forehead, cheeks, nose, upper lip area, and sometimes the chin. PIH, on the other hand, will appear in the exact location where the stress, injury, or inflammation occurred—anywhere on the body. Still, inflammation or stress ca cause symmetrically

distributed spots on the face, and researchers may refer to this type of pigmentation as melasma.

2.1 Melasma

Melasma is a challenging condition to treat due to its unpredictable course and common relapses. It is typically characterized by dark-brown symmetric patches with irregular borders. These occur most commonly on the face in a Centro facial and malar pattern. It is most prevalent among Fitzpatrick Skin Type III to VI, Hispanics, African Americans, Asians, or Middle Eastern females, and tends to present in patients between the ages of 20 and 40. Though less common, males account for 10 percent of cases and typically present with a malar pattern distribution (Valeria G-M, Alicia M-P, Noelani G.,2022). The exact pathogenesis of melasma is unknown. Sun exposure, oral contraceptives, pregnancy, medications (e.g., photosensitive drugs), genetic predisposition, steroids, depression, Ovarian tumors, hormone replacement therapy, inflammatory processes, intestinal parasites, certain cosmetics, and autoimmune disorders have all been found to aggravate and contribute to the clinical signs of melasma (Susruthi R., Mayra B d. C.M, Neelam A , N.,2019; Valeria G-M, Alicia M-P, Noelani G,2022; Zuzanna P., Danuta N., Jacek C. S.,2022).

Histologically, it is classified as involving the epidermis (this type being more responsive to treatment), dermis, or both (Valeria G-M, Alicia M-P, Noelani G.,2022).

The pathogenesis of melasma is highly complex; however, in recent years, numerous studies have shed a completely new light on it. Initially, pathologies were thought to be only related to melanocytes; however, we now know that the disturbances extend far beyond the skin pigment cells, as they also include the interaction of keratinocytes, abnormal melanocyte activation, aggregation of melanin and melanosomes in the epidermis and dermis, an increased number of mast cells, increased vascularization, basal membrane damage, skin extracellular matrix abnormalities and photoaging (solar elastosis) [18]. The patients show complex clinical and histological characteristics, suggesting the involvement of multiple pathogenetic pathways. The analysis of the transcriptional activity of melasma-related skin lesions showed that nearly 300 genes are differentially expressed in skin lesions and in the surrounding skin, which only emphasizes the complexity of etiopathogenesis (Zuzanna P., Danuta N., Jacek C. S.,2022).

Some of the causes of melasma are a trigger for different subcutaneous signaling and response pathways. Although their result is similar (i.e. melasma), the occurrences beneath the skin, in each pathway, are different from each other, and may dictate different treatment strategies accordingly. To easily navigate the variety of causes and stimulation pathways for melanogenesis, i have divided the causes of melasma into 3 classes, as shown in figure 2.

1. Excessive exposure to UV radiation

2. Hormonal factors

3. Inflammation, diseases and medication use

2.1.1 Excessive UV exposure

Sun exposure is the most important trigger for melasma. Ultraviolet radiation directly causes increased melanogenic activity. Melasma pigmentation usually improves in winter and worsens in summer (or immediately after intense sun exposure). Furthermore, in intertropical regions its prevalence in the population is increased. The use of a broad-spectrum sun protection reduces the intensity of the disease by 50% and reduces its incidence during pregnancy by more than 90% (Ana C. H, Luciane D. B. M., Hélio A. M.,2014).

Sun exposure activates several melanogenesis pathways. Ultraviolet light affects skin pigmentation by generating reactive oxygen species (Siddiq M., Yvette M M.,2022).

A significantly increased activity of Superoxide Dismutase (SOD) and a decrease in glutathione levels in patients with this disease indicate the presence of increased oxidative stress in melasma patients (Zuzanna P., Danuta N., Jacek C. S., 2022). In addition, another possible mechanism is the stimulation of vascular endothelial growth factor (VEGF) secreted from keratinocytes after exposure to ultraviolet radiation, which causes increased activity in melanocytes. UV light is linked to the downregulation of lipid metabolism-associated genes in melasma skin, which impairs the skin's barrier function and can contribute to the pathogenesis of the condition (Siddiq M., Yvette M M.,2022).

The formation of new vessels in healthy skin is negligible under normal conditions but can increase in certain pathological conditions such as chronic inflammation or after prolonged exposure to ultraviolet rays. Research has shown that skin with melasma is vascularized to a greater extent than skin without pathological changes. This is due to an increase in the number of mast cells, which induce vascular proliferation by secreting bFGF, vascular endothelial growth factor (VEGF) and transforming growth factor-β (TGF-β). VEGF is also upregulated in keratinocytes, and functional VEGF receptors are found on melanocytes (Zuzanna P., Danuta N., Jacek C. S.,2022). These angiogenic factors increase the size, density, and dilatation of vessels in affected skin, and present another therapeutic target when treating melasma (Susruthi R., Mayra B d. C.M, Neelam A, N., 2019).

It has been shown that UV radiation leads to upregulation of melanin-stimulating hormone (MSH) receptors - also known as melanocortin-1 receptors (MC1-R) - on melanocytes, which allows greater binding of the hormones and therefore more melanin production (Susruthi R., Mayra B d. C.M, Neelam A, N., 2019). UV radiation increases expression of microphthalmia-associated transcription factor (MITF). It is a key regulator in the melanin synthesis pathway that controls the expression of tyrosinase. UV radiation has also been shown to endogenously generate 1,2-diacylglycerols (DAGs), a type of second messenger, from phospholipids in the

plasma membrane of melanocytes via the phospholipase C and D (PLC and PLD) pathways. These DAGs activate the tyrosinase (Susruthi R., Mayra B d. C.M, Neelam A, N., 2019).

Ultraviolet radiation may lead to the production of multiple cytokines (interleukin-1, endothelin-1, α-MSH and ACTH) by keratinocytes, thus stimulating melanogenesis (Ana C. H, Luciane D. B. M., Hélio A. M.,2014).

Other factors produced under the influence of UVB are: growth factors, including stem cell factor (SCF), basic fibroblast growth factor (bFGF), interleukin 1 (IL-1), endothelin 1 (EDN1), inducible nitric oxide synthase (iNOS) , adrenocorticotropin (ACTH) and prostaglandin E2 (Zuzanna P., Danuta N., Jacek C. S.,2022).

The tumor suppressor protein p53 may also play a role in UV-induced melanogenesis. This protein upregulates POMC production in keratinocytes post UVB damage, which leads to increased melanin production. In addition, the protein also increases transcription of hepatocyte nuclear transcription factor-1alpha (HNF-1alpha), which induces tyrosinase downstream to increase melanin even in the absence of keratinocytes.

Although the above pathways induce melanogenesis in all skin, the response to UV light is exaggerated and expression of α-MSH is sustained in melasma lesions, augmenting the production of melanin (Susruthi R., Mayra B d. C.M, Neelam A, N.,2019).

UVB irradiation also increases plasmin production by keratinocytes. This enzyme leads to higher levels of arachidonic acid and alpha-MSH and therefore stimulates the melanin synthesis pathway. These factors all lead to hyperpigmentation of the affected skin.

Furthermore, it has been suggested that even visible light may play a role in the pathogenesis of melasma, especially in darker skin types (Fitzpatrick types IV-VI), by interacting with the opsin 3 sensor (Susruthi R., Mayra B d. C.M, Neelam A, N.,2019).

Numbers of mast cells are higher in melasma skin than in unaffected skin. UV exposure triggers the release of histamine from these mast cells, leading to downstream effects. Histamine binding at the H2 receptor activates the tyrosinase pathway and induces melanogenesis. This finding may help elucidate the link between the inflammatory process in UV radiation and the hyperpigmentation that follows (Susruthi R., Mayra B d. C.M, Neelam A, N.,2019).

Basement membrane abnormalities play a key role in melasma pathology. As described above, UV damage activates MMP2 and MMP9 to degrade type IV and VII collagen in the basement membrane. Cadherin 11, an adhesion molecule that is upregulated in melasma skin, can then mediate interaction between fibroblasts and melanocytes and promote melanogenesis. Cadherin 11 is also responsible for upregulating MMP1 and MMP2 expression, leading to further collagen degradation and accumulation of elastotic material in melasma skin. These effects may even be independent of UV irradiation.

Basement membrane damage also allows the movement of melanocytes and melanin granules down into the dermis, which contributes to the persistent and recurring nature of melasma. Therefore, trauma induced by lasers or any therapies that further aggravate the basement membrane may worsen the disease. Similarly, restoration of the basement membrane may limit recurrence (Susruthi R., Mayra B d. C.M, Neelam A , N.,2019).

Solar elastosis and photoaging Solar elastosis refer to the accumulation of abnormal elastic tissue in the dermis as a result of chronic sun exposure or photoaging. Melasma patients were found to have high levels of solar elastosis in the affected skin. In addition, histological analysis shows that melasma skin tends to have thicker, curled and more fragmented elastic fibers compared to normal skin (Susruthi R., Mayra B d. C.M, Neelam A , N.,2019).

Ultraviolet radiation causes the release and activation of MMP2 and MMP9 from mast cells. These cells secrete stem cell factor (SCF) which may diffuse and cause melanogenesis in the overlying epidermis. Also, levels of stem cell growth factor receptor, also known as c-kit, are also upregulated in melasma lesions. When c-kit binds to SCF it activates the tyrosine kinase pathway responsible for melanogenesis. And finally, MMP2 and MMP9 are associated with the degradation of collagen IV and VII in the basement membrane. Damage to the basement membrane also allows melanocytes and melanin molecules to migrate to the dermis, which contributes to the persistent and recurring nature of melasma (Susruthi R., Mayra B d. C.M, Neelam A , N.,2019; Zuzanna P., Danuta N., Jacek C. S.,2022).

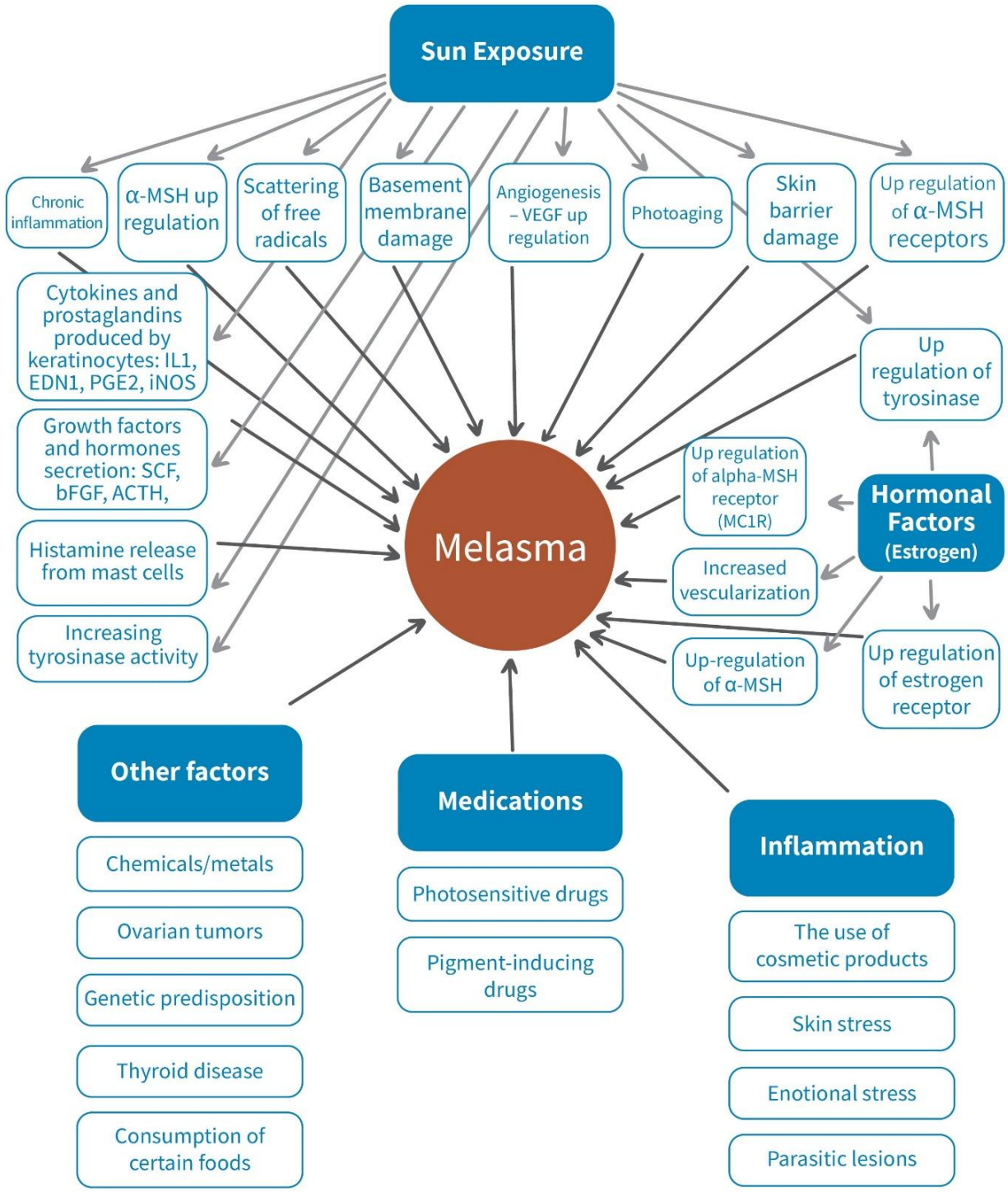

Figure 2. The Pathogenesis of Melasma

2.1.2 Hormonal Factors

Hormones appear to play an important role in the development of hyperpigmentation, with the epithelium of melasma lesions in women demonstrating increased expression of both estrogen and progesterone receptors (Siddiq M., Yvette M M.,2022).

In recent years, researchers have conducted studies on protein expression using immunohistochemistry in an attempt to understand and clarify the pathogenesis of melasma. An increased number of estrogen receptors in the dermis and progesterone receptors in the epidermis of melasma lesions was observed. Estrogen binding to its receptors on melanocytes and keratinocytes can activate tyrosinase and MITF pathways to induce melanin production

(Susruthi R., Mayra B d. C.M, Neelam A , N.,2019; Ana C. H, Luciane D. B. M., Hélio A. M.,2014). Sex hormones such as estrogen and progestin have been documented by several researchers as being associated with the appearance of melasma. Pregnancy, COC and hormone replacement therapy are the most cited triggering factors (Ana C. H, Luciane D. B. M., Hélio A. M.,2014).

Estrogens act on nuclear receptors, and in a non-genomic way also on melanocytes, and generate melanogenesis. They increase the expression of melanocortin type 1 receptors (MC1R) in melanocytes, which are involved in the pathophysiology of melasma. Furthermore, they promote the increased expression of the PDZK1 gene, which leads to the transcription of tyrosinase, although they do not change the number of melanocytes and/or keratinocytes. Estrogen increases the secretion level of MSH, melanogenesis stimulating hormone (Susruthi R., Mayra B d. C.M, Neelam A , N.,2019).

Estradiol promotes proliferation of epithelial cells through phosphorylation of extracellular kinases (ERK)1-2/mitogen activated protein (MAP) and activation of the Wnt/β-catenin pathway in keratinocytes, which promotes melanogenesis. Furthermore, estrogens lead to increased production of KGF in the epithelium, which also stimulates melanogenesis. Estrogens directly mediate melanogenesis through ER2 (estrogen receptors β) activation in melanocytes. Human melanocytes cultured with estrogens show increased MC1R expression, promoting upregulation of MITF, TRP1, and TRP2 through the blockade of protein kinase A (PKA). However, addition of ER2 antagonists inhibits melanogenesis (Ana C C. E et al,2022).

Muriel C (2019) reviewed findings from clinical studies designed to test the relationship between sex hormones and melasma through neovascularization. It was found that estradiol caused (via estrogen receptor) an increase in VEGF, proliferation and migration of endothelial cells, while it caused an increase in angiogenesis via GPER.

They concluded that E2 may be responsible for the increased number, density and size of blood vessels in melasma and the effect on pigmentation may be due to the high number of

blood vessels that secrete endothelin 1, a melanosis promoting factor. In addition, endothelial cells may also secrete NO due to an increase in eNOS and thus stimulate melanogenesis.

2.1.3 inflammation, diseases and drug use

The genetic component is considered to contribute to the formation and chronic nature of melasma, with some studies reporting a positive family history in up to 61% of affected patients (Siddiq M., Yvette M M.,2022).

> Some patients also report the appearance of melasma after stressful episodes and emotional disorders (for example: depression). Proopiomelanocortins (ACTH and MSH) are stress-related hormones that can activate melanocortin receptors in melanocytes, causing melanogenesis. There is also evidence that the melanocytes display an individual response to stress hormones, with the same hierarchy of the hypothalamic-pituitary axis (Ana C. H, Luciane D. B. M., Hélio A. M.,2014).

Melasma can appear, or worsen, due to cosmetic procedures that cause skin inflammation, such as peeling and light/laser treatments. A study on the onset of melasma associated with intense pulsed light (IPL) treatments concluded that patients with subclinical melasma may experience worsening due to the use of IPL axis (Ana C. H, Luciane D. B. M., Hélio A. M.,2014).

Inflammation can be caused by exposure to the sun (as mentioned before) and cosmetic procedures, but also by a variety of other internal and external factors. Interestingly, hyperpigmentation due to skin damage or inflammation is mainly considered PIH, but studies and reviews on melasma also note various inflammatory factors as one of the main manifestations of hyper melanosis. For example: axis (Ana C. H, Luciane D. B. M., Hélio A. M.,2014).indicate that endothelin-1, stem cell factor, c-kit, GM-CSF, iNOS and VEGF, besides a greater number of inflammatory cells and blood vessels, have been described as having a higher expression in melasma skin, compared to perilesional skin which supports the hypothesis that there is a greater inflammatory response in the affected skin.

The use of cosmetic products and the consumption of certain medications, such as anticonvulsants and other photosensitizing substances, have also been found to be risk factors for melasma. Also, a wide variety of chemicals such as arsenic, iron, copper, bismuth, silver, gold, and drugs such as antimalarials, tetracyclines, anticonvulsants, amiodarone, sulfonylureas, among others, may cause hyperpigmentation of the skin, by depositing in the surface layers or by stimulating melanogenesis (Ana C. H, Luciane D. B. M., Hélio A. M., 2014). Less frequently, melasma is caused by thyroid diseases, ovarian tumors, consumption of certain foods, parasitic lesions and even increased stress (Zuzanna P., Danuta N., Jacek C. S.,2022).

2.2 PIH

Post inflammatory hyperpigmentation (PIH) is an acquired hypermelanosis that occurs after skin inflammation or injury that can occur in all skin types, but more frequently affects patients with darker skin tones, including African Americans, Hispanics/Latinos, Asians, Native Americans, Pacific Islanders, and people of Middle Eastern origin (Erica C. D., Valerie D. C.,2010). In a study conducted in Singapore, the authors note that PIH tends to be more common in darker-skinned Asians, such as Malaysians and Indians, than in lighter-skinned Asians, such as the Chinese, suggesting that the degree of pigmentation rather than race/ethnicity may be the more significant contributor to the development PIH (Erica C. D., Valerie D. C.,2010).

Despite its high prevalence in dark skin (Fitzpatrick photo types III-VI), PIH can affect all skin photo types due to endogenous and exogenous injuries (Chi Z. et al.,2023).

Unlike melasma, this condition has no gender or age bias. It has been demonstrated that PIH appears in patients of all ages and without predominance in both sexes (Siddiq M., Yvette M M.,2022).

2.2.1 Epidermal and dermal PIH – the mechanism

A wide range of etiologies for PIH exists including infections such as dermatophytosis or viral exanthems, allergic reactions such as those from insect bites or a contact dermatitis, papulosquamous diseases like psoriasis or lichen planus, medication-induced PIH from hypersensitivity reactions, or cutaneous injury from irritants, burns, or cosmetic procedures [12]. Among the very common causes of PIH in dark skin are acne vulgaris, atopic dermatitis, and impetigo. A study in 2002 evaluating acne in skin of color found that 65.3 percent of African-American (N=239), 52.7 percent of Hispanic (N=55), and 47.4 percent of Asian (N=19) patients developed acne-induced PIH (Erica C. D., Valerie D. C.,2010).

One of the primary mechanistic explanations for the origin of post-inflammatory hyperpigmentation (PIH) highlights inflammation as a key contributor. Inflammatory processes can lead to damage in the basal layer of the epidermis, triggering melanocytes to release melanosomes containing pigment into the surrounding skin cells. These pigment granules may persist in the skin for prolonged periods, causing further discoloration of the epidermis. When PIH affects the epidermis, cytokines, chemokines, and reactive oxygen species released during the inflammatory response stimulate both melanocyte proliferation and melanin synthesis, as well as its transfer to adjacent keratinocytes. Physiological factors that promote these events include epidermal growth factor (EGF), interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor (TNF), as well as leukotrienes such as LTC4 and LTD4, prostaglandins E2 and D2, and thromboxane (Ewa M. et al ,2022; Erica C. D., Valerie D. C.,2010).

PIH may also be dermal. The process occurs following damage to the basal membrane and the basal keratinocytes, which lead to the release of melanin in large quantities in response to inflammation. The melanin flows into the upper dermis, where it is phagocytosed by macrophages, which subsequently become melanophages. They give the skin a dark blue-gray look. This type of hyperpigmentation can be prolonged or permanent (Siddiq M., Yvette M M.,2022; Ewa M. et al,2022).

2.2.2 PIH and skin phototypes

PIH is more common in dark skinned people. Several reasons have been raised in connection with its prevalence in dark skin, the main ones being:

i. The increased size and activity of the melanocytes
ii. Differences in the structure of the skin and different stratification of the epidermis
iii. Low level chronic systemic inflammation
iv. Expression levels of DEJ markers
v. Paracrine signaling of fibroblasts
vi. Weakened microvascular function

i. Larger and more active melanocytes

The degree of skin pigmentation is correlated with the size of the melanocytes; In dark skin, the cells are significantly enlarged and transport more melanosomes to the epidermis due to higher tyrosinase activity and have larger and more acidic dendrites compared to fair skin (Ewa M . et al, 2022).

ii. Differences in Skin Structure and Epidermal Layering

There are significant morphological differences in the epidermal layer between Caucasian and African skin phototypes. In Caucasian skin, proper layering and enhanced terminal differentiation are observed, leading to the formation of an intact skin barrier. In the African skin type model, a decrease in the expression and accumulation of structural proteins and ceramides is evident: keratin 14 (KRT14), keratin 10 (KRT10), filaggrin (FLG), filaggrin 2 (FLG2), choline transporter SLC44A5, γ-glutamylcyclotransferase (GGCT), and caspase-14 (CASP14). These specific profiles indicate a weaker skin barrier, and the molecular signatures overlap with those found in inflammatory skin disorders [16]. Further damage to the skin barrier is caused by the overactivity of melanocytes, which acidify the epidermis through secreted melanosomes. This acidification can influence the enzymatic activity of several pH-dependent proteins in the stratum corneum (Ewa M. et al, 2022).

iii. Chronic systemic inflammation

Individuals with darker skin may also be more prone to alterations in inflammatory profiles associated with chronic, low-grade expression of pro-inflammatory cytokines. Population-based analyses of plasma cytokine profiles reveal elevated levels of several inflammatory markers in circulation, such as IL-6 and C-reactive protein (CRP), in African Americans compared to Mexicans and light-skinned Hispanic Americans. The impact of ethnicity on circulating cytokine levels has also been reported for tumor necrosis factor-alpha (TNFα) among Mexican Americans, and for interleukin-8 (IL-8) and granulocyte colony-stimulating factor (G-CSF) in African Americans. Significant differences in IL-6, CRP, and fibrinogen (FBG) were also documented in another population-based study that included middle-aged and older Japanese and African American participants from the Midlife in Japan (MIDJA) and Midlife in the United States (MIDUS) health and aging surveys. The systemic average levels of all three pro-inflammatory markers were consistently lower in Japanese participants compared to African Americans throughout the lifespan, from ages 30 to 80 (Ewa M. et al, 2022).

iv. Expression levels of DEJ markers

The basement membrane, or dermal-epidermal junction (DEJ) is essential for maintaining skin homeostasis. The DEJ supports physical interactions and molecular signaling between keratinocytes and fibroblasts in the papillary dermis. There are differences in interactions between epidermal and dermal cells in light and dark skin. Skin biopsy studies have shown that the DEJ in African skin is significantly longer and more convoluted compared to Caucasian skin. However, DEJ from African skin biopsies contained lower levels of basement membrane markers, including type 4 collagen (COL4), laminin 5 (LAM5), type 7 collagen (COL7), and nidogen (NID1), compared to European skin. This suggests potential impairment in the organization, mechanical strength, and function of the DEJ in both light and dark photo types. Both melanocytes and keratinocytes rely on the mechanical support of the basement membrane. Changes in the basement membrane can affect the distribution, migration, and renewal of epidermal cells, and damage to this membrane is often observed in PIH. The susceptibility of the basement membrane to damage may also be explained by increased secretion activity of fibroblasts in the superficial dermis, particularly MMPs and their inhibitors, which are responsible for matrix remodeling involved in structural support (Ewa M. et al, 2022).

v. Paracrine signaling of fibroblasts

The secretory activity of fibroblasts also affects the physiology and morphogenesis of the epidermis. Darker skin is characterized by a thicker dermis with more macrophages and

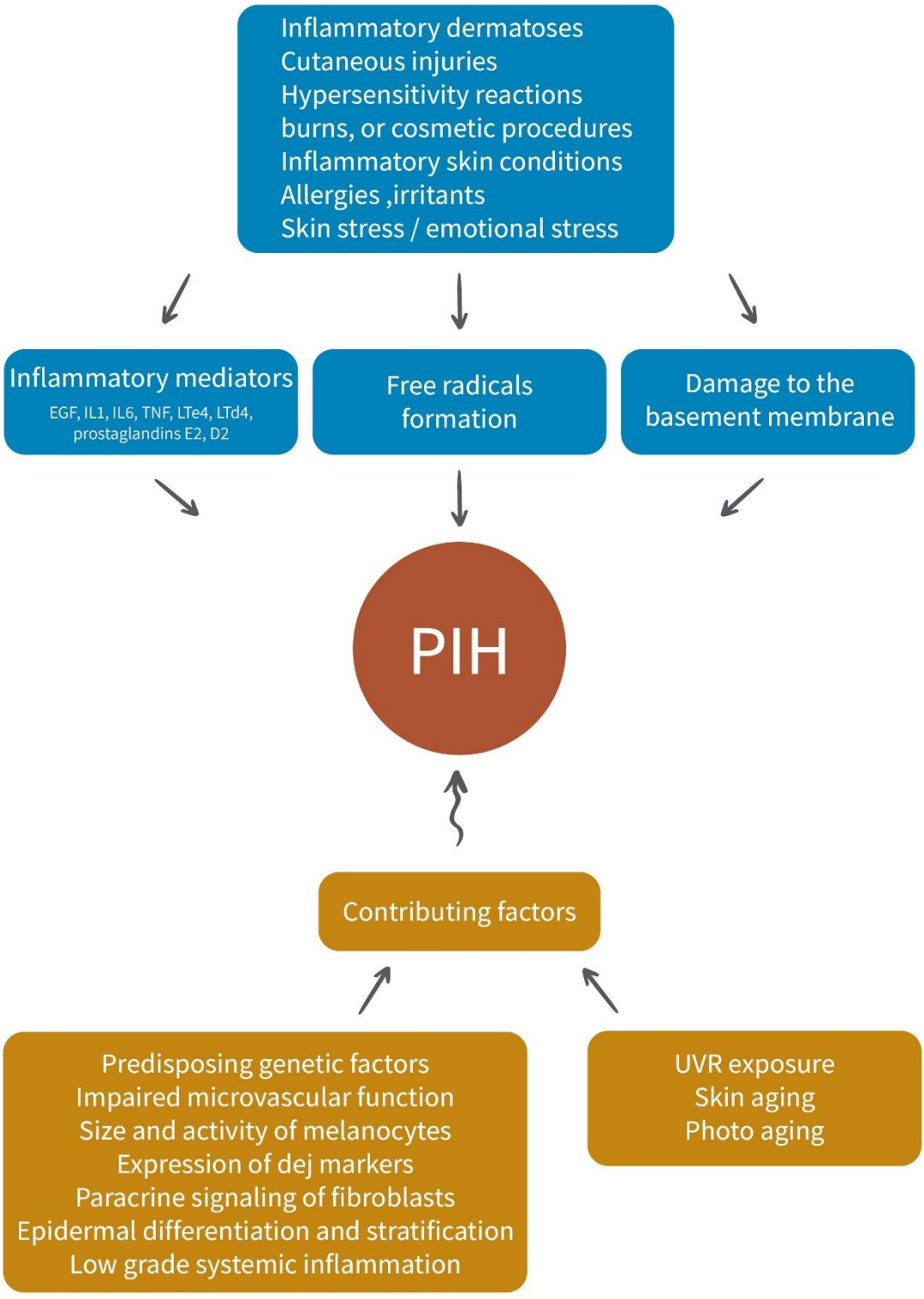

Figure 3. The Pathogenesis of PIH.

fibroblasts. Compared to the light-skinned types, the papillary fibroblasts in the dark skin also have an increased secretory activity, manifested by the production of higher amounts of

signaling molecules such as monocyte chemotactic peptide-1 (MCP-1), keratinocyte growth factor (KGF), matrix metalloproteinase 1 (MMP1) and tissue inhibitors of metalloproteinase protein 1 (TIMP-1) (Ewa M. et al, 2022).

vi. Weakened vascular function

Recently, several studies have demonstrated the increased cases of impaired cutaneous vascular and microvascular function in African American individuals, which were more prevalent compared to European Americans and detected in healthy young adults, therefore not caused by aging. Flow-mediated dilation (FMD) and local skin heating measurements indicated attenuated responses. Such characteristics are thought to be associated with increased oxidative stress, reduced cutaneous vasodilation mediated by (Ewa M. et al, 2022).

2.2.3 Aging and Photoaging – Contributing and Aggravating Factors for PIH

UVR exposure is a major external factor responsible for premature skin aging. Both UVB and UVA can cause DNA damage and inflammation mediated by oxidative stress. The accumulation of senescent fibroblasts in the dermis is particularly accelerated by UVA radiation (315-400 nm), which has longer wavelengths than UVB (280-315 nm) and is able to penetrate deeper skin layers. Chronic inflammation and photoaging are associated with pro-inflammatory cytokines/chemokines known as the Senescence-Associated Secretory Phenotype (SASP), secreted by senescent fibroblasts. This includes a broad array of growth factors, proteolytic enzymes, and cytokines, with interleukins IL-1, IL-6, and IL-8 found to be secreted by both fibroblasts and keratinocytes (Ewa M. et al, 2022).

3. Active ingredients

In this section, I will discuss the more established active ingredients for inhibiting melanin production. All the ingredients discussed are associated with several mechanisms of action. However, the primary and most well-documented mechanism is the disruption of tyrosinase enzyme activity.

3.1 Hydroquinone

Topical hydroquinone(HQ) is considered the gold standard for facial hyperpigmentation. Its main and most studied mechanism of action is the inhibition of melanin synthesis. It inhibits the conversion of L-3,4- dihydroxyphenylalanine (L-DOPA) to melanin by inhibiting tyrosinase due to its structural similarity to an analog of melanin precursor – the tyrosine amino acid (Siddiq M., Yvette M M.,2022; Valeria G-M, Alicia M-P, Noelani G., 2022; J. M. Gillbro, M. J. Olsson, 2011). Tyrosinase catalyzes the conversion of tyrosine into melanin precursors, such as dopaquinone and dopachrome. When HQ is present, tyrosinase preferentially oxidizes it over tyrosine, producing no melanin (Isabella M.F et al., 2023).

Although HQ is a poorer substrate for tyrosinase than tyrosine, it is effectively oxidized due to the generation of catalytic amounts of dopa, which acts as a cofactor for tyrosinase (Isabella M.F et al.,2023).

HQ is also thought to inhibit pigmentation by depleting glutathione, reducing DNA and RNA synthesis with concomitant melanosome degradation and melanocyte damage (J. M. Gillbro, M. J. Olsson, 2011).

Some other studies support these findings. They show that HQ can affect melanocyte metabolism. In cells treated with HQ, there was significant inhibition in DNA and RNA synthesis compared to control cell lines. Specifically, thymidine and uridine incorporation into DNA and RNA was inhibited in the presence of HQ, with the effect being more pronounced in melanocytes. It has been hypothesized that this disruption of cellular has a greater contribution to HQ′s hypopigmentation effects than its ability to inhibit tyrosinase (Isabella M.F et al., 2023). Furthermore, HQ inhibits the distribution of melanosome dispersion throughout the dendritic projections. This can limit pigmentation uptake among keratinocytes. HQ has also been shown to affect the cytoskeleton of melanocytes directly. Melanin-producing cells incubated with HQ have dramatic changes in morphology; they become smaller and more dendritic. HQ also influences cellular microtubule formation. Cells incubated with higher amounts of HQ have less microtubule formation and more clumping of cytoskeletal structures (Isabella M.F et al.,2023).

It has also been shown that HQ can scavenge and trap free radicals. Actin inside melanocytes is also influenced by HQ. The disruption of cellular cytoskeletal structures may help explain why HQ may have cytotoxic effects at higher doses (Isabella M.F et al.,2023).

The most common concentrations for HQ monotherapy are 2–4% with 4% having the strongest evidence for use in hyperpigmentation (Siddiq M., Yvette M M.,2022).

Topical HQ may lead to several side effects. Common short-term side effects include allergic or irritant contact dermatitis, hypopigmentation, and post-inflammatory hyperpigmentation (Valeria G-M, Alicia M-P, Noelani G.,2022). The most frequent side effect with chronic use is exogenous ochronosis resulting from homogentisic acid accumulation and deposition in the skin, which presents as erythema, papulonodules, colloid milia, and symmetric blue-black and/or gray-brown hyperpigmentation in sun-exposed areas. Other side effects include reduced elasticity of the skin, compromised wound healing, and peripheral neuropathy (Valeria G-M, Alicia M-P, Noelani G.,2022; J. M. Gillbro, M. J. Olsson,2011; Isabella M.F et al.,2023). This powerful skin lightening agent can lead to permanent loss of melanocytes due to the oxidative damage of the lipid membrane leading to irreversible loss of hereditary skin color. In addition, it was recognized that this substance is rapidly transported from the epidermis to the vascular system and undergoes detoxification within the liver into inert compounds (J. M. Gillbro, M. J. Olsson,2011). There has been some speculation regarding the

carcinogenicity of HQ due to DNA damage in rodents and a few reports of squamous cell carcinoma in human users. However, humans have insufficient evidence to confirm cancer or malignancy associated with topical use (Isabella M.F et al.,2023).

HQ is now also used in combination with other treatments to improve its effectiveness and reduce side effects. The most common combination is known as the Kligman formula, or triple combination therapy (TCT) in which HQ is combined with a topical retinoid and corticosteroids. Several randomized controlled trials (RCTs) in skin of color, as well as a Cochrane review, have evaluated the efficacy of TCT and found it to be more effective than HQ monotherapy (Siddiq M., Yvette M M.,2022).

3.2 Arbutin

Another common skin-lightening agent is arbutin, a derivative of hydroquinone (hydroquinone-O-b-D-glucopyranoside), which is found in cranberries, blueberries, wheat, and pears. Arbutin is used as an effective treatment for hyperpigmentation disorders and shows less cytotoxicity to melanocytes compared to hydroquinone. Unlike hydroquinone, arbutin competitively and reversibly inhibits melanogenesis by binding to tyrosinase without affecting the transcription of tyrosinase mRNA (J. M. Gillbro, M. J. Olsson,2011; Yong C B. et al.,2021; Rashmi S., Pooja A., 2013). The milder effect of arbutin compared to its parent compound, hydroquinone, may be due to its glycoside form, as the glycosidic bond must be cleaved before binding to the tyrosinase enzyme (J. M. Gillbro, M. J. Olsson, 2011).

Arbutin is a compound with a structure in which one molecule of D-glucose is attached to hydroquinone. It has been approximately 30 years since arbutin was deeply researched in a way that allows its use as a hydroquinone substitute for skin lightening purposes. Does arbutin, similarly to hydroquinone, affect the expression level of tyrosinase? It appears that the decrease in TYR activity in human melanocytes by arbutin does not result from a reduction in the expression level of this enzyme. Maeda et al. reported that arbutin at 0.5 mM reduced intracellular TYR activity by 50% but did not affect the mRNA expression level of TYR. Chakraborty et al. showed that arbutin (0.37 mM) lowered the melanin content in cells, but did not reduce the protein levels of TYR, TYRP-1, or TYRP-2. Therefore, arbutin may inhibit post-translational modification or maturation of newly synthesized TYR, or it may cause irreversible deactivation of already synthesized mature TYR (Yong C B. et al, 2021). The consensus view regarding the mechanism by which arbutin inhibits melanin synthesis in cells is that it inhibits the catalytic activity of already expressed TYR, or irreversibly inactivates it rather than suppressing new TYR synthesis. Many studies reported that arbutin did not affect mRNA and protein expression of TYR in the concentration range where it inhibited cellular

melanin synthesis. There are only a few reports that arbutin decreased the intracellular TYR protein level (Yong C B. et al, 2021).

Another proposed mechanism is neutralization of ROS. Oxygen radicals from various sources can promote melanogenesis or cause melanocyte death, leading to hyperpigmentation or hypopigmentation. Therefore, effective antioxidants are expected to reduce oxidative stress in cells, normalize the melanin production process and prevent melanocyte death (Yong C B. et al, 2021). Arbutin and α-arbutin may reduce ROS levels by directly scavenging free radicals or indirectly improving the antioxidant capacity of cells through activation of the Nrf2-ARE pathway. These antioxidant properties may contribute to the inhibitory action of arbutin and α-arbutin on melanin synthesis in cells (Yong C B. et al., 2021). Arbutin also inhibits the maturation of melanosomes (Rashmi S., Pooja A., K Vijay G, 2013).

There is controversy as to whether or not arbutin works by breaking down into hydroquinone and glucose. When arbutin is added to cosmetic products, hydroquinone can be produced at a different level depending on the storage conditions. Additionally, when arbutin is applied to the skin, hydroquinone can be produced by exposure to skin microorganisms or ultraviolet radiation (UVR). Therefore, the possibility remains that a small amount of hydroquinone, which may be produced as a breakdown product of arbutin, contributes to the inhibition of melanin synthesis or inactivation of TYR in cells. However, most evidence supports that arbutin has intrinsic properties that inhibit cellular melanogenesis and reduce cellular TYR activity independent of hydroquinone release (Yong C B. et al.,2021).

Deoxyarbutin is a locally synthesized derivative. Studies have shown it has improved lasting improvement, overall skin lightening, and a similar safety profile to hydroquinone (Rashmi S., Pooja A., K Vijay G, 2013).

An RCT comparing 1% arbutin to 1% ellagic acid in the treatment of melasma demonstrated the clinical effectiveness of arbutin, as evidenced by the clinical improvement of all 10 patients in the arbutin treatment group. Another prospective study examining the use of 7% alpha arbutin in combination with a dual-frequency Q-switched Nd: YAG laser (MedLite C6®, Cynosure®, Westford, Massachusetts) also demonstrated positive results in the treatment of melasma patients. However, this study was limited because arbutin was not studied separately from the laser treatment (Jasmine C. H, Kunal A., Rebat M. H.,2018).

A randomized, placebo-controlled, double-blind trial involving 102 women, aged 26-55, with melasma and solar lentigines, assessed the pigmentation-reducing efficacy of arbutin derived from *Serratulae quinquefoliae*. The treatment group (n = 54) applied a cream containing the

plant extract (final concentration of arbutin 2.51%) twice daily to the pigmented areas for 8 weeks. The results showed that the cream with the plant extract reduced melanin levels in the pigmented areas, compared to the control group (n = 48) which applied a placebo cream. Clinical improvement was observed in 75.86% of patients with melasma and 56% of patients with solar lentigines (Yong C B. et al, 2021).

Maeda et al. demonstrated that arbutin reduced TYR activity in a concentration-dependent manner in human melanocytes at concentrations between 0.1 and 1.0 mM without significantly reducing cell viability. Its inhibitory effect on cellular melanin synthesis was stronger than that of kojic acid or L-ascorbic acid at a fixed concentration (0.5 mM). Akio and colleagues reported that melanin content in cultured B16 murine melanoma cells was reduced by arbutin, and this effect was attributed to a decrease in intracellular TYR activity. Chio et al evaluated the depigmentation efficacy of aloesin and arbutin in a human study. After irradiation of the skin area of the forearm with UVR, a 10% solution of each substance was applied alone or together 4 times a day for 15 days. As a result, aloesin, arbutin, and their co-treatment reduced UVR-induced hyperpigmentation by 34%, 43.5%, and 63.3%, respectively, compared to placebo treatment (Yong C B. et al, 2021).

Although higher concentrations may be more effective, there is a higher risk of paradoxical hyperpigmentation (Rashmi S., Pooja A., K Vijay G, 2013).

3.3 Niacinamide

Niacinamide is a biologically active form of niacin (vitamin B3) and is found in yeast and root vegetables. Several benefits in terms of improved barrier function, reduced sebum production and improved appearance of photo-aged skin including hyper pigmentation, redness and wrinkles have been attributed to topical usage of niacinamide (J. M. Gillbro, M. J. Olsson, 2011).

It appears to reduce hyperpigmentation due to several mechanisms. The most documented is interference with the transfer of melanosomes from melanocytes to the adjacent keratinocytes (Valeria G-M, Alicia M-P, Noelani G.,2022; Chi Z. et al., 2023; J. M. Gillbro, M. J. Olsson, 2011 and more - see table no. 2). Rashmi S., Pooja A., K Vijay G. (2013) point out that niacinamide inhibits the PAR receptor, which is involved in the transfer of melanosomes to the skin cells. Another mechanism of action described by them is - interference with the interaction between melanocytes and keratinocytes - the same interaction that leads to the upregulation of melanin production or is a trigger for it. Zuzanna P., Danuta N., Jacek C. S.,(2022) document additional mechanisms of action: reduction in solar elastosis, and anti-inflammatory activity. Solar elastosis is found as one of the degenerative changes in the skin

tissue, which are associated with hyperpigmentation in people who have had prolonged exposure to the sun. Valeria G-M, Alicia M-P, Noelani G., (2022) also document the ability of niacinamide to reduce degenerative changes in the skin caused by prolonged sun exposure. Regarding the anti-inflammatory mechanism, Valeria et al (2022) state that niacinamide is considered an anti-inflammatory component without specifying that it is a mechanism in skin lightening.

In a double-blind study involving 27 patients with melasma, the efficacy of 4% niacinamide cream was compared to 4% hydroquinone (HQ). The reduction in MASI (Melasma Area and Severity Index) score was 62% and 70%, respectively, after eight weeks of treatment. Clinical photographs and colorimetric measurements showed statistically significant improvement, but no statistically significant difference between the two regimens. Clinical trials using 2% niacinamide demonstrated that it significantly reduced the total area of hyperpigmentation and increased skin brightness after four weeks of treatment. The study also showed that daily use of niacinamide with sunscreen was effective in reducing hyperpigmentation and enhancing the brightness of the underlying skin tone compared to sunscreen alone (Valeria G-M, Alicia M-P, Noelani G, 2022). In a nine-week, randomized, double-blind, left-right axilla, placebo-controlled trial, the efficacy of niacinamide 4% and desonide 0.05% emulsions were compared to placebo in the treatment of axillary hyperpigmentation. Twenty-four women of Phototypes III to V were included in the study. Niacinamide and desonide both induced significant colorimetric improvement compared to placebo, but desonide showed the best depigmenting effect overall (Jasmine C. H, Kunal A., Rebat M. H, 2018). A double-blind study showed 4% niacinamide had better results in reducing the MASI score compared to ATRA (all-trans-retinoic-acid) 0.05% (Valeria G-M, Alicia M-P, Noelani G, 2022). There are several studies, in which niacinamide is one of two to four lightening components, in which significant results were achieved in lightening. However, we cannot conclude from these studies about the exact contribution of niacinamide to the result. Such a study, for example, was cited by Valeria G-M, Alicia M-P, Noelani G, (2022) - a prospective, one-arm, open study of 33 patients evaluated the effectiveness of a new cream formulation containing nicotinamide 4%, arbutin 3%, bisabolol 1% and Retinaldehyde 0.05% and demonstrated a statistically significant decrease in the MASI score compared to baseline from week 4 to week 11. Although topical niacinamide is considered tolerable, it is associated with several mild side effects. Valeria G-M et al (2022) summarize the main side effects observed in clinical studies: mild burning, erythema and itching. These can improve along the way with continued use of the ingredient.

3.4 Tranexamic Acid

Tranexamic acid (Trans-4 (aminomethyl) cyclohexane carboxylic acid) is a lysine analogue, known to have antiplasmin activity. Plasmin is induced by several factors, including UV radiation. Plasmin inhibition is done by lowering the level of arachidonic acid, prostaglandins

and leukotrienes (which are melanogenic factors themselves) and down-regulates fibroblast growth factor (FGF) and alpha MSH, both of which signal hyper melanosis (Siddiq M., Yvette M M.,2022; Rashmi S., Shikha C., Vijay K.G,2012; Valeria G-M, Alicia M-P, Noelani G., 2022; Zuzanna P., Danuta N., Jacek C. S., 2022) and thus a decrease in activity the tyrosinase. Another mechanism reviewed in the literature is the inhibition of angiogenesis through a decrease in the expression of endothelin, VEGF and CD+ blood vessels (Valeria G-M, Alicia M-P, Noelani G,2022; Zuzanna P., Danuta N., Jacek C. S.,2022). Angiogenesis is one of the causes of the appearance and worsening of hyperpigmentation. Zuzanna P., Danuta N., Jacek C. S.(2022) and Valeria G-M, Alicia M-P, Noelani G.(2022) attribute to tranexamic acid (TXA) also a direct activity of inhibiting tyrosinase activity, although the mechanism is not entirely clear. Zuzanna P., Danuta N., Jacek C. S.(2022) mention additional mechanisms of action of TXA: downregulation of mast cells, which are also associated with hyperpigmentation, as well as a decrease in solar elastosis, which is also mentioned as being associated with hyperpigmentation.

Many studies have examined the use of topical TXA at 2-5% for the treatment of melasma in highly melanized skin. In a comparative split-face trial in patients with skin type III-V, 5% liposomal TXA was found to be as effective as 4% hydroquinone in improving the appearance of melasma lesions. Additionally, the TXA-treated arm had no adverse events compared to the hydroquinone-treated arm, which had three events of mild skin irritation. Similarly, promising results were observed in another split-face study comparing 3% TXA with dual therapy of 3% hydroquinone and 0.01% dexamethasone. During the 12-week study period, both sides showed a significant improvement in the MASI score of the lesions without a statistically significant difference between them. Again, the treatment was better tolerated on the side treated with TXA, and with much fewer side effects ($p<0.01$) than the side of the double hydroquinone and steroid treatment (Siddiq M., Yvette M M, 2022). 2% cream in an emulsion and lotion vehicle for the treatment of melasma was evaluated showing a statistically significant improvement at Week 4 and 8 according to MASI score (Valeria G-M, Alicia M-P, Noelani G, 2022). Valeria G-M et al (2022) cite additional studies on TXA: A 39-patient double-blind split-face trial study found that application of twice daily TXA 3% cream for 12 weeks was as effective as a solution of HQ 3% + dexamethasone 0.01% based on a statistically significant decreased in MASI score and clinical photographs for both therapies, without any differences between them based on efficacy. Another double-blinded RCT of 60 women with melasma, assessed the efficacy of TXA 5% cream, a higher concentration than the previous study, versus HQ 2% cream alone both applied twice daily for 12 weeks. Both regimens showed improvement according to MASI score, but no significant difference was found between them. A double-blinded split-face trial evaluated the efficacy of TXA 5% as a liposomal vehicle vs. HQ 4% cream which showed a statistically significant reduction in MASI score on week 12. Although

Ingredient	Mechanisms of action	Concentrations studied
Hydroquinone	Competitive Inhibition of Melanin Synthesis [18], [21], [11], [9] Depletion of Glutathione [21], [11], [9] Reduction of DNA and RNA Production [21], [11], [9] Degradation of Melanosomes and Damage to Melanocytes [21], [11], [9], [24] Scavenging of Free Radicals [9]	2%-5%
Arbutin	Tyrosinase inhibition [11], [10], [22], [15] Scavenging of Free Radicals [22] Irreversible Inhibition of Tyrosinase [22] Inhibition of Melanosome Maturation [15]	1%-7% Mostly 1%-4%
Niacinmide	Inhibition of Melanosome Transfer to Keratinocytes [21], [5], [11], [10], [24], [15] Anti-Inflammatory [24] Inhibition of Melanogenesis [24], [15] Reduction of Solar Elastosis [24] Disruption of Interaction Between Keratinocytes and Melanocytes [24]	2%-4%
Tranexamic Acid	Inhibition of Plasminogen [18], [21], [16], [24] Downregulation of Mast Cells [24] Reduction of Neoangiogenesis [21], [24] Decrease in Solar Elastosis [24]	2%-5%
Kojic Acid	Chelation of Copper Ions in the Tyrosinase Enzyme [2], [18], [19], [21], [11], [10], [12] Antioxidant [21], [10] Disruption of the Melanin Production Pathway [11], [10] Anti-Inflammatory [12], [8] Protection Against UV Radiation [12]	1%-4%
Azelaic Acid	Inhibition of Tyrosinase [18], [11], [10] Anti-Inflammatory [8], [5] Antioxidant [5], [10] Anti-Proliferative for Melanocytes [18], [11], [10]	15%-20%
Retinoids	Regulation of Cell Divisions [18, 23] Desquamation [21, 23] Increased Permeability of the Stratum Corneum [21] Anti-Inflammatory [18] Inhibition of the Enzyme Tyrosinase [21] Blocking of Tyrosinase Transcription [23] Dispersion of Melanin Granules in the Epidermis [21, 23]	Retinol: 0.2%-2% Retinoic Acid: 0.025%-0.1%

Table no. 2. Components Inhibiting Melanogenesis: Mechanisms of Action

a greater reduction in MASI score was seen with liposomal TXA 5%, no statistically difference between both treatments was reported.

In a double-blind clinical trial cited by (Susruthi R., Mayra B d. C.M, Neelam A, N., 2019), it was demonstrated that topical TXA is as effective as hydroquinone in reducing the mean score of the melasma area and the MASI index with fewer side effects. TXA can be used orally, topically and intradermally. Zuzanna P., Danuta N., Jacek C. S.(2022) point out that this substance can also be used locally, in the form of an injection as mesotherapy or in microneedling, but the oral prescription of TXA showed the best clinical results. When used topically, the most common side effects are transient erythema, scaling, and dryness, but it still has a theoretical risk of precipitating a thrombotic event (Siddiq M., Yvette M M., 2022)

3.5 kojic Acid

Kojic acid (KJA) is a natural metabolite produced by various fungal species(Siddiq M., Yvette M M., 2022). Tyrosinase contains a copper ion at its active site. Upon exposure to UV radiation, the copper ion activates tyrosinase, enhancing its activity. KJA captures the copper ion, preventing it from activating tyrosinase. By inhibiting the activity of tyrosinase, KJA can also prevent melanin production (Majid S., Masoumeh E., Khadijeh K, 2019). The chelation of copper ions is the most cited and documented mechanism for the activity of kojic acid, for example: (Amin M.T. et al., 2023; Siddiq M., Yvette M M.,2022; Susruthi R., Mayra B d. C.M, Neelam A, N.,2023; J. M. Gillbro, M. J. Olsson., 2011). However, additional mechanisms of action for this compound have been reported, such as: antioxidant properties (Valeria G-M, Alicia M-P, Noelani G., 2022; Jasmine C. H, Kunal A., Rebat M. H., 2018; Majid S., Masoumeh E., Khadijeh K.,2019); UV protection (Majid S., Masoumeh E., Khadijeh K.,2019); disruption of the melanin synthesis pathway (Jasmine C. H, Kunal A., Rebat M. H., 2018; J. M. Gillbro, M. J. Olsson., 2011; Majid S., Masoumeh E., Khadijeh K.,2019); and anti-inflammatory activity—inhibition of NF-κB in keratinocytes, thereby reducing melanogenic stimuli (Amin M.T. et al. , Majid S.,2023; Masoumeh E., Khadijeh K.,2023). Jasmine C. H, Kunal A., Rebat M. H.(2018) and Majid S., Masoumeh E., Khadijeh K.(2019) noted a property of enhancing skin permeability of KJA, which may be a contributing factor to its activity.

The effectiveness of different concentrations of KJA has been reported, in formulas where it was the only active ingredient or in combinations with other topical agents. In the RCT cited by Valeria G-M, Alicia M-P, Noelani G (2022), 80 patients received KJA 1% alone or in combination with 2% HQ and/or a strong steroid for 12 weeks. The greatest decrease in MASI score (71.87%) was reported with the combination of 2% KJA, HQ 2% and betamethasone. Jasmine C. H, Kunal A., Rebat M. H., (2018) conclude that the reports over the years about the effectiveness of KJA reveal mixed results. They conclude that based on previous work, KJA as a single treatment has shown modest efficacy, but has been shown to be more beneficial when combined with other ingredients. The following study, as well as several others, was

cited by them as an example of this: Draelos et al performed a 12-week double-blind study comparing a preparation containing KJA, emblica extract, and glycolic acid to 4% HQ in 80 multiethnic patients with facial dyschromia. The results showed the same effectiveness in skin lightening abilities between the two agents.

Majid S., Masoumeh E., Khadijeh K. (2019) cite several studies: in the first, the use of a formula containing 2% KJA, 10% glycolic acid and 2% HQ in 40 melasma patients for 12 weeks resulted in a 60% improvement. In another study, a combination of 1% KJA and 2% HQ was used in 80 patients for 12 weeks - with 71.9% improvement. A formula containing 2% KJA compared to 2% HQ in 50 patients, in a third 3-month study - resulted in greater improvement in the group treated with hydroquinone. In contrast, in a fourth study, in which 4% KJA was compared to 2% HQ in the treatment of 100 female melasma patients for 3 months - KJA was found to be more effective.

In many studies, various derivatives of KJA such as KA ester, KA laureate, KA dipalmitate and KA ethyl phosphonate with aldehyde have been reported to be more effective than KJA (Majid S., Masoumeh E., Khadijeh K., 2019)

As for tolerability on the skin and side effects, studies have shown that concentrations of KJA of 1% or less are safe and tolerated for a period of three months to two years, without significant side effects (Valeria G-M, Alicia M-P, Noelani G.,2022). The most common adverse reaction seen with KJA is irritant contact dermatitis; Accompanied by local irritation, edema, itching and pain (Valeria G-M, Alicia M-P, Noelani G.,2022).

3.6 Azelaic Acid

Azelaic acid (AzA) is a saturated dicarboxylic acid with 9 carbon atoms, derived from the fungus Pityrosporum ovale, and can be found in rye, wheat, and barley (Jasmine C. H, Kunal A., Rebat M. H., 2018). It is used to treat acne, rosacea, skin pigmentation, freckles, nevi, and senile lentigines (Jasmine C. H, Kunal A., Rebat M. H., 2018). The compound is capable of binding to amino and carboxyl groups and may prevent the interaction of tyrosine with the active site of tyrosinase, thereby functioning as a competitive inhibitor (J. M. Gillbro, M. J. Olsson, 2011). The competitive inhibition mechanism has also been proposed by (Siddiq M., Yvette M M., 2022; Jasmine C. H, Kunal A., Rebat M. H., 2018)., although it is not the only mechanism attributed to the compound. AzA has been suggested in several contexts as a treatment for acne-induced PIH, due to its well-established therapeutic role in acne treatment, alongside its anti-inflammatory properties, which enhance its efficacy as a melanin production suppressor (Chi Z. et al., 2023).

Melanocyte stimulation and the release of inflammatory factors also occur under UV radiation; azelaic acid can inhibit the UVB-induced expression and secretion of IL-1β, IL-6,

and TNF-α (Ewa M et al, 2022). AzA inhibits mitochondrial oxidoreductase, indicating that this compound is suitable for treating atypical and highly active melanocytes, with minimal impact on uninvolved skin (J. M. Gillbro, M. J. Olsson, 2011). Siddiq M., Yvette M M. (2022) mentions an additional mechanism of action - AzA induces selective cytotoxic and antiproliferative effects on melanocytes. A fourth proposed mechanism by Chi Z. et al., (2023); Jasmine C. H, Kunal A., Rebat M. H., (2018) is that AzA acts as an antioxidant.

In a mixed racial comparative study, patients with melasma were randomized to either 4% HQ or 20% AzA treatment groups.During the 24-week treatment period, 20% AzA was found to be as effective as 4% HQ in reducing the appearance of melasma lesions. Additional comparative studies between HQ and AzA have also produced similar results, with some studies finding AzA monotherapy to be even more effective than 2% HQ for the treatment of melasma (Siddiq M., Yvette M M., 2022). In a 16-week, baseline-controlled study of 20 patients with Fitzpatrick Skin Types IV to VI, 15% azelaic gel applied twice daily showed a reduction in acne and PIH. Patients experienced a 2-point improvement according to the investigator's global assessment score. Recently, a controlled trial performed in India studied 60 patients with epidermal melasma. Half of the participants were treated with a glycolic acid peel every three weeks and twice daily 20% AzA cream; the other half was treated with only AzA cream. The AzA/glycolic acid group showed a statistically significant decrease in MASI score compared to the AzA control group 12 weeks onwards. Another controlled trial performed in Poland found that dermocosmetics containing azelaic acid showed improvement in pigmentation measured with a skin colorimeter (Mexameter®, C+K Electronics, Cologne, Germany). Despite these studies, more well-designed clinical trials are still necessary (Jasmine C. H, Kunal A., Rebat M. H., 2018). Adverse effects associated with AzA are usually transient, usually appearing as erythema, irritation, dryness, burning, and itching (Siddiq M., Yvette M M., 2022). Another derivative of azelaic acid accepted in cosmetic treatments for skin renewal - PAD, which is considered more tolerable in the skin, does not seem to have been studied or documented as having the ability to lighten hyperpigmentation.

3.7 Retinoids

Retinoids are another topical treatment for pigmentary disorders that is effective both in combination with other substances and as monotherapy. The retinoids are derivatives of vitamin A. The most common are tretinoin, tazarotene and adapalene. They are effective for lightening the skin by regulating cell proliferation and due to their anti-inflammatory properties (Siddiq M., Yvette M M., 2022). According to (Valeria G-M, Alicia M-P, Noelani G., 2022) retinoids function as pigment lightening agents due to their ability to inhibit tyrosinase, decrease melanin transfer, accelerate cell turn-over of keratinocytes, increase permeability in the stratum corneum, and ultimately disperse melanin. Yong C. B. at al. (2021)

also point out some of the mechanisms mentioned above and add that retinoids also block the transcription of tyrosinase. The most common retinoid in cosmetic use is retinol. Retinoids that are defined as a drug, and are therefore not available in cosmetic preparations, are: tretinoin (all-trans retinoic acid - ATRA), isotretinoin, tazarotene, adapalene. There is a difference in the therapeutic results obtained from the various derivatives. The derivative commonly used in cosmetics, retinol, requires the use of much higher concentrations than those used in the medical derivatives, because only a small part of the topical retinol is converted into retinoic acid inside the cells.

Different studies show different levels of skin response to the same retinoid. I propose that one potential explanation for this may lie in variations in the skin phototypes of the subjects. In two RCTs, 38 individuals with fair skin and 28 African American participants were treated with 0.1% ATRA cream compared to placebo daily for 40 weeks (Valeria G-M, Alicia M-P, Noelani G, 2022). The researchers found a statistically significant improvement in pigmentation severity with the ATRA 0.1% treatment regimen compared to placebo after 40 weeks of treatment. In the fair-skinned group, a statistically significant improvement in pigmentation grade was noted by week 24 following ATRA treatment compared to placebo. However, in the African American group, the results revealed only marginal statistical significance. A recent single-center, single-blind study evaluated the efficacy of 4% HQ cream plus 0.02% ATRA over 24 weeks in 39 patients with mild to moderate epidermal melasma. A statistically significant reduction in MASI scores was reported from week 4 to week 24, with 87.9% of patients expressing satisfaction with the treatment's efficacy and improvement in quality of life (Valeria G-M, Alicia M-P, Noelani G, 2022).
Siddiq M., Yvette M M. (2022) cited studies in patients with dark skin tones, which indicated the effectiveness of retinoids. In a double-blind RCT of 54 Black women, it was found that 0.1% tretinoin is superior in lightening the skin of patients with PIH compared to placebo. An estimated effect of 40% lightening in hyperpigmented lesions was observed among those treated with 0.1% tretinoin cream compared to only 18% in the placebo group. Despite these promising results, patients in the tretinoin group did notice lightening of non-hyperpigmented skin, and 50% of the patients developed retinoid dermatitis. In another RCT of 28 women with melasma, it was found that topical 0.1% tretinoin caused a 32% improvement in the MASI score compared to only a 10% improvement in the vehicle control group. However, in this study, 67% of the patients in the treatment group developed retinoid dermatitis. This side effect of retinoid dermatitis was defined by Siddiq M., Yvette M M.,(2022) as concerning, as this condition may cause or exacerbate PIH in patients with darker skin. It was suggested that starting at lower doses and gradually increasing the concentration of the retinoid may help mitigate these side effects. Studies have also indicated that synthetic retinoids, such as 0.1% adapalene gel and 0.1% tazarotene cream, are equally effective in managing acne related to PIH in patients with ethnic skin tones with minimal side effects. In comparison to each other

in a multi-ethnic RCT, it was found that 0.1% tazarotene cream causes a statistically greater decrease (p<0.018) in the appearance of PIH lesions (Siddiq M., Yvette M M.,2022).

Additionally, these substances appear to be better tolerated than tretinoin for topical use, with only about 10% of patients in each group exhibiting mild side effects such as burning and dryness. In this study, no patients were reported to have developed retinoid dermatitis. Isotretinoin (ISO), a common retinoid, was the subject of further research cited by (Valeria G-M, Alicia M-P, Noelani G, 2022). In a 40-week RCT involving thirty Thai patients with moderate to severe melasma, one group applied 0.05% ISO gel daily while the other group applied a placebo mixed with broad-spectrum sunscreen daily. According to MASI measurements and colorimetry, no statistically significant difference was found between the two groups. Another study they cite was conducted on retinol (ROL). This was a 12-week open-label study designed to assess the safety and efficacy of a new formulation of HQ 4%/ROL 0.15% used twice daily. Significant improvement for 39%, 77%, and 77% of patients was reported at weeks 4, 8, and 12, respectively, based on colorimetry measurements and global assessment by the physician. A comparative study cited by Valeria G-M, et al (2022) compared two retinoids: adapalene (ADP) and ATRA. In an initial report of a 14-week RCT, the efficacy and patient satisfaction with ATRA 0.05% were evaluated against ADP 0.1% in 30 Indian women with melasma. Patients in the ATRA 0.05% group exhibited a MASI score reduction of 37% compared to a reduction of 41% with 0.1% ADP. Therefore, no statistical difference was reported between the two groups. Topical retinoids have been reported to cause redness, dryness, and peeling, while some resulted in sensations of burning and tingling. However, in most cases, they are well tolerated on the skin. Mild transient retinoid dermatitis was reported in 27% of patients treated with ISO gel for melasma (Valeria G-M, Alicia M-P, Noelani G., 2022).

4. Less Frequent Questions

Diving into the realm of active ingredients for melanin suppression and their mechanisms of action raises numerous questions that, to the best of my understanding, warrant further investigation. This section will include more questions than answers. Some of these issues have been partially discussed in a small number of studies, which may shed light on the subject or suggest a direction for answers. Other issues – i have not found any research addressing them. However, these points, raised as open questions, deserve separate and focused research due to their importance in hyperpigmentation treatment. In the discussion section, we will elaborate on most of these points and propose ways to investigate them, as well as direct attention toward new solutions.

1. What is the relationship (if any) between active ingredients used for hyperpigmentation treatment and the skin phototype of the patient? Does the differing impact on various skin phototypes stem from the nature of the ingredient? Is it related to concentration? To both?

2. In certain studies, the efficacy of whitening agents was evaluated in research that also included the use of light technologies such as IPL or laser. Is it possible that the ingredients were effective or suitable on their own, but the use of the device "spoiled" the results? (Considering that the choice of technology, intensity, and protocol must match the skin color and the type and state of pigmentation, and that there may not have been a complete match).

3. How can we assess the effectiveness of a single whitening ingredient amidst the numerous studies examining formulations that include multiple ingredients, such as vitamin C, arbutin, and kojic acid together in the same product? How can we determine the relative contribution of each ingredient, if any, to the achieved outcome?

4. Retinoids have demonstrated the ability to improve hyperpigmentation, with reviews presenting several possible mechanisms of action. The level of improvement in hyperpigmentation when used as monotherapy was only 30%-40%, despite the presentation of several possible mechanisms of action. Could it be that the primary mechanism through which it acts is exfoliation, which contributes to lightening existing spots but does not necessarily address the root cause—namely, the ongoing overproduction of melanin? Can retinoids be considered part of a complete treatment regimen that also includes effective melanin suppressants, rather than as monotherapy?

5. Given that a variety of melanin-suppressing ingredients affect at least seven stages of melanin production and the formation of hyperpigmentation, could it be that a combination of 2-3 ingredients, each affecting a different mechanism (or a different stage), would create a beneficial synergy, in the sense that 1+1 equals more than 2?

6. Is there a relationship, and what is the nature of the relationship, between anti-inflammatory ingredients (or their anti-inflammatory mechanisms) in PIH and the stage of PIH? Specifically, does the effectiveness of this mechanism increase when PIH is recent, or when the stress occurred recently, due to a more "agitated" inflammatory state and heightened immune activity with more inflammatory agents present in the tissue? Another thought on the same topic: would it be reasonable to

hypothesize that treatment for recent PIH, where the tissue is under higher stress (relative to its state after several months), should be more gentle, more graduated, with the emphasis on less irritating ingredients and concentrations to avoid "adding fuel to the fire" and causing further stress and irritation to the already triggered melanocytes?

7. Are physicians currently tailoring the Kligman formula (TCT) for each patient based on skin tone and pigmentation data? One limitation of the formula, commonly used by physicians, is that it allows for only one application per day due to the presence of the retinoid (which is suitable for evening use only). What are the implications of this when melanin suppression needs to be applied two or more times daily, based on individual circumstances? What does this mean for the suitability of the formula for individuals with darker skin who have reacted with irritation or lack of improvement to retinoids in some studies? What does it imply for the use of the formula in cases of pigmentation resulting from recent stress, when the skin is still irritated?

8. Are there ingredients that are more effective in treating melasma than in PIH? Are there ingredients that are more effective for lentigo pigmentation compared to types of hyperpigmentation arising from hyper melanosis? Are there ingredients that are more effective for epidermal pigmentation relative to mixed (epidermal and dermal) pigmentation?

9. Should the presence of a trigger (either external or internal) for hyper melanosis at the time of treatment (or research) dictate a different therapeutic strategy and selection of different ingredients (mechanisms of action)? Among the different types of pigmentation, we can differentiate between therapeutic cases according to the question: Is today, as of the day of the examination and treatment, the factor that activates the melanocytes and the cause of hyper melanosis still exists? Examples for existing factor: taking contraceptive pills or hormonal contraceptives; using certain medications, pregnancy, etc. Or is the pigmentation a result of a specific past event that has concluded, and we have no reason to believe that there is still hyperactivity of the melanocytes? Such as: post-acne; PIH due to a one-time skin trauma in someone who did not suffer from hyperpigmentation previously; the previous use of contraceptives that have since been discontinued; use of a photosensitizing drug or one associated with spot formation that has been stopped, etc. Will the current balanced level of melanin production at the start of treatment, based on our understanding and the patient's history, affect the selection of treatment ingredients? The treatment protocol?

10. Acne has been documented as one of the common causes of PIH in individuals with darker skin. Would it be advisable to develop specific acne treatment products for darker skin that include melanin-suppressing ingredients? Should such ingredients also be included in other therapeutic products for various skin issues that stimulate hyper melanosis in darker skin tones? Should they be incorporated into products for cosmetic/aesthetic treatments for individuals with darker skin?

11. To what extent can we rely on the results of comparative studies that utilized two whitening formulas, one of which includes an exfoliating ingredient among other components while the other does not? Does the difference in results favoring the formula with the exfoliant reflect the superiority of the other ingredients in that formula, or the fact that in the formula with the exfoliant, the other ingredients simply penetrate better, and additionally, the existing spot lightened due to the exfoliation process?

12. To what extent do practitioners (or researchers when deciding on the inclusion criteria in the study) consider the circumstances and course of pigmentation? The "story" of the pigmentation? The "story" of pigmentation does not define its type. It refers to all other data such as: duration of pigmentation/when it first appeared, causes—after what or due to what it appeared, previous treatment attempts and responses to them, patterns of exacerbation and remission, lifestyle, and levels of sun exposure or other factors related to hyper melanosis, medication usage, skin sensitivity, skin phototype, involvement of dermal pigmentation in addition to epidermal, and even the patient's willingness to adhere to the precise treatment protocol?

5. Discussion

Hyperpigmentation is a complex phenomenon, with knowledge, experience, and insights continually accumulating. Given the significant importance of this condition as an aesthetic concern affecting large segments of the population and impairing their quality of life, it is crucial to present new ideas constantly. We must critically review our practices, thoroughly assess what works best for us, and strive to improve therapeutic strategies. Openness to new ideas and the enhancement of current treatment protocols can lead us to a more updated, precise, and effective response to this significant aesthetic issue.

The review of well-documented active ingredients included in this work, along with an examination of the types and mechanisms of hyperpigmentation, combined with 20 years or

practical experience, has prompted me to raise several questions. I will elaborate on these topics here and attempt to offer insights.

The question of the relationship between melanin synthesis inhibitors and the patient's skin phototype is intriguing and familiar to some practitioners. I found very few comparative studies specifically designed to address this question. Are there ingredients whose concentrations should be reduced/adapted when treating darker skin? The study cited by Valeria G-M et al (2022) regarding the use of 0.1% ATRA retinoid, which is known to potentially irritate the skin, indicated that this concentration resulted in lightening effects in a light-skinned individuals, but a lack of significant response in a darker-skinned ones. The same ingredient and concentration in another study involving darker-skinned individuals, as cited by Siddiq M., Yvette M M. (2022), resulted in a 32% improvement, but 67% of the participants developed retinoid dermatitis, a phenomenon that the researchers consider concerning, as it can itself lead to the development or worsening of hyperpigmentation. it is possible that at the time the results were measured, the worsening of the spots had not yet occurred, but may have developed later, and this change was missed due to the lack of long-term follow-up measurements? Could it be that those patients, at some point, would no longer be able to use this formulation due to side effects, leading us to conclude that it is suitable only for short-term, limited use? Another ingredient known to some practitioners as one that should be moderated in darker skin is glycolic acid. At lower concentrations, up to 6-7% for daily use, it appears that darker skin can benefit from it. Higher concentrations for daily use might warrant consideration of alternatives. The studies presented here may support this.
Majid S., Masoumeh E., Khadijeh K. (2019) cited two similar studies involving kojic acid and hydroquinone over the same period. In one, a "stronger" formula was used: 2% kojic acid, 2% hydroquinone, and 10% glycolic acid. The less potent formula contained only 1% kojic acid and 2% hydroquinone. One might expect that higher concentrations of tyrosinase inhibitors, alongside an exfoliating agent, would yield better results. In practice, however, the "stronger" formula resulted in a 60% improvement, while the "gentler" one yielded a 71.9% improvement. These differences may be attributed to variations in pigmentation characteristics between the study groups. It may also be considered that 10% glycolic acid elicits different reactions in various skin phototypes, potentially reducing the overall improvement in pigmentation (as i have also experienced in practice). Another aspect i propose regarding skin tone and its relationship to ingredients is in relation to azelaic acid. Both according to studies and practical experience, individuals with darker skin tones seem to respond well to azelaic acid, although it may cause slight irritation in some cases. J. M. Gillbro, M. J. Olsson (2011) notes that it is suitable for treating highly active melanocytes, with minimal impact on uninvolved skin. This could explain the ingredient's affinity for darker skin, characterized by more reactive and active melanocytes. Additionally, its anti-inflammatory properties may specifically benefit individuals with darker skin, who tend to have a higher predisposition to chronic inflammation and exhibit more inflammatory markers.

Another topic worth discussing is research on formulas containing multiple lightening agents. When a positive outcome is achieved using a formulation with two, three, or four ingredients, it becomes challenging to determine the relative contribution (if any) of each ingredient to the overall result. It's possible that 90% of the effect came from just one ingredient, while the contribution of another in the formula may be negligible.

We propose that studies involving more than one ingredient should not only assess the full formulation but also evaluate the efficacy of each component individually as monotherapy. For example, a study conducted on arbutin and aloesin, as cited by (Yong C B. et al., 2021), included three groups: one group used a formulation containing both agents, which resulted in a 63.31% improvement; the second group used arbutin alone, showing a 43.5% improvement; and the group using only aloesin demonstrated a 34% improvement.

In question number 6, we raised the topic of the "duration" of the event that caused the inflammation, which ended in PIH. Skin injury or acute inflammation progresses through stages, the initial ones characterized by an intense immune response, damage to the epidermal barrier, and sensitivity and fragility of the tissue. This intense inflammatory response contributes, among other factors, to hyper melanosis. Should the treatment, when excess pigment begins to emerge while the tissue has not yet healed, be adjusted to the intensity/severity of the inflammation that is still present? Can the use of ingredients with potential to irritate the skin, or their high concentrations, act as "adding fuel to the fire" and irritate the skin triggering it to continue producing excess pigmentation? In studies concerning ingredients for treating PIH, was the condition of the tissue at the time of the study and the time elapsed since the inflammatory event taken into consideration?

We propose to examine the hypothesis that as long as the tissue remains "sensitive" and subclinical inflammation is still present, the treatment strategy should reflect the approach of "less is more." This means using less irritating ingredients/concentrations while prioritizing those that also possess anti-inflammatory properties. Additionally, emphasis should be placed in the early stages of inflammation on stronger tyrosinase inhibition (since hyper melanosis correlates with the intensity of inflammation) and less use of strong exfoliants (such as retinoids or glycolic acid). We recommend considering the avoidance of clinical peels, which themselves constitute stress, and certainly avoiding the use of lasers or IPL as part of the irritative-PIH treatment. To support this theory, it would be beneficial to conduct studies examining different treatment strategies according to the stage of inflammation and compare the outcomes.

Many dermatologists prescribe their hyperpigmentation patients the Kligman formula, or as it is known, the triple combination therapy (TCT). This formula, introduced by Professor Albert Kligman in the 1980s, has served as an ultimate prescription for 40 years, incorporating varying concentrations (depending on the case) of hydroquinone, tretinoin, and hydrocortisone. In light of the accumulating experience and knowledge, it may be worthwhile

to consider refreshing the ingredients and concentrations used thus far. Hydroquinone is associated with a wide range of side effects (as reviewed), particularly in individuals with darker skin tones. Tretinoin's efficacy in darker skin tones is not always high, and its tolerability poses a challenging issue, while hydrocortisone can lead to atrophy in the tissue. The combination of an intensive exfoliant like tretinoin necessitates caution regarding sun exposure, thus the product is prescribed for use in the evening. It may be beneficial to separate the formula into one containing melanin production inhibitors to be used 2-3 times daily, depending on the case. If the practitioner observes more significant hyper melanosis, the usage would indeed be 2-3 times a day rather than just once. Hydroquinone can be replaced with melanin suppressors that have significantly fewer side effects, or a low concentration of 1%-2% can be maintained to minimize adverse reactions and damage to melanocytes. The melanin suppression activity can be complemented with ingredients such as kojic acid, arbutin, niacinamide, tranexamic acid (which has been found to have efficacy similar to hydroquinone), or another well-documented ingredient.

The exfoliating component of the treatment could be tretinoin in lighter-skinned individuals. For darker skin types, Fitzpatrick 4-6, a combination of exfoliants that match the dark skin, or any gentler combination of tretinoin and an additional exfoliating agent, which will not provoke skin irritation, can be used. I propose investigating the issue of skin atrophy caused by hydrocortisone—how long usage is required before atrophy is observed, and to what extent the skin recovers after discontinuation. Given that large populations of individuals with darker skin use lightening prescriptions (including hydrocortisone) for prolonged periods, it may be worth considering additional alternatives. The Kligman prescription, in its current form, may not be suitable for use in treating PIH on skin that still exhibits signs of inflammation or sensitivity, for at least two reasons: (a) retinoids can irritate the skin, and (b) in inflamed skin, it is preferable to place greater emphasis on reducing melanin production; a once-daily application (as done with TCT) may not suffice.

Currently, substantial knowledge has accumulated that allows us to update the formula and make the treatment provided by dermatologists more tailored to the many important details that differentiate each patient from another.

Another important point, which I have not seen addressed in clinical studies or review articles, is the question: Is there currently (on the day treatment begins or on the day the clinical study starts) hyper melanosis present? The fact that hyperpigmentation has developed does not necessarily indicate ongoing hyperactivity of melanocytes and continued abnormal melanin production from the point of pigmentation appearance onward. In certain patients, the hyperpigmentation arose due to a specific event in skin that previously showed no hyperpigmentation. At a certain point in time, the level of melanin production may have returned to normal. In these individuals, hyperpigmentation can still be observed, but it is a result of a past event in skin where melanin production is currently normal. In other cases,

there is chronic and ongoing overproduction of melanin due to various reasons, including medication use, hormonal treatments, constant sun exposure, and combinations of multiple factors. Distinguishing between these two types of cases requires diagnosis and a thorough examination of the pigmentation data and patient history, each time anew. Should the treatment for these two categories of cases, as defined above, be different? I propose that it is worth considering a different treatment approach regarding the therapeutic emphasis. Validation of this approach will require pre-planned studies to test the hypothesis. The suggestion is that in cases where hyper melanosis is present at the current point in time, there should be greater emphasis on melanin suppression. This increased focus can be implemented by using more potent melanin inhibitors, ideally multiple ingredients in the formulation rather than just one, and with a higher frequency of use. This is to address the hyper melanosis existing at the time of treatment. Even after achieving satisfactory lightening of the spots, if current overproduction of melanin is diagnosed, it may be advisable to reduce the exfoliating agents in the treatment protocol while maintaining melanin production inhibitors. These latter ingredients should remain in the treatment protocol as long as the cause of hyper melanosis, or any other factor contributing to hyper melanosis, continues to exist. Additionally, aligning expectations with the patient should consider the fact that, specifically for them, the practitioner has reason to believe that chronic hyper melanosis exists. Discontinuing treatment with melanin suppressors after satisfactory results in cases of chronic hyper melanosis may lead to a recurrence of pigmentation and disappointment for the patients. The factor or factors contributing to hyper melanosis, which will determine the continuation of treatment with melanin suppressors, do not have to be the original cause of hyperpigmentation. They can change. The principle is to address the question of whether there is currently any factor contributing to hyper melanosis. Conversely, there are cases where hyper pigmentation arises from a past event in the patient's history, where currently, to the best diagnosis of the practitioner, there is no hyper melanosis. In such cases, therapeutic emphasis can be placed on exfoliating ingredients, with reduced use of melanin suppressors, and consideration can be given to discontinuing treatment upon achieving a satisfactory result.

Another point worthy of consideration is the comparative studies designed to evaluate the efficacy of ingredients for treating hyperpigmentation, where one group of participants is given tyrosinase inhibitors, while another group receives tyrosinase inhibitors in addition to an exfoliating agent. Such a study was conducted by Draelos et al. and cited byErica C. D., Valerie D. C. (2010).A formulation containing kojic acid, emblica extract, and glycolic acid was compared to a formulation containing 4% hydroquinone in 80 multi-ethnic patients. The results showed equivalent efficacy in skin lightening capabilities between the two agents. Can we conclude from this that kojic acid and emblica extract are effective in melanin suppression? To what extent? Glycolic acid can lighten the spots to some degree on its own. In addition, it increases skin permeability to the other two agents, giving them an advantage over

hydroquinone, which was used alone without any exfoliating agent to aid its penetration or provide additional lightening effects. As mentioned above, it is also difficult to deduce the efficacy (if any) of emblica extract alone or kojic acid alone, since they were part of a formula. To evaluate the efficacy of melanin suppressors or to compare them with each other, it may be advisable not to include exfoliating ingredients in the study, as they can create a bias in the interpretation of the results.

The final topic to be discussed is the issue of personalization and the precise tailoring of treatment for hyperpigmentation. To what extent do the findings from existing studies support a personalized approach to the ingredients and treatment strategies? Numerous factors influence an individual patient's response to pigmentation treatment. A variety of factors must be considered when developing a treatment strategy for hyperpigmentation. These include the duration of the pigmentation, its course (what caused it to worsen, if it did? Was there spontaneous regression, or was it non-spontaneous, and what contributed to that?), previous treatments and ingredients the patient has used, their personal response to these treatments, what "worked for them" and what did not; the initial trigger for the development of pigmentation, whether there are multiple triggers; the extent of the patient's exposure to factors that could exacerbate pigmentation; what medications and products they are currently using; whether there are ingredients to which their skin is more sensitive; in which layers of the skin the pigmentation is located; and more. When selecting a study population, most of these questions are not considered. A heterogeneous study population may yield findings that reflect some average but do not provide what will yield effective treatment outcomes in individual cases. This is because each patient is a complex interplay of circumstances, genetics, and factors. On one hand, it is challenging to gather a study population with completely homogeneous data; if achieved, the findings will reflect what is applicable only to very specific individuals whose data are similar to those of the study population. On the other hand, excessive variability in the study population, as often occurs, may lead to results that distort our understanding of certain ingredients, which may be perfectly suited for some patients but not beneficial, or even harmful, for others. This is due to differences in skin tone, circumstances of the pigmentation, or other important data.

We propose that an attempt be made to establish more specific inclusion criteria in research populations so that the population will be more homogeneous in its data. The findings may then be more relevant to individuals similar to the subjects, leading to treatment strategies that are closer to personalized care, precise, and with the potential to yield better therapeutic outcomes.

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