Nutrition for Healthy Skin

Jean Krutmann • Philippe Humbert
(Editors)

Nutrition for Healthy Skin

Strategies for Clinical and Cosmetic Practice

Springer

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Foreword

The intimate relationship between dermal demand of nutrients and adequate supply from the blood circulation seems to have been understudied. In fact, the title of this book can be read in two ways; balanced nutrition is necessary for maintaining healthy skin, and there are nutritive aspects to restore healthy conditions after a disease state has developed. Skin care has an important metabolic and nutritive component. Maintenance and restoration are integral processes in skin health.

It is satisfying to see that the two editors, Professors Jean Krutmann from Düsseldorf, Germany, and Phillippe Humbert from Besancon, France, have been able to compile the pertinent aspects by attracting contributions from the world leaders in this subject area. Three major sections make up the treatise: Nutrition and Skin, and its scientific basis; Functional Food, addressing botanicals and other micronutrients as well as probiotics and; thirdly, Aspects of Clinical Dermatology, culminating in the topic of beauty from inside.

The need for scientifically sound information on this subject area is particularly urgent, since the general public is being supplied with suggestions from the news media and, increasingly, from the Internet with material which is not always based on sufficient scientific evidence. The present treatise will also be good for delineating the problems and limitations in current knowledge. The authors, the editors, and the publisher can be congratulated to a timely opus!

Duesseldorf, Germany — Helmut Sies

Preface

The relationship between nutrition and skin has become a “hot” topic that is exciting researchers and clinicians worldwide. New insights into the effects of orally applied, biologically active molecules on skin functions have stimulated a continuously growing interest in the development of nutritional supplements and, most importantly, functional food products to benefit human skin. This monograph attempts to provide an up-to-date overview regarding all aspects of nutrition and skin. It includes in-depth, critical discussions of the molecular basis as well as current concepts propagated for nutrition-based cosmetic, preventive, and therapeutic dermatological strategies. The explosion of knowledge in this field over even the last few years is remarkable with consequences for practicing dermatologists, patients, cosmetic and nutritional industry, and consumers in general. To capture the depth and breadth of this learning, we have recruited leading experts from multiple subdisciplines. All authors are internationally recognized, and we are very grateful for their excellent contributions. We hope that this book will serve you as a state-of-the-art reference and will further stimulate your interest in this fascinating area.

Duesseldorf, Germany — Jean Krutmann
Besançon, France — Philippe Humbert
March 2010

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Part I

Nutrition and Skin: The Scientific Basis

Cutaneous Changes in Nutritional Diseases

1

Matthias Schmuth and Peter O. Fritsch

Core Messages

- Abnormal nutrition causes cutaneous changes that are either due to insufficient food supply; i.e., inadequate intake of nutrients, vitamins, and minerals, or to excess calory intake.
- In countries with inadequate food supply, protein-energy malnutrition (*marasmus*, *kwashiorkor*) is common and children ≤ 5 years are at highest risk. In 2001, approximately 50% of childhood deaths were indirectly or directly attributable to inadequate nutrition.
- In countries with adequate food supply, the most common nutritional abnormalities are obesity due to excess food consumption, and malnutrition due to psychological (anorexia nervosa, bulimia) or medical conditions (metabolic disease, chronic illness, hospitalization), affecting both children and adults.
- Skin changes provide important clues for lack or overabundance of individual nutritional components and can help clinicians to correctly detect, diagnose, and consequently treat nutritional disease, which can be confirmed by laboratory testing.
- While the importance of individual components for normal function of the skin is undisputed, there are many compensatory mechanisms in place. Nutritional disease is rarely the result of the deficiency of a single nutrient.
- While substitution of deficient nutritional components usually results in rapid resolution of symptoms, toxic effects of overload have become more common with the increasing popularity of dietary supplementation. This is particularly common with lipophilic vitamins (A, D, E, and K) because they accumulate in the tissue.

1.1 Nutritional Deficiencies

1.1.1 Marasmus and Kwashiorkor

Nutritional deficiencies can be exogenous or endogenous. The primary exogenous reason is insufficient intake of nutrients. Endogenous etiologies include intestinal or metabolic disease that interferes with the absorption and delivery of nutrients to the cellular machinery (e.g., intestinal malabsorption, gastrointestinal and metabolic disease, infections, cancer) (Table 1.1). With prolonged nutritional deficiencies, energy storage is exhausted and energy supply lags behind. Because of their increased nutritional needs during the growth phase, children ≤ 5-years old are particularly susceptible to the developmental and physiologic consequences of poor nutrition.

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Table 1.1 Causes of nutritional deficiency

Exogenous (inadequate food intake)	Endogenous (inadequate food absorption/metabolization)
Poverty	Intestinal malabsorption
Old age	Gastrointestinal disease
Alcoholism	Metabolic disease
Psychiatric disorders	Chronic systemic disease
Diets (e.g., "fad diets," "allergy diets")	Cancer
Child neglect	Recurrent infections
	AIDS (inadequate intake, e.g., due to candida esophagitis)

Table 1.2 Differential diagnosis of hyperkeratotic papules on the extremities due to nutritional deficiency

Vitamin A deficiency (phrynoderma)
Vitamin C deficiency
Essential fatty acid deficiency

Marasmus is due to insufficient (although balanced) nutritional quantities. Marasmus is not only due to decreased overall caloric supply, but also results from a deficit in essential nutritional components (e.g., vitamins, essential amino acids, minerals). Therefore, the cutaneous changes of marasmus are multifold. Aside from a decrease in the subcutaneous fat, the dermal and epidermal layers are thinned which gives the skin an aged appearance. In addition, there is dryness of the skin, sometimes to the degree of ichthyosis-like scaling. Vitamin A and C deficiency result in follicular hyperkeratosis (see below, Table 1.2). Because of anemia and vasoconstriction, the skin color is pale, while in sun-exposed areas there is spotty hyperpigmentation. The hair is dry, loses color ("premature graying"), and hair loss (telogen effluvium) is common. The growth of the nails is delayed, and the nail plates may show longitudinal ridging. Marasmus is corrected by carefully restoring protein-calorie intake and by supplementation of vitamins, essential fatty acids, and zinc according to their respective blood levels.

Kwashiorkor occurs if normal carbohydrate consumption is coupled with insufficient protein intake; i.e., chronic malabsorption such as in cystic fibrosis. It is most common in infants in third world countries as soon as their mothers discontinue breast feeding. Kwashiorkor can also occur in children receiving a calorie-rich diet that is poor in proteins of animal origin [4]. These children show the cutaneous changes of marasmus (see above), and in addition develop diffuse edema due to hypoalbuminea, and increased vulnerability of the skin (e.g., to mechanical trauma), which results in erosions and blisters in areas of friction. A further characteristic of kwashiorkor is a reddish-brown scaly dermatitis ("flaky paint"), and dusky erythematous plaques with a waxy appearance in pressure-exposed areas (diaper area, over bony prominences) with a thickened, pigmented stratum corneum on histology. Depigmentation of the skin can be observed (predominantly in the perioral area and on the lower legs). Moreover, depigmentation of the hair to a reddish color is often observed. Correction of kwashiorkor must be undertaken carefully; electrolyte imbalances need to be taken into account, combined with supplementation of vitamins, essential fatty acids, and zinc as above.

In both marasmus and kwashiorkor, individual hair shafts show pigmented areas alternating with depigmented areas ("signe de la bandera" or "flag sign"), reflecting intermittent periods of food availability. In fact, because of overlapping features, a clear distinction between marasmus and kwashiorkor can not always be made with certainty. In these cases, the term *protein-calorie malnutrition* is used instead. Generally, chronic nutritional deficiencies increase the susceptibility to opportunistic infections by causing a secondary immune deficiency. Particularly problematic are mixed infections of the skin with fusiform bacteria and spirochetae (e.g., bacterium fusiforme, spirochäta refringens) causing necrotizing ulcerative gingivitis, noma, or cancrum oris which can be life-threatening. In adults, similar treatment-recalcitrant ulcerations occur on the lower legs following insect bites.

Most commonly, malnutrition is due to inadequate food availability, but it is also seen in individuals with medical conditions, particularly in hospitalized patients, which often can simply be ascribed to poor logistics (negligence of nutritional needs in patients waiting for a complex diagnostic workup). Other reasons are individuals voluntarily subjecting themselves to unusual diets and individuals with excessive alcohol consumption [3]. *Anorexia nervosa* and *bulimia* are psychiatric disturbances that lead to physical disturbances. Cutaneous changes associated with these disorders are manifold including dry skin, pruritus, patchy hyperpigmentation, freckles, lanugo hair, brittle terminal hair and nails, and paronychia. Russell's sign refers

to callus formation on the hand used to elicit vomiting, which is another diagnostic clue. Early recognition is desirable, because the mortality is much lower with early intervention.

1.1.2 Essential Fatty Acid Deficiency

Malnutrition is a common cause of essential fatty acid (e.g., linoleic, linolenic, and arachidonic acid) deficiency. Patients present with diffuse eczematous skin changes that can be pruritic and preferentially affect the periorificial areas. With long-standing essential fatty acid deficiency, there can also be depigmentation and alopecia (telogen effluvium). In children, there is growth failure. Essential fatty acid deficiency is associated with impaired wound healing, capillary fragility, abnormal liver, and kidney function, and neurologic damage. The differential diagnosis includes zinc deficiency (see below), and necrolytic migratory erythema. Plasma levels of linoleic, linolenic, and arachidonic acids are decreased. In contrast, palmitoleic and oleic acids are increased, and there is abnormal presence of 5,8,11-icosatrienoic acid in plasma. Therapeutic fatty acid supplementation is effective.

1.1.3 Vitamin Deficiencies

Vitamins are cofactors in metabolism; nutritional vitamin deficiency results in metabolic disturbances. In Western societies, this is mostly due to impaired intestinal absorption (e.g., in inflammatory bowel disease, inherited metabolic disease, parenteral nutrition, following surgery), or due to alcoholism. Because the deficiency usually involves multiple vitamins, it is often difficult to determine the relative role of individual vitamins [1].

Vitamin A deficiency causes ichthyosis-like skin changes with generalized fine scaling and a thickening of the outermost skin layer, the stratum corneum ("phryonoderm"), which is particularly pronounced in the follicular openings, causing follicular hyperkeratosis [2]. This is often associated with effluvium and fragility of the hair. One of the earliest signs of vitamin A deficiency, however, is impaired night vision and the inability to see in bright light. Metaplasia of the conjunctival epithelium in vitamin A deficiency has been called keratoconjunctivitis sicca (Bitot macules), which can progress to keratomalacia, permanent scarring and blindness. Finally, vitamin A deficiency is associated with an increased incidence of epidermal neoplasias (anticarcinogenic activity of vitamin A). The differential diagnosis of vitamin A deficiency includes lichen pilaris, ichthyosis vulgaris, Darier disease, and other vitamin deficiencies (see biotin, vitamin C deficiency below). Extracutaneous manifestations include growth failure and mental retardation. For diagnosis plasma retinol levels are measured. Vitamin A supplementation resolves ophthalmologic symptoms within days and cutaneous changes within weeks.

Vitamin B1 (thiamin) is involved in carbohydrate metabolism, and B1 deficiency is known as *beriberi*. It is seen with gastrointestinal disease, a diet restricted to polished rice, alcoholism, pregnancy, lactation, and diabetes mellitus. Mucocutaneous changes include edema and glossitis with glossodynia. Predominant are neurologic symptoms including peripheral neuropathy, confabulation (Korsakoff's syndrome), and encephalopathy (Wernicke). Low urinary aneurin excretion is used as a diagnostic test. Supplementation is effective.

Vitamin B2 (riboflavin) can be due to a poor diet, but can also be caused by medications that impair its absorption (galactoflavin, phenothiazines, tricyclic antidepressants). Cutaneous changes that indicate vitamin B2 deficiency include seborrheic dermatitis-like scaling on the face (nasolabial folds), head, and genitocrural region. In addition, these patients present with cheilitis, perleche, pallor, and atrophy of the tongue

Table 1.3 Differential diagnosis of cheilitis due to nutritional deficiency

Zinc deficiency – genetic – acquired
Biotin deficiency
Vitamin B2 deficiency*
Vitamin B6 deficiency
Vitamin B12 deficiency*
Folic Acid deficiency
Zinc deficiency*
Iron deficiency*

* in association with angular involvement (perleche)

(Table 1.3). Ophthalmologic involvement includes blepharitis, conjunctivitis, and corneal vascularization. Vitamin B2 is a cofactor of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which are involved in many redox reactions. On blood testing patients show a normochromic anemia. Decreased erythrocyte glutathione reductase activity confirms the diagnosis. The differential diagnosis includes seborrheic dermatitis and zinc deficiency. In mild cases, the recommended treatment is riboflavin 3–10mg daily per mouth, in refractory cases 2 mg daily via the intravenous route.

Vitamin B3 (niacin) deficiency causes pellagra. Pellagra is characterized by a triad consisting of changes in skin, nervous system, and the gastrointestinal tract ("3D's": dermatitis, dementia, diarrhea). An early symptom is diarrhea. At later stages, patients report increased UV sensitivity (face, sun-exposed distal upper extremities) and sun-burn-like pruritic or burning erythematous macules, and occasionally blisters (Table 1.4). The facial rash at times resembles the butter fly rush of lupus erythematodes, but is always associated with other components of the triad. Quite characteristic is the sparing of the forehead as well as eczema of the neck and upper chest that can resemble a necklace (Casal's necklace) (Fig. 1.1). Here the skin is erythematous to brown (or black), scaly. Sometimes there is an eczema craquele-like appearance with fissures and occasionally there are crusts. These lesions are common on the dorsal hands (Fig. 1.2), and can also be found on the feet and in the genitocrural region. Glossitis and stomatitis can also be present. Neurologic symptoms include peripheral polyneuropathy, encephalopathy, and depression. The differential diagnosis includes contact eczema, photo-induced dermatitis, and porphyria cutanea tarda. Pellagra is a clinical diagnosis, there are no laboratory markers. Niacin is a component of nicotinamide-adenine-dinucleotide (NAD) catalyzing redox reactions. A frequent setting for niacin deficiency is a niacin-deficient diet, which has occurred with the introduction of corn as a major food that only contains bound niacin that cannot be used by the human body. This is exemplified by endemic pellagra in geographic areas with predominant corn consumption, e.g., in South America. Aggravating factors include alcoholism, long-standing antibiotic therapy, isoniazid, 5-fluoruracil, inflammatory bowel disease, abnormalities of tryptophan metabolism (carcinoid), and Hartnup disease (see below). As with the other vitamin deficiencies, vitamin B3 supplementation will resolve the symptoms, but exogenous niacin can release histamine causing urticaria and worsening of preexistent asthma. Niacinamide is the preferred choice for supplementation, because it avoids these adverse effects.

Fig. 1.1 Pellagra: butter fly rash and Casal's necklace

Fig. 1.2 Pellagra: scaly, erthematous to brown plaques on the dorsal hands

Table 1.4 Differential diagnosis of photosensitive eruptions due to nutritional deficiency

Vitamin B3 deficiency (pellagra)
Hartnup disease

Vitamin B6 (pyridoxine) deficiency is usually accompanied by other deficiencies and is associated with seborrhoic dermatitis-like skin changes in perioricial distribution (eyes, nose, mouth) as well as cheilitis and glossitis. Pyridoxine is a cofactor of enzymes involved in amino acid metabolism (e.g., transaminases, synthetases, hydroxylases) and the metabolization of linoleic acid into arachidonic acid. Associations have been described with drugs such as isoniazid, penicillamine, hydralazine hydrochloride, oral contraceptives, phenelzine sulfate, cycloserine, and with uremia and liver cirrhosis. Diagnosis is made by measuring pyridoxine serum levels. Supplementation is effective.

Lack of *vitamin B12* (cyanocobalamin), because of decreased intrinsic factor, is known for causing pernicious anemia, but can rarely also be due to strict vegetarian diet. Aside from its hematologic consequences (megaloblastic anemia), occasionally vitamin B12 deficiency also is associated with atrophic glossitis, angular cheilitis, mucositis, and symmetric acral (dorsal fingers and toes) and flexural hyperpigmentation. Poliosis, vitiligo, and alopecia areata occur with increased frequency. The differential diagnosis for the hyperpigmentation includes Addison's disease. Intramuscular supplementation is effective (1 mg per month), resolving symptoms within 2–12 weeks.

Folic acid deficiency has similar mucocutaneous changes to vitamin B12 deficiency including hyperpigmentation and glossitis, but cheilitis and mucosal erosions have also been described. Decreased serum folate is diagnostic; oral supplementation is effective.

Vitamin C deficiency is the cause of scurvy. In the past, this was common among sailors and other people without access to fresh fruits and vegetables for extended periods of time. Although vitamin C deficiency has become much less common today, it is still encountered in the setting of urban poverty where it preferentially affects the very young and the aged (exacerbated by general malnutrition, mental incapacity, alcoholism). It is also seen with fad diets. Cutaneous changes of vitamin C deficiency include follicular hyperkeratosis on the extensor surfaces of the extremities, which characteristically show perifollicular hemorrhage (Fig. 1.3). The propensity for hemorrhage is due to fragile blood vessels, which is particularly pronounced in newborns and infants that present with petechia (over mechanical pressure points) and intestinal as well as urinary tract bleeding. In children, subperiosteal hemorrhage with radiographic alterations and pseudoparalysis has been described. Adults with long-standing vitamin C deficiency report impaired wound healing, bleeding gums, gingivitis, gingival hypertrophy, and loss of teeth. General symptoms of scurvy include fatigue, muscle weakness, myalgia, arthralgia, diarrhea, and anemia. The onset is approximately 1–3 months after onset of insufficient vitamin C intake. Long-standing, severe vitamin C depletion can result in diffuse edema, oligura, anemia, dyspnea, and neuropathy. Supplementation with vitamin C is usually successful, if the deficiency is recognized early enough. Left untreated, the condition can lead to death. Low vitamin C serum levels are diagnostic. The recommended dose for vitamin C supplementation in individuals with deficiency varies between 100–1,000 mg of ascorbic acid per day. Infants should be treated with 50 mg of ascorbic acid up to four times per day.

Fig. 1.3 Scurvy: follicular hyperkeratoses with perifollicular hemorrhage

Vitamin D deficiency is not associated with cutaneous changes (it primarily causes bone disease; i.e., rachitis in children and osteomalacia in adults). Vitamin D deficiency has also been associated with higher susceptibility to infections; i.e., tuberculosis.

Vitamin E deficiency is not associated with cutaneous changes (it primarily causes neurologic abnormalities).

Biotin (vitamin H) deficiency causes an exfoliative dermatitis on acral skin, cheilitis, and/or periorificial dermatitis. If occurring in newborns, the disease may present with erythroderma and alopecia. The most common extracutaneous feature is enteritis. Other extracutaneous findings include metabolic acidosis, developmental delay, hearing loss, paraesthesias, seizures, and conjunctivitis. Biotin deficiency is associated with impaired cellular immunity, there is a predisposition for infections; i.e., candida dermatitis. Biotin is a cofactor of carboxylases (biotinidase, holocarboxylase). Decreased serum biotin levels can be acquired or genetic. The differential diagnosis includes essential fatty acid deficiency. Hyperamonemia and organic aciduria are used for screening; the definitive diagnosis is established by assaying carboxylase synthetase activity in fibroblasts. Supplementation is effective.

Vitamin K deficiency, in severe cases, can lead to hemorrhage of the skin and mucous membranes. Clinical hemorrhage together with a prolonged prothrombin time leads to the diagnosis. This is seen in newborns or in later life in individuals with malabsorption, cystic fibrosis, liver disease, and drugs (warfarin, salicylates, cephalosporins). Treatment consists of parenteral vitamin K (1 mg newborns, 2 mg children, 5–10 mg adults).

1.1.4 Trace Element Deficiencies

Zinc deficiency is known for the classic triad of dermatitis, alopecia, and diarrhea. However, only 20% of patients present with all three components of the triad at a given time. Zinc deficiency can be hereditary or acquired. H*ereditary zinc deficiency (acrodermatitis enteropathica)* is an autosomal recessive intestinal abnormality of zinc absorption due to a mutation in a zinc transport protein. Human milk contains a zinc transport protein that is much less abundant in cow's milk. Therefore, infants typically develop cutaneous changes days to weeks after being switched to bottle feeding (cow milk). Following intestinal absorption, zinc is bound to albumin. Whereas 99% of body zinc is intracellular, zinc storage is poor (total body zinc 2–3 g) and depletion occurs rapidly; i.e., within a month (zinc deficiency = <70mg/dl). Patients initially present with a perioral erosive dermatitis and perleche that progresses to involve the entire face, scalp, acral sites, and the diaper area. This can be accompanied by ulcerations of the oral mucous membranes and glossitis. The periorificial distribution is helpful in making the diagnosis. Palmar erythema, sometimes with anular or collarette-like scaling, may be present. If the dermatitis is accompanied by alopecia (telegon effluvium) and/or photophobia, zinc deficiency needs to be considered. Conversely, telogen effluvium alone without accompanying skin changes cannot be ascribed to zinc deficiency. Other differential diagnoses of zinc deficiency include seborrheic dermatitis or other eczematous eruptions. At times, patients present with persistent cutaneous infections, e.g., candida dermatitis, paronychia, as well as with onychodystrophy, blepharitis, and conjunctivitis. There is an immunodeficiency, preferentially due to functional impairment of T cells. Before the advent of zinc supplementation, affected individuals, primarily newborns and infants, would die from infections. The predominant extracutaneous symptom is diarrhea with electrolyte imbalance of variable degree. Long-standing zinc deficiency also leads to delayed wound healing, growth retardation, anorexia, anemia, hypogonadism, and altered mental status. Individuals with zinc deficiency are frequently infertile. If they conceive, infants may show malformations.

The cutaneous changes of *acquired zinc deficiency* are similar, but usually milder than those of hereditary deficiency. Because of its relatively mild symptoms, acquired zinc deficiency may be underdiagnosed. It can develop relatively quickly with an unbalanced diet (exclusive high fiber content interferes with absorption), parenteral nutrition lacking sufficient zinc supplementation, malabsorption (including cystic fibrosis), or abnormal intestinal loss of zinc. Chronic diarrhea is a common cause and can lead to a vicious cycle where diarrhea compromises zinc absorption and zinc deficiency in turn causes diarrhea. Other disease associations include chronic renal failure, malignancy, drugs, alcoholism, HIV infection, and pregnancy (Table 1.5). Zinc is a critical component of many enzymes. An important consequence of zinc deficiency is poor incorporation of essential fatty acids into eicosanoids. Skin histology shows a "pallor" of the upper epidermis. In more pronounced cases, there may be vacuolar degeneration of the upper epidermis,

Table 1.5 Causes of acquired zinc deficiency

Poor Intestinal Zinc Absorption	Malabsorption
	Chronic liver and pancreatic disease
	Other gastrointestinal disease
	Alcoholism
	Unbalanced diet (e.g., exclusive high fiber)
	Parenteral nutrition lacking zinc supplementation
Increased Zinc Excretion	Liver cirrhosis
	Renal disease
	Diabetes mellitus
	Dialysis
Increased Catabolism	Cancer
	Chronic recurrent infections, AIDS
	Trauma, burns
Decreased Serum Albumin	Nephrotic syndrome
	Liver cirrhosis

Table 1.6 Differential diagnosis of periorificial dermatitis due to nutritional deficiency

Zinc deficiency – genetic – acquired
Essential fatty acid deficiency
Biotin deficiency
Vitamin B2 deficiency
Vitamin B6 deficiency
Glucagonoma syndrome
Pseudoglucagonoma syndrome

epidermal hyperplasia, and hyperkeratosis. In later stages, there is epidermal atrophy with flattening of the rete ridges and dermal fibrosis. The differential diagnosis of zinc deficiency (see Table 1.6) includes abnormal amino acid absorption, biotin deficiency, essential fatty acid deficiency, and the glucagonoma syndrome ("necrolytic migratory erythema"), all of which may show similar histologic changes. Serum zinc levels are measured for diagnosis. Because alkaline phosphatase is zinc-dependent, it can serve as an additional surrogate marker. Supplementation of zinc can be achieved by the oral or intravenous routes (1–2 mg/kg/day in the acquired, 3 mg/kg/day in the hereditary form).

Iron deficiency is associated with pallor of the skin, dry/scaly skin, perleche, glossitis, dull, shaggy hair, in the case of long-standing deficiency with telogen effluvium and coilonychia [5]. Blood smears typically show microcytic, hypochromic anemia. Serum iron is decreased, being tightly regulated between intestinal absorption, protein-bound transport (transferrin), and intracellular storage (ferritin). Supplementation is effective.

Copper deficiency (*Menkes Syndrome, Kinky hair Syndrome, Steely hair disease*) is due to X-chromosomal recessive mutations in the Cu(2+)-transporting ATPase (ATP7A). Patients present with saggy and hypopigmented skin, there are follicular hyperkeratoses, and the hair is sparse, hypopigmented, and brittle (pili torti, monilethrix, occasionally trichorrhexis nodosa). In addition patients lack eyebrows and lashes. Extracutaneous changes include neurodegenerative changes. At birth and for the first few months, infants appear normal, but subsequently develop hypotonia, seizures, and failure to thrive resulting in death by 2–3 years of age. Another feature is tortuous, elongated arteries due to immature elastin fibers. Copper is a component of enzymes important for elastin, collagen, and melanin synthesis, e.g., lysyl hydroxylase, tyrosinase, etc. Total body copper content is 80 mg, 90% of which is associated with ceruloplasmin, the remainder with other plasma proteins, mainly albumin. Due to fluctuations, serum copper and ceruloplasmin are unreliable predictors in the neonatal period. However, because the lack of copper impairs the function of enzymes of catecholamine synthesis and metabolism, there is a distinctive increase in the dihydroxyphenylacetic acid to dihydroxyphenolglycol ratio that is of diagnostic value. Despite the defective transport protein, intramuscular copper injections can be effective if commenced within days after birth, particularly in individuals with residual ATP7A activity.

Selenium deficiency presents with a whitish discoloration of the nails and effluvium. It has been reported in patients receiving parenteral nutrition. Extracutaneous features include cardiomyopathy, muscle pain, and weakness. Because selenium is essential for glutathione peroxidase, low activity of this enzyme and low plasma selenium are diagnostic. Supplementation is effective (2 mg/kg/day).

1.2 Excess Nutrition

1.2.1 Obesity

In Western societies excess nutrition has become a significant problem (Table 1.7). Overweight (body mass index 25–29.9) and obese (body mass index ≥ 30) individuals have an increased general morbidity, predominantly from metabolic and cardiovascular disease. There are several characteristic skin changes that are more common in overweight individuals, and can be used as markers for individuals at risk for internal disease. In overweight and obese individuals, pseudoacanthosis nigricans is an indicator for insulin resistance and metabolic syndrome. The skin folds of obese individuals are subject to increased friction, they are commonly hyperpigmented (inner thighs, submammary region) and carry skin tags (achrocordon). The enlarged surface between the folds creates a niche for microbial growth, which is further exacerbated by sweating. Over time, this commonly leads to intertiginous eczema, secondary overgrowth of bacteria, erythrasma, dermatophyte, and yeast infections. Other skin findings associated with obesity include hyperhidrosis, striae distensae, stasis dermatitis, venous hypertension, and leg ulcers.

1.2.2 Hypervitaminoses

With the increased popularity of vitamin supplementation, excess vitamin intake has become more common (often triggered by aggressive advertisements promoting the vitamin's beneficial effects). Because lipophilic vitamins (A, D, E, K) can accumulate in tissue, these are more prone to having toxic effects. Syndromes due to excess hydrophilic vitamins are not as well described.

Table 1.7 Causes of excess nutrition

Exogenous (excess food intake)	Endogenous (abnormal metabolism)
Social	
Depression	Genetic
Anxiety	
Iatrogenic	

Several meta-analyses have failed to demonstrate sustained beneficial effects of vitamins A, B6, B12, C, E, and beta-carotene on carcinogenesis and cardiovascular disease.

Hypervitaminosis A develops with excess supply of vitamin A. Today, this is seen with long-term vitamin (over)supplementation. The skin findings of hypervitaminosis A include pruritus, generalized scaling, dry mucous membranes, alopecia (telogen effluvium), cephalea, nausea, increased serum transaminases, and lipids. Hyperostoses similar to those seen with retinoid medication have been described. Hypervitaminosis A is also characterized by an orange-yellowish skin tint. In contrast to generalized jaundice from hyperbilirubnemia, there is sparing of the sclera, eyelids, ears, and axillary folds. The same pattern of skin discoloration is seen with excessive beta-carotene consumption (the natural provitamin of vitamin A contained in carrots, red palm oil, etc.) which is used for self-tanning. The skin color is particularly evident in the palms and soles (depends on the thickness of the epithelium; i.e., mucous membranes are less affected). This is quite common in children and vegetarians, it is sometimes also seen with renal disease, diabetes mellitus, and thyroid disease (myxedema) due to a decreased ability to convert beta-carotene into vitamin A in these diseases. Serum carotenoid levels are increased. Patients should be educated about the limitations of photoprotection by beta-carotenes (cf. Chap. 6).

Historically, hypervitaminosis A was seen in inuit populations who consumed polar bear liver that contains excessive amounts of vitamin A causing hypervitaminosis A. Therefore, inuits have learned to be very careful about eating polar bear liver while hunting.

Hypervitaminosis C develops after long-standing dietary intake of vitamin C which then interferes with vitamin B12 metabolism and bears the symptoms of its deficiency (see above). The combination of vitamin C with estrogen medication can lead to kidney (oxalat) stones.

Hypervitaminosis D, e.g., in patients with renal disease supplemented with vitamin D, can result in anorexia, cephalea, vomiting, diarrhea, hypercalcemia, and calcium deposition in the skin (*calcinosis cutis*).

Hypervitaminosis E is rare and only manifests after very high vitamin E consumption, causing gastrointestinal upset, cephalea, and icterus in premature neonates.

1.2.3 Trace Element Deposition

Dietary *zinc* supplementation can be toxic if overdosed causing nausea, vomiting, upper intestinal hemorrhage, vertigo, and neutropenia. In these cases, serum zinc levels are markedly increased. Therefore, monitoring of serum zinc levels and blood counts are warranted with long-standing zinc supplementation.

Because normally 95% of nutritional iron is not absorbed (mucosa block), *iron overload* is usually due to an inherited abnormality in iron absorption (primary hemochromatosis) or to parenteral iron overload (secondary hemochromatosis). Iron is deposited in many tissues including liver, heart, and skin. Diffuse bronze-color hyperpigmentation of the skin with a predilection of sun-exposed areas can facilitate the early diagnosis of hemochromatosis. The hyperpigmentation not only derives from cutaneous iron deposits, but also from an induction of melanogenesis. Other cutaneous changes include ichthyosis-like scaling, alopecia, and coilonychia. Organ involvement consists of the classic triad of diabetes mellitus, cardiomyopathy, and liver cirrhosis (which in turn has the characteristic cutaneous findings of palmar erythema, teleangiectasia, etc.). Therapy consists of deferroxamine and bloodletting.

High content of either *lead* or *mercury* in food is associated with a bluish-gray discoloration of the gums.

Aluminum intoxication can cause porphyria-like bullous skin changes.

Arsenic is well known as a skin carcinogen that increases the incidence of Bowen disease and basal cell carcinoma. The nails show whitish lines (Mees lines). Extracutaneous consequences of arsenic ingestion are lung cancer, vomiting, diarrhea, hepatic/renal damage, as well as peripheral neuropathy.

Argyrosis is the term for cutaneous deposition of silver metal. In cases of chronic silver consumption, the skin has a diffuse grayish color with a predilection of sun-exposed areas, but also involving the sclerae, mucous membranes, and finger nails (typically toe nails are not affected). Silver deposits can be visualized by dark field or electron microscopy. No therapy is available. Argyrosis has become rare since many of the silver-containing medications (e.g., for the treatment of rheumatoid arthritis) have been discontinued.

Chrysiasis, the deposition of gold in the skin, is similar to argyrosis. Only the color of gold deposits is somewhat different; i.e., diffuse bluish-gray. In contrast to argyrosis, the mucous membranes are typically not affected in chrysiasis. Other cutaneous changes associated with gold intake are maculopapular, vesiculobullous, and urticarial eruptions, occasionally also an erythema multiforma-like rash. Gold-containing medications (e.g., for the treatment of rheumatoid arthritis) have become rare.

1.3 Abnormalities of Amino Acid Metabolism

Hartnup disease is an autosomal recessive disorder of intestinal and renal amino acid transport presenting with amino aciduria. Patients show a sun-burn-like photosensitive eruption reminiscent of pellagra, sometimes blistering, onset is at <13 years. Post-inflammatory hypopigmentation is a common residual. The differential diagnosis of the skin changes includes pellagra and lupus erythematodes. The primary extracutaneous feature is intermittent ataxia, sometimes also nystagmus and tremor. A high-protein diet or oral nicotinamide supplementation have been reported to be beneficial (nicotinamide is photoprotective, cf. Chap. 11.).

Phenylketonuria is an autosomal recessive abnormality of phenylalanine metabolism; i.e., lack of downstream metabolic product tyrosine and accumulation of phenylalanine. Paucity of tyrosine results in diffuse hypopigmention of the skin and hair of affected individuals (“blond and blue eyed”). Other cutaneous changes include eczematous (early onset atopic dermatitis, but also unspecific dermatitis) and scleroderma-like skin lesions. Phenylalanine accumulation is toxic for the brain and causes mental retardation, developmental delay, microcephaly, seizures, and behavioral and psychiatric problems. Urinary screening for phenylalanine accumulation has been widely established for approximately 40 years. Strictly speaking this is not a nutritional disease, but it is the prototype of an inherited condition that can be cured by dietary restriction of phenylalanine together with supplementation of tyrosine and other amino acids. Incompliant adults experience recurrences of the dermatologic manifestations. Individuals resuming the diet may show darkening of their hair.

Tyrosinemia is a rare autosomal recessive disorder of tyrosine metabolism with accumulation of tyrosine

metabolites in the liver, kidneys, and central nervous system. Tyrosinemia type II (Richner-Hanhart syndrome, oculocutaneous tyrosinemia) is characterized by photophobia, corneal ulcerations with onset during the first year of life (the latter can be misinterpreted as herpes keratitis). A painful focal palmoplantar keratoderma occurs during early childhood or may be delayed until adolescence. Although again not a nutritional disease in the strict sense, a diet restricted in tyrosine and phenylalanine will clear the keratitis, keratoderma, and will ameliorate or prevent cognitive impairment.

Arginine-Succininc Acid disease is due to mutations in the gene encoding for arginine succinase and manifests with fragile hair (trichorhexis nodosa) and neurologic symptoms.

Alkaptonuria is an autosomal recessive defect of the enzyme homogentisic acid (or alkapton) oxidase. Cutaneous changes include a grayish-blue discoloration of the ears and the axillae (ochronosis). There can also be arthritis and darkening of the urine (due to oxydation).

Eosinophilia-Myalgia-Syndrome stands for a scleroderma-like disorder with woody induration of the distal extremities that historically was observed in individuals who consumed large quantities of contaminated L-tryptophan.

1.4 Nutrition, Skin Physiology, and Skin Pathology

In this chapter, the characteristic skin findings that are reproducibly associated with either lack or abundance of individual nutrients, vitamins, or trace elements are described. These observations allow us to deduce the importance of individual nutritional components for skin physiology. The substitution of deficient components rapidly reversesas associated skin changes. In contrest the prophylactic supplementation of nutrients to enhance skin physiology has yielded disappointing results. The available studies not only fail to consistently prove beneficial effects of vitamin supplementation on skin physiology, but meta-analyses even indicate an increased risk for cardiovascular disease with high doses of vitamins B6, B12, C, and E. Thus, to date it is still controversial if prophylactic vitamin supplementation can have sustained beneficial effects. One possible explanation is that the metabolism of cutaneous tissues, in particular the epidermis, is remarkably autonomous; i.e., many metabolic reactions in the epidermis occur independent from the rest of the body. Furthermore, while several of the vitamins mentioned above are potential oxygen radical scavengers, systemic delivery is unlikely to achieve a sufficient concentration in the epidermis to effectively prevent free radical formation.

Similarly complex is the evidence for nutritional supplementation effects on skin pathology. For example, there is a decade-long discussion about the dietary factors that may elicit or exacerbate acne vulgaris, but suggestive data has not been replicated. Approximately 10% of children with severe atopic dermatitis experience flares upon food allergen exposure. However, diagnostic testing and recommendations for avoidance of individual food ingredients should regularly be reevaluated (retested), because of the risk of developing nutritional deficiency due to unnecessary food restriction. Thus, the goal of testing is to identify food that is tolerated in order to reduce the risk for nutritional deficiency. Current evidence does not support a major role for maternal dietary restrictions during pregnancy or lactation for infants with atopic disease. However, there is moderate evidence from meta-analyses that prophylactic (but not therapeutic) use of dietary probiotics may be beneficial for atopic dermatitis; these findings warrant replication. For treating psoriasis, the supplementation of omega-3-fatty acids has been proposed to have beneficial effects by modulating eicosanoid metabolism, but again the replication of the data is not sufficient. Finally, not only psoriasis, but also leg ulcers have been shown to be associated with nutritional deficiencies. Yet, it remains to be established if poor nutrition is a direct cause or merely an associated bystander of these skin pathologies.

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Skin Aging

Jean Krutmann

2

Core Messages

- Skin aging is caused by
  (i) UV radiation,
  (ii) Infrared radiation,
  (iii) tobacco smoke and
  (iv) traffic related particulate matter
- Damage to macromolecules such as mtDNA and proteins in dermal fibroblasts drives chronic skin aging

2.1 Introduction

For decades it has been appreciated that aging is the consequence of both genetic and environmental influences. Genetic factors are evident, e.g., in the >100-fold variation among species in the rate of aging; and recent studies of fruit flies, worms, and even mice have identified specific longevity genes whose modification can greatly alter lifespan [22]. Conversely, a role for environmental factors can be deduced both from epidemiologic and laboratory-based experimental data. Such influences include ionizing radiation, severe physical and psychological stress, overeating versus caloric restriction, and in the case of skin ultraviolet irradiation.

In this regard, skin is no exception as skin aging results from intrinsic (genetic, endocrinologic) and extrinsic (environmental) factors. In this chapter I will focus on extrinsic skin aging for the following reasons: (a) The overall topic of this chapter is functional food for skin or, in other words, manipulation of skin aging by nutrition-based strategies; (b) It has already been shown for topical approaches (sunscreens, cosmeceuticals, etc.) that extrinsic skin aging can be effectively manipulated. (iii) And thus, nutrition-based anti-skin-aging strategies will be most effective if they are directed against extrinsic skin aging.

Extrinsic and intrinsic skin aging can be clearly distinguished at a clinical, histological, and molecular level. The two most prominent clinical signs of extrinsic skin aging are the formation of coarse wrinkles and an increase in the number of pigment spots (Fig. 2.1). Interestingly, ethnic differences exist, because, e.g., Caucasian women develop earlier and more severe skin wrinkling whereas Japanese women show more lentigines at a younger age. Among all environmental factors, solar ultraviolet (UV) radiation is most important for extrinsic skin aging, a process accordingly also termed photoaging.

Within recent years substantial progress has been made in elucidating the underlying molecular mechanisms. From these studies it is now clear that both UVB (290–320 nm) and UVA (320–400 nm) radiation contribute to photoaging. UV-induced alterations at the level of the dermis are best studied and appear to

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Fig. 2.1 Coarse wrinkle (**a**) and pigment spot (**b**) formation in extrinsic skin aging

be largely responsible for the phenotype of photoaged skin. It is also generally agreed that UVB acts preferentially on the epidermis where it not only damages DNA in keratinocytes and melanocytes but also causes the production of soluble factors including proteolytic enzymes which then in a second step affect the dermis; in contrast UVA radiation penetrates far more deeply on average and hence exerts direct effects on both the epidermal and the dermal compartments (Fig. 2.1). UVA is also 10–100 times more abundant in sunlight than UVB, depending on the season and time of day. It has therefore been proposed that, although UVA photons are individually far less biologically active than UVB photons, UVA radiation

may be at least as important as UVB radiation for the pathogenesis of photoaging [4].

It should be noted that extrinsic skin aging is not exclusively due to solar UV irradiation. Accordingly, also other wavelengths within the solar spectrum, most notably near infrared radiation (IRA; 770–1,400 nm), have been shown to contribute to skin aging, in particular to the formation of coarse wrinkles [25]. The relative contribution of IRA to photoaging is currently not known, but likely to be very relevant. Accordingly, IRA radiation constitutes one-third of the energy that is being emitted by the sun and that reaches the earth surface and thus human skin. Also, IRA radiation deeply penetrates into the human skin with 50% of the energy reaching the dermis, and at a molecular level, the magnitude of IRA radiation-induced collagen breakdown appears to be similar to that caused by UVA radiation.

It is also important to realize that at least two other environmental factors contribute to extrinsic skin aging independent of solar radiation [33]. Accordingly, exposure to tobacco smoke is well known to cause wrinkle formation, elastosis, and teleangiectasia, whereas exposure to traffic-related, airborne particulate matter significantly increases the number of pigment spots (= lentigines) [40].

In the past, the pathogenesis of extrinsic skin aging has been a major research focus and most work has been done with UV radiation. Despite all these efforts the exact mechanisms by which UV radiation causes premature skin aging is not completely clear. In these studies a number of molecular pathways have been described to explain one or more of the key features of photoaged skin. Some of these models are based on irradiation protocols which use single or few UV exposures, whereas others take into account the fact that photoaging results from chronic UV damage and as a consequence employ chronic repetitive irradiation protocols. Still others rely on largely theoretic constructs rather than on experimental observations.

2.2 Mechanisms of UV-Induced Photoaging

Of interest is the fact that most if not all age-accelerating environmental factors damage DNA either directly or indirectly, often through oxidative damage [28]. In addition, rate of aging among species correlates inversely with rate and fidelity of DNA repair [20] and most progeroid syndromes for which the genetic lesion has been identified have impaired DNA replication and/or DNA damage responses [28]. In combination with the fact that cumulative DNA damage accompanies chronologic aging [41], these observations suggest that both the indisputable heritable component and the environmental component of aging result in large part from changing DNA status during the individual's life. The next section of this chapter summarizes the current evidence that damage to mtDNA is of major importance in photoaging and in fact might drive and promote photoaging in a chronic fashion, i.e., over decades [5, 25]. The subsequent sections provide detailed information now available with regard to specific aging targets and signaling pathways responsible for photoaging-associated morphologic and functional changes in skin. These include UV-induced alterations of connective tissue components, vascularization patterns, inflammatory cells, protein oxidation and IRA radiation-induced retrograde signaling cascades. At the end I will present a unifying concept that reconciles the most recent findings in an attempt to provide a novel and comprehensive model to explain photoaging and a framework for the development of nutrition-based strategies to prevent, delay, or reverse skin aging.

2.3 Mitochondrial DNA Mutations and Photoaging

Mitochondria are organelles whose main function is to generate energy for the cell. This is achieved by a multi-step process called oxidative phosphorylation or electron transport chain. Located at the inner mitochondrial membrane are five multi-protein complexes that generate an electrochemical proton gradient used in the last step of the process to turn ADP and organophosphate into ATP. This process is not completely error free and ultimately this leads to the generation of reactive oxygen species (ROS), making the mitochondrion the site of the highest ROS turnover in the cell. In close proximity to this site lies the mitochondrions' own genomic material, the mitochondrial (mt)DNA. The human mtDNA is a 16,559-bp-long, circular, and double-stranded molecule of which four

to ten copies exist per cell. Mitochondria do not contain any repair mechanism to remove bulky DNA lesions; although they do contain base excision repair mechanism and repair mechanisms against oxidative damage, the mutation frequency of mtDNA is approximately 50-fold higher than that of nuclear DNA. Mutations of mtDNA have been found to play a causative role in degenerative diseases such as Alzheimer's disease, chronic progressive external ophthalmoplegia, and Kearns-Sayre syndrome [14]. In addition to degenerative diseases, mutations of mtDNA may play a causative role in the normal aging process with an accumulation of mtDNA mutations accompanied by a decline of mitochondrial functions [42]. Recent evidence indicates that mtDNA mutations are also involved in the process of photoaging [4, 25].

Photoaged skin is characterized by increased mutations of the mitochondrial genome [1, 7, 44]. Intraindividual comparison studies have revealed that the so-called common deletion, a 4,977 base pair deletion of mtDNA, is increased up to tenfold in photoaged skin, as compared with sun-protected skin of the same individual. The amount of the common deletion in human skin does not correlate with chronological aging [24], and it has therefore been proposed that mtDNA mutations such as the common deletion represent molecular markers for photoaging. In support of this concept, it was shown that repetitive, sublethal exposure to UVA radiation at doses acquired during a regular summer holiday induces mutations of mtDNA in cultured primary human dermal fibroblasts in a singlet oxygen-dependent fashion [3]. Even more important, in vivo studies have revealed that repetitive three-times daily exposure of previously unirradiated buttock skin for a total of 2 weeks to physiological doses of UVA radiation leads to an approximately 40% increase in the levels of the common deletion in the dermal, but not epidermal compartment of irradiated skin [5]. Furthermore, it was shown that, once induced, these mutations persist for at least 16 months in UV-exposed skin. Interestingly, in a number of individuals, the levels of the common deletion in irradiated skin continued to increase with a magnitude up to 32-fold. It has been postulated for the normal aging process as well as for photoaging that the induction of ROS generates mtDNA mutations, in turn leading to a defective respiratory chain and, in a vicious cycle, inducing even more ROS and subsequently allowing mtDNA mutagenesis independent of the inducing agent [21]. It is the characteristic of vicious cycles that they evolve at ever-increasing speeds. Thus, the increase of the common deletion up to levels of 32-fold, independent of UV exposure, may represent the first in vivo evidence for the presence of such a vicious cycle in general and in human skin in particular (Fig. 2.3).

The mechanisms by which generation of mtDNA mutations by UVA exposure translates into the morphologic alterations observed in photoaging of human skin are currently being unraveled. In general, a cause–effect relationship between premature aging and mtDNA mutagenesis is strongly suggested by studies employing homozygous knock-in mice that express a proof-reading-deficient version of PolgA, the nucleus-encoded subunit of mtDNA polymerase [38]. As expected, these mice developed a mtDNA mutator phenoytpye with increased amounts of deleted mtDNA. This increase in somatic mtDNA mutations was found to be associated with reduced lifespan and premature onset of aging-related phenoytypes such as weight loss, reduced subcutaneous fat, alopecia, kyphosis, osteoporosis, anemia, reduced fertility, and heart enlargement.

In addition, recent studies demonstrate that UVA radiation-induced mtDNA mutagenesis is of functional relevance in primary human dermal fibroblasts and apparently has molecular consequences suggestive of a causative role of mtDNA mutations in photoaging of human skin as well [2]. Accordingly, induction of the common deletion in human skin fibroblasts is paralleled by a measurable decrease of oxygen consumption, mitochondrial membrane potential, and ATP content, as well as an increase of MMP-1, while TIMP remains unaltered, an imbalance that is known to be involved in photoaging of human skin (see below). These observations suggest a link not only between mutations of mtDNA and cellular energy metabolism, but also between mtDNA mutagenesis, energy metabolism, and a fibroblast gene expression profile that would functionally correlate with increased matrix degradation and thus premature skin aging. In order to provide further evidence for the role of the energy metabolism in mtDNA mutagenesis and the development of this "photoaging phenotype," the effect of creatine was studied in these cells. This applied the hypothesis that generation of phosphocreatine, and consequently ATP, is facilitated if creatine is abundant in cells. This would

allow easier binding of existing energy-rich phosphates to the energy precursor creatine. Indeed, experimental supplementation of normal human fibroblasts with creatine normalized mitochondrial mutagenesis as well as the functional parameters, oxygen consumption and MMP-1, while an inhibitor of creatine uptake abrogated this effect [2].

The studies discussed above always required the UV radiation-induced formation of mtDNA mutations prior to functional analysis and thus it was not possible to differentiate between functional consequences resulting from mtDNA mutagenesis and those which were UV-induced but occurred independent of damage to mtDNA. In recent studies this problem has been addressed by employing unirradiated dermal fibroblasts. Mitochondrial DNA was partially depleted from these cells in order to generate phenocopies of large-scale deletion bearing fibroblasts [35]. Subsequent analysis of their gene expression pattern showed striking similarities to that expressed by dermal fibroblasts in photoaged skin, indicating that the presence of mtDNA deletions in skin fibroblasts resulted in functional alterations which were of pathogenic relevance for photoaging. This assumption was further corroborated and extended by recent studies in which primary human skin fibroblasts from patients with the mitochondriopathy Kearns-Sayre Syndrome (KSS) were used [29]. These cells constitutively carry large amounts of UV-inducible large-scale mutations of mtDNA such as the common deletion. They were used to generate three-dimensional dermal equivalents by seeding them into collagen gels. Interestingly, within 6 weeks after contraction of gels, KSS, in comparison to normal dermal equivalents, showed many features reminiscent of photoaging. These include an overexpression, both at the mRNA and protein level, as well as an increased activity of matrix metalloproteinase-1 (see next paragraph), a rarefication of collagen fibers, an increased amount of fragmented collagen fibers, an increase in oxidized proteins, signs of neovascularization, and an overexpression of lysyl oxidase-1 [29]. Taken together these studies strongly indicate that the presence of large-scale deletions of mtDNA in human dermal fibroblasts is causally related to photoaging because it leads to an altered gene expression pattern in these cells and subsequently to structural and functional alterations of the human dermis which are characteristic for photoaged human skin [25].

2.4 Connective-Tissue Alterations in Photoaging: The Role of Matrix Metalloproteinases and Collagen Synthesis

Photoaged skin is characterized by alterations of the dermal connective tissue. The extracellular matrix in the dermis mainly consists of type I and type III collagen, elastin, proteoglycans, and fibronectin. In particular, collagen fibrils are important for the strength and resiliency of skin, and alterations in their number and structure are thought to be responsible for wrinkle formation.

In photoaged skin, collagen fibrils are disorganized and abnormal elastin-containing material accumulates [36]. Biochemical studies have revealed that in photoaged skin levels of types I and III collagen precursors and cross-links are reduced, whereas elastin levels are increased [9, 37].

How does UV radiation cause these alterations? In principle it is conceivable to assume that UV radiation leads to an enhanced and accelerated degradation and/or a decreased synthesis of collagen fibers and our current knowledge indicates that both mechanisms may be involved.

A large number of studies unambigously demonstrate that the induction of matrix metalloproteinases (MMPs) play a major role in the pathogenesis of photoaging. As indicated by their name, these zinc-dependent endopeptidases show proteolytic activity to degrade matrix proteins such as collagen and elastin. Each MMP degrades different dermal matrix proteins, e.g., MMP-1 cleaves collagen type I, II, III, whereas MMP-9, which is also called gelatinase, degrades collagen type IV, V, and gelatin. Under basal conditions, MMPs are part of a coordinate network and are precisely regulated by their endogenous inhibitors, i.e., tissue-specific inhibitors of MMPs (TIMPs), which specifically inactivate certain MMPs. An imbalance between activation of MMPs and their respective TIMPs could lead to excessive proteolysis.

It is now very well established that UV radiation induces MMPs without affecting the expression or activity of TIMPs [17, 31]. These MMPs can be induced by both UVB and UVA radiation, but the underlying photobiological and molecular mechanisms differ depending on the type of irradiation. In a very simplified scheme, UVA radiation would mostly act

indirectly through the generation of reactive oxygen species, in particular singlet oxygen, which subsequently can exert a multitude of effects such as lipid peroxidation, activation of transcription factors and generation of DNA-strand breaks [31]. While UVB radiation-induced MMP induction has been shown to involve the generation of ROS as well [43], the main mechanism of action of UVB is the direct interaction with DNA via the induction of DNA damage. Recent studies have indeed provided evidence that enhanced repair of UVB-induced cyclobutane pyrimidine dimers in the DNA of epidermal keratinocytes through topical application of liposomally-encapsulated DNA repair enzymes on UVB-irradiated human skin prevents UVB radiation-induced epidermal MMP expression [15].

The activity of MMPs is tightly regulated by transcriptional regulation and elegant in vivo studies by Fisher et al. have demonstrated that exposure of human skin to UVB radiation leads to the activation of the respective transcription factors [16]. Accordingly, UV exposure of human skin not only leads to the induction of MMPs within hours after irradiation, but already within minutes, transcription factors AP-1 and NFkB, which are known stimulatory factors of MMP genes, are induced. These effects can be observed at low UVB dose levels, because transcription factor activation and MMP-1 induction could be achieved by exposing human skin to one-tenth of the dose necessary for skin reddening (0.1 minimal erythema dose). Subsequent work by the same group clarified the major components of the molecular pathway by which UVB exposure leads to the degradation of matrix proteins in human skin Low-dose UVB irradiation induced a signaling cascade which involves upregulation of epidermal growth factor receptors (EGFR), the GTP-binding regulatory protein p21Ras, extracellular signal-regulated kinase (ERK), c-jun amino terminal kinase (JNK), and p38. Elevated c-jun together with constitutively expressed c-fos increased activation of AP-1. Identification of this UVB-induced signaling pathway does not only unravel the complexity of the molecular basis which underlies UVB radiation-induced gene expression in human skin, but also provides a rationale for the efficacy of tretinoin (all-trans-retinoic acid) in the treatment of photoaged skin. Accordingly, topical pretreatment with tretinoin inhibited the induction and activity of MMPs in UVB-irradiated skin through prevention of AP-1 activation.

In addition to the destruction of existing collagen through activation of MMPs, failure to replace damaged collagen is thought to contribute to photoaging as well. Accordingly, in chronically photodamaged skin, collagen synthesis is downregulated as compared to sun-protected skin [18]. The mechanism by which UV radiation interferes with collagen synthesis is not yet known but in a recent study evidence has been provided that fibroblasts in severely (photo)damaged skin have less interaction with intact collagen and are thus exposed to less mechanical tension, and it has been proposed that this situation might lead to decreased collagen synthesis [39].

2.5 UV-Induced Modulation of Vascularization

There is increasing evidence that cutaneous blood vessels may play a role in the pathogenesis of photoaging. Photoaged skin shows vascular damage which is absent from intrinsically aged skin. In mildly photodamaged skin, there is venular wall thickening, while in severely damaged skin the vessel walls are thinned and supporting perivascular veil cells are reduced in number [10]. The number of vascular cross-sections is reduced [23] and there are local dilations, corresponding to clinical teleangiectases. Overall, there is a marked change in the horizontal vascularization pattern with dilatated and distorted vessels. Studies in humans as well as in the hairless skh-1 mouse model for skin aging have demonstrated that acute and chronic UVB irradiation greatly increases skin vascularization [6, 45].

The formation of blood vessels from preexisting vessels is tightly controlled by a number of angiogenic factors as well as factors which inhibit angiogenesis. These growth factors include basic fibroblast growth factor, interleukin-8, tumor growth factor-beta, platelet-derived growth factor, and vascular endothelial growth factor (VEGF). VEGF appears to be involved in chronic UVB damage because UVB radiation-induced dermal angiogenesis in Skh-1 mice is associated with increased VEGF expression in the hyperplastic epidermis of these animals [45]. Even more important, targeted overexpression of the angiogenesis inhibitor Thrombospondin-1 does not only prevent UVB radiation-induced skin vascularization and endothelial cell proliferation, but significantly reduces dermal photodamage and wrinkle formation. These studies suggest that UVB radiation-induced angiogenesis plays a direct biological role in photoaging.

2.6 Photoaging as a Chronic Inflammatory Process

In contrast to intrinsically aged skin, which shows an overall reduction in cell numbers, photoaged skin is characterized by an increase in the number of dermal fibroblasts, which appear hyperplastic, but also by increased numbers of mast cells, histiocytes, and mononuclear cells. The presence of such a dermal infiltrate indicates the possibility that a chronic inflammatory process takes place in photoaged skin and in order to describe this situation the terms heliodermatitis and dermatoheliosis have been coined [26]. More recent studies have shown that increased numbers of CD4+ T-cells are present in the dermis whereas intraepidermally, infiltrates of indeterminate cells and a concomitant reduction in the number of epidermal Langerhans cells have been described [13, 19]. It is currently not known whether the presence of inflammatory cells represents an epiphenomenon or whether these cells play a causative role in the pathogenesis of photoaging, e.g., through the production of soluble mediators which could affect the production and/or degradation of extracellular matrix proteins.

2.7 Protein Oxidation and Photoaging

The aging process is accompanied by enhanced oxidative damage. All cellular components including proteins are affected by oxidation [27]. Protein carbonyls may be formed either by oxidative cleavage of proteins or by direct oxidiation of lysine, arginine, proline, and threonine residues. In addition, carbonyl groups may be introduced into proteins by reactions with aldehydes produced during lipid peroxidation or with reactive carbonyl derivatives generated as a consequence of the reaction with reducing sugars or their oxidation products with lysine residues of proteins.

Within the cell, the proteasome is responsible for the degradation of oxidized proteins. During the aging process this function of the proteasome is diminished and oxidized proteins accumulate. In addition, lipofuscin, a highly cross-linked and modified protein aggregate is formed. This aggregate accumulates within cells and is able to inhibit the proteasome. These alterations mainly occur within the cytoplasm and lipofuscin does not accumulate in the nucleus.

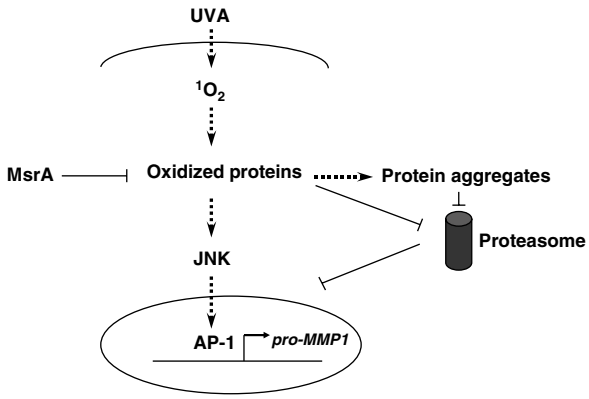

Fig. 2.2 Role of protein oxidation in photoaging of human skin. Ultraviolet radiation generates oxidized proteins through a singlet oxygen-dependent mechanism. Oxidized proteins as well as resulting protein aggregates inhibit proteasomal function. This leads to an accumulation of transcription factors such as AP-1. The resulting increase in MMP-expression eventually leads to collagen degradation and wrinkle formation

In biopsies from individuals with histologically confirmed solar elastosis, an accumulation of oxidatively modified proteins was found specifically within the upper dermis [30]. Protein oxidation in photoaged skin was most likely due to UV irradiation, because repetitive exposure of human buttock skin on 10 days to increasing UV doses as well as in vitro irradiation of cultured dermal fibroblasts to UVB or UVA radiation caused protein oxidation. The functional relevance of increased protein oxidation in UV-irradiated dermal fibroblasts, in particular with regard to the pathogenesis of photoaging, has recently been unraveled. In these studies, it was observed that increased protein oxidation that may result from exposure of human fibroblasts to UVA radiation inhibits proteasomal functions and thereby affects intracellular signaling pathways which are involved in MMP-1 expression (Fig. 2.2).

2.8 Infrared a Radiation-Induced Retrograde Signaling

Similar to UVB or UVA, IRA radiation is a potent regulator of gene expression in human dermal fibroblasts [12]. In particular, there is no more doubt that IRA radiation causes an imbalance between MMP-1 versus TIMP-1 expression in favor of MMP-1 [32] and at the same time decreases COL 1A1 and COL 1A2

expression [11] and thereby leads to a rarefication of collagen fibers and eventually to wrinkle formation. Importantly, the signaling mechanisms involved in IRA radiation-induced gene regulation differ completely from those induced by UVB or UVA radiation [34]. Accordingly, IRA radiation is primarily absorbed by cupper atoms in complex IV of the mitochondrial respiratory chain. The first detectable signaling event is the subsequent intramitochondrial generation of ROS. This intramitochondrial signal is then transmitted to the cytoplasm where it causes an increase in calcium levels, followed by an activation of MAPKs and the subsequent intranuclear transcriptional activation of IRA-responsive genes (Fig. 2.3). The importance of intramitochondrial ROS production for the elicitation of this retrograde signaling response is emphasized by the fact that mitochondrially targeted antioxidants are highly effective in blocking this signaling cascade in vitro and in preventing IRA-radiation-induced MMP-1 upregulation in vivo in human skin [35].

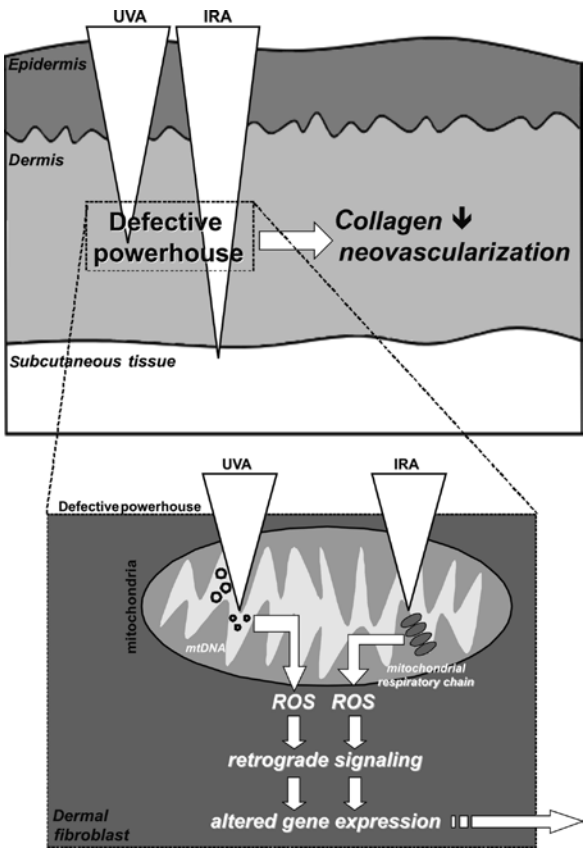

Fig. 2.3 Defective powerhouse model of cutaneous ageing. UVA and IRA via different mechanisms lead to the disruption of the mitochondrial function ("defective powerhouse") which results in changes in the dermal compartment of the skin and leads to photoageing. *Insert /lower panel*: Repetitive UVA irradiation results in the increased formation of large-scale deletions of mtDNA (*left*). Also, even a single dose of IRA leads to a disruption of the mitochondrial electron transport chain (*right*). Both events cause an increased production of ROS and thereby initiate retrograde signalling responses

2.9 Concluding Remarks: The Defective Powerhouse Model of Photoaging of Human Skin

From the above it is evident that major progress has been made recently in identifying molecular mechanisms involved in photoaging. In this regard, skin has proven to serve as an excellent model organ to understand basic mechanisms relevant for extrinsic aging.

Despite all this progress, however, a general, unifying concept linking the different mechanisms and molecular targets described in the previous paragraphs is still missing. In other words, the critical question to answer is: How do mitochondrial DNA mutagenesis, neovascularization, protein oxidation, downregulation of collagen synthesis, and increased expression of matrixmetalloproteinases together cause photoaging of human skin? Which of these mechanisms are of primary importance and responsible for inducing others? Are some or all of the above-mentioned characteristics of photoaged skin merely epiphenomena and, if so, to what extent causally related to premature skin aging?

The current state of knowledge does not allow to answer these questions in a definitive manner. Nevertheless I have proposed a hypothesis which tries to reconcile most of the research discussed above in one model [25].

I envision photoaging of human skin to be initiated and driven by UV radiation-induced mitochondrial DNA mutagenesis in the dermis of human skin. I believe that the persistence of UV radiation-induced mitochondrial DNA mutations and the resulting vicious cycle with further increases in mitochondrial DNA mutations leads to a situation which can best be described as a "defective powerhouse" where inadequate energy production leads to chronic oxidative stress (Fig. 2.3). In the dermis, functional consequences of direct DNA damage and aberrant ROS production in human dermal fibroblasts could be (a) an altered gene

expression pattern which would affect neovascularization and collagen metabolism and possibly also the generation of an inflammatory infiltrate and (b) the oxidation of intracellular proteins, inhibition of the proteasome, and again an altered gene expression pattern with detrimental consequences for collagen metabolism. Evidence supporting this model has recently been generated in human-skin-equivalent models employing dermal fibroblasts which constitutively carry large amounts of UV-inducible mtDNA deletions [29]. Ongoing studies will answer the question whether dermal mtDNA mutagenesis is also of importance for epidermal photoaging (= inside – outside mechanism), or whether epidermal changes are due to direct UV-induced effects, e.g., DNA damage in combination with indirect ROS-induced damage, which would be expected to cause the well-documented UV signature mutations in p53 [8] leading to poorly regulated growth and differentiation of epidermal cells associated with discrete premalignant actinic keratosis and diffuse photoaging (outside–inside mechanism).

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Non-melanoma Skin Cancer

3

Mark Berneburg

Core Messages

- Non-melanoma skin cancer is the most frequent cancer in man.
- Carcinogens are of chemical (arsenic), viral (HPV), and physical (UV) nature.
- Ultraviolet (UV) radiation is the most important carcinogen.
- Multistep carcinogenesis includes initiation, promotion, and malignant conversion with protective mechanisms being DNA repair, cell cycle control by p53 and apoptosis, senescence-associated growth control, and immunosurveillance.
- The most important non-melanoma skin tumors include seborreic keratosis, actinic keratosis, squamous cell carcinoma, keratoacanthoma, and basal cell carcinoma.
- The most important preventive measure is sun protection.
- Treatment of choice is histologically controlled micrographic surgery. Alternatives are cryotherapy, imiquimod, and photodynamic therapy.

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3.1 General Overview

3.1.1 Skin Carcinogenesis

Multistep carcinogenesis: The transformation of normal to neoplastic cells is a process which lasts many years and takes place in a number of steps. Of these steps, many but not all are currently understood. Furthermore, exactly which steps need to take place in order to generate a certain type of tumor is even more unclear. According to our current understanding, the multistep carcinogenesis comprises the following stages.

Tumor initiation: An initiating mutation in the genome of a cell causes the irreversible transformation of this cell. With this, the phenotype is unchanged but growth control mechanisms such as terminal differentiation, senescence-associated growth control, cell cycle arrest, and apoptosis are perturbed.

Tumor promotion: Initiated cells show a disturbed reaction to differentiation signals. They do not show differentiation upon these stimuli but they proliferate instead. This provides the cells with a proliferative advantage. Cells showing promotion often develop papilloma. Promotion is an important event for carcinogenesis which, if not occurring, prevents the development of aggressively invasive tumors. If the carcinogenic stimuli still exist, or even simply under the proliferative competition of the now existing multiple transformed cells, genomic instability in the form of point mutations, deletions, and chromosomal aberrations occur frequently, particularly during the multiple mitoses found in the developing tumors.

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Malignant conversion: The still benign tumors can subsequently develop into malignant tumors with aggressive invasive tumors and the ability to metastasize.

3.1.2 Protective Mechanisms of Carcinogenesis

The organism has developed multiple mechanisms that protect from malignant transformation of cells. These are highly effective being the reason why carcinogenesis is a process which often takes several decades. Tumors have to develop mechanisms to escape all of these protective mechanisms.

DNA repair mechanisms. Multiple mechanisms are known which are highly effective in removing mutagenic lesions from the genome. The importance of these mechanisms is shown by the highly increased tumor incidence in genetic disease with missing repair mechanisms.

Cell cycle control by p53 and apoptosis. The protein p53 checks if a genomic lesion can be repaired. If so, the cell cycle is arrested and repair can take place. If this is not the case, the cell will undergo apoptosis.

Senescence-associated growth control. A transformed cell can be stopped from further proliferation by growth control that is associated with a senescence phenotype of the cell. With this the cell remains in the transformed status but does not proliferate any further.

Immunosurveillance. Tumor cells often express markers that allow the immune system to detect these cells as transformed. Once these cells are identified they can be removed from the organism by inflammatory reactions.

3.1.3 Carcinogens of the Skin

Chemical carcinogens. Chemical carcinogens very often bind to specific regions of the genome and induce mutations upon replication. For instance dimethylbenzanthracen (DMBA) leads to an adenosine-tyrosine transversion in the H-ras gene.

For the skin arsenic is one of the most important chemical carcinogens. It has been used in viniculture as well as in therapeutic situations (psoriasis). It leads to typical keratoses and later leads to basal cell carcinoma of the skin.

Due to strict regulations, these and other carcinogens (tar, pesticides) nowadays only play a reduced role in carcinogenesis.

Viral carcinogens [2]. It has become increasingly evident that the so-called high-risk human papilloma virus (HPV) play an important role in carcinoma of the genitouretal tract. The mechanism relies on complex binding of the viral proteins E7 with retinoblastoma protein or E6 with p53. Disintegration of both complexes results in depletion of these tumor suppressor genes. Viral carcinogens have been shown to play a role in immunosuppressed individuals as well as in the disease epidermodysplasia verruciformis (i.e., HPV types 5 and 8).

Ultraviolet radiation. UV radiation is by far the most important carcinogen of the present time. This is not only shown in animal studies but also in humans. The strongest chain of evidence exists for squamous cell carcinoma of the skin with basal cell carcinoma and keratoacanthoma following. For seborrheic keratoses and malignant melanoma evidence increases which also secures UV radiation as causative agent (Fig. 3.1).

UV radiation leads to the so-called pyrimidine dimers. If these dimers are not removed by the repair mechanism nucleotide excision repair this will lead to specific fingerprint point mutations (C to T transversions). It has been shown that in over 90% of all squamous cell carcinoma as well as actinic keratoses the p53 gene contains these fingerprint mutations

Fig. 3.1 Multiple seborrheic keratoses on the trunk. Initial lesions show skin-colored maculae and later stages are characterized by elevation and cauliflower-like structure with a rough but greasy appearance

Fig. 3.2 Two squamous cell carcinoma on the head of a 72-year old patient. Chronically sun-exposed skin with poikilodermatic appearance and multiple actinic keratoses that can better be felt than seen. Frontoparietal region with a 3.5 cm large, sharply demarcated, erythematous, partly hyperkeratotic plaque. Parietooccipital region with a similar lesion that, according to the patient, bleeds after minimal trauma

(Fig. 3.2). These mutations in p53 lead to the fact that the cell cycle of the affected cell is not halted in order to allow DNA repair or induce apoptosis. Thus, this cell clone can proliferate uninhibited without growth control (tumor initiation).

Furthermore, UV radiation suppresses immunourveillance of the affected cells (UV radiation as complete carcinogen) and it also suppresses the growth of surrounding cells, thus giving the transformed cells an even bigger proliferative advantage.

Ionizing radiation shows similar carcinogenic potential as UV. Today tumors that were induced by ionizing radiation have become less frequent since exposure is continuously decreasing.

Genetic factors of carcinogenesis. Pigmentation is genetically determined. Furthermore, it becomes more and more evident, that also antioxidative capacity is genetically determined. In addition to this, rare diseases with different underlying defects underscore the role of genetically determined susceptibility to allow carcinogenic growth. Syndromes with defective DNA repair mechanisms (xeroderma pigmentosum [3–5], ataxia telangiectasia, Fanconi anemia) with defective tumor suppressor genes (Neurofibromatosis, Li-Fraumeni-syndrome), with other instabilities of the genome upon physical and chemical carcinogens (Bloom-syndrome) are examples for this.

3.2 Different Types of Non-melanoma Skin Cancer

3.2.1 Seborrheic Keratosis

The seborrheic keratosis is a benign papilloma of the skin characterized by its typical morphology. It is a characteristic sign of aged skin and probably the most frequent differential diagnosis to the malignant melanoma.

Epidemiology. Seborrheic keratoses are very frequent in the second half of life. Their numbers can reach up to hundreds in one individual.

Pathogenesis. Recent publications indicate a role for mutations in the fibroblast growth factor gene (FGF) 3 as well as for UV radiation [10, 13].

Clinical appearance. Seborrheic keratoses can be found anywhere with predilection areas being the face and the trunk. They begin as skin-colored maculae until they become more pigmented and elevated. Their surface usually shows a cauliflower-like structure with a rough but greasy appearance.

Histology. Massive acanthosis and orthohperkeratosis is sharply demarcated from the surrounding normal skin. The normally vertically oriented differentiation is replaced by a mixture of differently differentiated regions such as multiple horn cysts in the epidermis and melanocytes and melanin in varying degrees. Focal atypic areas can be found in irritated seborrheic keratoses but otherwise atypia is not regularly found.

Treatment. Surgical removal including curretage, shave excision, or complete excision, if wanted. Keratolytic topical treatments are usually not effective.

Differential diagnosis. Compound nevi, melanoma, and pigmented basal cell carcinoma.

The seborrheic keratosis is the classic differential diagnosis to melanoma and the most frequent lesion presented to the dermatologist for melanoma prevention. Therefore, careful clinical inspection and dermoscopic evaluation is prudent. If in doubt, excision with subsequent histology is necessary.

3.2.2 Clear Cell Acanthoma

A rare benign tumor, usually at the lower limbs of elderly patients [9]. Clinically slightly elevated erythematic nodules.

Histology. Glycogen containing epidermal cells with a clear appearance.

Differential diagnosis. Bowen's disease

3.2.3 Dyskeratoma (Syn. "Warty Dyskeratoma")

A rare benign tumor of the head and neck, clinically characterized by 1–2 cm small, slow growing, round nodules with central horn material. Histologically it is a horn cyst with dyskeratotic cells and "corps ronds" in its center.

3.2.4 Actinic Keratoses

Epidemiology. Actinic keratoses usually appear in multiple lesions in chronically UV-exposed skin, depending on the skin type and the cumulative UV dose. Men are usually more affected.

Clinical appearance [14, 19]. Forehead, nose, earlobes, cheeks, lower lips, hands, and in men the bald head are areas of predilection. Actinic keratoses begin as 1–5 mm large flat roughness of the skin which can be better felt than seen. Their surface is skin colored to erythematous. In the course of years these keratoses grow, become more elevated and adapt an irregular, rough, and hard surface. If these keratoses are removed, bleeding will occur. After further growth, horny tumors can develop.

Histology. Excessive compact orthohyperkeratosis, fokal parakeratosis, missing regularity of the stratum granulosum, as well as dyskeratotic cells and nuclear atypia throughout the whole epidermis. Tumoracantholysis.

Prognosis. The numbers vary greatly but progression into squamous cell carcinoma from actinic keratoses is estimated to occur in 20% [17]. Stringent photoprotection and therapy keeps the risk low, particularly since squamous cells that developed from actinic keratoses show very low rates of metastasis.

Differential diagnosis. Seborrheic keratosis, Lentigo simplex, and chronic discoid lupus erythematodes.

Actinic cheilitis. The actinic cheilitis is the actinic keratosis of the lip. Importantly, actinic cheilitis is more dangerous since carcinoma of the lip tend to metastasize earlier than those of the skin.

3.2.5 Bowen's Disease [6]

A relatively frequent precancerous lesion. It is usually a single lesion. Areas of predilection include trunk and extremities. Sun exposure, chemical carcinogens, in particular arsenic, and human papilloma virus play a causative role.

Clinical appearance. An erythematous macule with sharp but irregular border and rough keratotic surface. Usually there are no subjective symptoms such as itching or burning. Hyperkeratosis or pigmentation can occur.

Histology. Thickened epidermis, loss of normal cell differentiation toward outer cell layers. Cell atypia, multiple mitoses and dyskeratotic cells.

Differential diagnosis. Plaques of chronic eczema, psoriasis, superficial basal cell carcinoma, and extramammary Paget. Bowen's disease is often misinterpreted as eczema by patients as well as physicians but particularly the missing lichenification, sharp demarcation, and missing symptoms give the right clue.

3.2.6 Bowenoid Papulosis

Multiple flat lichen planus-like lesions caused by human papillomavirus of the genitoanal region. Usually found in young adults with less dysplasia than erythroplasia. Progression into carcinoma of the genitoanal region can occur.

3.2.7 Erythroplasia

Singular lesion at the glans penis or preputium or rarely female labia in elderly patients. As with bowenoid papulosis, human papillomavirus is often found. Clinically, dark erythematous maculae with sharp but polycyclic irregular border. Slow growth over years without symptoms.

Differential diagnosis. Diabetic balanitis, Soor-balanits

Therapy of actinic keratoses and other carcinomata in situ. Generally, surgical excision should be carried out. Under special circumstances, other non-surgical treatments such as cryotherapy, chemical peeling, photodynamic therapy, and imiquimod can be employed. These treatments will collectively be discussed together with all tumor treatments available.

3.2.8 Squamous Cell Cancer

Epidemiology. Squamous cell cancer is among the most frequent types of cancer in man [1, 9]. Within the last few years, the incidence has risen between 3% and 6% per year.

Etiology. The most important etiologic factor in the development of squamous cell cancer is the cumulative dose of UV radiation. Furthermore, smoking plays an important role. Human papilloma virus are also becoming increasingly important in the development of virally induced squamous cell carcinoma.

Clinical appearance. Squamous cell carcinoma develop from actinic keratoses. They can manifest with diffusely infiltrating, superficial, deeply exulcerating, or nodular-exophytic growth. The lesions commonly show a rough texture, infiltration of the basis and an irregular cover with relatively little subjective symptoms. Often verrucous keratotic areas can be seen. Metastasis takes place lymphogenic depending on tumor thickness [7, 8] although this occurs only in very late stages of the disease. The metastases of the lymph nodes are big, hard, and often infiltrate surrounding structures. Squamous cell carcinoma of the skin are usually less aggressive than those of the mucous membranes or internal organs.

Histology. Epidermally differentiated tumors, which infiltrate the surrounding tissue diffusely or in columns [16]. Often intense inflammatory infiltrate. The epidermal differentiation is usually intact, manifesting in cornification and few dyskeratotic cells. Dedifferentiation correlates with higher aggressiveness. Dedifferentiation is graded according to Broders in four groups with grade IV as complete dedifferentiation. The higher the dedifferentiation, the more monotonous cell masses with mitoses and nuclear atypia prevail. Demarcation of tumor from surrounding stroma becomes less and destructive growth more visible. Differentiation from sarcoma can be difficult. Then keratin and vimentin markers can be of help.

Treatment. Squamous cell carcinoma will be removed by surgical excision with a safety margin of 1 cm. All therapies available will be discussed below.

Differential diagnosis. Keratoacanthoma, basal cell carcinoma, depigmented melanoma, different adnexal tumors. Squamous cell carcinoma are often surrounded by multiple actinic keratoses.

3.2.9 Keratoacanthoma

Keratoacanthoma are relatively frequent benign tumors of the hair follicle with characteristic clinical appearance and histology.

Epidemiology. Keratoacanthoma are rare in patients of higher skin types and approximately twice as frequent in men as in women. Their incidence is about half the incidence of squamous cell carcinoma. Mostly elderly patients are affected.

Pathogenesis. Keratoacanthoma are tumors stemming from the infundibulum of the hair follicle upon proliferation. This is why these tumors show such a high rate of mitosis. Subsequently, they differentiate to form excessive amounts of keratotic material leading to a typical cornu cutaneum which then goes into the next cycle: degradation. These cycles are interpreted in analogy to the hair cycle. This is also supported by the fact that often in keratoacanthoma surrounding hair follicles can also be affected.

Clinical appearance. Classical keratoacanthoma occur almost exclusively in sun exposed areas such as the head, face, neck, and forearms. They appear as singular tumor which grows within weeks or months showing a regular erythematous or skin-colored nodule of smooth surface with a crater-like indention and the cornu cutaneum in its center. When the cornu

cutaneum is lost, a hypotrophic scar remains. Regular keratoacanthoma usually do not exceed a maximum size of approximately 3 cm in diameter. Rarely multiple keratoacanthoma can appear (Ferguson-Smith tumors, or eruptive keratoacanthoma) or they can manifest as gigantic keratoacanthoma.

Histology. The differentiation between keratoacanthoma and squamous cell carcinoma can be difficult. The classic histological picture is that of a cup-shaped tumor. Columns of differentiated epidermis surround the central column filled with cornified debris. At the base the keratinocytes are enlarged and they cornify without much granular layer. Inflammatory cells are found in the dermis and pseudocarcinomatous infiltration presenting as columns of epidermis which reach into the dermis. Mitoses and cell atypia are frequent.

Treatment. Since the lesion will disappear spontaneously, theoretically, no treatment is necessary. However, in order to secure the diagnosis and to rule out squamous cell carcinoma surgical excision should be carried out. Under special circumstances retinoids or radiotherapy can be considered.

Prognosis. The prognosis is very good but it is not clear whether squamous cell carcinoma can develop from keratoacanthoma.

3.2.10 Basal Cell Carcinoma

The basal cell carcinoma is the most frequent tumor of the skin with a characteristic clinical and histological appearance mostly of the elderly patient. Its growth is locally destructive but it only very rarely develops metastases.

Epidemiology. The basal cell carcinoma occurs worldwide, is most frequent in patients with fair skin, and more frequent in men than in women [11]. The incidence of this tumor is rising with a doubling within the last 20 years. The tumor usually appears after the age of 40. It inversely correlates with Fitzpatrick skin types and directly with the exposure to UV radiation. Mortality rates are low.

Pathogenesis. UV radiation is an important pathogenetic factor although the correlation is not as direct as for squamous cell carcinoma. Arsenic is known to be important in the pathogenesis. Multiple basal cell carcinoma occur in the genetic disease basal cell nevus syndrome. For both, basal cell nevus syndrome and for sporadic basal cell carcinoma, a causative role of alterations in the sonic hedgehog signaling pathway has been described.

Clinical appearance. Basal cell carcinoma can appear in any location with the exception of skin without hair follicles such as palms, soles, the genital area, and the mouth. In about 90% of cases, basal cell carcinoma appear on the head with nasolabial fold, nose, periorbita,l and preauricular region as the most frequent locations. But they can also develop in skin areas where direct exposure to UV radiation is not as high. These include the retroauricular region, scalp, and the trunk. Therefore, the distribution does not follow the cumulative UV exposure as strictly as is the case for squamous cell carcinoma.

Basal cell carcinoma are characterized by a typical, one to several millimeter in diameter, hemispheric, solid, skin-colored, and sometimes translucent nodule with a mother-of-pearl reflectance which is surrounded and infiltrated by telangiectasia. Initially there is no keratosis (Fig. 3.3). A basal cell carcinoma consists of

Fig. 3.3 Typical nodular basal cell carcinoma of the nasolabial fold with telangiectasia, mother-of-pearl appearance and central ulceration

a single or multiple collar-shaped nodules. Growth of these tumors is slow in both vertical and radial direction. Later, central atrophy leads to shrinkage and exulceration.

3.2.10.1 Clinical Subtypes

Nodular basal cell carcinoma. Most common type of basal cell carcinoma with clinical appearance as described above.

Cystic basal cell carcinoma. Translucent cystic appearance. If they are punctured, serous liquids can be discharged.

Pigmented basal cell carcinoma. Growth like nodular basal cell carcinoma but with brown to black color. This is not a melanocytic lesion.

Superficial basal cell carcinoma, basal cell carcinoma of the trunk. This rare subtype can mostly be seen on the trunk, with mild infiltration, nummular or with polycyclic border often resembling a psoriatic plaque. This subtype can appear after exposure to arsenic. The fibroepithelioma Pinkus resembles a seborrheic keratosis and is histologically characterized by multiple columns of basal cell carcinoma in the surrounding stroma.

Skleordermiform or cicatricial basal cell carcinoma. This subtype can appear like a scar with basaloid columns in the skin. Basaloid nodules and telangiectasia are often missing instead a strong fibrotic stromal response causes the scar-like appearance. Since the streaks can branch far underneath the skin, excision is often incomplete resulting in frequent recurrence of this tumor.

Ulcerating basal cell carcinoma. While superficial erosion or ulceration can often be seen, extensive or deep ulceration only occurs after years of growth. These ulcerations can either be horizontal (Ulcus rodens) or radial in growth (Ulcus terebrans). If no treatment is carried out basal cell carcinoma will destructively grow into any of the surrounding tissues.

Metastasis of basal cell carcinoma. This can be seen in lymph nodes, lung, or bone tissue. However, this is extremely rare.

Histology. Basal cell carcinoma are built of basaloid cells with basophilic plasma. Mitoses and cellular atypia are rare. The tumor consists of sharply demarcated strands or columns of cell nests with the peripheral cells growing in a palisade order. Surrounding stroma is fibrotic, particularly when it is a cicatricial basal cell carcinoma. Pigmented basal cell carcinoma show melanocytes and sometimes pseudoglandular structures can be found (adenoid or keratotic basal call carcinoma).

Differential diagnosis. The classical basal cell carcinoma is easy to diagnose. But small nodular basal cell carcinoma have to be distinguished from milia, hyperplasia of sebacous glands, actinic or seborrheic keratoses, adnexal tumors (i.e., trichoepithelioma) as well as dermal nevi. Pigmented basal cell carcinoma have to be differentiated from histiocytoma, pigmented nevi, and melanoma; superficial basal cell carcinoma have to be distinguished from Bowens disease, psoriasis, nummular eczema; cystic from adnextumors. Ulcerated basal cell carcinoma can resemble squamous cell carcinoma and other ulcerated tumors such as melanoma and Paget's disease.

3.2.11 Prevention of UV-Induced Skin Cancer

Since UV radiation is the central causative carcinogen, protection from excessive single doses as well as high cumulative doses of UV is the most important measure to prevent the development of non-melanoma skin cancer [22]. This should include avoidance of the sun at peak times (between 10:00 h and 14:00 h), long-sleeved clothing and hats that also protect neck and ears (textile UV protection), as well as application of topical sun cream lotions protecting from both UVB and UVA. In this regard, not only the DNA damaging effects of UVA and UVB play a role but it has also been shown that oxidative DNA damage also plays an important role in skin carcinogenesis. Therefore, not only mere protection from UVA and UVB appears sensible but also antioxidative strategies. Antoxidants can be applied topically or orally.

3.2.12 Treatment of Non-melanoma Skin Cancer [11, 18, 21]

The treatment of first choice should always be surgical excision. This is particularly the case if the dignity or

the exact diagnosis of the lesion to be treated is unclear. Excised tumors have to be evaluated by histological examination. This not only allows to secure the exact diagnosis of the excised lesion but it also provides the physician with the crucial information whether the whole tumor is excised. Next to surgery, a number of non-surgical therapies can be used in order to treat non-melanoma skin cancer if the diagnosis of the lesion is clear. These include cryotherapy, imiquimod, and photodynamic therapy. These treatments will be discussed together with the different surgical methods.

3.2.12.1 Surgical Methods

Curettage. The superficial removal of tumor tissue allows histological examination with a minimum of invasiveness. Particularly seborrheic keratoses can be removed with this method.

Complete excision of tumor. The complete surgical removal of the tumor remains the gold standard for any tumor entity. When histologically controlled excision (Mohs surgery) is performed, the safety margin can be kept as small as possible. Thus, the scars can be kept at a minimum but complete excision with the maximum security to avoid recurrence is achieved.

Cryotherapy. Particlularly for treatment of single or low numbers of actinic keratoses, this simple but effective treatment is an option. Often this treatment is combined with curettage for treatment of seborreic keratoses.

Imiquimod. This treatment is applicable for the treatment of basal cell carcinoma. Treatment with imiquimod is carried out for 3 days a week over a period of approximately 6 months. This can be used for treatment of tumors in patients which are reluctant to undergo surgery.

Photodynamic therapy. This treatment also avoids surgical excision at the cost of missing histological examination. However, advantages are high efficiency and scar-free treatment. Particularly photodynamic therapy is an option for the treatment of patients with field cancerization.

Treatment of metastasized tumors. As described above, most of non-melanoma skin tumors rarely or never metastasize. The risk that this occurs nevertheless is greatest in squamous cell cancers, particularly when they stem from the head and neck region. Should this occur, further treatment modalities such as chemotherapy with bleomycin have to be taken into account. Retinoids have also been reported to be effective for treatment of metastasized squamous cell carcinoma, particularly in conjunction with chemotherapy. Squamous cell carcinoma are particularly radiosensitive. This treatment can also be considered when patients of high age with this tumor or with basal cell carcinoma have to be treated.

Take Home Pearls

- Non-melanoma skin cancer is the most frequent malignancy in man.
- The role of UV radiation as the most important carcinogen is increasingly well understood.
- Measures to protect from UV radiation and its damaging cellular effects are the most important preventive strategies against skin cancer.

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Skin Barrier Function

4

Peter M. Elias and Joan S. Wakefield

Core Messages

- There is a strong rationale for the deployment of strategies to restore barrier function in atopic dermatitis (AD).
- Based upon the mechanisms described in this chapter, these approaches could range from a simple reduction in the pH of stratum corneum alone (hyperacidification), to applications of serine protease inhibitors; general moisturization measures; or finally, specific lipid replacement therapy.
- Moisturizers are widely used in AD, and have been shown to reduce topical steroid usage. Moisturizers supplemented with either botanical ingredients or anti-inflammatory lipids, or incomplete lipid mixtures have recently been approved by the FDA as therapeutic devices, but their clinical efficacy to date has not exceeded that of low-potency steroids.
- Of various 'barrier repair' approaches, the most specific for AD is a triple-lipid, ceramide-dominant, barrier repair formulation. Though still preliminary, these studies suggest that pathogenesis-based therapy, directed at the lipid biochemical abnormality that is responsible for the barrier defect in AD, could comprise a new paradigm for the therapy of AD.

Abbreviations

AD	Atopic Dermatitis
AMP	Antimicrobial Peptides
Cer	Ceramides
FLG	Filaggrin
FFA	Free Fatty Acids
GC	Glucocorticoids
hBD	Human B-Defensins
hCAP	Human Cathelicidin
IL	Interleukin
IV	Ichthyosis Vulgaris
LB	Lamellar Bodies
LEKTI	Lymphoepithelial Kazal-Type Trypsin Inhibitor
NS	Netherton Syndrome
PAR2	Plasminogen Activator Type 2 Receptor
SP	Serine Protease
SC	Stratum Corneum
t-UCA	*Trans*-urocanic Acid

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4.1 Overview of Barrier Function in Normal Skin

4.1.1 Cutaneous Barrier Function

The stratum corneum (SC), long considered an inconsequential end-product of epidermal terminal differentiation, is now increasingly appreciated as the mediator of several key protective (defensive) functions of the skin (Table 4.1) [26], and also as a biosensor that recognizes and responds to changes in external humidity, pH, atmospheric O_2, water content, thermal stimuli, UV- and visible-light irradiation, and microbial challenges (Table 4.2). The structure of the SC is unique, consisting of multiple layers of anucleate, proteinaceous corneocytes embedded in an expanded, lipid-enriched extracellular matrix (forming a dual-compartment model analogous to "bricks and mortar" [22]). The extracellular lipids are also unusual in that they are organized into broad membrane multilayers that lack phospholipids, while being enriched in ceramides, free fatty acids (FFA), and cholesterol [23].

The extracellular lipids are delivered to the extracellular domains of the SC through secretion of epidermal lamellar body (LB) contents [24, 65]. These lipids are secreted as polar precursors, along with their respective lipid hydrolases, β-glycocerebrosidase, steroid sulfatase and secretory phospholipase A, IIA + F, which generate Cer, cholesterol, and FFA, respectively [47]. But the SC interstices contain much more than lipids and lipid hydrolases. At least two antimicrobial peptides, the hCAP18 carboxyterminal product, LL-37 [9], and the β-defensin (hBD2) [70] are co-secreted from LB, as are a set of proteases and protease inhibitors, which regulate corneocyte cohesion/desquamation. Finally, LB deliver a structural protein, corneodesmosin, which coats the extracellular surface of corneodesmosomes (CD), rendering these junctions transiently resistant to proteolysis. Thus, a spectrum of LB-derived products accounts for a set of protective functions that largely localize to the extracellular spaces.

Yet, the corneocytes ("bricks") also contribute to several epidermal protective functions, including permeability barrier homeostasis. Mechanical/frictional resistance is the most widely recognized function of the corneocytes, largely attributed to the rigid, highly cross-linked cornified envelope [74, 79]. In addition, the corneocyte cytosol generates filaggrin proteolytic products, which are further deiminated into acidic metabolites that collectively mediate SC hydration

Table 4.1 Defensive functions of the stratum corneum

Function	Localization	Morphologic basis	Biochemical basis	How signaled
Permeability barrier + Xenobiote penetration	Matrix	Lamellar bilayer	Cer:Chol:FFA (1:1:1)	Δ TEWL ? ⊣TRPV4
Antimicrobial defense	Matrix ? Cytosol	Lamellar bilayer ND	LL-37, hBD2 RNase 5, 7, psoriasin	Δ TEWL ?
Cohesion/desquamation	Matrix	Corneosdesmosomes	Protease/antiprotease cholesterol sulfate;	Local Δ in pH
Mechanical/rigidity	Corneocyte	Cornified envelope	Isopeptide (γ-glutamyl x-linking), Ca++	TGase1 activation
Hydration	Corneocyte matrix	Corneocyte lipid env Sebaceous glands	ω-OH-ceramides FLG → "NMF" Glycerol	TRPV4; tonFAT→TAUT,GABA; AQP3
UV defense	Corneocyte cytosol	–	FLG → UCA	↓ RH → TRPV4 ?
Antioxidant defense	Surface → Extracellular matrix	Sebaceous glands	Vitamin E, CoQ	?

Table 4.2 Epidermis/stratum corneum as a biosensor (1)

External stressor	Sensor →Signal	Regulated change(s)
↓ External Humidity	TRPV4 → ↓ Ca^{++} influx	↑ DNA, lipid synthesis
↑ Osmotic pressure	tonEBP/NFATS	↑ GABA, TAUT, Na-dependent myoinositol transporter
↑ Tactile stimulation	ATP dependent → ↓ [Ca]i	↑ oxytocin production
↑ Heat	TRPV3	?
↑ pH	TRPV1	? ↑ NHE1 expression
↓ Oxygen	NO synthase	↑ Erythropoietin production
↓ Light (Circadian sensor)	↑ Melatonin production → MT3 (Quinone reductase II); RXRα	↑ Free radicals; ↑ Redox enzymes
↓ Electrical potential	?	Lamellar body secretion
Permeability Barrier Disruption	Neurotransmitters (dopamine, glutamate, NO, ATP) Ca^{++}gradient; serine proteases; IL-1 α/β release	↑ PAR2 → ↑ TRPV1 & 4 ↑ Lamellar body secretion ↑ Lipid/DNA synthesis; ↑ cornification
↑ Extremely high frequency radiation	Eccrine glands-helical array antennae	↑ Sweating
Visible light	Rhodopsin receptor	?
↑ UV–B	p53	↑ POMC → ACTH, αMSP (↑ Pigment)
Microbial Pathogens	Toll-like receptors	↑ LL-37, hBD2 & 3, psoriasin

Epidermis/stratum corneum as a biosensor (2)

External Stressor	Sensor → Signal	Downstream change(s)
Microbial Pathogens		
S. aureus	TLR2	MAPK → NFkB → IL-1, TNF α → hBD2+3
Viral	Double-stranded RNA → TLR3	**?** → ↑ LL-37
Gram-	LPS → TLR4	MAPK → NFkB →cytokines → →hBD2+3
E. coli	flagellin → TLR4	↑ Psoriasin

(osmolytes) [76]. One of these osmolytes, *trans*-urocranic acid, is a potent UV filter, which upon irradiation is isomerized to *cis*-urocanic acid, a potent photo-immunosuppressive molecule thought to account for the local development of non-melanoma skin cancer [52]. Finally, the corneocyte is important for the permeability barrier by three unrelated mechanisms: (1) The vertical stacks of cells "force" the extracellular matrix into a highly elongated pathway, which slows both water loss and ingress of xenobiotes [72]. (2) The corneocyte is a necessary scaffold for the supramolecular organization of the extracellular lipids into elongated, multi-lamellar structures [25]. (3) The acidic metabolites of FLG likely acidify the SC [58], activating Cer-generating lipid-processing enzymes, while inactivating potentially degradative serine proteases [40]. These observations demonstrate the interdependence of the two SC compartments.

4.1.2 Co-regulation and Interdependence of Permeability Barrier and Antimicrobial Defense

While it is sometimes convenient to categorize cutaneous protective functions as discrete processes, this exercise in taxonomy fails to recognize the extent to

which these functions are intertwined [26, 27]. The geometric structural coherence, low water context, hydrophobicity, and reduced acidity of the SC are critical not only for permeability barrier homeostasis, but together they also create an environment hostile to microbial pathogens [28]. There is also substantial biochemical overlap, since both epidermal- and sebaceous gland-derived FFA exhibit potent antibacterial, antiviral, and antiyeast activities (op. cit.). Moreover, FFA, like at least two antimicrobial peptides (AMP), LL-37 and hBD2 [9, 70], localize to the extracellular matrix, and these AMP are delivered, along with phospholipid precursors of FFA, to the matrix by LB secretion [1]. Since most pathogens attempt to breach the SC via the SC interstices [66], both FFA and AMP normally are arranged in the appropriate location to block pathogen ingress [28]. Finally, we recently provided two types of direct evidence for the interdependence of these two functions [2]: (1) Acute permeability barrier abrogation upregulates not only lipid metabolic processes leading to barrier recovery, but also the expression of mBD3 and CRAMP (murine homologues of hBD2 and LL-37, respectively). (2) Mice with transgenic ko of CRAMP exhibit not only increased susceptibility to cutaneous infections, but also abnormal permeability barrier function, further attributable to defective LB and lamellar bilayer structure. Together, these studies demonstrate the co-localization, co-regulation, and interdependence of permeability barrier homeostasis and cutaneous antimicrobial defense.

4.1.3 Stratum Corneum as a Dynamic Sensory Interface

Though its cells (corneocytes) are "dead", i.e., lack nuclei and energy-dependent synthetic machinery, the SC nevertheless is replete with multiple types of metabolic activity. For example, the cohesion/desquamation of normal SC is exquisitely self-regulated so that corneocytes are shed individually (invisibly) from the skin surface in normal human skin. Yet, it is not loss of cells, but rather barrier requirements that regulate terminal differentiation, leading to new corneocyte formation [16]. Endogenous SC proteases regulate not only this process, but also the release of the prestored, pro-inflammatory cytokines, IL-1α and IL-1β, from the corneocyte cytosol [68]. In fact, a wide range of signaling mechanisms has been identified recently, which further demonstrate how the SC, often in conjunction with receptors on the membranes of the outer epidermis, serves as an exquisite biosensor.

While details about some of these signaling mechanisms have been clarified recently, others still remain largely unexplored. Best studied of all the signaling mechanisms is the epidermal calcium gradient, which restricts LB secretion under basal conditions (rev in [31]). Loss of the Ca^{++} gradient, as occurs with acute barrier disruption, stimulates a wave of LB secretion [63, 64], the first in a series of metabolic responses that leads to restoration of barrier function [26, 31]. Yet, changes in SC function also signal metabolic events in the underlying epidermis by a set of receptors located in the outer epidermis. For example, barrier disruption leads to elevations in SC pH, which not only activate serine proteases (SP) leading to SP-PAR2 activation [16], but external pH changes also likely activate the transient vanilloid receptor 1 (TRPV1) [19]. It is likely, but not yet known, whether activation of TRPV1 could, in turn, upregulate the sodium-proton antiporter, NHE1, whose expression is also known to be regulated by changes in SC pH [39]. Changes in *SC hydration* that occur in response to either reductions in external humidity alone or to accelerated water loss due to barrier abrogation could signal epidermal metabolic response by activating TRPV4. However, the epidermis also contains other signaling mechanisms that respond to changes in osmolarity, e.g., tonEBP/NFATS, a transcriptional mechanism that upregulates transmembrane osmolyte transporters [83], and glycerol transporters, such as AQP3, could also be activated.

4.1.4 External Stressors Alter Permeability Barrier Function

Permeability barrier function in normal skin changes not only in response to alterations in external humidity, but also to UV-B exposure. We showed several years ago that sustained exposure to a reduced relative humidity (RH) upregulates epidermal metabolic processes leading to superior barrier function [17]. Single, erythemal doses of UV-B provoke transient, dose-dependent abnormalities in barrier function [43], attributable to passage of a band of apoptotic, secretion-incompetent keratinocytes outward through the stratum granulosum–SC interface [46]. The transient nature of the defect is further attributable to a T-cell-driven stimulation of epidermal proliferation, which rapidly restores

secretion-competent cells to the outer epidermis [43]. In contrast to erythemal UV-B, we showed recently that sub-erythemal doses of UV-B instead *improve* epidermal permeability barrier homeostasis and upregulate antimicrobial peptide expression in parallel [48].

4.1.5 Sources, Function, and Pathophysiology of Stratum Corneum Acidification

The fact that the surface of normal skin is highly acidic (≈5.0) has been appreciated for several decades. Its putative function was assumed to be antimicrobial, since the normal cutaneous microflora proliferate at this pH, while conversely, in vitro studies showed that a variety of microbial pathogens grow preferentially at a neutral pH [56, 57]. Since eccrine and sebaceous glands secrete acidic metabolites, such as lactic acid and free fatty acids, respectively, the acidic pH of SC initially was ascribed to surface deposition of these and other exogenous products.

4.1.5.1 Endogenous Sources of Stratum Corneum Acidification

More recent studies from our lab have demonstrated instead that much of the acidity of SC derives from three endogenous sources: (1) The total hydrolysis of cellular and secreted phospholipids during terminal differentiation leads to the generation of large quantities of nonessential free fatty acids (FFA), which are required not only as structural components of the extracellular lamellar bilayers [61, 62], but also as bulk acidifiers of the SC (accounting for ≈1 pH unit) [32]. (2) A second endogenous mechanism, the sodium-proton exchanger, NHE1, is inserted in the apical membranes of outermost cells of the stratum granulosum. Though studies in NHE1 transgenic knockout mice show that NHE1 only accounts for one-fourth unit of bulk SC pH, this mechanism selectively acidifies membrane microdomains where pH-sensitive events leading to barrier formation and desquamation are initiated [6]. (3) A third endogenous mechanism, i.e., the hydrolysis and deinimation of filaggrin-derived amino acids into polycarboxylic acids, such as *trans*-urocanic acid (from histidine by histidase) [58], theoretically could contribute to bulk acidication, but its physiologic role is unclear, since histidase-deficient (Peruvian) mice display a normal surface pH, and since neither histidase-deficient mice nor humans display functional abnormalities [32]. Together, these endogenous mechanisms account for much (but not all) of the acidity of normal SC. Since none of these three mechanisms differs in darkly versus lightly pigmented subjects, the melanocyte-derived mechanism described below represents yet another likely contributor to bulk acidification, at least of the SC of pigmented subjects.

4.1.5.2 Role of pH in Regulating Function

We have identified at least two key epidermal functions that are impacted by changes in pH of SC. Most importantly, permeability barrier homeostasis is regulated primarily through the acidic-pH-dependent activation of two ceramide (Cer) generating enzymes, β-glucocerebrosidase (β-GlcCer'ase) and acidic sphingomyelinase (aSMase), which both display pH optima ≈ 5.0 [38, 40]. Accordingly, the reduced barrier competence of both neonatal and aged skin was subsequently linked, at least in part, to an elevated SC pH, leading to reduced activities of these lipid-processing enzymes [7, 12, 34]. Interestingly, the biochemical basis for the pH abnormality in neonatal and aged skin differs: in neonates, it could be attributed to delayed activation of secretory phospholipase [33], while the pH abnormality in aged skin correlated with a decline in NHE1 expression [12]. Developmental (age-related) abnormalities in acidification provoke abnormalities not only in barrier function, but also in SC integrity/cohesion, but by another mechanism, i.e., at an elevated pH, SC serine proteases, specifically kallikrein 5 (SC tryptic enzyme) and kallikrein 7 (SC chymotryptic enzyme) become more active. The net result of a sustained increase in these SP is not only degradation of corneodesmosomes (CD) and their constituent proteins, desmoglein 1 (DSG1), desmocollin 1 (DSC1), and corneodesmosin, but also degradation of the Cer-generating hydrolases (β-GlcCer'ase and aSMase) [41, 42]. The direct role of pH in orchestrating these divergent downstream events was demonstrated with the topical applications of "superacids" (more acidic than 1 N H_2SO_4) and "superbases" (more basic than 1 N NaOH) [40, 41].

The consequences of a pH-driven increase in SP are still more far-reaching – this mechanism activates the G-protein coupled receptor, plasminogen activator receptor type II (PAR2), which in turn inhibits lamellar

body (LB) secretion [41], while simultaneously inducing terminal differentiation (physiologic apoptosis = cornification of outermost SG cells) [16]. Thus, the reduced pH of normal SC, which is itself regulated in response to changes in external pH by an increase in NHE1 expression [39], regulates multiple downstream epidermal functions by divergent mechanisms.

4.1.5.3 Pathophysiology of an Elevated Stratum Corneum pH

Diseased (inflamed) skin inevitably displays an elevated SC pH [32], which is not simply a passive marker of disease, but actually a driver of inflammation through a pH →↓SP-driven activation and release of IL-1α/β from corneocytes [68], initiating the "cytokine cascade" ("outside–inside" pathogenesis of inflammatory dermatoses) [29]. In addition, by modifying barrier function, the flawed barrier allows continuous access of external xenobiotes (e.g., allergens), and increased colonization by microbial pathogens, which further exacerbate inflammation by superantigen and/or toxin-initiated mechanisms [30]. Conversely, we have shown that maintenance of an acidic pH alone largely prevents emergence of an atopic dermatitis (AD)-like dermatoses in mice repeatedly challenged with the hapten, oxazolone [45]. Thus, SC acidification is not only of physiologic and pathophysiologic importance, but it also could comprise an effective preventive strategy for inflammatory skin disease.

4.1.6 Permeability Barrier Function in Relation to Pigmentation

4.1.6.1 Enhanced Barrier Function in Darkly Pigmented Skin

Several years ago, we reported superior permeability barrier homeostasis and SC cohesion in a small cohort of darkly pigmented (type IV/V) than in lightly pigmented (types I/II) subjects [73]. Moreover, these differences appeared to be independent of country of origin or ethnicity, suggesting that the differences were pigment-type dependent. We have now confirmed these differences in two much larger cohorts of Sinhalese and Germans with similar occupations (1° nursing personnel) and gender (1° female). Moreover, to exclude latitude-dependent differences in humidity as a co-variable (though it should favor inferior barrier function!) [17], we demonstrated the same differences among another cohort of darkly versus lightly pigmented humans living at the same (temperate) latitude (i.e., San Francisco) (Fig. 4.1a and b) [37]. The differences in permeability barrier function in SC were paralleled by marked

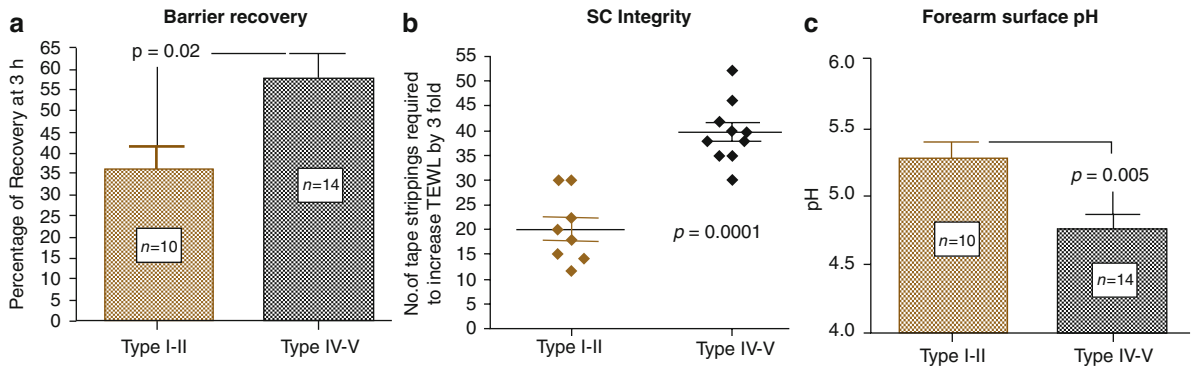

Fig. 4.1 Epidermal functional differences among divergent pigment groups are independent of geographic location and occupation: Barrier recovery (**a**), epidermal integrity (**b**) and forearm surface pH (**c**) were assessed in a cohort of age- and gender-matched subjects with type I-II and IV-V skin, living in the same geographic location (San Francisco, California). SC integrity (**b**) was assessed as the number of D-squame tape strippings required to increase TEWL 3-fold over baseline. TEWL was assessed immediately and 3 hrs after barrier disruption and expressed as % recovery (**a**). Surface pH (**c**) of the volar forearm and dorsum of the hand was measured in the same 2 groups using a flat glass electrode. Results shown represent means ± SEM

differences in SC integrity (resistance to sequential tape stripping). Together, these results confirm enhanced epidermal function in darkly pigmented epidermis.

4.1.6.2 Enhanced Epidermal Function of Darkly Pigmented Skin Correlates with a Lower SC pH

The darkly pigmented cohorts exhibited about a half unit lower surface pH than did their lightly pigmented age/occupational/gender equivalents (Fig. 4.1c), and the reduced acidity extended to all levels of the SC, as shown by fluorescence lifetime imaging (FLIM) microscopy [6, 37]. Further mechanistic studies showed that the lower SC pH results in greater activation of the Cer-generating, lipid-processing enzymes, β-GlcCer'ase and aSMase, while it conversely reduced activity of potentially harmful serine proteases (SP). As a result, CD and their constituent proteins persisted high into the SC, but SC thickness did not increase in darkly pigmented individuals due to increased activity of the acidic-pH-dependent aspartate protease, cathepsin D [37]. In addition, epidermal lipid content and cornified envelope thickness increased significantly in darkly pigmented skin, but the extent to which these differences account for enhanced function has not yet been determined. Finally, the importance of the lower pH for improved function was shown directly by experiments in which the SC pH of a cohort of lightly pigmented subjects was lowered to levels comparable to the darkly pigmented subjects, using either topical buffers or topical polyhydroxyl acids (PHA). This adjustment alone enhanced both barrier function and SC integrity to levels comparable to darkly pigmented subjects (Fig. 4.2a and b). Together, these results show that pH differences alone largely account for enhanced epidermal function in darkly pigmented skin.

4.1.6.3 Lower pH of Melanocyte Dendrites Could Account for Lower pH of Pigmented Skin

We next assessed two mechanisms whereby melanocytes could influence epidermal acidification and function. First, we assessed whether the activities or expression of the three endogenous acidifying mechanisms differ, i.e., NHE1, $sPLA_2A$ & F (the only $sPLA_2$ isoforms expressed in the outer epidermis), and histidase. None of these mechanisms differed in lightly versus darkly pigmented skin, suggesting that melanocyte-derived paracrine signals do not upregulate these endogenous acidifying mechanisms (not shown). These findings shifted our focus to a second mechanism, i.e., whether melanocytes directly acidify the outer epidermis. Accordingly, melanocytes from darkly pigmented subjects displayed a significantly lower cytosolic pH than did the cytosol of melanocytes from lightly

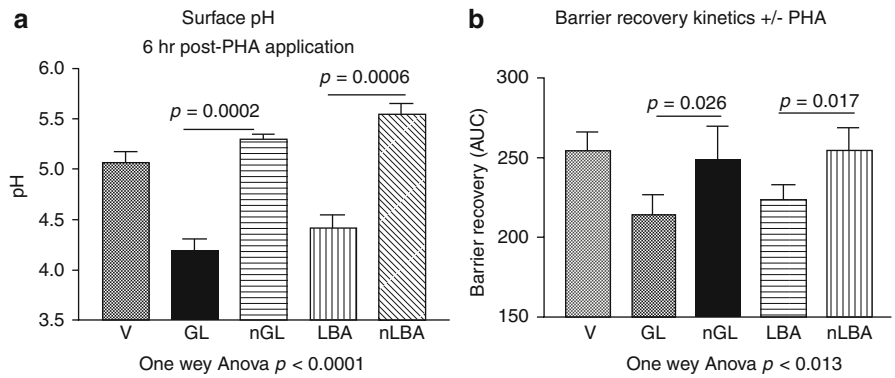

Fig. 4.2 Single PHA applications decrease SC pH and accelerate barrier recovery in lightly pigmented subjects: (**a**) A single application of either lactobionic acid (LBA), or gluconolactone (GL), 5% in propylene glycol/ethanol: 70/30, neutralized LBA (nLBA), neutralized GL (nGL) or vehicle (V) was applied on the forearm skin of human volunteers ($n = 16$). While basal values were identical on all test sites prior to application (not shown), significant decreases in surface pH were observed at 1, 6, and 24 h following a single application of either LBA or GL compared to the vehicle (1 + 24 h not shown). (**b**) TEWL was measured before and at 0, 3, 24, and 48 h following acute barrier disruption by repeated cellophane tape stripping on the forearms of type I-II subjects. Acidification of SC pH by either PHA (LBA or GL) significantly improved barrier recovery in comparison to either vehicle or nLBA/nGL-treated skin sites

pigmented cells, assessed by dual channel confocal microscopy, using the pH-sensitive dye, SNARF-5F [37]. Moreover, these pigment-type-determined differences were even more dramatic in dendritic processes, which were about 1/3 pH unit more acidic than in darkly pigmented melanocytes. Since melanosomes persist much higher in the epidermis in darkly pigmented than in lightly pigmented subjects, likely releasing their load of protons at or near the stratum granulosum–SC junction, these results could provide a cellular mechanism whereby melanocytes could (further) acidify the outer epidermis of darkly pigmented subjects.

4.2 Barrier Failure in Atopic Dermatitis

Like the permeability barrier, the *antimicrobial barrier* is also compromised in AD. Colonization by *Staphylococcus aureus* is a common feature of AD [4, 28]. And while colonization is highest on lesional skin, colony counts often are high on clinically normal skin of AD patients [5]. Moreover, overt secondary infections are well-recognized complications in the management of AD. Furthermore, colonization by superantigen producing *S. aureus* strains further exacerbates disease in severe AD through generalized augmentation of IgE production, as well as through development of specific IgE-directed toward staphylococcal exotoxins (rev. in [19]). In addition, patients with atopic dermatitis are also susceptible to widespread cutaneous viral infections, including molluscum contagiosum, *Herpes simplex* (Kaposi's varicelliform eruption), and life-threatening Vaccinia. Widespread dermatophytosis (tinea corporis) and *Malassezia* infections also occur in AD, and the latter, like *S. aureus*, can stimulate specific IgE production. Taken together, these observations point to loss of a competent antimicrobial barrier in AD.

Whereas both permeability and antimicrobial dysfunction are well-recognized features of AD, only recently has the likely reason become clear; i.e., these two functions are both co-regulated and interdependent [2] Moreover, failure of the permeability barrier in itself favors secondary infection; and conversely, pathogen colonization/infection further aggravates the permeability barrier abnormality [2, 28] (Fig. 4.3). For example, as with water egress, pathogen ingress occurs via the extracellular domains [66]. Moreover, an impaired permeability barrier alone predisposes to pathogen colonization, not only because of the increase in surface pH [32], but also because levels of FFA and the Cer metabolite, sphingosine, which exhibit potent antimicrobial activity [8, 66], are reduced in AD (cited in [28]). Surface proteins on *S. aureus* also can down-regulate epidermal FFA production [13], thereby aggravating both permeability and antimicrobial function in parallel, a strategy that could further facilitate microbial invasion. In addition, members of two key families of antimicrobial peptides (AMP), the human cathelicidin (hCAP) product, LL-37, and human β-defensins (hBD) 2 and 3, are down-regulated in a TH2-dependent fashion in AD [67, 69]. Notably, both the hCAP aminoterminal fragment, cathelin [86], and hBD3 [55] display robust activity against *S. aureus*. LL-37 is required for

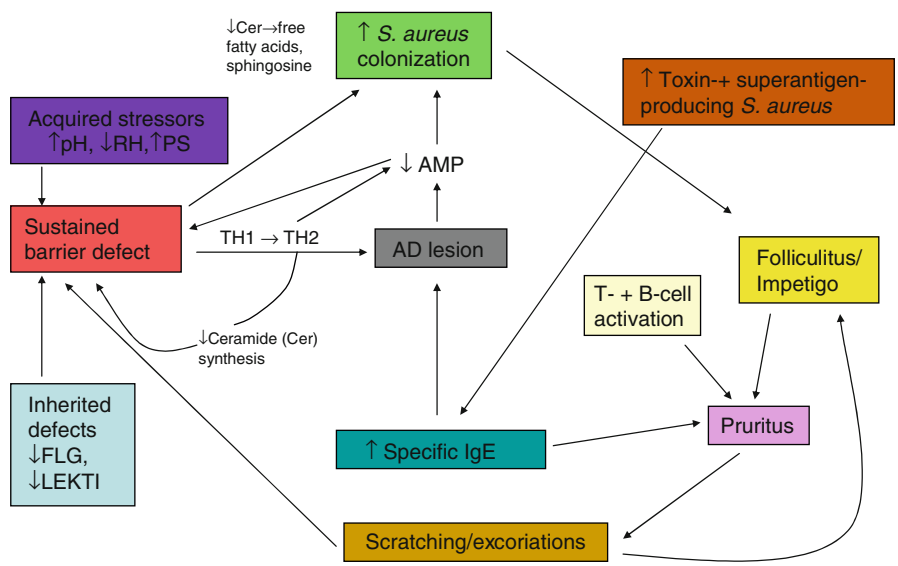

Fig. 4.3 Pathogen colonization further aggravate the barrier abnormality in AD. (Modified from Elias et al., JACI Rev, 2008)

normal epidermal permeability barrier function (notably, LL-37 is also important for the integrity of extracutaneous epithelia) (cited in [2]). Thus, it is likely that decreased LL-37 amplifies the barrier defect in AD.

As noted above, non-toxigenic strains of *S. aureus* that colonize AD can be replaced by enterotoxin-generating strains [60], which in turn, could aggravate AD by at least three mechanisms (Fig. 4.3): (1) Toxigenic strains are more likely to produce clinical infections than are non-toxigenic strains [60]. (2) Some toxins stimulate pruritus and production of specific IgE [5]. And (3) some toxins serve as "superantigens" that stimulate T and B cell proliferation, as well as immunoglobulin class-switching to allergen-specific or "superallergens" that stimulate IgE production [5, 36]. Activated T cells produce IL-31, which also induces pruritus [78]. Finally, clinical infections, particularly folliculitis, are notoriously pruritic, even in non-atopics, eliciting an "itch-scratch" vicious cycle that creates additional portals of entry for pathogens (Fig. 4.3). It is self-evident that excoriations create further defects in the permeability barrier, representing yet another potentially important vicious cycle in AD pathogenesis.

Finally, several other critical defensive functions of the SC are also compromised in AD, including (Table 4.1):(1) *SC integrity* (cohesion), reflected by excess scale (abnormal desquamation); and (2) diminished *SC hydration*, reflected by life-long cutaneous xerosis in these patients, even after overt inflammation recedes [21, 77, 81]. Like the defective permeability and antimicrobial barriers, SC hydration declines in both lesional and non-lesional AD skin, with severity paralleling disease activity [11, 81]. Decreased SC hydration is not merely of cosmetic concern; it alone suffices to stimulate epidermal hyperplasia and early evidence of inflammation, such as mast cell degranulation, even in normal skin [18]. Thus, AD can be viewed as a disease of broad barrier failure.

4.3 Basis for Abnormal Barrier Function in Atopic Dermatitis

4.3.1 Inherited Abnormalities

Based primarily upon inherited abnormalities either in filaggrin (FLG) production in Northern Europeans, the development of AD is now increasingly linked to a primary defect in the structure and function of the SC. The strongest evidence for a primary structural abnormality of SC underlying the pathogenesis of AD derives from the recent link between loss-of-function mutations in the gene encoding, filaggrin (FLG), and AD [38, 50, 71, 84]. Up to 50% of European kindreds with AD reveal single or double allele, or compound mutations in FLG on chromosome 1q21. Although 15 different mutations have been reported, the two most common (R501X and 2282del4) account for the majority of cases [75], and because of their proximal location on the *FLG* gene, they also predict more severe, loss-of-function [38, 85]. Yet, while the logic for the link between excess SP activity and the barrier abnormality in AD seems clear, how loss of FLG (an *intracellular* protein) provokes a permeability barrier abnormality (almost always an *extracellular* defect) is not known. Loss of this quantitatively important protein could alter corneocyte shape (e.g., flattening) sufficiently to disrupt the organization of the extracellular lamellar bilayers. Alternatively, or in addition, FLG is generated during cornification as its precursor protein, profilaggrin, which is then proteolytically processed into FLG during the abrupt transition from the granular cell layer to corneocyte. Whereas FLG initially aggregates keratin filaments into keratin fibrils, subsequently, it is itself proteolytically degraded into amino acids, which are further deiminated into polycarboxylic acids, such as pyrrolidine carboxylic acid and *trans*-urocanic acid (t-UCA) [76]. These metabolites, in turn, act as osmolytes, drawing water into corneocytes, thereby accounting in large part for corneocyte hydration. Hence, the most immediate result of FLG deficiency in AD is decreased SC hydration, leading in turn to a steeper water gradient across the SC, which likely "drives" increased transcutaneous water loss. Thus, decreased SC hydration, leading to increased water loss, is the first and most obvious cause of barrier dysfunction in AD. However, neither corneocyte flattening nor decreased SC hydration alone would suffice to enhance antigen penetration, which is best explained by another consequence of FLG deficiency, i.e., decreased downstream production of acidic metabolites resulting from FLG proteolysis. Indeed, t-UCA, in particular, is a purported, endogenous acidifier of the SC [58]. Decreased generation of FLG products in turn could result in an initial increase in the pH of SC in AD, sufficient to increase the activities of the multiple serine proteases (SP) in the SC, which all exhibit neutral-to-alkaline pH optima [10]. Such a pH-induced increase in SP activity, if prolonged, could precipitate multiple

downstream structural and functional alterations that could converge with acquired abnormalities in SP/anti-protease expression.

The most compelling case for the role of excess SP activity in the pathogenesis of AD comes from Netherton syndrome (NS), an autosomal recessive disorder due to loss-of-function mutations in *SPINK5*, the gene encoding the SP inhibitor, lymphoepithelial Kazal-type trypsin inhibitor (LEKTI) [82]. NS is characterized by severe AD, mucosal atopy, and anaphylactic reactions to food antigens (cited in [25, 26]). Residual LEKTI expression in NS correlates inversely with excess SP activity within the outer epidermis [42], resulting in a severe permeability barrier defect and dramatic thinning of SC due to unrestricted, SP-dependent degradation of lipid-processing enzymes and corneodesmosome-constituent proteins, respectively [42, 54]. Pertinently, several European, American, and Japanese case-control studies of patients, with AD or mucosal atopy, have been found an increased frequency of single nucleotide polymorphisms (Glu420Lys) in *SPINK5* [82]. Conversely, a British case-control study described putative, gain-of-function polymorphisms (AACCAACC vs. AACC) in the 3' region of *KLK7*, which encodes the serine protease SC chymotryptic enzyme or KLK7. Moreover, transgenic mice forced to express human KLK7 display a severe AD-like dermatosis. Yet, the incidence of both these polymorphisms is quite high in unaffected normals, and it is not yet known whether either of these SNPs alters expression of its respective protein product(s). Nevertheless, in experimental animals, a net increase in SP activity, achieved by a variety of means, has been shown to compromise barrier function through accelerated degradation of both corneodesmosomes (accounting for flawed SC integrity) and degradation of extracellular lipid-processing enzymes, i.e., β-glucocerebrosidase and acidic sphingomyelinase. SP-mediated degradation of the extracellular hydrolytic enzymes would in turn, result in a failure to generate Cer, a characteristic lipid abnormality in AD [20, 51].

4.3.2 Acquired Stressors Could Further Aggravate Barrier Function in AD

Acquired pH-dependent increases in SP activity could also contribute to AD pathogenesis. That *FLG* mutations alone do not suffice as shown in ichthyosis vulgaris (IV), where the same single or double allele *FLG* mutations reduce FLG content, but inflammation (i.e., AD) does not always occur. Certain stressors could elicit disease by aggravating the barrier abnormality by provoking an incremental increase in pH of the SC, leading to a further amplification of SP activity. Such a barrier-dependent increase in pH (and SP activity) likely accounts for the precipitation of AD following the use of *neutral-to-alkaline soaps*, a well-known exogenous stressor of clinical AD [15].

Prolonged exposure to a *reduced environmental humidity*, as occurs in radiant-heated homes in temperate climates during the winter, is also a well-known risk factor for AD. Under these conditions, transcutaneous water loss would accelerate across a defective SC, aggravating the underlying permeability barrier abnormality, and amplifying cytokine signaling of inflammation. Because FLG proteolysis is regulated by changes in external humidity [76], sustained reductions in environmental relative humidities could further deplete residual FLG in single-allele *FLG*-deficient patients.

Sustained *psychological stress* (PS) aggravates permeability barrier function in humans [3, 35], and PS is both a well-known precipitant of AD, and cause of resistance to therapy. In the case of PS, however, the likely mechanism differs from either surfactant use or decreased environmental humidities. In experimental animals, psychological stress induces an increase in endogenous glucocorticoids (GC), which in turn alter permeability barrier homeostasis, SC integrity, and epidermal antimicrobial defense [1]. The putative mechanism for the negative effects of psychological stress is GC-mediated inhibition of synthesis of the three key epidermal lipids that mediate barrier function, i.e., Cer, cholesterol, and FFA. Accordingly, a topical mixture of these three lipids largely normalizes all of these functions, even in the face of ongoing PS or GC therapy [1].

4.4 "Outside–Inside," Then Back to "Outside" Pathogenic Mechanism in AD

One important downstream consequence of an increased pH, and a pH-driven increase in SP activity, is generation of the primary cytokines, IL-1α and IL-1β, from

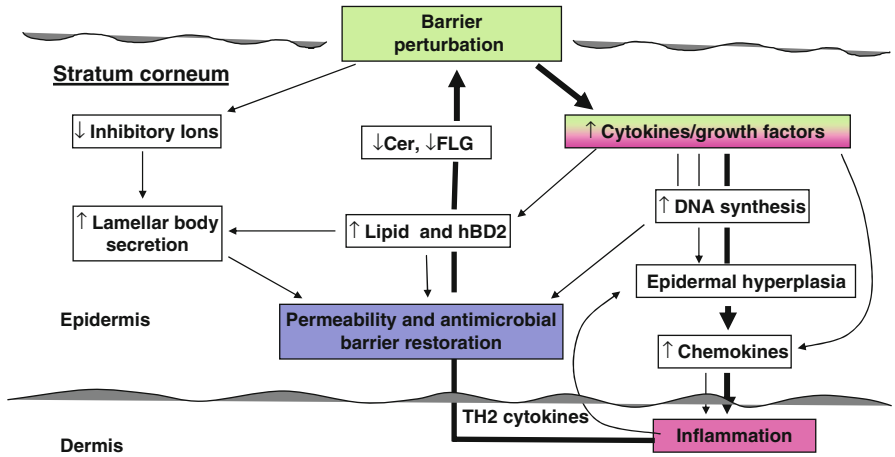

Fig. 4.4 "Outside–inside" initial provocation could be followed by "back-to-outside" vicious cycle. (Modified from Elias et al., JACI Rev, 2008)

their 33kDa pro-forms, which are stored in large quantities in the cytosol of corneocytes. The putative pH-induced increase in SP activity would generate 17kDa active forms of these cytokines, the first step in the cytokine cascade that we propose is a primary contributor to inflammation in AD ("outside–inside" hypothesis: Fig. 4.4). Sustained antigen ingress through a defective barrier, leading to a TH2-dominant infiltrate, would then become the second cause of inflammation in AD [50]. Accordingly, correction of the barrier abnormality alone should ameliorate both causes of inflammation in AD.

Yet, despite accumulating evidence in support of a primary, barrier-initiated pathogenesis of AD, recent studies suggest specific mechanisms whereby TH2-generated cytokines could also further aggravate AD. Exogenous applications of the TH2 cytokine, IL-4, impede permeability barrier recovery after acute perturbations [59]. The basis for the negative effects of IL-4 could include: (1) the observation that exogenous IL-4 also inhibits ceramide (Cer) synthesis [44], providing yet another mechanism accounting for decreased Cer; (2) IL-4 also was shown recently to inhibit expression of keratinocyte differentiation-linked proteins, most notably FLG [49]; and (3) desmoglein 3 expression is also inhibited by exogenous IL-4 [53]. Together, these observations provide acquired mechanisms that could further compromise barrier function in AD [49, 53]. Thus, primary inherited barrier abnormalities in AD ultimately stimulate downstream paracrine mechanisms that could further compromise permeability barrier function, completing a potential "outside–inside–outside" pathogenic loop in AD (Fig. 4.4) [29].

4.5 A Potential New Therapeutic Paradigm for AD

Together, the converging pathogenic features described above create a strong rationale for the deployment of strategies to restore barrier function in AD. Based upon the mechanisms described above, these approaches could range from a simple reduction in the pH of SC alone (hyperacidification) to applications of serine protease inhibitors; general moisturization measures; or finally, specific lipid replacement therapy. Moisturizers are widely used in AD, and when used under nursing supervision have been shown to reduce topical steroid usage [14]. Moisturizers supplemented with either botanical ingredients (e.g., Atopiclair) or anti-inflammatory lipids (e.g., MimyX), or incomplete lipid mixtures have recently been approved by the FDA as therapeutic devices, but their clinical efficacy to date has not exceeded that of low-potency steroids. Of various "barrier repair" approaches, the most specific for AD is a triple-lipid, Cer-dominant, barrier repair formulation (EpiCeram® cream, Ceragenix Corp.).[1] This formulation demonstrated efficacy that was comparable to a mid-potency steroid (fluticasone propiate 0.5%, Cutivate® cream)

[1]Dr. Elias is a co-inventor of this University of California patented technology. He is a consultant to Promius Pharmaceuticals and to PediaPharm Inc., which markets this technology in the United States and in Canada, respectively.

in an investigator-blinded, multicenter clinical trial of pediatric patients with moderate-to-severe AD [80]. Though still preliminary, these studies suggest that pathogenesis-based therapy, directed at the lipid biochemical abnormality that is responsible for the barrier defect in AD, could comprise a new paradigm for the therapy of AD.

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Part II

Functional Food for Skin Works: Intervention Studies in Humans and Animal Models

Botanical Antioxidants for Skin Protection: An Overview

5

Farrukh Afaq and Hasan Mukhtar

Core Messages

- The concept of photoprotection is gaining attention as a viable approach to reduce the occurrence of skin cancer and photoaging.
- Accumulating data indicates that botanical antioxidants possess anti-proliferative, anti-inflammatory, anti-angiogenic and anti-carcinogenic effect in the skin by targeting multiple signaling pathways.
- Since multiple pathways are involved in photoaging and photocarcinogenesis process, a mixture of several botanical antioxidants working through different mechanisms could be an effective strategy.
- The use of skin care products supplemented with several effective botanical antioxidants in conjunction with the use of sunscreens may be an effective approach for skin protection.

5.1 Introduction

Earth is continuously irradiated by light photons coming from the sun of which 56% are infrared light photons (780–5,000 nm), 39% visible light (400–780 nm), and 5% UV light (280–400 nm). UV radiation impending from the sunlight is divided into three categories dependent on wavelength, UVC (200–280 nm), UVB (280–320 nm), and UVA (320–400 nm). UVC is the most biologically damaging region of UV radiation; however, it is filtered out by the ozone layer of the Earth's atmosphere, and therefore, its role in human pathogenesis is minimal. UVB radiation, and to a lesser extent, UVA are responsible for various skin disorders including skin cancers [1, 2]. It is well documented that UV radiation is absorbed by chromophores (such as DNA, RNA, proteins, melanin, *trans*-urocanic acid, etc.) in the skin [3]. Absorption of UV photons by these chromophores present in the skin results in different photochemical reactions and secondary interactions involving ROS that result in damaging effects. UV irradiation to skin results in erythema, edema, hyperplasia, hyperpigmentation, sunburn cells, immunosuppression, photaging, and photocarcinogenesis [4, 5]. UVB irradiation to skin has direct effects on biomolecules, for example, the formation of cyclobutane pyrimidine dimers (CPDs), 8-hydroxy-2'-deoxyguanosine (8-OHdG) and pyrimidine (6–4) pyrimidone photodimers, protein oxidation, photoisomerization of *trans*- to *cis*-urocanic acid, and generation of reactive oxygen species (ROS) [3, 6, 7]. These effects of UVB may result in a variety of skin disorders including skin cancer and premature aging.

Skin covers an enormous surface area of 1.5–2.0 m^2 and is the organ that is most accessible to sunlight and directly suffers from the deleterious effects of UV radiation. UV radiation is the main cause for the vast

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majority of cutaneous malignancies diagnosed in the humans, more so in Caucasian populations [4, 5]. According to an estimate by the American Cancer Society, approximately 1.2 million new cases of skin cancer are identified each year in the USA and these accounts for 40% of all new cancer cases that are diagnosed [8]. Similar trend is also noted in natives of many countries with predominantly Caucasian populations. The basal cell carcinomas (BCCs) and squamous cell carcinomas (SCCs), grouped together as non-melanoma skin cancers (NMSC) are the most frequently diagnosed cutaneous malignancies and account for approximately 80% and 16% of all skin cancers, respectively. Melanoma, the most malignant form of skin cancer, accounts for about 4% of new cancer cases diagnosed in the USA [8]. It is imperative to mention that among all the cancers, unquestionably skin cancer is most preventable.

Kligmann and Kligmann coined the term photoaging to describe modifications that occur after years of cutaneous exposure to UV radiation [9]. Wrinkles, dryness, and alterations in pigmentation are directly associated with photoaging and considered its most salient cutaneous manifestations [10]. Losses in skin tone and elasticity, along with increased skin fragility, are also observed in photodamaged skin. In its most basic form, photodamage skin consists of the deposition of abnormal elastin as amorphous, blue-gray aggregates within the superficial dermis, often referred as elastosis [11]. There is increased generation of ROS in skin when it is exposed to UVB radiation and these ROS are thought to be critical mediators of the photoaging process [4]. These ROS causes oxidative stress and oxidative photodamage of proteins and other macromolecules in the skin. Studies have also shown that ROS can modify proteins to form carbonyl derivatives that accumulate in the papillary dermis of photodamaged skin [12]. UV radiation increases tropoelastin protein and mRNA expression in the three-dimensional reconstituted human skin and in the epidermis of human skin [13, 14]. UV irradiation increased the levels of elastin transcript containing exon 26A and its encoded elastin isoform in cultured human keratinocytes and in the epidermis of human skin [15]. In photoaged skin an increase of collagen synthesis and greater matrix metalloproteinase expression were observed [16]. Wrinkles in photoaged skin resulted from decreased de novo synthesis and increased degradation of type I and type III collagen fibers [17].

Over the years changes in lifestyle patterns have led to a significant increase in the amount of UV radiation that people receive and this has led to a surge in the incidence of skin cancer and photoaging. Since these trends are likely to continue in the foreseeable future, the adverse effect of UV has become a major human health concern. In recent years, botanical antioxidants have gained considerable attention because of their skin photoprotective effects [1, 18]. Botanical antioxidants have also been shown to reduce the incidence of ROS-mediated photocarcinogenesis and photoaging. In general, these botanical antioxidants modulate cellular signaling pathways known to be deregulated by solar UV radiation [1, 18]. This has generated a great interest in using botanical supplements rich in antioxidants to delay photocarcinogenesis and prevent photoaging. This chapter presents an overview of some of the selected botanical for skin protection against UV radiation. The adverse biological effects of UV radiation and the postulated mechanism(s) of action of botanical antioxidants are summarized in Fig. 5.1.

5.2 Green Tea/EGCG

Tea from the plant *Camellia sinensis,* of the Theaceae family, in fact, is the most popular beverage consumed by the humans and it contains polyphenolic constituents known as "catechins." The four major polyphenolic constituents present in green tea include (-)-epicatechin (EC), (-)-epicatechin gallate (ECG), (-)-epigallocatechin (EGC), and (-)-epigallocatechin-3-gallate (EGCG). All of these polyphenols act as antioxidants with different potency and can scavenge ROS. Among these, EGCG is the most abundant polyphenol and is believed to be the most active. Pretreatment of normal human epidermal keratinocytes (NHEK) with green tea polyphenol (-)-epigallocatechin-3-gallate inhibited UVB-induced oxidative-stress-mediated phosphorylation of mitogen-activated protein kinases (MAPKs) [19]. In addition, EGCG was found to inhibit UVB-induced phosphorylation and degradation of IκBα and activation of IKKα and nuclear factor kappa B (NF-κB) in a dose- and time-dependent manner in NHEK [20]. Treatment of cultured human keratinocytes with EGCG inhibited UVB- and UVA-induced NF-κB nuclear translocation and interleukin-6 (IL-6) secretion [21].

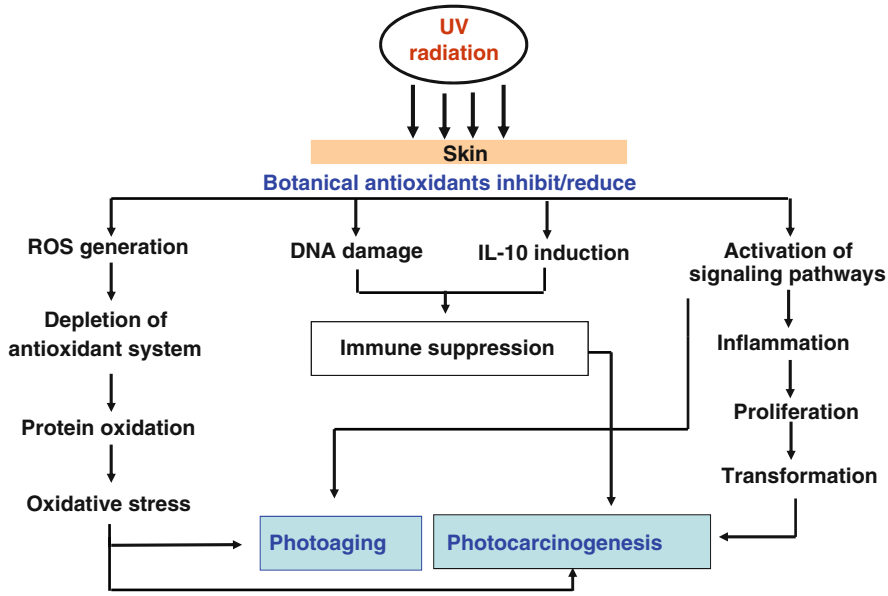

Fig. 5.1 Botanical antioxidants have the ability to inhibit/reduce the adverse biological effects of UV radiation

Topical application of green tea polyphenols (GTP) to SKH-1 hairless mice before multiple UVB exposure resulted in a significant decrease in skin edema and infiltration of leukocytes. Furthermore, GTP also inhibited UVB-induced phosphorylation of MAPKs, activation of NF-κB/p65 and IKKα, and degradation and phosphorylation of IkBα [22]. Treatment of mouse skin with EGCG prior to a single dose of UVB irradiation inhibited hydrogen peroxide and nitric oxide production both in the dermis and epidermis. In addition, EGCG pretreatment also inhibited UVB-induced infiltration of leukocytes and depletion of antigen-presenting cells [23]. Topical application of hydrophilic ointment containing EGCG (2%) to rat prior to UVA showed significant decrease in sunburn cells and dermo-epidermal activation [24]. Supplementation of EGCG (1,500 ppm) in normal diet significantly increased skin tolerance by increasing minimal erythema dose (MED) and reduced UV-induced perturbation of epidermal barrier function and sunburn severity in female HWY/Slc hairless rats [25]. We have shown that topical application or oral feeding of green tea to SKH-1 hairless mice afforded protection against photocarcinogenesis [26]. Cooney et al., [27] have demonstrated that oral administration of a green tea infusion as the sole source of liquid sustenance to SKH-1 mice inhibited UVB-induced sunburn lesions, UVB-induced initiation of skin tumors, and UVB-induced formation of skin tumors. Decaffeinated green tea also had a marked inhibitory effect on UVB-induced skin carcinogenesis in DMBA-initiated SKH-1 mice [28]. Oral administration of green tea to SKH-1 hairless mice for 2 weeks stimulated UV-induced increases in apoptotic sunburn cells in the epidermis. Furthermore, oral administration of green tea to UV-pretreated high-risk mice for 23 weeks inhibited skin tumorigenesis, decreased the size of the parametrial fat pads, and decreased the thickness of the dermal fat layer away from tumors and directly under tumors. [29]. Pretreatment of SKH-1 hairless mice with green tea for 2 weeks enhances UV-induced increases in epidermal p53, p21(WAF1/CIP1), and apoptotic sunburn in the epidermis [30]. When green tea was given to mice as the sole source of drinking fluid starting immediately after discontinuation of UVB treatment enhanced the rate and extent of disappearance of the mutant p53-positive patches [31]. Furthermore, mice treated with green tea during chronic UVB irradiation changed the mutation profile of the p53 gene in early mutant p53 positive epidermal patches [32].

Topical application of GTP to C3H/HeN mice, 30 min prior to, or 30 min after, exposure to a single dose of UVB, resulted in significant protection against local and systemic suppression of contact hypersensitivity and inflammation in mouse dorsal skin. These protective effects were dependent on the dose of GTP employed; increasing the dose resulted in an increased protective effect [33]. In addition, topical application of EGCG before a single low-dose UVB exposure to

C3H/HeN mice protected against UVB-induced immunosuppression and tolerance induction by blocking UVB-induced infiltration of CD11b+ cells into the skin, reducing IL-10 production in skin as well as in draining lymph nodes, and markedly increasing IL-12 production in draining lymph nodes [34]. Application of EGCG prevented UV-induced suppression of the contact hypersensitivity in wild-type (WT) mice but did not in IL-12 KO mice. EGCG reduced or repaired UV-induced DNA damage in WT mice skin faster as observed by reduced number of CPDs-positive cells and also reduced the migration of CPDs-positive antigen-presenting cells from the skin to draining lymph nodes. EGCG was also able to repair UV-induced CPDs in XPA-proficient cells but not repair in XPA-deficient cells, demonstrating that nucleotide excision repair mechanism is involved in DNA repair [35].

Studies have shown that EGCG at the nontoxic dose of ≤10 μM, attenuated the UVB-induced fibroblast toxicity by blocking ROS production. In addition, EGCG treatment inhibited UVB-induced production of collagenases, activation of matrix metalloproteinase (MMP)-1,-8 and -13, activation of apoptosis signal-regulating kinase-1, and phosphorylation of MAPKs in dermal fibroblasts [36]. These results suggest that EGCG has abilities to impede UVB-induced collagenolytic MMP production via interfering with the MAPK-responsive pathways. Oral feeding of GTP to SKH-1 hairless mice inhibited UVB-mediated oxidative damage of the protein macromolecules, and expression of MMPs that degrade extracellular matrix proteins [37]. Feeding of green tea extract to Sprague-Dawley rats remarkably inhibited the age-associated increase of the fluorescence in the aortic collagen [38]. EGCG treatment reduced UVA-induced skin damage and protected from the decrease of dermal collagen in hairless mouse skin. In addition, EGCG treatment blocked the UV-induced increase of collagen secretion and collagenase mRNA level and the promoter-binding activities of activator protein-1 (AP-l) and NF-κB in cultured human epidermal fibroblasts [39].

Topical application of EGCG before UVB exposure to human skin inhibited UVB-induced infiltration of leukocytes, erythema, and myeloperoxidase activity. Preapplication of EGCG significantly reduced prostaglandin (PG) metabolites, particularly PGE_2 when compared to UVB alone group [40]. EGCG was also found to protect human skin from UV-induced oxidative stress. Topical application of EGCG to human skin before a single exposure to a 4x minimal erythema dose (MED) of UV irradiation inhibited UV-induced markers of oxidative stress. In addition, EGCG was found to restore the UV-induced decrease in glutathione levels and glutathione peroxidase enzyme [41]. Topical application of human skin with an extract of green tea 30 min prior to solar-simulated radiation (2MED) resulted in a dose-dependent inhibition of the erythema response evoked by UV radiation. Studies were also conducted to determine the effect of polyphenols from green tea extract. The EGCG and ECG polyphenolic fractions were most efficient at inhibiting erythema, whereas EGC and EC had little effect. Furthermore, skin treated with green tea extracts reduced the number of sunburn cells and protected epidermal Langerhans cells and also reduced the DNA damage from UV radiation [42]. Treatment of human skin with varying doses of GTP before a single dose of UVB exposure (4.0 MED) decreased dose-dependently the formation of UVB-induced CPDs in both epidermis and dermis. This study suggests that inhibition of UVB-induced CPDs by GTP treatment may be, at least in part, responsible for the inhibition of photocarcinogenesis [43]. A double-blinded, placebo-controlled trial of green tea was conducted in 40 women with moderate photoaging and was randomized to either a combination regimen of 10% green tea cream and 300 mg twice-daily green tea oral supplementation or a placebo regimen for 8 weeks. Participants treated with a combination regimen of topical and oral green tea showed histological improvement in elastic tissue content but clinically significant changes could not be detected. This study suggests that longer supplementation may be required for clinically observable improvements. [44].

5.3 Pomegranate

Pomegranate (*Punica granatum* L of family Punicaceae.) fruit widely consumed fresh and in beverage as juice or wine has been used for centuries in ancient cultures for its medicinal purposes. Pomegranate is a rich source of two types of polyphenolic compounds: anthocyanins (such as delphinidin-3-glycoside, delphinidin-3,5-diglycoside, pelargonidin-3-glycoside, pelargonidin-3,5-diglycoside, cyanidin-3-gylcoside, cyanidin-3,5-digylcoside) and hydrolyzable tannins (such

as punicalin, pedunculagin, punicalagin, gallagic and ellagic acid esters of glucose). The other flavanoids present in pomegranate include quercetin, kaempherol, and luteolin glycosides. Pomegranate possesses strong antioxidant, anti-inflammatory, and anticancer properties [45].

Studies from our laboratory have demonstrated that treatment of NHEK with pomegranate fruit extract (PFE) prior to UVB radiation inhibited UVB-mediated phosphorylation of ERKl/2, JNK1/2, and p38 proteins. PFE pretreatment of NHEK also resulted in inhibition of UVB-mediated degradation and phosphorylation of IκBα, activation of IKKα, nuclear translocation and phosphorylation of NF-κB/p65 at Ser^{536} [46]. In another study, we have shown that pretreatment of NHEK with PFE inhibited UVA-mediated phosphorylation of signal transducers and activators of transcription (STAT)-3, AKT and ERK1/2 [47]. Treatment of HaCaT cells with PFE prior to UVB exposure protected cells from UVB-mediated decrease in cell viability, and also inhibited UVB-mediated decrease in endogenous glutathione levels, lipid peroxidation, and expression of MMP-2 and MMP-9 [48]. We further showed that delphinidin, one of the major anthocyanins present in pomegranate, protected NHEK from UVB-mediated decrease in cell viability, induction of apoptosis, increase in lipid peroxidation, formation of 8-OHdG, decrease in PCNA, increase in poly (ADP-ribose) polymerase (PARP) cleavage and activation of caspases, lipid peroxidation, decrease in cell viability, induction of apoptosis, and increase in PARP and activation of caspases [49]. Topical application of delphinidin to SKH-1 hairless mouse skin inhibited UVB-mediated apoptosis and markers of DNA damage such as CPDs and 8-OHdG. These results suggest that treatment of HaCaT cells and mouse skin with delphinidin inhibited UVB-mediated oxidative stress and reduced DNA damage, thereby protecting the cells from UVB-induced apoptosis [49].

Oral feeding of PFE to SKH-1 hairless inhibited single UVB-exposure-mediated epidermal hyperplasis, infiltration of leukocytes, hydrogen peroxide generation, and lipid peroxidation. In addition, PFE protected against UVB-induced DNA damage by inhibiting CPDs and 8-OHdG formation in the mouse skin [50]. In another study, oral feeding of PFE to SKH-1 hairless inhibited multiple UVB-exposure-mediated epidermal hyperplasia, infiltration of leukocytes, and oxidation of proteins. PFE consumption further protected the mouse skin against the adverse effects of UVB radiation by modulating UVB-induced signaling pathways including NF-κB, MAPKs, c-Jun as well as decreased expression of gelatinase (MMPs-2 and -9), and stromelysin (MMP-3) [51]. Inhibition of UVB-mediated proliferative pathways provided further evidence of the photochemopreventive role of the extract and its potential to attenuate UVB-induced oxidative stress and in turn stress-induced molecular pathways associated with a high risk of carcinogenesis. More recently, we reported that PFE also protected against UVB-induced skin tumorigenesis in SKH-1 hairless mice by modulating NF-κB, STAT-3, and hypoxia inducible factor-α leading to a decrease in inflammatory and angiogenic responses [52]. These data suggest that oral consumption of PFE affords protection against photocarcinogenesis by modulating cell signaling pathways. These results provide a molecular basis for the photochemopreventive effect of PFE.

The individual effect of pomegranate juice, oil, and extract was determined against UVB-mediated damage using reconstituted human skin model. Pretreatment of reconstituted human skin model with pomegranate juice, oil, and extract resulted in inhibition of UVB-induced protein oxidation, sunburn cell formation, DNA damage, phosphorylation of c-Jun, protein expression of c-Fos, and various MMPs protein expression [53]. Topical and oral administration of pomegranate to humans was shown to augment the protective effect of sunscreens and afforded protection from UVB [54]. A double-blind, placebo-controlled clinical trial indicated that oral intake of PFE inhibited UV-induced pigmentation in the human skin [55].

5.4 Resveratrol

Resveratrol (3,5,4'-trihydroxystilbene), a polyphenolic flavonoid that belongs to the stilbene family of phytoalexins, is found in the seeds and skins of grapes, red wine, peanuts, and berries. Epidemiological studies indicate that the French have relatively lower risk of cardiovascular diseases despite consuming a diet relatively rich in fat. In reality, the high concentration of resveratrol in red wine consumed by this population is frequently cited to account for the "French paradox." Studies have shown that resveratrol possesses potent

antioxidant, anti-inflammatory, and antiproliferative properties [3].

We have shown that pretreatment of NHEK with resveratrol inhibited UVB-mediated activation of NF-κB and IKKα, phosphorylation and degradation of IκBα [56]. In another study, topical application of resveratrol to SKH-1 hairless mice prior to UVB irradiation resulted in the inhibition of UVB-induced skin edema, generation of H_2O_2, infiltration of leukocytes, lipid peroxidation, cyclooxgenase-2 and ornithine decarboxylase enzyme activities, and protein expression [57]. Shannon et al. [58] have demonstrated that resveratrol imparts its protective effect against multiple UVB exposure via modulations in the cki-cyclin-cdk network and MAPK pathway. Further, topical application of resveratrol to SKH-1 hairless mouse skin prior to UVB irradiation resulted in inhibition of UVB-induced cellular proliferation, phosphorylation of survivin, and up-regulation of proapoptotic Smac/DIABLO protein [59]. Topical application of resveratrol to mouse skin with resveratrol has been shown to result in significant inhibition in tumor incidence and delay in the onset of tumorigenesis [60]. In recent years, resveratrol has generated great interest because of its properties to delay skin aging. Exposure of cultured human skin keratinocytes to UV resulted in down-regulation of sirtuin 1 (SIRT1) in a time- and dose-dependent fashion. Treatment of these cells with resveratrol inhibited UV-induced down-regulation of SIRT1. Furthermore, activation of SIRT1 by resveratrol negatively regulated UV-induced p53 acetylation [61].

5.5 Silymarin

Silymarin a polyphenolic flavonoid is isolated from the milk thistle plant, *Silybum marianum L. Gaertn.* Milk thistle belongs to the aster family (Asteraceae or Compositae) that includes daisies, thistles, and artichokes. Silymarin is a mixture of several flavonolignans, which includes silybin, silibinin, silidianin, silychristin, and isosylibin. Treatment of irradiated HaCaT with silymarin also resulted in concentration-dependent diminution of UVA-caused oxidative stress. Silymarin treatment also reduced glutathione depletion and ROS production as well as lipid peroxidation in irradiated HaCaT cells. Formation of UVA-induced DNA single-strand breaks and caspase-3 activity was also significantly decreased by treatment with silymarin [62]. Treatment of JB6 C141 cells and $p53^{+/+}$ fibroblasts with silymarin resulted in a dose-dependent inhibition of cell viability and induction of apoptosis in an identical manner. These results suggest that silymarin-induced apoptosis is primarily p53-dependent and mediated through the activation of caspase-3 [63]. Treatment of HaCaT cells with silibinin, a major component of silymarin, restored UVB-caused depletion of survivin, concomitant with up-regulation of NF-κB DNA binding activity. Further, silibinin treatment up-regulated UVB-induced ERK 1/2 phosphorylation and increased duration of S phase, possibly providing a prolonged time for efficient DNA repair [64].

The antioxidant and anticarcinogenic effects of silymarin and its active constituent silibinin in mouse model of photocarcinogenesis have been established. Topical application of silymarin prior to exposure to UVB significantly reduced tumor incidence, tumor multiplicity, and average tumor volume per mouse. Furthermore, topical application of silymarin to SKH-1 hairless mice inhibited UVB-induced skin edema, sunburn cell formation, apoptosis, depletion of catalase activity, and induction of COX-2 and ODC activities and mRNA expression. These studies suggest that topical application of silymarin provides substantial protection against UVB-mediated damage in mouse skin, possibly via its strong antioxidant properties at different stages of UVB-induced carcinogenesis [65]. Topical application of silibinin prior to, or immediately after, UV irradiation to SKH-1 hairless mice afforded protection against UV-induced damage in epidermis by a decrease in CPDs formation and an up-regulation of p53-p21/Cip1 [66]. Studies have shown that silibinin activates DNA-protein kinase-dependent p53 pathway for apoptosis in response to UVB-induced DNA damage [67]. Topical treatment of silymarin to C3H/HeN mice inhibited UVB-induced suppression of contact hypersensitivity response to contact sensitizer dinitrofluorobenzene. Silymarin treatment also resulted in significant reduction of UVB-induced immunosuppressive cytokine interleukin-10 producing cells and its production. Furthermore, topical treatment of silymarin also resulted in significant reduction of the number of UVB-induced H_2O_2 producing cells and inducible nitric oxide synthase expressing cells concomitant with decrease in H_2O_2 and nitric oxide production [68]. Treatment of C3H/HeN mice with

topically applied silymarin reduced the UVB-induced enhancement of the levels of the immunosuppressive cytokine, IL-10, in the skin and draining lymph nodes and enhanced the levels of the immunostimulatory cytokine, IL-12 [69]. Recently, it has been shown that treatment of C3H/HeN mice with silymarin inhibited UV-induced oxidative stress through inhibition of infiltrating $CD11b^+$ cells [70].

5.6 Genistein

Genistein (5,7,4'-trihydroxyisoflavone) is a soy-derived isoflavone that has attracted much attention because it possesses antioxidant and anticancer effects in skin. Although soybeans contain a number of ingredients with demonstrated anticancer activities, the most potent is genistein. Treatment of mouse skin with genistein before UV reduced sunburned cells [71]. Pretreatment of irradiated cultures with genistein blocked UVB-stimulated PGE_2 synthesis. Furthermore, UVB-induced phosphorylation of tyrosine residues of EGFR was blocked by the tyrosine kinase inhibitor genistein [72]. Treatment of HaCaT cells with genistein augmented the induction of c-*jun* and *junB* by UVB irradiation [73]. Topical application of 0.5% solutions of genistein protected pig skin from solar-simulated ultraviolet-induced photodamage, as measured by sunburn cell formation and/or erythema [74]. Genistein also had a powerful potential to reduce the inflammatory edema reaction and suppressed contact hypersensitivity induced by moderate doses of solar-simulated UV radiation in hairless mice [75]. Treatment of human reconstituted skin with genistein prior to UVB radiation dose-dependently preserved cutaneous proliferation and repair mechanics, as evidenced by the preservation of proliferating cell populations with increasing genistein concentrations. Genistein also inhibited UV-induced DNA damage, evaluated with CPDs immunohistochemical expression profiles, and demonstrated an inverse relationship with increasing topical genistein concentrations [76]. Treatment of hairless mouse skin with genistein prior to UVB exposure significantly inhibited UVB-induced H_2O_2 and MDA in skin and 8-OHdG in epidermis. Suppression of 8-OHdG formation by genistein has been corroborated in purified DNA irradiated with UVA and UVB. These results suggest that UVB irradiation elicit a series of oxidative events, which can be substantially inhibited by genistein through either direct quenching of ROS or indirect anti-inflammatory effects [77].

5.7 Curcumin

Curcumin, a natural compound extracted from the rhizome of *Curcuma longa* of family Zingiberaceace, is a major yellow pigment in turmeric that imparts a yellow color to food and is widely used as a spice. The varied biological properties of curcumin and lack of toxicity even when administered at higher doses makes it attractive to explore its use in skin cancers and other skin-related diseases. Curcumin has been shown to possess a wide range of pharmacological activities that include antioxidant, anti-inflammatory, antiproliferative, anticarcinogenic, antimicrobial, and wound healing properties and owing to these it has a wide range of clinical applications [78]. The molecular basis for the chemopreventive effect of curcumin is due to its effect on several targets including transcription factors, apoptotic genes, growth regulators, angiogenesis regulators, and cell adhesion molecules [79]. Treatment of HaCaT cells with curcumin inhibited COX-2 mRNA and protein expressions as well as activation of p38 MAPK and JNK. The DNA binding activity of AP-1 transcription factor was also markedly decreased with curcumin treatment in UVB-irradiated HaCaT cells. These results collectively suggest that curcumin may inhibit COX- 2 expression by suppressing p38 MAPK and JNK activities in UVB-irradiated HaCaT cells [80]. Studies have shown that curcumin photosensitizes UVB-mediated apoptosis of HaCaT cells through activation of caspase pathways [81]. Curcumin at low concentrations (0.2–1 μg/ml) inhibited the proliferation-associated MAPKs ERK1/2 and protein kinase B when applied in combination with UVA or visible light. Combination of curcumin and UVA light induced apoptosis in human skin keratinocytes represented by the increase of fragmented cell nuclei, release of cytochrome *c* from mitochondria, activation of caspases-9 and -8, and inhibition of NF-κB activity. Furthermore, epidermal growth factor receptor, an upstream regulator of both kinases, was inhibited indicating that apoptosis is induced by blocking survival- and proliferation-associated signal cascades at the receptor level [82].

5.8 Sulforaphane

Sulforaphane, an isothiocyanate found in cruciferous vegetables, such as broccoli and broccoli sprouts, exerts anticancer effects via multiple mechanisms. Sulforaphane possesses antioxidant, proapoptotic, anti-inflammatory, antiproliferative and antihistone deacetylase properties [83–86]. In addition, it is also one of the most potent naturally occurring phase 2 enzyme inducer [87, 88]. Studies have shown that treatment of HCL14 cells to sulforaphane dose-dependently reduced the UVB-induced AP-1 activation, and this appears to be at least, in part, due to the direct inhibition of AP-1 DNA binding activity [89]. Recent studies have shown that the UV-radiation-induced skin carcinogenesis in "initiated high-risk mice" was substantially inhibited by topical application of broccoli sprout extracts containing sulforaphane [90]. Topical application of broccoli sprout extracts containing sulforaphane to the skin of mice and healthy human subjects elevates NAD(P)H:quinone oxidoreductase 1, a representative cytoprotective enzyme [88]. In another study, this group had shown that topical application of sulforaphane-rich extracts of broccoli sprouts protected against UVR-induced inflammation and edema in mice, and reduced susceptibility to erythema arising from narrow-band 311-nm UVR in humans [91]. These studies suggest that broccoli sprout extracts containing sulforaphane may help reduce UV-induced skin damage and skin cancer.

5.9 Lycopene

The most abundant carotenoid in tomatoes is the red pigment lycopene, followed by phytoene, phytofluene, ζ-carotene, γ-carotene, β-carotene, neurosporene, and lutein [92]. Lycopene is a well-established potent antioxidant, and its anticancer properties have been shown in cultured cells and animal models. Studies have shown that single exposure of a small area of one volar forearm to a dose of solar-simulated light resulted in reduction in skin lycopene concentration compared with an adjacent non-exposed area. This study suggests a role of lycopene in mitigating oxidative damage in tissues [93]. Dietary intake of tomato paste rich in lycopene for 10 weeks protected against UV-induced erythema formation in humans [94]. Using multilamellar liposomes as a vehicle to deliver lycopene to skin fibroblasts, Eichler et al. [95] showed that lycopene was capable of decreasing UV-induced formation of thiobarbituric acid-reactive substances. Studies have shown that supplementation with tomato-based products increases lycopene levels in human serum and protects against UV-light-induced erythema [96]. Topical application of lycopene dose-dependently inhibited UVB-induced ornithine decarboxylase and myeloperoxidase, and significantly reduced bifold skin thickness [97].

5.10 Lutein/Zeaxanthin

Lutein and zeaxanthin are carotenoids found in green leafy vegetables with potent antioxidant properties. In a recent study, mice were given either a lutein/zeaxanthin-supplemented diet or a standard nonsupplemented diet. Dorsal skin of female SKH-1 hairless mice was exposed to UVB radiation with a cumulative dose of 16,000 mJ/cm^2 for photoaging and 30,200 mJ/cm^2 for photocarcinogenesis. The skin fold thickness and number of infiltrating mast cells following UVB irradiation were significantly less in lutein/zeaxanthin-treated mice when compared to irradiated animals fed the standard diet. There was increased tumor-free survival time, reduced tumor multiplicity, and total tumor volume in lutein/zeaxanthin-treated mice in comparison with control irradiated animals fed the standard diet [98]. In a clinical trial, lutein and zeaxanthin were administered orally, topically, or in combination. It was found that the combined oral and topical administration of lutein and zeaxanthin provided the highest degree of antioxidant protection. Oral administration of lutein provided better protection than that afforded by topical application of this antioxidant when measured by changes in lipid peroxidation and photoprotective activity in the skin following UV light irradiation [99]. The effect of orally administered lutein and zeaxanthin in the cutaneous response to UVB irradiation was investigated. Female hairless SKH-1 mice receiving 0.4% and 0.04% lutein plus zeaxanthin-enriched diet for 2 weeks were exposed to single doses of UVB radiation. Orally administered 0.4% lutein and zeaxanthin decreased significantly the edematous cutaneous response as determined by the reduction of the UVB-induced increase of

ear bifold thickening. Dietary carotenoids were efficient in reducing the UVB-induced increases in the percentage of PCNA, bromodeoxyuridine and terminal dUTP nick end-labeling-positive cells [100]. There was significant inhibition of MMP-1 expression and MMP-2 protein levels in dermal fibroblasts, without altering TIMPs expression. It significantly inhibited MMP-1 expression in melanoma cells while stimulating TIMP-2. There was no alteration of fibroblast or melanoma cell viability or membrane integrity. Lutein improved cell viability, membrane integrity, and inhibited elastin expression, though more significantly in the UVB-exposed fibroblasts [101]. It has been reported that mice fed dietary lutein demonstrated significant inhibition of ear swelling owing to UVB radiation compared to mice fed the standard laboratory diet. Mice exposed to UVB radiation four times at daily intervals and then sensitized to dinitrofluorobenzene at the site of irradiation showed a decreased CHS response upon challenge. This suppression by UVB radiation was significantly inhibited by lutein feeding. There was no effect of lutein when UVB radiation was given at a single dose of 10,000 J/ m^2 to inhibit the induction of CHS at a distant, non-irradiated site. Lutein accumulated in the skin of mice following diet supplementation and was shown to decrease ROS generation following UVR exposure [102].

5.11 Conclusion

A large number of studies support the notion that botanical antioxidants exert anti-inflammatory, anticarcinogenic and antiphotoaging effects based on laboratory and epidemiological studies. The botanical antioxidants discussed in this chapter abrogate dysfunction of the cellular signaling pathways, disturbances in the apoptotic machinery, and various biochemical processes induced or mediated by the solar UV radiation. This suggests the possibility that specific botanical antioxidants might be used to target defined and established molecular events for the prevention and treatment of a variety of human skin disorders, including skin cancer and photoaging. There is also evidence that UV-dependent skin damage could be prevented by increasing the basal antioxidative protection level systematically by regular intake botanical antioxidants. Therefore, the use of botanical antioxidants as dietary sources, and/or supplementing skin care products or sunscreens with these botanical antioxidants for daily use may be an effective approach for reducing UV-induced photodamage and other skin disorders.

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Systemic Photoprotection by Carotenoids

6

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Core Message

› Carotenoids exhibit specific antioxidant activity and also influence cellular signaling and gene expression at the cellular level. They modulate skin properties when ingested with supplements or dietary products and protect against sunburn. Dietary intervention is not comparable to effects sunscreen with a high sun protection factor. However, a suitable diet may contribute to basal protection and thus increase the defense against UV light-mediated damage to skin.

6.1 Introduction

A balanced diet providing all of the required nutrients is essential for the proper function of any organ or tissue. Apart from lipids, carbohydrates, proteins, minerals, and vitamins numerous other compounds including secondary plant constituents are ingested with the food. Some of them are biologically or pharmacologically active and are thought to contribute to the beneficial health effects associated with an increased intake of fruit and vegetables. Antioxidant activity, impact on cellular signaling and gene expression, modulation of membrane properties, or protein modification are among the potential mechanisms underlying the health effects of these micronutrients. Targeting nutrients to specific tissues where they accumulate and exhibit selected activities is a new approach in functional nutrition [3].

In the present chapter, the influence of selected carotenoids on skin properties is described. Several studies have shown that antioxidant micronutrients are active when applied topically as active ingredients of a cream, lotion, liquid, or gel. However, here we will summarize the results from some of our human intervention studies with dietary supplements or food rich in carotenoids and discuss some basic mechanisms possibly underlying these effects. Additionally, we describe basic in vitro work on lutein and the phenolic carotenoid 3,3'-dihydroxyisorenieratene (Fig. 6.1).

6.2 Carotenoids: Properties

Carotenoids represent a class of lipophilic secondary plant components of which the most prominent is β-carotene. Based on epidemiological observations, fruits and vegetables which are rich sources of carotenoids are thought to provide health benefits by decreasing the risk of various diseases, particularly certain cancers and eye diseases [11]. In higher plants, carotenoids are part of the light-harvesting complex and serve as colorants for petals. Only plants, bacteria, algae, and

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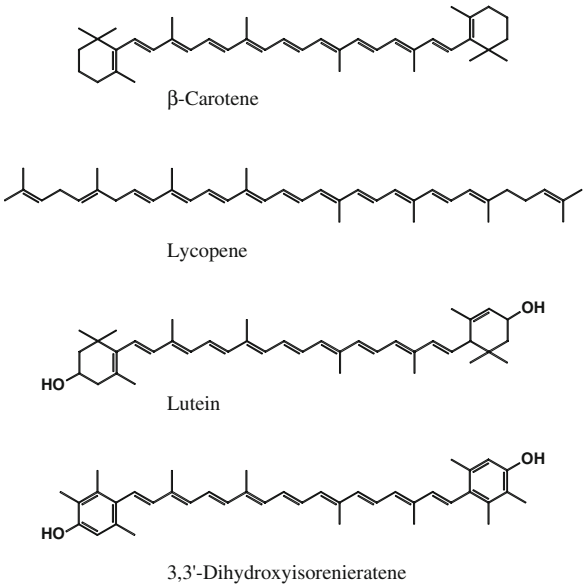

Fig. 6.1 Structures of selected carotenoids

some fungi are cable of synthesizing carotenoids de novo. However, considerable amounts are ingested with the diet and accumulate in the human organism. Especially fruit and vegetables are rich sources of various carotenoids. In human blood and tissues, the carotenoid pattern is dominated by α- and β-carotene, β-cryptoxanthin, lutein, zeaxanthin, and lycopene (Fig. 6.1). The first three in this line are precursors of vitamin A and their importance as a vitamin source is established since many years. There is evidence that β-carotene has a higher affinity to the cleaving enzymes as compared to other provitamin A compounds and appears to be the superior source for vitamin A. Lutein and zeaxanthin are responsible for the yellow color of the macula lutea, the yellow spot of the eye; no other major carotenoids are found in this tissue. Macular carotenoids likely play a role in the protection of the retina against light-induced damage. There is some evidence that an increased dietary intake of lutein and zeaxanthin is associated with a lowered risk for age-related macular degeneration [21]. While protecting ocular tissue against photooxidative damage carotenoids may act either as filters for damaging blue light or as antioxidants scavenging reactive oxygen species.

Most carotenoids efficiently scavenge singlet molecular oxygen ($^1O_2$) and excited triplet states [20]. Carotenoids inactivate singlet oxygen via physical or chemical quenching. The efficacy of physical quenching exceeds that of chemical quenching by far and involves the transfer of excitation energy from $^1O_2$ to the carotenoid, resulting in ground state oxygen and an excited triplet state carotenoid. The energy is dissipated between the excited carotenoid and the surrounding solvent to yield a nonreactive ground state carotenoid and thermal energy. In the process of physical quenching the carotenoid remains intact, so that it can undergo further cycles of singlet oxygen quenching. Among the carotenoids, lycopene is the most efficient singlet oxygen quencher. In contrast to most other carotenoids, lycopene is an acyclic compound which contains 11 conjugated double bonds arranged linearly in the *all-trans* form. Lycopene is the colorant of the tomato and found in relatively high concentrations in all tomato products which are the major source of lycopene for the human organism. Further sources of lycopene are watermelon, guava, rosehips, and pink grapefruit. Intake of lycopene with the diet is inversely associated with the risk of prostate cancer. Several preclinical studies provide evidence that lycopene has antitumor effects, suggesting potential preventive and therapeutic roles for the compound [15].

All of the carotenoids mentioned here efficiently scavenge peroxyl radicals, especially at low oxygen tension. The interaction of carotenoids with free radicals has been studied in various systems; in some cases, carotenoids are superior to other dietary antioxidants such as α-tocopherol or vitamin C.

During the biosynthesis of carotenoids from the key C5-isoprene unit to structurally complex xanthophylls a number of products are formed which are found in various amounts in the plant. Among them are phytoene and phytofluene, precursors of lycopene, with only three or five conjugated double bonds, respectively. Both compounds are colorless but absorb UV light. They are present in human plasma and their levels considerably increase upon ingestion of a suitable source, e.g., tomatoes [1]. Plasma concentration of phytofluene may increase to levels up to 1 μmol/L, whereas phytoene levels are considerably lower. Based on their structural features both compounds may act as UV absorbers and contribute to photoprotection of the skin.

Cell culture studies support the idea that carotenoids modulate cellular signaling independent of their vitamin A activity [16]. They affect direct signaling via gap junctions, influence cell cycle progression and membrane receptor-dependent pathways, and influence the regulation of gene expression via ligand-dependent transcription factors. Such biological effects likely play

a role in skin protection and may have impact on skin structure and texture.

6.3 Carotenoids: Skin Effects

The skin is the largest organ of the body and functions as a protective barrier that interfaces with the environment. It is made up of two distinct layers epidermis and dermis, underlying is the subcutaneous tissue. The major cells of the dermis are the fibroblasts, producing the extracellular matrix with collagen as the predominant compound; other important structural components are elastin and glucosaminoglycans. UV exposure induces the expression of selected matrix metalloproteinases (MMPs) which degrade collagen and elastic fiber finally leading to premature skin aging [5]. Cutaneous texture is affected by the thickness and smoothness of the epidermis, quality of the fibers, and the amount of fluid in dermal connective tissue. Blood vessels are embedded in the dermis and are important for the supply of the entire skin with oxygen and nutrients and the removal of waste. The visible, primary reaction following extended sun exposure is skin reddening (sunburn). Sunburn or solar erythema is an acute cutaneous inflammatory reaction as a result of excessive ultraviolet radiation. As a consequence of photochemical damage inflammatory pathways are stimulated in the exposed skin. UVB irradiation is considered to be the major cause of typical sunburn, which starts to develop a few hours subsequent to irradiation, culminating about 18–24 h post-irradiation. Individual sensitivity toward erythematogenic UV exposure is characterized by the minimal erythemal dose which is defined as the lowest dose of UV radiation that will produce a detectable erythema 24 h after exposure.

In addition to the application of topical sunscreens several micronutrients are used as so-called oral sun protectants. It has been postulated that protection against UV-induced skin damage is most efficient when a combination of topical and oral factors is applied [7]. Following UV irradiation, reactive oxygen species are formed in the light-exposed tissue. Among them are reactive compounds such as singlet molecular oxygen, superoxide radical anion, or peroxyl radicals damaging cellular lipids, proteins, and DNA. Such photodamaging reactions are thought to be involved in the pathobiochemistry of erythema formation, premature aging of the skin, or the development of photodermatoses [17]. Exposure to UV radiation is related to an increased mortality from non-melanoma skin cancer whereas the association with cutaneous melanoma is still unclear [2]. Dietary antioxidants as a part of the antioxidant network scavenge reactive oxygen species and may help to protect biologically important molecules (for mechanical details: see above). If not regenerated antioxidants are consumed in most of these reactions. Several human studies have shown that carotenoid levels in plasma and skin decrease upon UV irradiation; lycopene is lost preferentially as compared to other carotenoids [14]. Thus, protective effects of a supplementation with carotenoids against UV-induced photooxidation have been postulated.

Among the oral sun protectants β-carotene is the most popular. Based on the outcome of seven human intervention studies a meta-analysis was performed estimating the effects of β-carotene in the prevention of UV-induced erythema (sunburn) [10]. This analysis provides evidence that a supplementation with β-carotene is associated with a protection against the development of a sunburn reaction. The protection is only moderate compared to the efficacy of a modern sunscreen and has been estimated to be comparable to a sun protection factor of three to four. In contrast to topical sunscreens, protection with systemic β-carotene builds up over a period of several weeks which has also been shown as a result of the meta-analysis. Short-term studies showed no effects even when high doses of the carotenoid were applied. For example, no effect of a supplementation with β-carotene was measured when 90 mg/day of the carotenoid were given for a period 23 days [6]. Although plasma levels increased significantly it was stated, “no clinically or histologically detectable protection was observed upon treatment.” Apparently the efficacy of β-carotene is independent of the source. Synthetic β-carotene as well as β-carotene from natural sources is efficient. β-carotene levels in skin and serum are increased following the supplementation with carotenoids derived from the alga Dunaliella salina [18]. Erythema formation was significantly diminished when 24 mg of β-carotene per day were supplied. Protection was even more efficient when the carotenoid from the alga was combined with vitamin E. From all the successful intervention studies with β-carotene it can be estimated that a minimal dose of about 10–15 mg/day is needed to provide protection against sunburn. The efficient dose may vary depending on

differences in the bioavailability of the carotenoid from various formulations.

Safety concerns have been raised for β-carotene supplements when they are taken over several years at non-physiological dose levels [11]. A higher cumulative index for lung cancer has been observed in smokers and asbestos workers. Consequently a study was performed where β-carotene was partially substituted by other carotenoids. It has been demonstrated that a carotenoid mixture consisting of β-carotene, lycopene, and lutein, with 8 mg of each compound provides a photoprotective effect comparable to that 24 mg of β-carotene alone [8].

In contrast to many other carotenoids, lycopene can be found in only in a few food items. The major sources in the human diet are tomatoes and tomato products. More than 80% of lycopene consumed in the USA comes from that sources but apricots, papaya, pink grapefruit, guava, and watermelon also contribute to dietary intake. Lycopene is an efficient antioxidant and also suitable to prevent UV-induced skin damage. This has been shown in an intervention study with lycopene-rich tomato paste providing about 16 mg/day of the carotenoid which was ingested together with 10 g of olive oil over a period of 10 weeks [19]. Serum levels of lycopene increased upon supplementation; the other carotenoids did not change. At week 10 of the study erythema formation induced with a solar light simulator was lower in the group that consumed tomato paste as compared to controls. However, no significant difference between groups was found at week 4 of treatment, demonstrating that the protective compound must accumulate over a longer period of time. These data also showed that it is feasible to achieve protection against UV-light-induced sunburn by ingesting carotenoids from a dietary source.

A number of other products with high levels of lycopene were investigated in a human intervention study [1]. Lycopene sources were a carrot juice from the variety Nutri Red, a supplement from tomato extract (Lyco-Mato), a drink with tomato extract (Lyco-Guard), and synthetic lycopene providing 10, 9.8, 8.1, and 10.2 mg lycopene per day; treatment was for 12 weeks. Carrots usually do not contain lycopene but the variety Nutri Red is of purplish red color and contains quite high amounts of this carotenoid. In addition to 10.2 mg of lycopene about 5 mg of β-carotene were provided with the carrot juice in this study. Precursor molecules in the biosynthesis of lycopene are phytoene and phytofluene. Both compounds were present in all of the sources except of the synthetic lycopene. Also in this study, photoprotection was measured as prevention of erythema formation. Upon intake of lycopene from the different sources increases in lycopene serum levels were observed. Photoprotective effects were determined for all kinds of intervention, however, not statistically significant with synthetic lycopene. It has been speculated that other components present in dietary sources may contribute additionally to the protection. Especially phytoene and phytofluene may be important. Both are non-colored carotenoids because they carry only three and five conjugated double bonds in their structure, respectively. Their absorption maxima are at wavelength of the UVB and UVA range with quite high extinction coefficients. They may directly absorb damaging UV light thus contributing to photoprotection.

The claim that carotenoids are oral sun protectants is supported by these and several other human intervention studies; for summary see [17]. It should be noted that UV-induced erythema was evaluated as a measure of "sunburn" in most of these studies which reflects a delayed inflammatory response of cutaneous tissue following UVB irradiation. Only few data from human studies provide evidence for a decrease in UV-induced damage to DNA or other skin constituents. No clear correlation has been shown up till now between the intake of carotenoids or carotenoid-rich food and the risk for skin cancer [13].

Function and structure of the skin is influenced by dietary factors and several micronutrients are used as constituents of creams for topical application [4]. There is evidence from other studies that nutrients like specific lipids have impact on skin properties when ingested regularly. In a human intervention study over 12 weeks with a supplement mixture containing carotenoids, vitamin E, and selenium, increases skin density and thickness were measured [9]. Also skin surface parameters such as scaling and roughness were improved. The biochemical mechanisms responsible for these effects are poorly understood. It has been suggested that under stress conditions systemic application of antioxidants contributes to the maintenance of a healthy skin barrier.

6.4 Carotenoids: New Developments

Lycopene and β-carotene are the dominating carotenoids in the human organism; however, there are more than 50 other carotenoids which are constituents of the

human diet. A very interesting compound with an unusual structure is the 3,3′-dihydroxyisorenieratene (DHIR). In this carotenoid, the polyenic backbone is substituted with phenolic endgroups which can be easily oxidized to the respective quinone [12]. DHIR is present in the bacterium *Brevibacterium linens* which is used in dairy industry for the production of various red smear cheeses. The antioxidant activity of the compound exceeds that of other carotenoids like astaxanthin, cryptoxanthin, zeaxanthin, or lutein. It acts as a bifunctional radical scavenger due to the presence of a polyenic and phenolic substructure whereas its activity for singlet oxygen quenching is in the range of other polyenic carotenoids. Further studies in liposomes revealed that DHIR is superior to lutein preventing UV-induced lipid oxidation. Following UVA exposure, the expression of the enzyme heme oxygenase-1 is increased in human skin fibroblasts which is taken as a biomarker of photooxidative damage. Upon preincubation of cells with DHIR, heme oxygenase-1 expression was significantly lowered; only little effects were observed with lutein. A major DNA damage following UVB exposure is the photochemically induced formation of cyclobutane pyrimidine dimers. After exposure of human fibroblasts to UVB light thymidine dimers are formed and can be visualized with specific antibodies. In cells preincubated with DHIR less dimer formation was observed compared to the irradiated solvent control. Because the formation of pyrimidine dimers is a typical photochemical reaction in DNA strands and not related to photooxidation it is likely that UVB absorbing properties of the phenolic end groups in DHIR are responsible for the effect. The experiments underline the multifunctional properties of DHIR as a radical scavenger, quencher of singlet oxygen, and UV-absorbing compound. Further studies are requested to evaluate the possible use of this compound in humans.

6.5 Conclusion

The carotenoids lycopene and β-carotene are examples for dietary constituents with beneficial effects on skin health and properties proven in intervention studies and measured with objective methods. Dietary intervention is not comparable to effects that can be achieved with especially designed sun protectants. The efficacy of protection against sunburn is by far not comparable to the use of a sunscreen with a high sun protection factor. However, a suitable diet may contribute to basal protection and thus increase the defense against UV light-mediated damage to skin. There is evidence that also other skin parameters can be modulated with a suitable diet. In vitro data support the idea that other carotenoids may also be suitable for photoprotection and might be integrated into the concept of dietary defense against UV-induced damage.

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Systemic and Topical Use of Green Tea Polyphenols for Healthy Skin

7

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Core Messages

- Green tea contains polyphenols which appear to exert photoprotective effects in mice.
- Green tea polyphenols (GTP), and particularly (-)-epigallocatechin-3-gallate (EGCG) inhibit UVB-induced and chemical carcinogenesis and photoaging in animal models.
- Human studies demonstrate that GTP reduces acute UVB-induced effects including erythema and DNA damage.
- Human studies further demonstrate increases in elastic tissue content with chronic GTP consumption.
- GTP have been demonstrated to exert numerous cellular, biochemical, and molecular effects on treated cells, and activates several signal transduction pathways.
- GTP are thought to exert their photoprotective mechanisms via upregulation of the cytokine IL-12 and stimulation of nucleotide excision repair.

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7.1 Introduction

Tea, a popular beverage with its origins in southeast Asia, generally refers to an infusion derived from processed leaves, leaf buds, and internodes of the plant *Camellia sinensis*. There are four types of *C. sinensis* teas commonly available on the market – black, oolong, green, and white – which differ in their modes of processing, and in the case of white tea, maturity. ("Red tea" generally refers to an infusion derived from the South African Rooibos plant.) Green tea is produced from fresh leaves of the plant; unlike the black and oolong varieties, green tea is derived from fresh *C. sinensis* leaves that are steamed and dried at high temperatures before any oxidation and polymerization of polyphenolic compounds has taken place.

Green tea has long been employed as a traditional medicine in southeast Asian cultures, and has recently gained prominence for health benefits observed in some, though not all, epidemiological studies, including protective effects against malignancy in several organ systems [55]. These effects are thought to derive primarily from water-soluble polyphenolic constituents of green tea, epicatechins, [44, 45, 55, 109], although there is evidence that other compounds present in green and/or black tea have chemopreventative effects, including caffeine, flavandiols, flavanoids, phenolic acids, and the alkaloids theobromine and theophylline [42, 68, 69, 71]. Of the four major epicatechins in green tea, (-)-epicatechin (EC), (-)-epicatechin-3-gallate (ECG), (-)-epigallocatechin (EGC), and (-)-epigallocatechin-3-gallate (EGCG) (Fig. 7.1), EGCG, which is present in the greatest amount [109], has been shown in comparative studies to have the greatest photoprotective activity, followed by ECG; EGC and EC are less active. The total content of

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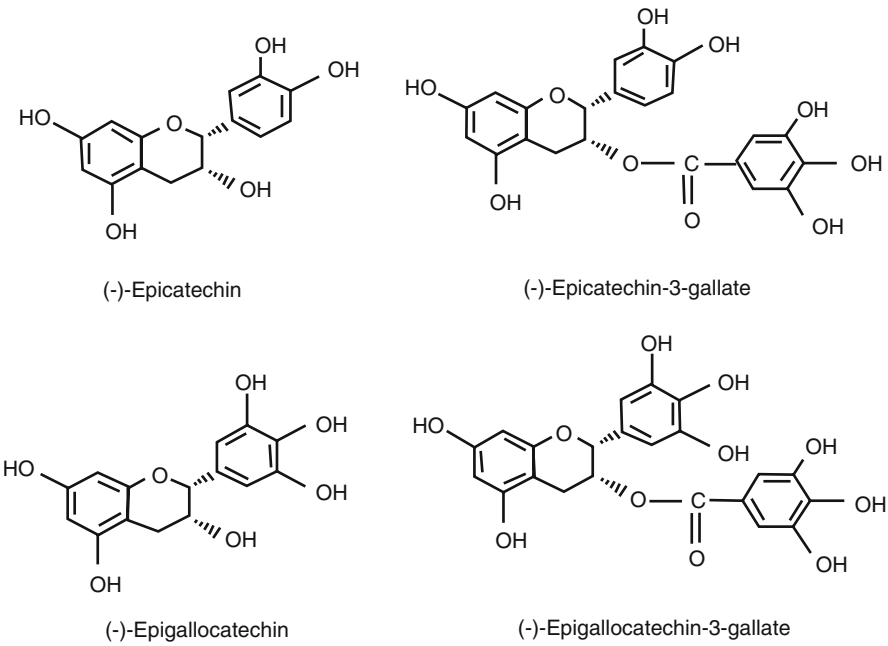

Fig. 7.1 Structures of the four major green tea polyphenols

polyphenols in tea leaves varies from approximately 20–40%, depending on the subspecies of the plant and geographic location. The polyphenols are readily extracted from green tea leaves by water or organic solvents such as methanol and ethanol. The polyphenols present in green tea are also found in black tea, but in smaller amounts due to the oxidation in the manufacturing process. Black tea additionally contains theaflavins and thearubugins which also have photoprotective activities [42, 67, 71, 109].

At present, there is no conclusive epidemiological evidence of the protective effect of tea consumption against the development of human cancers. This may be attributed to variables related to individual differences in tea preparation and consumption patterns, and seasonal and geographic differences in tea production. Because it is not easy to change the dietary habits of an individual, ingesting green tea products in oral formulations may be more acceptable for chronic use in healthy populations. Chow et al. have recently reported the pharmacokinetics and safety of two oral green tea polyphenol formulations (EGCG and Polyphenon E, a defined mixture of green tea polyphenols (GTP)) after single-dose administration [24]. Peak plasma EGCG levels of 200–400 ng/ml (0.4–0.8 μM) can be achieved after the administration of these formulations at doses equivalent to the EGCG content in 8–16 cups of green tea (depending on the cup size).

7.2 Systemic Versus Topical Administration of Green Tea

The animal studies discussed below suggest that oral administration of GTP can provide skin protection in rodents. However, similar studies in human beings did not achieve such effects, possibly because the human dermis provides a stronger barrier to absorption from the vasculature as compared to rodents, who possess a weaker barrier [39]. Topical application of a high concentration of EGCG (10% in hydrophilic ointment) demonstrated toxicity in SKH-1 mice, manifest in erythema and papular lesions, within days of application [95], but no such effects were observed in treated human skin [25].

With any topical preparation, one must consider that observed photoprotective effects may be mediated by a barrier effect, like the mechanism of action of

traditional sunscreens, rather than by pharmacologic activity of the studied compound. With regard to green tea, there are several reasons to avoid this conclusion. First, spectrophotometric analysis demonstrates that these agents do not absorb wavelengths within the UVA or UVB spectra. More importantly, these compounds are effective when given systemically rather than topically, and finally, even in the topical formulations, they afford protection against at least some of the sequellae of UV irradiation even when applied subsequent to the exposure event [52].

7.3 Evidence from Animal Models

Earlier animal studies of green tea extracts on hairless mice focused on oral consumption or topical application of brewed green tea, green tea extracts, or GTP against UV or chemical-induced carcinogenesis [27]. Oral consumption of brewed green tea at concentrations similar to human consumption (1.25% and 2.5%) significantly inhibited UVB- and TPA-induced tumorigenesis [103]. Oral administration of decaffeinated green tea demonstrated similar anticarcinogenic effects. Later studies showed that oral administration of green tea in mice not only decreased skin tumorigenesis, but also reduced fatty tissue in the dermis [26]. It also inhibited UVB-induced ornithine decarboxylase and cyclo-oxygenase (COX) activities [5], enzymes closely associated with the promotion stage of UV carcinogenesis. Similar effects of oral and peritoneal administration of green tea resulted in inhibition of the growth of UV-induced skin papillomas [103] or TPA-induced COX2 expression in rodent models [63]. EGCG decreased approximately 50% of nonmalignant and malignant tumors per mouse in SKH-1 hairless mice which were exposed to UVB twice a week for 20 weeks prior to topical treatment with EGCG. This suggested that the effect of EGCG was not just caused by a sunscreen/antioxidant effect [68].

When applied topically, GTP also inhibited DMBA-initiated, TPA-induced carcinogenesis and benzo[a] pyrene- and TPA-induced tumor initiation in CD-1 mice [41, 54]. Topical application of GTP reduced TPA-induced inflammation, ornithine decarboxylase activity, hyperplasia, and hydrogen peroxide (H_2O_2) formation, suggesting GTP serve as both an antioxidant and a regulator of enzymatic activities [41]. In other studies, TPA-induced elevation of COX and lipoxygenase were significantly inhibited by topical application of GTP to Sencar mice [50].

7.4 Evidence from Human Studies

Very few studies have evaluated the effect of GTP in human skin. EGCG penetrates the epidermal barrier in mice but is not as effective in human beings [30]. In a human biopsy study, when green tea extracts and individual polyphenols were applied topically on the skin before exposure to UV irradiation, they caused a dose-dependent inhibition of the erythema response induced by UV radiation [33]. Green tea extracts also reduced the number of sunburn cells and DNA damage. Among the GTP, EGCG and (-)-EC-3-gallate (the polyphenols that possess a gallate group) were effective, whereas EGC and EC (the polyphenols that lack a gallate group) were ineffective [33]. Application of EGCG before UVB exposure also reduced UVB-induced erythema and UVB-induced infiltration of leukocytes [53]. Thus, GTP, when applied topically, have photopreventive effects.

A combination of psoralen and UVA (PUVA) therapy has been used for treatment of certain skin diseases, such as psoriasis [61]. However, prolonged treatment with PUVA increases the risk of skin cancer, especially squamous cell carcinoma [34]. A study using several in vitro and in vivo models, including in vivo studies in human beings, found that topical application of green tea extract almost completely inhibited PUVA-induced erythema; the same extract also inhibited DNA damage caused by PUVA, suggesting that GTP may be able to protect epidermal keratinocytes from PUVA-induced carcinogenesis [15, 111]. Another interesting finding was that green tea may help to protect hair follicles from γ-ray-induced apoptosis [60]. A number of human trials using GTP as chemopreventive agents are currently underway, to develop formulations of these agents in sunscreen and other protective skin applications for use against UVB-induced carcinogenesis [31, 65].

7.5 The Role of Green Tea in Photocarcinogenesis

As skin has the greatest exposure to sunlight, the damaging effects of ultraviolet (UV) radiation are observed most frequently in this organ [76]. One adverse effect of excessive sun exposure is the development of nonmelanoma skin cancer.

Skin cancer presents an enormous burden to the US health care system. The incidence of skin cancer is estimated to be greater than one million new cases yearly, and while their visibility allows more rapid diagnosis and treatment than other cancers, over 10,000 Americans die from skin cancer yearly [98]. In excess of $650 million is spent yearly in treatment of nonmelanoma skin cancer [19]. Skin cancers are also among the most preventable.

The first studies to assess the photoprotective effects of GTP were conducted in animal models of skin cancer [102]. In SKH-1 hairless mice, chronic exposure to UVB radiation generates the development of premalignant papillomas (analogues of human actinic keratoses) and cutaneous squamous cell carcinomas. Wang, et al. found that when these animals were fed extracts of green tea in their drinking water (0.1% w/v) or when green tea extracts were applied to their skin before each UV treatment, the latency to tumor development was prolonged and the incidence of UV-induced tumors was reduced in a dose-dependent manner [102]. These results were confirmed with regard to tumor incidence, multiplicity, and tumor growth or size compared to controls in studies with oral administration of GTP [13, 46, 55, 74]. Topical EGCG as well as oral GTP were also shown to induce partial regression or inhibition of tumor growth in established skin papillomas in mice [104]. Subsequent experimentation in mice has shown that the EGCG component of green tea alone is highly effective at controlling UV-induced tumor formation [35]. Further studies have shown that topical administration of GTP or EGCG in hydrophilic ointment appears to be significantly more effective than other vehicles and even more effective than oral administration, perhaps due to greater concentrations of EGCG delivered to the putative site of action [99]. UV-induced skin tumorigenesis has been divided into initiation, promotion, and progression stages [84], and GTP have been demonstrated to exert protective effects in each of these stages in mice [27, 103, 105], but, importantly, long-term oral administration or topical application of GTP or EGCG did not result in signs of visible toxicity in laboratory animals. Although a small clinical trial demonstrated that short-term application of a high-dose topical formulation of EGCG was ineffective at causing regression of actinic keratoses [65], a recent trial demonstrated that a low-dose topical formulation of EGCG significantly reduced UV-induced p53 expression and apoptosis in human keratinocytes [82].

7.6 The Role of Green Tea in Photoaging

Another adverse effect of solar UV radiation is photoaging of the skin. Photoaging represents a major source of financial outlay for consumers. Over $12.4 billion was spent on US retail sales of cosmeceuticals, and anti-aging expenditures are expected to exceed $16.5 billion by 2010 [23]. The rising costs of photodamage have led to increased interest in the development of better methods of prevention. Currently, prevention strategies focus on sun avoidance and sunscreen use, but these approaches are limited by modest efficacy [37], inadequate application [8], and poor patient compliance [1]. Among the agents that have received attention for their photoprotective qualities is green tea.

In an animal model of photoaging, UVA-irradiated SKH-1 hairless mice, topical application of EGCG resulted in an observable reduction in the amount of skin wrinkling, as well as a reduction of matrix metalloproteinases (MMP)-2, (MMP)-3, (MMP)-7 and (MMP)-9, which contribute to photodamage by degrading collagen, and a reduction in skin protein oxidation, another hallmark of photoaging [100]. In an 8-week double-blind, placebo-controlled trial of a combination oral and topical green tea supplementation, green-tea-treated patients showed histologic improvement in elastic tissue content, and while no clinical differences were observed, it was noted that longer supplementation may be necessary for clinically observable improvements [22].

7.7 The Role of Green Tea in the Sunburn Response

The human sunburn reaction, a painful erythema response to acute overexposure to UV radiation, is reduced in a dose-dependent manner both in murine models [52] and human subjects [33] with application of

a preparation of GTP or with EGCG extract; this result was also obtained via oral administration in mice [52].

7.8 The Role of Green Tea in Photoimmunosuppression

Another cause of untoward effects of UV radiation stems from its induction of immunosuppression. UVA and UVB cause local immunosuppression to treated areas by functionally inhibiting the resident skin antigen-presenting cells, and systemic immunosuppression by inducing keratinocytes to release immunosuppressive cytokines such as interleukin (IL)-10, as well as tumor necrosis factor (TNF)-α and transforming growth factor (TGF)-β. [76] In a murine allergic contact hypersensitivity model, UVB exposure suppresses the afferent phase of the contact hypersensitivity response. Topical application of GTP, either before or immediately after UVB exposure, reverses the immunosuppressive effects of UVB [51, 52]. UV-induced immunosuppression is mediated in part by an increase in levels of the cytokine IL-10 and a reduction in production of IL-12, a cytokine that augments the development of Th1 type T cells that secrete IFNγ [38, 73, 90]. In mice, EGCG application appears to switch that balance, reducing IL-10 levels and increasing IL-12, [51] to the extent that contact hypersensitivity (CHS) response was reestablished in EGCG-treated mice irradiated at a UV dose sufficient to cause CHS suppression [80]. This effect was blocked by intraperitoneal injection of anti-IL-12 monoclonal antibodies, confirming the key role of IL-12 in EGCG-mediated immunoprevention. The increase in IL-12 that occurs when EGCG is applied prior to UV irradiation mediates its effect at least partially through the induction of DNA repair enzymes. Moreover, in IL-12 deficient mice, EGCG inhibits neither the UVB-induced sunburn response nor carcinogenesis. Recent in vitro studies have extended that model of IL-12 mediated reduction in UVB-induced DNA damage to human living skin equivalents [89].

7.9 The Role of Green Tea in Other Skin Disorders

The anticancer and photoprotection potentials of green tea are well studied, but green tea may also provide an alternative treatment for other skin disorders, such as psoriasis, skin cancer, actinic keratosis, cherry angiomas, Bateman's purpura, chondrodermatitis nodularis helicis, seborrheic keratosis, and rosacea [72]. Abnormalities in the differentiation process of keratinocytes are involved in the pathogenesis of several of these disorders. In pathologic conditions such as psoriasis, in which cornification is altered, the normal expression pattern of caspase 14 is absent [66]. EGCG is able to induce caspase 14 expression in exponentially growing NHEKs within 24 h, subsequent to p57 induction [39]. EGCG may be an effective treatment in psoriasis through this pathway, since human psoriatic tissue lacks nuclear translocation of caspase 14 [101]. Induction of caspase 14 expression and its nuclear localization by green tea may promote differentiation and skin barrier formation, which may lead to new treatments for other skin disorders that lack normal differentiation.

7.10 Cellular Effects

7.10.1 *Modulation of Keratinocyte Apoptosis*

EGCG appears to exert paradoxical effects in the context of acute versus chronic UV exposure. Histologic examination of skin after acute UV irradiation typically reveals a large number of apoptotic keratinocytes, known as "sunburn cells." In vivo pretreatment of murine [52] and human [25, 33, 82] skin with a topical EGCG preparation resulted in a reduced of number of apoptotic keratinocytes; the histologic finding was confirmed with TUNEL staining. These in vivo studies were confirmed by studies of cultured human keratinocytes [25, 106] and a human living skin model [89] exposed to UVB radiation in vitro. Closer study revealed that the anti-apoptotic effect was mediated by increased expression of the anti-apoptotic molecule Bcl-2, and a decrease in the pro-apoptotic protein Bax [25]. This finding might have lead to the concern that EGCG might rescue damaged keratinocytes at risk for transformation. Recent work, however, has shown that the anti-apoptotic effect results from EGCG-induced upregulation of the cytokine IL-12, which induces nucleotide excision repair (NER); since NER is able to proceed apace in the EGCG-treated skin, fewer cyclobutane

pyrimidine dimers (CPDs) remain, and there are fewer mutations remaining to trigger Bax-mediated apoptosis [89] and UV-induced inflammation [78]. EGCG-treated IL-12 knockout mice did not demonstrate rapid removal of sunburn cells after UV exposure [79], and EGCG-treated, UVB-irradiated fibroblasts with a mutation in the NER component XPA (xeroderma pigmentosum complementation group A) did not repair CPDs as well as their wild-type counterparts [79]. GTP-mediated IL-12 induction of NER at least partially explains the anticarcinogenic effect of GTP, as EGCG-treated IL-12 knockout mice do not exhibit the inhibition of photocarcinogenesis seen in wild types [79].

The conclusion that the effect of EGCG is entirely reparative rather than protective is supported by the recent finding that EGCG pretreatment of UV-irradiated skin does not alter gene expression [82], and the mechanism of EGCG-mediated photoprotection is post-transcriptional. However, it should be noted that recent findings demonstrating photoprotective effects in rats with EGCG pretreatment only, and not posttreatment, are not consistent with this hypothesis [91].

In the context of chronically UV-irradiated tissue, GTP have been found to exert the opposite effect, generating apoptosis in premalignant papillomas and invasive squamous cell carcinomas [25, 68]. The basis of this effect is not yet fully understood but it seems likely to result from activation of pro-apoptopic pathways triggered by NER proteins [14] in response to irreparable DNA damage.

7.10.2 Keratinocyte Proliferation

UV irradiation typically leads to increased proliferation of keratinocytes, evident histologically as epidermal hyperplasia. Incorporation of BrdUrd, a thymidine analogue, into proliferating cells during the S-phase is used to measure the rate of cell proliferation. UV-irradiated mice pretreated with green tea demonstrated only a small inhibitory effect upon BrdUrd incorporation into epidermal DNA, and no difference in mitotic index [70]. However, in studies of normal keratinocytes, EGCG has been found to significantly suppress proliferation and activate differentiation pathways in keratinocytes [11].

7.10.3 Inflammatory Cell Infiltration

A hallmark of the sunburn response, and a key event in the UV-induced tumorigenesis pathway, is the development of an inflammatory cell infiltrate consisting of neutrophils and macrophages. In particular, the CD11b+ macrophage, which migrates into the skin following UV radiation, is a potent source of hydrogen peroxide and nitric oxide that may play a role in UVB-induced mutagenesis and tumor promotion [81]. The CD11b protein is an α-chain integrin expressed on neutrophils, monocytes, NK cells, and a subset of CD8+ T cells [58]. In addition, Langerhans cells, resident epidermal antigen-presenting cells, are downregulated by UV irradiation.

Application and consumption of GTP has been observed, by histology, to markedly reduce the UV-induced inflammatory response in mice [56] and in humans [53]. EGCG additionally inhibits neutrophil migration in rats in vitro (in Boyden chambers) and in vivo [96]. The putative mechanism of this effect stems from direct binding of EGCG to cell surface CD11b, which thereby inhibits both neutrophil migration [58] and infiltration of CD11b+ macrophages [56]. Finally, UVB exposure produces a marked diminution of epidermal Langerhans cell densities. Topical application of green tea prior to UV irradiation returns Langerhans cell densities back to close to that which is seen in normal skin [33] by a mechanism that is not yet understood.

7.11 Biochemical Effects

7.11.1 Cytokine Modulation, Angiogenesis, and Inflammation

As noted above, in the context of skin UV irradiation, EGCG appears to upregulate the proinflammatory cytokine IL-12, which, at least in part through its effects on NER, reverses the immunosuppressive effects of UV on cell-mediated immunity. EGCG additionally inhibits UVB-induced production of IL-10, a cytokine that downregulates cell-mediated immunity, by reducing the migration of IL-10 producing CD11b+ macrophages into the epidermis [51].

In the context of EGCG downregulation of UV-induced tumorigenesis, it was observed that

topical application of EGCG to UV-induced skin tumors resulted in a reduction of matrix metalloproteinase (MMP)-2 and (MMP)-9 protein expression and activity, perhaps by upregulating tumor inhibitor of matrix metalloproteinase (TIMP)-1 expression. EGCG was further found to inhibit the expression of vascular endothelial growth factor (VEGF) in UV-induced skin tumors [75]. MMPs promote tumor growth and invasion of skin tumors, and VEGF stimulates the development of the tumor vasculature. These cytokines are also proinflammatory cytokines. Indeed, other proinflammatory cytokines such as TNF-α, IFN-γ, IL-2, and IL-12 p40 are downregulated by EGCG in atopic dermatitis-like skin lesions in mice, and this has been postulated to result from EGCG-mediated inhibition of macrophage migration inhibitory factor (MIF), [86] a proinflammatory cytokine that is essential for activating T cells after antigenic stimulation [94].

7.12 Molecular Effects

7.12.1 DNA Damage

DNA is a chromophore for UV radiation, and alterations induced by UV exposure can induce mutagenic, tumorigenic, and lethal effects in skin cells, attributable to the direct generation of pyrimidine dimers by UVB. Bipyrimidine site mutations have been linked to inactivating mutations in the tumor suppressor gene p53, a gene which is upregulated in the initial phases of skin cancer [112]. p53 mutations have been shown to lead to photocarcinogenesis [29], and p53 degradation appears to play a role in photoaging as well [77]. DNA damage also appears to trigger a "stress response" characterized by inflammation [110], immunosuppression [98], and melanogenesis [4]. The generation of cyclobutane pyrimidine dimers, among other DNA damaging effects of UV radiation, plays a role in the initiation stage of photocarcinogenesis and is crucial in precipitating UV-induced immunosuppression [62].

GTP have been found to be highly effective at decreasing DNA damage and cyclobutane pyrimidine dimers in UV-irradiated skin [33, 57]. In vitro studies using cultured human cells demonstrated a dose-dependent reduction in UV-induced DNA damage in EGCG-treated cells [83]. In vivo studies with topical GTP in mouse epidermis [18], as well as humans [33], demonstrated a significant, and in the human subjects, dose-dependent inhibition in UVB induction of DNA damage in the epidermis, dermis, and even deep dermis [57]. As mentioned above, it is now thought that EGCG does not prevent UV-induced mutations directly, but upregulates production of the cytokine IL-12, which stimulates nucleotide excision repair to mend the damaged DNA [89]. This would explain the seemingly contradictory finding of decreased apoptosis despite an increase in p53- and p21-positive cells in normal EGCG-treated mouse skin exposed to UV radiation [70].

7.12.2 Reactive Oxygen Intermediates

UV radiation exposure can overwhelm the body's sophisticated mechanisms to defend against oxidative stress. This can result in an increase in reactive oxygen intermediates and a depletion in endogenous antioxidant enzymes such as catalase and glutathione peroxidase [48, 99]. In vitro studies in mouse keratinocytes demonstrated green tea polyphenols can inhibit UV-induced lipid peroxidation in mouse epidermal microsomes [49]. In another study, EGCG decreased UVB-induced intracellular release of hydrogen peroxide and the consequent phosphorylation of MAPK proteins [47]. In vitro studies in human HaCaT keratinocytes treated with EGCG demonstrated protection from UVA-induced damage, measured by DNA single-strand breaks at alkali labile sites [97]. In vivo studies in SKH-1 hairless mice demonstrated that topical or oral GTP or EGCG prevents depletion of glutathione, glutathione peroxidase, and catalase, decreases UV-induced lipid peroxidation, and inhibits UVB-induced protein oxidation [48, 99, 100]. In vivo studies with topical EGCG in human volunteers demonstrated a significant decrease in post-UVB exposure hydrogen peroxide and nitric oxide production, an inhibition of UV-induced decrease in glutathione and glutathione peroxidase levels, as well as a decrease in dermal and epidermal lipid peroxidation [48].

GTP also provided an effective deterrent against photocarcinogen-induced oxidative stress. Topical application of GTP or EGCG before UVB exposure in SKH-1 hairless mice significantly prevented UVB-induced depletion of the antioxidant enzymes glutathione

peroxidase, catalase, and glutathione; inhibited UVB-induced oxidation as measured by lipid peroxidation and protein oxidation; and inhibited UVB-induced activation of MAPK family members extracellular signal-regulated kinase (ERK) 1 and 2, c-jun N-terminal kinase (JNK), and p38 [3, 99].

The in vitro effects may result from direct EGCG scavenging of reactive oxygen species[93], or an electron transfer mechanism by which catechins may reduce base damage and single-strand DNA breaks [7]. These processes may be less relevant in vivo due to limited bioavailability (although perhaps relevant to skin preservation ex vivo, as seen in EGCG promotion of preservation and "take" of rat skin grafts [59]). At least some of the in vivo effects have been shown to result from decreased infiltration of CD11b+ macrophages into UV-irradiated skin [53, 56], a major source of reactive oxygen intermediates in UV-irradiated skin [81].

Some studies have shown in vitro that high concentrations of EGCG (200 μM) generate reactive oxygen species in tumor cells, but reduce reactive oxygen species to background levels in normal epithelial cells. These findings, if demonstrable in vivo, would make EGCG quite relevant for tumor prevention and even therapy [107, 108].

7.13 Signal Transduction Pathways

UV exposure activates several cellular signal transduction pathways, which transmit signals from the plasma membrane to the nucleus to initiate a cellular response [16]. These responses are implicated in photoaging, enhanced cell survival and proliferation, skin cancer growth and invasion, and some kinds of DNA damage. GTP have been found to modulate several major signal transduction pathways, including p53 and cell cycle regulatory molecules, MAP kinase, NF-κB, AP-1 and phosphatidylinositol 3-kinase/Akt and p70 S6-K.

7.13.1 Apoptosis, p53, and Cell Cycle Regulatory Pathways

Green tea, when administered orally to SKH-1 hairless mice, causes an increase in p53- and $p21^{waf1/cip1}$-positive cells induced by UV exposure [70]. Transcriptional activation of p53, $p21^{waf1/cip1}$, MDM2, and the proteins of the Bcl-2 family of proteins is crucial to DNA repair and apoptosis of severely damaged cells, and plays an important role in endogenous photoprotection [17, 32, 43, 64].

7.13.2 Mitogen-Activated Protein Kinases (MAPK)

MAPK, a family which includes the extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase/stress-activated protein kinases (JNK/SAPK), and p38 proteins, are important regulators of the activator protein (AP)-1 and Nuclear factor (NF)-κB transcription factors [16], and are activated by oxidative stress. They also play an important role in activating collagenolytic MMP (matrix metalloproteinase) production which has been implicated in skin photoaging. Normal human epidermal keratinocytes treated with EGCG demonstrate inhibition of UV-induced phosphorylation of the MAPK family proteins [47]. In addition, green tea, when applied topically to mouse skin, causes a time-dependent marked inhibition of UV-induced phosphorylation of ERK1/2, JNK and p38 proteins of the MAPK family [99]. Indeed, EGCG demonstrates inhibition of collagen destruction and collagenase activation in UV-irradiated human dermal fibroblasts [9]. In addition, the JNK and p38 pathways have been demonstrated to be essential for the activation of p57 and caspase 14, which, as mentioned above, play an important role in promoting differentiation and skin barrier formation in psoriasis and other inflammatory skin disease [40].

7.13.3 Nuclear Factor-κB

NF-κB, a ubiquitously expressed member of the Rel family of transcription factors, regulates genes involved in inflammation, immunity, cell cycle progression, apoptosis, and oncogenesis[10, 12, 36]. In A431 human epidermoid carcinoma cells, EGCG inhibits constitutive expression and TNF-α-mediated activation of NF-κB more effectively than in normal human epidermal keratinocytes [6]. Treatment of normal human epidermal keratinocytes with EGCG protected

against NF-κB activation, resulting in a time-dependent inhibition of UVB-mediated degradation and phosphorylation of IκBα and activation of IKKα [2]. In vivo, mouse skin treated with GTP demonstrated inhibition of UVB-induced activation of NF-κB, activation of IKKα, and phosphorylation and degradation of IκBα [3].

7.13.4 AP-1

The AP-1 family of transcription factors regulates the expression and function of a number of the cell cycle regulatory proteins, including cyclin D1, p53, p21, p19, which are involved in cell proliferation and survival [92]. In the human keratinocyte cell line HaCaT, EGCG inhibits UVB-induced expression of c-fos, an AP-1 heterodimer, and AP-1 activation [21]. Since p38 and ERK have been shown to be required for UVB-induced c-fos expression in HaCaT cells [20], it is likely that the EGCG effect on c-fos results from inhibition of MAPK proteins.

7.13.5 Phosphatidylinositol 3-Kinase/Akt and p70 S6-K

Phosphatidylinositol 3-kinase and its downstream effectors are important in many biological activities, including protein synthesis, cell growth, cell motility, and apoptosis [87]. In mouse epidermal cells, EGCG inhibits UVB-triggered epidermal growth factor receptor (EGFR) activation of PI3K and its downstream effector, Akt, also known as protein kinase B, as well as the downstream effector of Akt, p70 S6-kinase [28].

7.13.6 Proteasome Activation

In tumor cell lines, ECG and EGCG irreversibly inhibit the activity of the 20S proteasome, resulting in the accumulation of $p27^{Kip1}$ and IκBα, and consequent G1 growth arrest of the cell cycle. The 20S proteasome, a constituent of the ubiquitin–proteasome pathway, is responsible for degrading p53, pRB, p21, $p27^{Kip1}$, IκBα and Bax, proteins whose loss or mutation have been implicated in skin tumorigenesis. Evidence has been presented to implicate the chymotrypsin-like activities of this molecule in tumor cell survival, and its inhibition by GTP may be crucial to its chemopreventative effects [85].

7.13.7 Signal Transducer and Activator of Transcription (STAT)-3 Signaling

In human keloid fibroblasts, EGCG has been demonstrated to inhibit collagen production and proliferation via the STAT3 signaling pathway, an oncogene and latent transcription factor that plays a role in cell survival, proliferation, migration, inflammation, and immune response, and is an important signaling mediator for keloid pathology [88].

7.14 Conclusion

Polyphenolic extracts of green tea are well-tolerated compounds that demonstrate efficacy in protection against UV skin carcinogenesis. They have the potential, both in oral and topical formulations, to synergize with current photoprotective measures in preventing and repairing the adverse effects of overexposure to the sun.

Take Home Pearls

- Green tea polyphenols, in topical or oral preparations, are potentially efficacious agents for photoprotection.
- Green tea polyphenols may help prevent or reverse photodamage even after sun exposure.
- Green tea polyphenols may prove useful in combating the effects of photoaging.
- Green tea polyphenols may have utility in promoting skin barrier function in the context of inflammatory skin diseases.

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Flavonoid-Rich Nutrients for the Skin

8

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Core Message

› Flavonoids comprise a family of plant pigments which are used in cosmetics and dermatology. In addition to antioxidant properties they exhibit other biological activities which likely contribute to their cutaneous effects. In-vitro and in-vivo studies provide evidence that selected flavonoids contribute to photoprotection and modulate skin properties either after topical application or systemically after ingestion.

8.1 Introduction

Flavonoids comprise a group of secondary plant constituents widespread in nature and are easily recognized as flower pigments. However, their occurrence is not restricted to flowers but include all parts of the plant. They are available from dietary sources such as cocoa, green tea, soy, berries, or other fruits like apples, lemons, cherries, plums, and peaches [7]. Many formulations used as ointment, lotion, or cream in clinical dermatology and cosmetics contain this class of compounds as active ingredients [2]. Edible and nonedible plants are sources for the preparation of flavonoid-rich extracts that have been widely used as topical medication for wound healing, anti-aging, and in the treatment of skin disorders.

Some examples for such botanic medicals are arnica, calendula, ginkgo biloba, echinacea, ginseng, grape seed, green tea, lemon, lavender, rosemary, soy, jojoba, aloe vera, or papaya-based preparations. Arnica is promoted for cutaneous application to support wound healing, recovery from sunburn or irritation following injuries and burns, acne, and eczema. Calendula extracts may contribute to moisturize, recondition, and smooth skin. It has been postulated that Calendula preparations stimulate the biosynthesis of collagen, thus lessening the appearance of wrinkles. Green tea extracts have gained popularity as ingredients of skin care preparations to delay skin aging and prevent UV-induced skin lesions and cancer [11]. In contrast to topical respectively transdermal delivery, systemic distribution of bioactive micronutrients has been shown as an alternative pathway to provide compounds which modulate skin properties [19].

8.2 Flavonoids: Structure and Occurrence

Flavonoids represent a subgroup of naturally occurring polyphenols with over 5,000 individual compounds already known [3]. Their structure is based on 15-carbon

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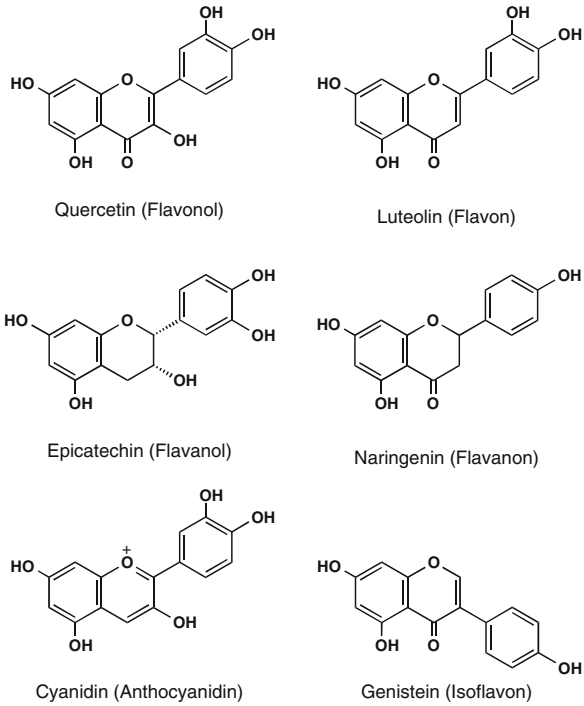

Fig. 8.1 Structures of selected flavonoids

skeleton with a chroman ring as key element carrying a second aromatic ring B at the C2 or C3 position. Based on the substitution pattern of the C ring flavonoids can be further subdivided (Fig . 8.1) into flavonols, flavons, flavanols, flavanons, and anthocyanidins all substituted with the B ring in 2 position. In contrast, isoflavons carry the B ring at the carbon 3 atom of the chroman system. The aromatic rings A and B are usually substituted with a varying number of hydroxyl groups which are relevant with respect to the antioxidant properties of the compounds. Flavonols like quercetin, kaempferol, myricetin, or isorhamnetin and flavones such as luteolin and apigenin are the most common phenolics in plant-based foods, e.g., onions, tomatoes, apples, grape, berries, tea, and red wine. They also occur in herbs and medical plant like ginkgo biloba or thyme (Thymus vulgaris). Flavanons as naringenin and hesperitin are typically present in citrus fruits, e.g., lemons and oranges where peel and membranous parts have the highest concentrations. Flavanols, particularly catechin and epicatechin, are found in green or black tea, wine, and cocoa products. They also occur as gallate esters and polymeric procyanidins. Apart from the free phenolic compounds numerous glycosides have been identified as derivatives of the flavonoids with rutinose, glucose, arabinose, galactose, or rhamnose. Examples are rutin, hesperidin, or naringin the bioactive glycosides of quercetin, hesperitin, and naringenin, respectively. Anthocyanins are hydrophilic, versatile, and plentiful pigments are present in lots of colored plants and are responsible for the red, purple, and blue colors of many fruits and vegetables as for example purple cabbage, beets, blueberries, cranberries, bilberries, pomegranate, or plums. Soy products contain high amounts of the isoflavons genistein and daidzein, which are structurally related to estrogen and reveal their activity as photoestrogens via hormone receptor-dependent pathways. Red clover contains also considerable amounts of genistein and is used in skin care as a component of creams. Further sources with relevance in dermatology are ginkgo biloba extract, oregano, and sage. Plants may contain various flavonoids as glycosides and aglycons from different subgroups but the pattern is typical and can be used to classify a plant family. They play different roles in the ecology of plants including coloration to attract pollinating insects, feeding repellants, or photoprotection.

8.3 Flavonoids: Biochemical Properties

Flavonoid-containing phytomedicals are marketed as anti-inflammatory and anti-allergic remedies and a flavonoid-rich diet is suggested to play a role in the prevention of several kinds of cancer and cardiovascular disorders [4]. Many of the alleged effects have been linked to the antioxidant properties of flavonoids. Numerous in vitro experiments applying various systems to determine antioxidant activity have proven that they are very efficient antioxidants, scavenging reactive oxygen species (ROS) and reactive nitrogen species (RNS) such as the superoxide anion, peroxyl radicals, singlet oxygen, hydroxyl radicals, nitric oxide, or peroxynitrite. Reactive intermediates formed in prooxidative reactions are able to damage biologically important molecules like DNA, lipids and proteins, processes that have been linked to the initiation or progression of degenerative diseases [23]. A structural feature directly related to the antioxidant properties of flavonoids is the presence of aromatic hydroxyl groups [24]. Most efficient for radical scavenging is a substitution pattern with two hydroxyl groups in the B ring orientated in ortho position. Antioxidant

activity is even more pronounced when additional hydroxyl groups are located at the 3 and/or 5 carbon atom of the A and C ring and/or carbons 2 and 3 are connected to each other with a double bond. Quercetin represents a flavonoid which satisfies all of the determinants for optimal antioxidant activity. But also chelating properties of the flavonoids are likely involved in antioxidant activity. Some of the compounds form stable complexes with iron and copper ions either lowering the load of free metal ions or inducing metal-dependent radical formation in the vicinity to the antioxidant flavonoid where they can be directly inactivated.

Bioavailability plays an important role in the evaluation of the biological efficacy of flavonoids. It has been shown that bioavailability differs greatly between the various polyphenols, and the most abundant polyphenols in our diet are not necessarily those that have the best bioavailability profile [14]. In this context it should also be noted that a number of flavonoids exhibit a strong first pass effect. Bioavailability studies with selected flavonoids demonstrate that hydroxyl groups of the molecules are efficiently glucuronidated, sulfated, or methylated during gut and liver passage and consequently the levels of the parent compounds at least in the blood are quite low. Since the presence of free hydroxyl groups is a prerequisite for radical scavenging it has been discussed if direct antioxidant activity of flavonoids in vivo plays a major role in their mode of action. Indirect antioxidant effects could be mediated inhibiting prooxidant enzymes including cyclooxygenases and lipoxygenase, monooxygenases, xanthinoxidase, or mitochondrial succinate dehydrogenase and NADPH-oxidase. Vice versa they may stimulate enzymes involved in antioxidant defense such as superoxide dismutase or glutathione peroxidase. Flavonoids biological effects have also been attributed to non-antioxidant bioactivities modulating cellular signaling at various levels from gene expression to postranslational modification of bioactive proteins [21]. Phytoestrogen activity of isoflavons has already been mentioned. The compounds bind and transactivate human estrogen receptors, thus, directly interfere with the expression of estrogen-dependent proteins. Flavanol-rich foods exert cardiovascular health benefits probably via improving endothelial dysfunction which is characterized by a decreased bioactivity of nitric oxide. In human studies it has been shown that the ingestion of flavanol-rich food is correlated with increased levels of circulating NO and augmented microcirculation. Similar effects were achieved with the pure flavanol (−)-epicatechin [9]. Although the mechanism of action is not clear yet it is likely that flavanols trigger biochemical pathways responsible for the regulation of NO activity in endothelial tissue. Antioxidant and non-antioxidant properties may also account for anti-inflammatory properties of selected flavonoids like quercetin. The pathways address regulatory elements including the redox-sensitive transcription factor NfκB, inducible nitric oxide synthase, or cyclooxygenase a key enzyme for prostaglandine synthesis. Thus, flavonoids likely reveal their biological activities via a number of different biochemical mechanisms and the one which is relevant for a specific activity has to be attributed and evaluated depending on the compound and the affected biological system.

8.4 Flavonoids in Photoprotection

Irradiation with UV or visible light induces photooxidative reactions via intermediate formation of singlet molecular oxygen in processes triggered by suitable sensitizers. In subsequent reaction cascades secondary reactive oxygen intermediates including the superoxide radical anion and other reactive oxygen species are generated. ROS generation in the skin is followed by oxidation of cellular macromolecules and decrease of endogenous antioxidants. This has been associated with photoaging and skin cancer [17]. Because of their antioxidant activity flavonoids may provide protection against cutaneous photodamage and protect important biomolecules [6]. DNA bases are directly affected by light. Formation of cyclobutane pyrimidine dimers is a major DNA damage found in tissues exposed to UVB light. Because of the presence of aromatic ring systems flavonoids absorb UV light and may prevent direct light-induced DNA damage by this pathway.

The isoflavone genistein is an example for a potent antioxidant [19]. The compound exhibits additionally estrogen activity and inhibits protein tyrosine kinases. Antioxidant and anticarcinogenic effects have been proven in skin and enhanced activities of antioxidant enzymes have been determined in the skin of SENCAR mice after treatment with the isoflavone. Treatment of animal skin with genistein prior to UVB exposure resulted in significant inhibition of UVB-induced H_2O_2,

malondialdehyde, and 8-hydroxy-2-desoxyguanosine (8-OH-dG) production. Following topical application of genistein to hairless mice a diminished response after exposure to solar-simulated UV radiation was determined, measured as reduced inflammatory edema reaction. When applied topically prior to UV radiation, genistein protected against photodamage in human reconstituted skin model. The compound also inhibited tumor development in hairless mice that were chronically exposed to UV radiation. Treatment of the human keratinocyte cell line NCTC 2544 with genistein prevented UVA-induced enhancement of the DNA-binding activity of signal transducer and activator of transcription-1 (STAT-1) by acting as a tyrosine kinase inhibitor, thus limiting lipid peroxidation and increases in ROS formation.

Silymarin is a mixture of several flavonolignans, including silybin, silibinin, silidianin, silychristin, and isosylibin which are found as major flavonoids in the milk thistle plant. Like other flavonoids they are efficient antioxidants as shown in several model systems [12]. A possible mechanism underlying protective effects of silymarin has indirectly been shown by demonstrating modulatory activity of the compounds on the activation of transcription factors NF-kB and AP-1 in cell culture. Topical application of silymarin protects against photocarcinogenesis in mice. When applied to hairless mice it significantly lowered tumor incidence, tumor multiplicity, and average tumor volume resulting from UVB irradiation. Topical application of silymarin was found to significantly inhibit UVB-induced skin edema, formation of sunburn and apoptotic cells, depletion of catalase activity, and induction of COX and ODC activities and ODC mRNA expression.

Flavonoids play an important role in the protection of higher plants exposed to high fluxes of solar radiation. It has been shown that they contribute significantly to the prevention of UVB-induced damage in apple fruits where the protection was correlated with quercetine respectively quercetine glycoside levels [18]. Quercetin and rutin were tested as potential topical sunscreen factors in humans and found to provide protection in the UVA and UVB range [5].

Polyphenols from green tea, respectively green tea extracts rich in polyphenols, have been extensively studied in terms of protection against photodamage and photocarcinogenesis induced by UV radiation [1, 25]. Flavan-3ols including (−)-epicatechin (EC), (-)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC) and (−)-epigallocatechin-3-gallate (EGCG) are the major flavonoids in green tea and all of them are potent antioxidants at least in vitro. After UV exposure the expression of several metalloproteinases (MMPs) including MMP-2, MMP-3, MMP-7, and MMP-9 is induced as a so-called delayed response. UV-induced expression of MMPs in mouse skin is inhibited by oral administration of green tea polyphenols indicating that the compounds prevent premature skin aging. (−)-Epigallocatechin 3-gallate and green tea polyphenol fraction effectively prevent carcinogenesis in UVB radiation-treated mice when administered either in the diet or topically. Topical application of EGCG antagonizes immunosuppressive effects following UV radiation.

The protection correlates with inhibition of macrophage and neutrophil infiltration, lower levels of IL-10 in irradiated mouse skin, and a marked increase in levels of IL-12. Green tea polyphenols also protect against UV-mediated DNA damage and apoptosis as shown in human keratinocytes and skin equivalents. The effect is correlated with induction of IL-12 secretion suggesting that this regulatory cytokine plays an important role mediating the effects of green tea flavanols. When EGCG is topically applied to mice skin it inhibits hydrogen peroxide and nitric oxide formation both in the dermis and epidermis, following irradiation with a single dose of UVB. Photooxidative damage also affects proteins leading to protein–protein cross links or the formation of protein carbonyl derivatives. In a study with mice it was demonstrated that protein cross-linking (collagene) was diminished by green tea extract. As shown in hairless mice, treatment of EGCG diminishes skin roughness and sagginess as a result of UV exposure and protects against the loss of dermal collagen. Following topical application of green tea polyphenols, UV-induced erythema and the formation of DNA pyrimidine dimers in human skin was diminished. Pretreatment of small areas of photoprotected buttock skin of six human subjects with EGCG before exposure to a single dose of four MED prevented erythema formation. The effect was associated with a decreased generation of hydrogen peroxide and nitric oxide. UV-induced depletion of glutathione and glutathione peroxidase was restored upon treatment. In a study with 118 patients suffering from atopic dermatitis, the consumption of three cups of oolong tea (a combination of green and red tea) for 6 months decreased the severity of the disease [22].

There is evidence that regular tea consumption in general is associated with a diminished risk for squamous cell carcinomas of the skin [16]. After adjustment for several confounders, regular tea consumption was associated with an about 30% lower risk for SCC. The strongest inverse associations between SCC and tea consumption were observed in the highest categories of duration of consumption and daily amount of consumption.

Monomeric flavanols, such as (−)-epicatechin and (+)-catechin which also occur in green tea, and their oligomers, the procyanidins, represent a major class of flavonoids in cocoa and chocolate products [13]. Vasodilatory effects of flavanol-rich cocoa have been demonstrated in humans measuring pulse wave amplitude or flow-mediated dilation of the brachial artery. There is evidence from in vitro and in vivo studies that cocoa flavanols provide vasodilatory effects via NO-dependent mechanisms. It has been suggested that apart from parent cocoa flavanols, flavanol metabolites are active constituents responsible for vasodilation [10, 20].

Microcirculation within the skin is an important factor for thermoregulation, nutrient and oxygen supply, and determines skin condition and appearance. After consumption of a single dose of cocoa polyphenols provided with the intake of a cocoa powder rich in flavanols, increasing (−)-epicatechin plasma levels were observed with highest levels at 1 h [15]. With elevated concentrations, cutaneous blood flow was also increased to about 1.7-fold compared to baseline. The maximal effect was observed 2 h after ingestion of the cocoa product. However, no effects were found following supplementation with a low-flavanol cocoa powder. Very similar effects are observed with other vasoactive agents like acetylcholine or sodium nitroprusside where skin blood vessels respond to topical or systemical application . The effects are in the same range as with cocoa flavanols.

Vasodilatory activity of cocoa intake on skin was also determined in a long-term human intervention study [8]. It has been suggested that a prolonged increase in dermal blood flow is related to several other effects of long-term consumption of cocoa products on skin. In a 12-week intervention study two groups of women consumed either a high-flavanol (326 mg/day, containing 61 mg epicatechin and 20 mg catechin) or low-flavanol (27 mg/day, containing 6.6 mg epicatechin and 1.2 mg catechin) cocoa powder dissolved in 100 mL water. As measured after 6 and 12 week of supplementation with the high flavanol cocoa beverage, an increase in blood flow was in both cutaneous and subcutaneous tissues. However, no change in blood flow was found in the low flavanol group. The maintenance of skin integrity requires an optimal supply with nutrients and improved blood flow likely affects various skin properties. In the intervention study with high and low cocoa polyphenol products over 12 weeks it was further shown that ingestion of cocoa flavanols improves skin texture, mainly density, thickness, roughness, and scaling. Also skin hydration was improved, whereas transepidermal water loss was decreased. All of the observed effects were moderate but statistically significant. Additionally protection by dietary cocoa flavanols against UV-induced skin erythema was determined in this study. Changes in skin reddening after UV exposure were determined as a measure of prevention. Compared to baseline, skin response was decreased by 15% after 6 week of intervention, and the effects were even more pronounced, to 25%, after 12 weeks. No significant change in UV sensitivity was found in a group of volunteers receiving the cocoa beverage low in flavanols.

8.5 Conclusion

Flavonoids and flavonoid-rich products can protect skin against UV-induced damage at the molecular and cellular level and may as well improve overall skin quality. Regular intake or topical application confer significantly to photoprotection and help maintaining skin health by improving skin structure and function. The photoprotective effects are moderate but contribute to permanent, overall protection. They are similar to those that have been reported for other dietary constituents like carotenoids.

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Omega-3 Fatty Acids and Skin

9

Suzanne Margaret Pilkington and Lesley Elizabeth Rhodes

Core Message

› Using nutrients such as omega-3 polyunsaturated fatty acids (n-3 PUFAs) for the promotion of skin health and treatment of skin disorders is a novel concept. These bioactive fatty acids have a high safety profile and could potentially be used as an adjuvant or alternative to traditional therapy. Evidence exists to suggest that n-3 PUFA exert their protective effects in biological tissues via a range of mechanisms, from alteration of receptor mediated signaling at cellular membranes to eicosanoid signaling and transcriptional activation. A more detailed examination of the activities of n-3 PUFA in the skin is required to enable dissection of the molecular actions of these fats. This could lead to the development of targeted natural treatments in addition to enhancing our understanding of how diet impacts upon skin function.

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9.1 Introduction

Over 70 years ago, Burr and Burr discovered that exclusion of fat from the diet resulted in a new "deficiency disease" which involved reduced growth rate, reproductive failure, impaired barrier function, and scaliness of the skin [1]. This founded the concept of essential fatty acids (EFAs), i.e., fats that cannot be synthesised by higher animals and therefore must be obtained from the diet. These EFAs can be divided into two main families, the omega-6 (n-6) and omega-3 (n-3) polyunsaturated fatty acids (PUFAs). It has become known that in addition to EFA deficiency, the ratio of n-6 to n-3 PUFAs is of physiological significance, with higher n-6 PUFA levels generally associated with deleterious effects. Therefore, attention is shifting to the n-3 PUFAs and their therapeutic potential. Dietary intake of n-3 PUFAs is essential for healthy growth and development and there is substantial evidence that these fatty acids can also help prevent and/or treat disease, including cardiovascular disease, hypertension, arthritis, diabetes, and autoimmune/inflammatory conditions [2–5]. Omega-3 PUFAs show evidence of benefit in inflammatory conditions of the skin including polymorphic light eruption (PLE) and psoriasis, and also potential for prevention of skin cancer [6–10]. Evidence suggests they are involved in the regulation of many cellular processes including cell proliferation, gene expression, and apoptosis. A significant property of n-3 PUFAs is their ability to competitively inhibit metabolism of n-6 PUFAs by cyclooxygenase (COX) and lipoxygenase (LOX) enzymes, resulting in decreased levels of lipid-derived mediators of inflammation, and potentially a reduction in longer-term deleterious effects.

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9.2 Classification and Nomenclature

Fatty acids are straight chain hydrocarbons with a carboxylate (COOH) group at one end, and a methyl (CH_3) group at the other end. Nomenclature indicates the position of the first double bond in relation to the CH_3 end. Thus, in n-3 PUFAs, the first point of unsaturation is at the third carbon from the CH_3 end of the hydrocarbon chain (Fig. 9.1), and the same principle applies to fatty acids of the n-6 and n-9 families.

9.3 Sources

Higher animals do not have the capability to synthesise n-3 fatty acids, as they do not have the machinery to insert a double bond at the third carbon from the methyl end of the fatty acid chain. Only algae, plants, and certain fungi can synthesise n-3 fatty acids, while the marine animals that feed on algae elongate and desaturate these to the long-chain PUFAs, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) (Fig. 9.2). Thus, humans generally obtain short-chain PUFA from plants sources and longer-chain PUFA from marine sources.

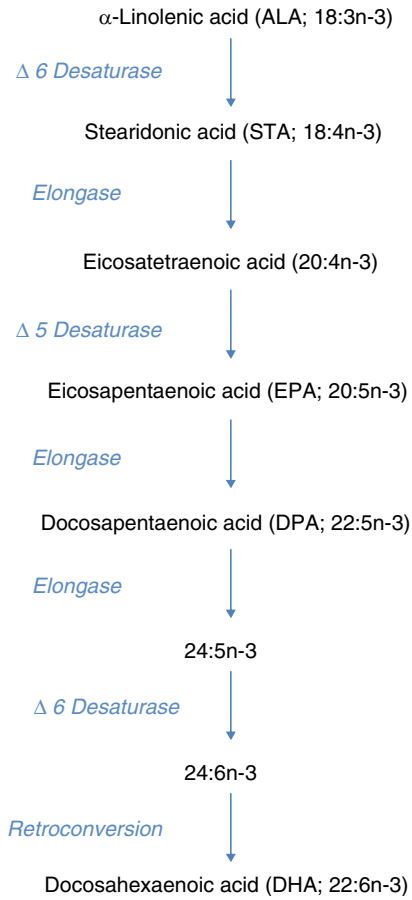

Fig. 9.2 Metabolic conversion of α-linolenic acid (ALA) to the longer more unsaturated fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)

9.3.1 Short Chain n-3 PUFAs

9.3.1.1 ALA

The primary source of α-linolenic acid (ALA;18:3n-3) in the human diet is PUFA-rich vegetable oils such as soya bean and canola oils (Table 9.1); ALA is also found in nuts, seeds, and vegetables although usually in relatively small amounts (0.1–1.7%), with the exception of

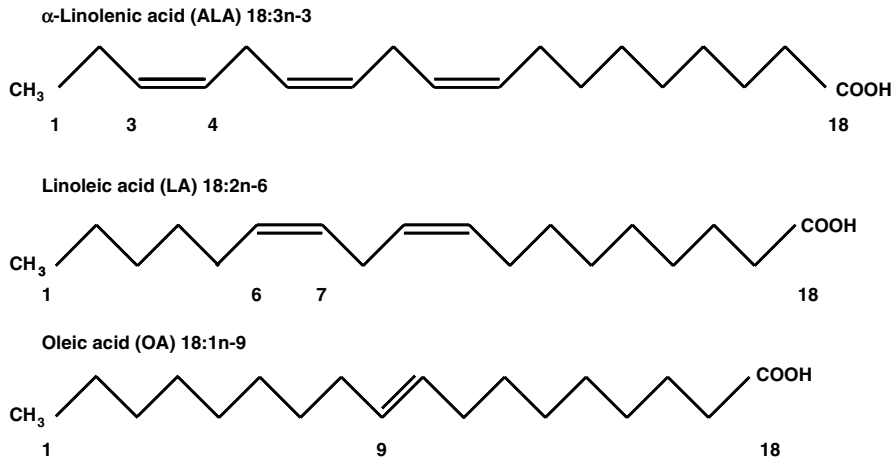

Fig. 9.1 Chemical structure of representative n-3 (α-linolenic acid), n-6 (linoleic acid), and n-9 (oleic acid) fatty acids

Table 9.1 Dietary sources of α-linolenic acid (ALA) (g/100 g)

Source	ALA g/100 g
Linseed oil	55.3 g
Canola oil	8.6 g
Soya bean oil	7.6 g
Walnut	6.8 g
Purslane	0.4 g

Table 9.2 Land and marine sources of Stearidonic acid (STA) (g/100 g)

Source	STA g/100 g
Canola oil (GMO)	23 g
Echium plantagineum	13 g
Lappula intermedia	17.7 g
Lithospermum avense	17.4 g
Ribes nigrum (blackcurrant)	3 g

butternuts and walnuts that contain 8.7 and 6.8 g of ALA per 100 g edible portion [11]. The richest vegetable source of ALA is Purslane or *Portulaca oleracea*, a wild plant native to India and the Middle East, which contains 4 mg of ALA per gram of wet plant. ALA is metabolised to EPA and DHA in mammals, however only 0.2% of plasma ALA is converted to EPA [12], most likely due to a rate limiting Δ6 desaturase enzyme [13], making ALA an inefficient source of EPA.

9.3.1.2 STA

Stearidonic acid (STA; 18:4n-3) is the immediate metabolic derivative of ALA (Fig. 9.2). There are few available dietary sources, the richest terrestrial source of STA known being the seed oil from plants of the Boraginaceae family, of which *Echium*, *Lappula*, and *Lithospernum* appear to be the best (Table 9.2) [14] (*Echium Plantaginium* seed lipids contain ~13% STA) [15]. STA is also found in blackcurrant seed oil (~3% of fatty acids), in genetically modified vegetable oils, such as canola (which can contain STA levels of up to 23%) [16], and is also present in marine algae [17]. Micro algae such as *Isochrysis,* and macro algae such as *Micelophyclus simples,* are rich sources of n-3 fatty acids, with STA comprising 26.8% and 20.1% of total fatty acids respectively [14]. Synthesis of EPA from STA has shown to be approximately four times more efficient than from ALA [18, 19].

9.3.2 Long-Chain n-3 PUFAs: EPA and DHA

EPA (20:5n-3) and DHA (22:6n-3) are the long chain, biologically potent derivatives of ALA metabolism. In contrast to ALA, they are mainly found in marine animals and oily fish, including mackerel, sardines, herrings, and salmon, where they comprise 30–50% of fatty acids (Table 9.3) [15]. EPA is also found in liverwort, ferns, mosses, and micro algae, and can comprise up to 4% of the fat of wild animals that forage on such plants. Oily fish is the principal source, but in the longer term, the overfished wild stocks may not meet the demands of the global population. In addition, there are also concerns about heavy metal contamination of fish [20, 21], however, most fish oil supplement manufacturers now use molecular distillation to remove these contaminants.

Table 9.3 Dietary sources of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (g/100 g)

Source	EPA+DHA g/100 g
Mackerel	2.5 g
Herring	1.7 g
Salmon	1.2 g
Tuna	0.4 g

9.4 Recommended Dietary Intakes

Studies of Palaeolithic nutrition suggest that our ancestors lived on a diet low in saturated fats and with approximately equal amounts of n-6 to n-3 PUFAs (1–2:1) [11]. This ratio has significantly increased in today's Western diet to ~6–15:1 [11]. A recent dietary survey of British adults aged 19–64 years revealed an average daily intake of 1.7–2.0 g/day of n-3 PUFA and 9.4–12.9 g/day of n-6 PUFA [22]. These elevated levels of n-6 PUFAs in our diet reflect modern agriculture, where crops and livestock are intensively farmed, and animals are fed on n-6 rich grains, resulting in meat and vegetable produce with higher n-6 fatty acid

Table 9.4 Recommended intakes of omega-3 (n-3) polyunsaturated fatty acids(PUFAs)

Organisation	n-3 PUFA intake	Reference
American Heart Association (AHA) 2006	Consume 2 portions (~8oz) of oily fish/week	[25]
International Society for the Study of Fatty Acids and Lipids (ISSFAL) 2004	**ALA** 0.7% energy **EPA + DHA** 0.5 g/day	[26]
UK Scientific Advisory Committee on Nutrition (SACN) 2004	**EPA + DHA** 0.45 g/day	[27]
World Health Organisation (WHO)/ Food and Agriculture Organisation (FAO) of the United Nations 2003	**EPA + DHA** 0.4–1.0 g/day	[28]
US National Academies of Science, Institute of Medicine (IOM), 2002	**ALA** 0.6–1.2% energy (up to 10% of which can come from **EPA + DHA**)	[29]
Committee on Medical Aspects of Food Policy (COMA), UK Department of Health 1994	**EPA + DHA** 0.1–0.2g/day	[30]

content [23]. This is also seen in some farmed fish [24]. Recommendations for daily intake of n-3 fatty acids vary between organisations, e.g., for EPA and DHA intakes range from 0.1 to 1.0 g/day; and there is a lack of clarity in this area (Table 9.4).

9.5 Omega-3 PUFA in the Skin

9.5.1 Omega-3 Content of Skin

Omega-3 PUFAs, principally ALA and EPA, can be detected in low levels in human skin, while the n-6 PUFAs, linoleic acid (LA), and arachidonic acid (AA) are more abundant, comprising 12% and 3.5% of epidermal fatty acids respectively [31–33]. Omega-3 and n-6 PUFA exist in both membrane bound and free fatty acid forms. Keratinocyte membrane n-6 fatty acids are found linked with ceramides in the granular layer and stratum corneum, forming lamellae that fill intercellular spaces and playing a critical role in maintenance of the epidermal water barrier [34]. Omega-3 PUFAs are incorporated into epidermal phospholipids in a specific manner, with EPA preferentially incorporated into phosphatidylcholine (PC) and lesser amounts in phosphatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidylserine (PS). In contrast, DHA is preferentially incorporated into PE.

9.5.2 Omega-3 Delivery to the Skin

Dietary n-3 fatty acids are transported in chylomicrons from the small intestine to the liver where they are processed into triglycerides. They are then transported to the tissues in the bloodstream by very low-density lipoprotein (VLDL). In addition to passive diffusion of long-chain fatty acids across the plasma membrane, fatty acid transport proteins (FATPs) with acyl CoA synthetase activity also play an important role in fatty acid uptake. Indeed, this applies to n-6 PUFA uptake and maintenance of stratum corneum (SC) lipids [35], but may also be relevant to n-3 PUFA uptake. Six mammalian FATPs are known, and in adult mouse skin, FATP1 and -3 were found to be predominantly expressed in keratinocytes [36]. FATP4 has been found to be expressed in fibroblasts, and is also localised to the stratum granulosum and stratum spinosum in murine skin [37]. Fatty acid binding proteins (FABPs) are a large family of homologous cytosolic proteins, which may also facilitate PUFA uptake and fatty acid mediated signalling. Epidermal FABP (e-FABP) expression has been demonstrated in keratinocytes, in addition to adipocytes and macrophages [38–40]. Epidermal-FABP knockout mice present skin barrier dysfunction, indicating a role for e-FABP in maintenance of the skin lipid barrier. However, the precise mechanisms of n-3 PUFA transport in the epidermis are unknown.

9.5.3 Metabolism of n-3 PUFAs

9.5.3.1 Synthesis of Long-Chain n-3 PUFAs

Long-chain n-3 PUFAs are synthesised from the parent fatty acid, ALA (18:3n-3), via a series of desaturation and elongation processes (Fig. 9.2). These occur at the endoplasmic reticulum (ER), where Δ5- and Δ6-desaturases are associated with the membrane [41]. Initially, Δ6 desaturation of ALA occurs to produce STA (18:4n-3); desaturation and elongation occur at a position close to the carboxyl terminal, resulting in preservation of the relationship between the first double bond and the methyl terminal. Subsequently, STA is elongated via the addition of two carbons to produce eicosatetraenoic acid (ETA; 20:4n-3), which is then desaturated to EPA by Δ5-desaturase. Subsequent elongations and desaturations occur, followed by a peroxisomal β-oxidation, retroconversion step where two carbons are removed and DHA is produced. However, evidence of low Δ5- and Δ6- desaturase activity in the human epidermis suggests that the longer-chain fatty acids may not be synthesised locally, and that the skin may be dependent on hepatic provision of long-chain PUFAs [13]. There is no interconversion between the n-3, n-6, and n-9 families, and they compete for the same enzymes for their metabolism. Hence, n-3 and n-6 fatty acids suppress metabolism of each other, and both suppress metabolism of n-9 fatty acids. This relationship between fatty acids is used to determine EFA deficiency by calculating the ratio of n-9:n-6 PUFAs (triene:tetraene ratio), where a ratio of 0.4 indicates deficiency [42].

9.5.3.2 Metabolism of PUFAs to Eicosanoids

Omega-3 and -6 PUFAs including EPA and AA are metabolised in the skin by COX and LOX enzymes to produce extracellular signalling molecules including prostaglandins (PGs), thromboxanes, leukotrienes, hydroxy fatty acids, and lipoxins (Fig. 9.3). Collectively these fatty acid metabolites are termed eicosanoids. At low concentrations, they play critical roles in cell homeostasis while at higher concentrations they are involved in inflammation, cell proliferation, and tumorigenesis [43–46]. Following release from membrane phospholipids by phospholipases, e.g., PLA_2 the PUFAs undergo oxidative transformation by COX and LOX enzymes [47]. Free n-3 PUFAs are metabolised to three-series prostanoids, i.e., prostaglandins, thromboxanes, and prostacyclins, and five-series leukotrienes (Fig. 9.3) that are generally less potent than AA-derived eicosanoids. The major products of EPA metabolism by COX are PGE_3, $PGF_{3\alpha}$, and PGD_3. In addition, EPA is converted via 15-LOX to 15-hydroxyeicosapentaenoic acid (15-HEPE) and lipoxins, by 12-LOX to 12-HEPE, and by 5-LOX to leukotriene B_5 (LTB_5), LTC_5, and LTD_5 [43, 48]. In the skin, DHA may also be metabolised to a range of mediators including 17-hydroxydocosahexaenoic acid

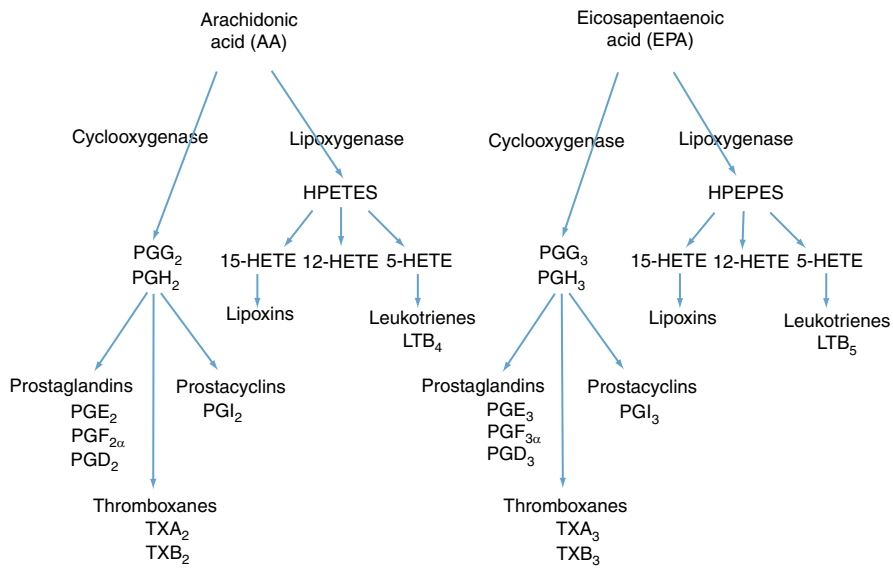

Fig. 9.3 Metabolism of EPA and AA by cyclooxygenase and lipoxygenase enzymes

(17-HoDHE) [49]. In addition to COX and LOX metabolism, PUFAs can be metabolised by cytochrome (CYP) P450 enzymes, and can also undergo non-enzymatic oxidation, resulting in production of a wider range of lipid mediators. The CYP450 4F subfamily enzymes can efficiently metabolise n-3 PUFAs to their hydroxylated derivatives [50]. Pathways of eicosanoid inactivation include ω- and β- oxidation, and conjugation with glucuronide, sulphate, and glutathione.

9.6 Activities of Omega-3 PUFAs

9.6.1 Modulation of Cell Signalling

In recent years it has been realised that lipid organisation in cell membranes is more complex than displayed in the fluid mosaic model [51]. Plasma membranes are composed of distinct microdomains, i.e., caveolae and lipid rafts rich in unsaturated fatty acids, which are recognized to play important roles in cell signalling. Incorporation of omega-3 PUFAs into these lipid microdomains increases membrane unsaturation and fluidity, impacting on the localization of signalling proteins, which in turn may alter downstream signalling events. This is illustrated in T lymphocytes where treatment with omega-3 PUFAs resulted in displacement of integral signalling proteins, subsequently inhibiting T-cell activation [52, 53]. Omega-3 PUFAs also inhibit palmitoylation of Src kinase Fyn in T cells, preventing its localization to lipid microdomains [54], and these effects are thought to be partly responsible for the anti-inflammatory properties of n-3 PUFA. Ras GTPases have also been shown to be compartmentalised to lipid rafts and caveolae and to be displaced by n-3 PUFAs [55, 56]. As ras signalling is commonly constitutively active in transformed cells, n-3 PUFAs may hold potential for anti-ras cancer therapies.

Stimulation of cells via extracellular signals results in hydrolysis of membrane phospholipids to produce secondary messengers. For example, phosphatidylinositol 4,5 biphosphate (PIP2) is cleaved by phospholipase C (PLC) to produce diacylglycerol (DAG) and inositol triphosphate (IP3), which stimulate protein phosphorylation and calcium release, respectively. DAG activates protein kinase C (PKC), which subsequently phosphorylates mitogen activated protein kinases (MAPKs) leading to induction of gene expression downstream. In lymphocytes, EPA and DHA have been shown to curtail PKC activation, inhibiting cell proliferation [57–59]. EPA has also been shown to reduce formation of platelet activating factor (PAF), a pro-inflammatory molecule [60].

9.6.2 Modulation of Gene Expression

Omega-3 PUFAs modulate expression of a range of genes, with evidence of effects on transcription factors nuclear factor kB (NFkB) and activator protein-1 (AP-1), peroxisome proliferator activated receptors (PPARs), and COX-2. Omega-3 PUFAs suppress COX-2 expression in tumour cells, [61, 62] resulting in reduced cell proliferation and reduced angiogenesis; [63] n-3 PUFAs have also been shown to suppress tumour growth via mechanisms independent of the COX pathway [64]. This is in contrast to n-6 PUFAs, which are known to increase COX-2 expression [65]. Long-chain omega-3 PUFAs have also been shown to decrease the expression of the oncogenes ras and B-cell lymphoma-2 (bcl-2), inhibiting proliferation of cancer cells and encouraging apoptosis [66, 67]. In mouse epidermal cells in vitro, EPA and DHA inhibited TPA-induced transformation by preventing activation of AP-1 [68], a transcription factor commonly activated in tumours, which promotes transcription of genes involved in cell proliferation and metastasis. Another key transcription factor influenced by changes in PUFA content of cells is NFkB. This transcription factor plays an important role in epidermal homeostasis, but is also upregulated in response to stress and inflammatory signals, transcribing many genes involved in inflammation and carcinogenesis [69, 70]. In a human promonocytic cell line, AA and PGE_2 were found to upregulate activity of NFkB, compared to EPA that had no significant effect [71]. In this instance, DNA binding of NFkB was unaffected suggesting a reduction in free AA was responsible for decreased activity [71], while other studies have suggested EPA may inhibit NFkB activation by preventing phosphorylation of inhibitor of kB-a (IkB-α) [72].

Peroxisome proliferator activated receptors (PPARs) are nuclear receptor proteins that modulate gene expression. There are three PPAR isomers, PPARα, -β/δ, and -γ, all of which are expressed in the epidermis and are involved in keratinocyte proliferation and/or differentiation [73–76]. PPARs are stimulated by peroxisome

proliferator proteins, fatty acids, and fatty acid metabolites [77–79]. In this way, these molecules are able to regulate gene expression, as once stimulated PPARs associate with peroxisome proliferator response elements (PPREs) in gene promoter regions [79]. In epithelial cells, stimulation of COX-2 expression by a range of fatty acids and eicosanoids appears to be facilitated by PPAR binding to a PPRE in the COX-2 promoter region [80]. Also, in human keratinocytes EPA and GLA are observed to upregulate COX-2 expression via PPARγ [81]. These and other studies suggest that gene expression may be regulated by the n-3:n-6 ratio in the diet.

9.6.3 Modulation of Pro-inflammatory Mediators

Omega-3 PUFAs are strongly associated with anti-inflammatory effects. A key mechanism is their behaviour as partial agonists [48], through their competition with n-6 PUFA for metabolism by COX and LOX resulting in production of less potent mediators [82]. For example, PGE_3 is significantly less effective than PGE_2 at inducing IL-6 secretion in macrophages [83]. Similarly, LTB_5 derived from EPA is a less potent chemoattractant for neutrophils than LTB_4, due to a weaker binding affinity with neutrophil receptors [84]. In addition, when neutrophils are active during inflammation the 5-HEPE product is metabolised further to 5-oxo-EPE, an eosinophil attractant, and in vitro this EPA metabolite is only one tenth as chemotactic for inflammatory cells as its analogous AA product, 5-oxo-ETE [85]. Omega-3 PUFAs also regulate expression of endothelial adhesion molecules such as vascular adhesion molecule-1 (VCAM-1), E-selectin, and inter-cellular adhesion molecule-1 (ICAM-1), reducing the ability of leukocytes to migrate across the vascular endothelium [5, 86, 87]. There is also evidence of a modulatory role for n-3 PUFAs on inflammatory cytokine production, potentially mediated via NFkB. In humans, dietary supplementation with n-3 PUFAs decreases the production of pro-inflammatory cytokines interleukin-1a (IL-1α), -1β, tumor necrosis factor-a (TNF-α), and IL-6 from mononuclear cells [88–90] and inhibits T-cell mitogenesis [91]. However, conflicting reports are seen regarding their effects on cytokine secretion and more modest intakes of n-3 PUFAs showed little effect on IL-1α, IL-1β, and TNF-α expression [92]. Omega-3 PUFAs have also shown potential in reducing expression of ultraviolet (UV)B-induced cytokines. In human keratinocytes and fibroblasts, UVB-induced expression of IL-8, a powerful neutrophil attractant, was inhibited by EPA and DHA [93]. EPA has also been shown to reduce UVB-induced IL-6 expression in human keratinocytes, but unexpectedly enhanced TNFα and IL-1α [94]. Interestingly, in human skin in vivo, n-3 PUFAs appeared to reduce the inflammatory response to UVB, but through cytokine independent mechanisms [95]. These contrasting reports may be partially explained by differences in concentrations of PUFAs employed. Moreover, results are often difficult to reproduce in vivo due to the complex homeostatic mechanisms at work in the whole organism.

Resolution of inflammation has been regarded as largely a passive process, where the cellular response decreases due to fading of the inflammatory stimulus. However, with the isolation of novel lipid mediators from inflammatory exudates, it has become apparent that resolution is a more active process [96–98]. The first family of molecules found to have anti-inflammatory and pro-resolving abilities were the lipoxins, which are lipoxygenase metabolites of AA [99]. In addition, novel compounds generated from n-3 PUFAs have been collected in inflammatory exudates in other systems, namely resolvins and protectins [97, 100, 101].

9.6.4 Modulation of Immune Function

In vitro and in vivo, n-3 PUFAs are seen to inhibit major histocompatibility unit- II (MHC II) expression on macrophages and monocytes [102, 103], decrease antigen-presenting activity to lymphocytes [104, 105], and downregulate the Th1 response associated with chronic inflammatory disease [106]. Omega-3 PUFAs may modulate immune function through effects on and independent of eicosanoid production, e.g., through influencing membrane fluidity and phospholipid signalling as discussed above. In mononuclear cells, LA, dihomo-gamma-linolenic acid (DGLA), and AA comprise approximately 8%, 10%, and 20% of total fatty acids respectively. Considerably lower levels of n-3 PUFAs are present, with negligible ALA and approximately 0.5% and 3% of EPA and DHA respectively [107]. In individuals with higher intakes of EPA and DHA, significantly elevated levels of these fatty acids are observed in association with a reduction in AA [108].

Polyunsaturated fatty acids are known to be involved in the immunological regulation of UV-carcinogenesis. Omega-6 PUFAs exert their principal effects at the promotion stage of tumorigenesis, enhancing UV-induced immunosuppression [109], characterised by a shift from a T helper 1 (Th1) to Th2 (suppressor T-cell) type response. This has been illustrated by the reduced ability of mice to reject transplanted tumours after UV irradiation [110]. Induction of EFA deficiency can halt UV-carcinogenesis, and both dietary and topical EPA have been shown to inhibit systemic and local UV-induced immune suppression, respectively [111, 112]. This could reflect decreased PGE_2 levels, as suppressor T-cell function has been shown to be PGE_2 dependent [113].

9.6.5 Modulation of Oxidative Stress

Due to their highly unsaturated structure, PUFAs, in particular n-3 fatty acids, are prone to oxidation by free radicals. This is a self-propagating reaction, resulting in formation of lipid peroxides that can be damaging to cell membranes. While susceptibility of unsaturated lipids to oxidation has raised questions as to whether elevated n-3 PUFA content could be harmful, in fact, anti-tumour activity exhibited by n-3 PUFAs has been attributed to lipid peroxidation inducing apoptotic pathways [114]. In human skin exposed to ultraviolet radiation (UVR), thiobarbituric acid reactive substances (TBARS) produced as a result of lipid peroxidation are seen to increase after supplementation with fish oil, in association with decreased erythemal sensitivity to UVR [32]. This has prompted the suggestion that n-3 PUFAs may exert protective effects by acting as a free radical buffer, protecting more vital cellular structures from damage [32, 115]. Peroxidation of the n-6 PUFA, AA, results in the production of F(2)-isoprostanes, which prove reliable indicators of oxidative stress in vivo [116, 117], and notably, mice supplemented with fish oil exhibited reduced levels of F2-isoprostanes compared to controls [118]. Inconsistent reports regarding impact of n-3 PUFAs on oxidative status may be due to differences in experimental protocols and variations in baseline fatty acid and antioxidant levels [9].

9.6.6 Modulation of Apoptosis

Evidence exists for a role of n-3 fatty acids in the modulation of apoptosis. This may occur though alteration of activity of apoptotic mediators such as bcl-2 [119, 120]. In various cancer cell lines, EPA and DHA increase apoptosis and caspase activity [121, 122]. Similar effects are reported in endothelial cells [123, 124], suggesting part of their anti-tumour activity may involve inhibition of angiogenesis. In melanoma cells, DHA stimulates cell cycle arrest and apoptosis in association with decreased phosphorylation of the tumour suppressor protein, retinoblastoma (pRb) [125]. As PUFAs are particularly susceptible to oxidation, lipid peroxidation is also a suggested mechanism of n-3 PUFA-induced apoptosis, with associations with inhibition of DNA synthesis, cell division, tumour growth, and induction of tumour cell death [126, 127]. Furthermore, n-3 PUFA-induced apoptosis can be inhibited by antioxidants [128, 129]. Increased concentrations of lipid radicals generated from cellular n-3 PUFA may result in activation of mitochondrial pathways, causing tumour cell apoptosis [130].

9.7 Beneficial Effects of Omega-3 PUFAs in the Skin

9.7.1 Photoprotection

9.7.1.1 Acute UVR Damage

Exposure to UVR induces the sunburn response in skin, clinically evident as erythema and oedema, with a dermal leukocyte infiltration observed histologically. A host of inflammatory mediators including eicosanoids are involved in regulating this response [45, 131]. Modulation of these signalling molecules through n-3 PUFA supplementation has proved effective in protecting against UVR-induced skin inflammation [8, 132]. Two studies of dietary supplementation with mixed EPA and DHA in healthy humans resulted in a decrease in erythemal sensitivity to UVR [32, 132]. This effect was further demonstrated with purified EPA (95%) in a double-blind randomised study of 42 healthy volunteers [9]. Elevated levels of EPA were observed in the skin along with reduced UVB-erythemal sensitivity, which is proposed to be attributable to the reduction in PGE_2 levels observed [9]. In addition, this study showed significant protection by EPA of UVB-induced cutaneous p53 expression and UVB-induced single strand breaks in peripheral blood lymphocyte DNA,

suggesting potential for protection against longer-term UVR effects. In a study of 13 patients with the common photosensitivity disorder, PLE, fish oil supplementation for 3 months resulted in decreased sensitivity to papule provocation and reduced PGE_2 levels in unirradiated and irradiated skin [6]. Supplementation with the short-chain n-3 PUFA, ALA, provided some protection against UVR damage in hairless mice, in association with reduced PGE_2 [133]. Little work has been performed with topical n-3 PUFAs in UVR protection, however, Puglia et al. showed that application of sardine oil (11.2% EPA, 23.6% DHA) to human skin after UVB irradiation decreased erythema by 24.5% compared to control [134].

9.7.1.2 Chronic UVR Damage

Studies in mice show a correlation between dietary intake of n-6 PUFAs and occurrence of UV-induced skin cancers, while in contrast, n-3 PUFAs offer protection. In hairless mice, corn oil rich diets, high in n-6 PUFAs, reduced latency and increased numbers of skin tumours after exposure to UVR, whereas dietary menhaden oil, rich in n-3 PUFAs, increased latency and decreased tumour multiplicity [8, 109, 135]. Interestingly, Black et al. observed that on crossing animals from n-6 to n-3 PUFA rich diets after UV treatment, tumour incidence was not ameliorated, suggesting that n-3 PUFAs may exert anti-carcinogenic effects at the UVR initiation phase [109]. In further support of the relationship between PUFAs and skin cancer, epidemiological study of the Nambour community in Australia showed a trend for association between high intakes of n-6 PUFA and incidence of squamous cell carcinoma (SCC) [104, 136]. A case-control study found a statistically significant inverse relationship between risk of non-melanoma skin cancer and fish consumption [137], and a case-control study of SCC found higher n-3 PUFA intakes were associated with reduced risk of cancer [138].

Many strands of evidence suggest that a major protective action of n-3 PUFAs in photocarcinogenesis occurs at the promotion stage, including reduction of photoimmunosuppression [139]. Angiogenesis is also inhibited by n-3 PUFAs, but enhanced by n-6 PUFAs [140, 141], and is associated with decreased expression of angiopoietin-2 and matrix metalloproteinase (MMP)-9 and a shift in eicosanoid production towards PGE_3 [141]. Cyclooxygenase-2 is commonly over-expressed in skin cancer, stimulating hyperproliferation and angiogenesis and inhibiting apoptosis [142–144]. Elevated levels of lipoxygenase products are also observed in skin cancers [145, 146]. Inhibition of COX and LOX enzymes by non-steroidal anti-inflammatory drugs has proven effective in reducing tumorigenesis in animal models [147], suggesting the possibility of application of n-3 PUFAs as a safer potential anti-cancer agent.

Repeated UVR exposure also causes the long-term damage of photoageing, characterised by deep wrinkles, uneven pigmentation and reduced elasticity of the skin. This is partially attributable to connective tissue remodelling by MMPs. Kim et al. found that addition of EPA to fibroblasts in vitro resulted in reduced MMP-1 expression, via inhibition of mitogen activated kinase (MAPK) pathways [148]. In vivo, in young human skin, topical application of EPA (2% EPA in polyethylene glycol) inhibited expression of MMP-1 and -9 and COX-2 after UV irradiation, while application of EPA to aged skin increased extracellular matrix expression in association with an increase in transforming growth factor-b1 (TGF-β1), - β2, and β3 [149].

9.7.2 Psoriasis

In psoriatic lesions, AA is seen to be elevated 20-fold compared to levels in uninvolved epidermis [150, 151]. Phospholipases A_2 and C are required for release of AA from epidermal lipid pools, and these enzymes are found to be significantly increased in psoriatic skin [152]. Lipoxygenase metabolism of AA is higher in psoriatic plaques, and this is characterised by the presence of high levels of the chemoattractant LTB_4, and 12-hydroxy eicosatetraenoic acid (12-HETE) [153, 154]. Hence, reducing the n-6/n-3 PUFA ratio might be anticipated to improve psoriasis. Ziboh et al. found that oral supplementation with EPA and DHA increased n-3 PUFA levels in serum lipids in association with a mild to moderate improvement in psoriatic skin [155]. Maurice et al. found that in psoriasis patients taking dietary fish oil, there was a small reduction in erythema and scaling and a significant reduction in LTB_4 along with an increase in the less potent mediator LTB_5 [156]. However, other studies have shown no significant improvement in psoriatic lesions after n-3 PUFA supplementation [157, 158]. Reports of the effects of topical application of n-3 PUFAs are also mixed [159–161], while a study of intravenous administration of n-3 PUFAs appeared effective

in guttate psoriasis, in association with an increase in the ratio of EPA:AA-derived leukotrienes [162]. Omega-3 PUFAs may have a role as an adjuvant therapy to UVB in the treatment of psoriasis [163].

9.7.3 Atopic Dermatitis

There are mixed reports regarding association between the n-3:n-6 PUFA ratio and atopic dermatitis (AD). In patients with AD, plasma levels of LA and ALA are seen to be normal, whilst, levels of their metabolites, AA, DGLA, EPA, and DHA have been reported to be low [164–166]. Thus it was hypothesised that activity of the Δ6-desaturase enzyme is impaired in eczema [167]. On the other hand, Laitinen et al. found that serum levels of EPA were higher in infants with atopic dermatitis than in controls [168]. A second hypothesis relates the increasing incidence of AD to the increase of n-6 PUFAs, particularly LA, in the diet in the last century. This may elevate production of $PGE_{2,}$ which subsequently skews the immune response towards the Th2 phenotype, with increased IgE production from B cells (Fig. 9.4) [169]. The reported lower level of AA in AD does not correlate with the latter hypothesis; an alternate explanation may be that there is a high rate of metabolism of AA rather than low rate of synthesis [170].

In a mouse model of AD, n-3 PUFA supplementation reduced inflammation through inhibition of Th1 and Th2 responses and through upregulation of IL-10 [171]. In a double-blind randomised study of 53 patients with atopic eczema who consumed 5.4 g of DHA daily for 4 weeks, the n-3:n-6 plasma PUFA ratio increased and there was clinical improvement in terms of decreased SCORAD (scoring of atopic dermatitis) [172]. In contrast, other studies have shown saturated fats and n-6 PUFAs to improve clinical scoring equally to n-3 PUFAs [173, 174]. These findings were attributed to increased clinician guidance and the placebo effect. Therefore, as with psoriasis, no firm conclusions can be made with respect to the benefit of n-3 PUFA supplementation.

9.7.4 Acne Vulgaris

Epidemiological studies show 79–95% of adolescents in Westernised societies suffer from acne vulgaris,

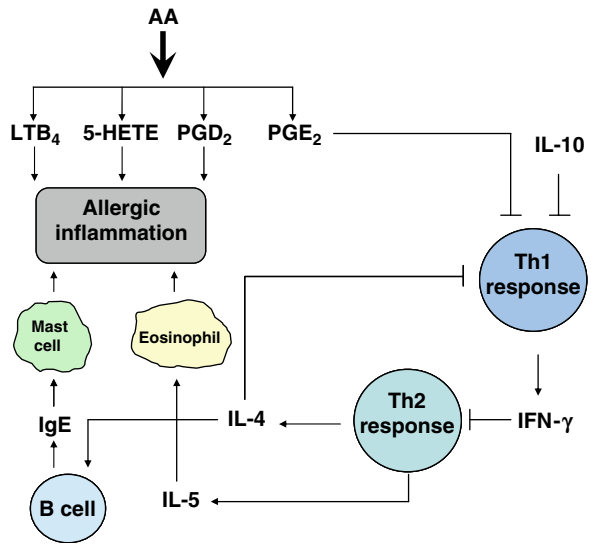

Fig. 9.4 Role of AA in allergic inflammation. Eicosanoids produced from AA metabolism are involved in the induction of atopy. PGE2 is immunosuppressive as it inhibits the Th1 type response. This promotes production of IL-4, which reinforces inhibition of Th1 cell activity and promotes the Th2 type response by stimulating release of IgE from B cells. IL-10 is also implicated in the allergic response, where it may downregulate the Th1 response. Subsequently the immune response is skewed towards a Th2 type phenotype characteristic of atopic inflammation. This diagram is modified from Ref. [105]

while there is a considerably lower incidence in non-Westernised societies, prompting some to suggest that diet may a contributory factor [175]. In addition to the low glycaemic load in non-Westernised societies, omega-3 PUFA intake is generally quite high. In Inuit populations living traditionally and consuming a large amount of fish, acne was completely absent, however, on transition to modern living, acne reached prevalence comparable to Western communities [175]. Interleukin-1 and LTB_4 have both been implicated in the initiation of acne lesions [176] and in patients with acne, treatment with zileuton, a 5-LOX inhibitor, reduced acne lesions by 70% after 3 months [177]. As n-3 PUFAs are known to be effective in reducing the production of LTB_4 this provides a feasible mechanism [178, 179].

9.7.5 Wound Healing

Wound healing is a complex process comprising inflammation, proliferation, and remodelling stages. Wounding stimulates release of pro-inflammatory cytokines, IL-1β,

IL-6, and TNF-α from neutrophils, mast cells, macrophages, and endothelial cells, which are involved in initiating wound repair [180]. Increased levels of n-3 PUFAs have been shown to affect the production of these pro-inflammatory cytokines in plasma and in skin cells [91, 94]. However, it is not clearly understood what influence n-3 PUFAs may have in the wound-healing process, and variable results have been reported in the limited number of studies carried out in *vivo* in humans. A recent double-blind randomised study in humans reported that supplementation with marine n-3 PUFAs (1.6 g EPA and 1.1 g DHA daily) for 4 weeks, resulted in an elevated in IL-1β in blister fluid and increased time to wound closure when compared to control [181]. In contrast, addition of EPA to fibroblasts increased collagen formation and recoverage area in a wound healing model in *vitro* [182]. In a mouse ear wound model, application of cod liver oil resulted in significantly faster epithelialisation and neovascularisation of wounds compared to saline treatment [183]. However, topical application of n-9 fatty acids to cutaneous wounds in mice induced faster healing than n-6 and n-3 fatty acids, with n-3 PUFAs significantly delaying wound closure [184]. Hence, there is currently little evidence to support application of n-3 PUFAs in wound healing.

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Potential Benefits of Soy for Skin, Hair, and Nails

10

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Core Messages

- Dietary consumption of soy has been shown to have beneficial effects on skin health.
- Topical applications of soy-based active ingredients may help protect the skin from photoaging and photocarcinogenesis.
- The potential benefits of soy for skin health may derive from both soy protein components and the soy isoflavones.
- Soy may support skin health through a variety of mechanisms including antioxidant functions, anti-inflammatory processes, reduction in DNA damage, suppression of extracellular matrix (ECM) proteases, and up-regulation of ECM components.
- Soy appears to have beneficial effects for hair growth and hair appearance.
- Initial information suggests that soy consumption may support stronger, healthier appearing fingernails.

10.1 Introduction

The effects of soy on diet and health have been topics of intense research for the last 20 years or more. Much of this research has suggested that soy consumption can have beneficial effects on several aspects of human health. Regular inclusion of soy and/or soy isoflavones in the diet has been reported to modestly improve plasma lipid profiles, improve bone health, reduce menopausal symptoms, enhance cognitive function, and potentially reduce the risk of breast and prostate cancers. The health benefits of dietary soy have been attributed to its isoflavones as well as to the biological actions of its constituent proteins. These potential health benefits of soy consumption have been extensively reviewed elsewhere [9, 30] and will not be discussed in this chapter.

In addition to its potential health benefits, soy-based ingredients are being used in an ever-growing number of cosmetic products. While most of these products are designed for topical application, it is becoming clearer that dietary soy consumption may beneficially impact dermatological health. In this chapter we will review the evidence regarding the potential benefits of soy and soy isoflavones for skin, nail, and hair health.

10.2 Biology of Soy

10.2.1 Soy Isoflavones

Soy has been reported to contain several potentially beneficial components, including phytic acid, saponins, isoflavones, and various protein fractions. However, the vast majority of the research to date has focused on

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soy protein in its entirety or the specific isoflavones present in soy. While soy has been shown to have a variety of health benefits related to both the protein and isoflavone components of the soybean, many of the beneficial effects of soy have been attributed to its isoflavones.

Isoflavones are phytochemicals belonging to the flavonoid class, which also includes flavonols, flavones, flavonones, anthocyanins, and proanthocyanidins. Isoflavones are structurally characterized by two benzene rings linked to a central heterocyclic pyran ring and are produced in a specific pathway of flavonoid biosynthesis [12].

The three primary isoflavones present in soy are found in either glycoside or aglycone forms. The glycoside forms include the β-glycosides (genistin, daidzin, and glycitin) as well as the acetyl- and malonyl-glycosides [47, 48]. The glycoside forms are the predominant isoflavone form in the soybean; however it is the aglycone forms (genistein, daidzein, and glycitein) that are the best studied and have reported health benefits.

After consumption, the soy isoflavone glycosides are hydrolyzed to their respective aglycone forms in the small intestine by β-glucosidases of the intestinal brush border membrane [11]. After conversion to the aglycone form, the soy isoflavones are either absorbed or further metabolized by intestinal bacteria to such metabolites as p-ethyl phenol, O-desmethylangolensin, and equol [4, 37, 58]. Conversion of daidzein to equol is of particular interest since equol has been implicated in a variety of health benefits; however, only about 30% of the population produces equol [39]). The bioavailability of the isoflavones is highly variable and may depend on a number of factors including form of soy intake, intestinal microflora populations, and inter-individual differences [9]. The metabolism, absorption, and bioavailability of soy isoflavones are thoroughly reviewed elsewhere [33, 38, 59].

10.2.2 Mechanisms of Action

The isoflavones in soy have a variety of biological actions. They are probably best known for their ability to bind estrogen receptors (ER), especially in regard to human health benefits. However, soy isoflavones also have a number of non–ER-mediated biological functions.

10.2.2.1 Estrogen Receptor Binding

The isoflavones present in soy are able to both bind and activate both ERα and ERβ [20, 29], though with much less potency than estradiol. It has been demonstrated that the binding of the soy isoflavones to ERβ is more potent than their ability to bind ERα [20, 29]. It has been suggested that the soy isoflavones may act more like a selective estrogen receptor modulator (SERM) than an estrogen since genistein binds to the ERs more like raloxifene, a SERM, than estradiol [36]. In addition to potentially having different ER-mediated actions at different tissue sites, isoflavone actions may be affected by the estrogen environment. It has been suggested that isoflavones may act as estrogen antagonists in a high estrogen environment, but act as estrogen agonists in a low estrogen environment [17]. Many of the purported health benefits of soy, especially in relation to menopausal symptoms, osteoporosis, and cardiovascular disease, have been suggested to be due to the isoflavones' ability to bind to ERs.

10.2.2.2 Non–ER Binding Activities

While we generally think of the ER-mediated actions of soy and soy isoflavones first, soy and/or soy isoflavones can affect biological systems in a variety of other ways. Genistein, the predominant soy isoflavone, has long been known to be a potent tyrosine kinase inhibitor [2] and as such may inhibit cell proliferation and differentiation. Despite genistein's ability to inhibit tyrosine kinase, it has been postulated that this mechanism of action may be irrelevant to genistein's potential health benefits due to the doses required to observe these effects [26].

It has also been reported that soy and soy isoflavones have antioxidant functions, which may be partly responsible for the health benefits of soy. Research has demonstrated that soy isoflavones can reduce lipid peroxidation [5, 56], scavenge oxidative free radicals [34], reduce oxidative damage to DNA [57], and up-regulate the expression of antioxidant genes [7]. However, not all studies have supported the antioxidant functions of soy. Vega-Lopez et al. [46] reported that soy has minimal antioxidant functions.

Soy isoflavones may also suppress some steroidogenic enzymes. Early studies demonstrated that soy isoflavones can weakly inhibit aromatase [1, 31], the

steroidogenic enzyme responsible for the conversion of androgens to estrogens. Other studies have demonstrated that soy isoflavones inhibit 3β- and 17β-hydroxysteroid dehydrogenase [21, 25], 5α-reductase [14], and additional steroidogenic enzymes. These enzyme inhibitory actions of the soy isoflavones may have beneficial effects in regard to cancer risk.

10.3 Soy for Skin Care

10.3.1 In Vitro Studies

The vast majority of in vitro research on the potential benefits of soy for skin health to date has examined the effects of soy isoflavones on skin aging and photocarcinogenesis, both of which have been attributed to solar ultraviolet (UV) radiation exposure. Liu et al. [24] reported that 10-μM genistein treatment of UVA-exposed human epidermoid carcinoma cells suppressed the production of 8-hydroxy-2′-deoxyguanosine (8-OHdG), a form of oxidative DNA damage [24]. The ability of genistein to scavenge free oxygen radicals was thought to be the mechanism by which it was able to reduce oxidative DNA damage in this system.

Recent research has confirmed the antioxidant and DNA protective effects of soy isoflavones. High doses of genistein (>100 μM) have been shown to block UV irradiation-induced apoptosis in human epidermal carcinoma A431 cells [10]. The protective effect of genistein was likely due to a reduction in intracellular oxidative stress since genistein pretreatment suppressed the UV irradiation-induced increase in intracellular reactive oxygen species (ROS). Moore and coworkers [28] demonstrated in a full thickness, three-dimensional human reconstituted skin model that pretreatment with genistein (10, 20, and 50 μM) caused a dose-dependent reduction in UVB-induced DNA damage and a preservation of the skin's histological architecture. These authors also reported that genistein treatment suppressed UVB-induced reduction in proliferating cell nuclear antigen (PCNA), suggesting that genistein dose-dependently maintained the skin cells' ability to proliferate and repair in the face of UVB radiation. It has recently been reported that soy isoflavones possess antioxidant properties in skin through inhibition of UVB-induced hydrogen peroxide (H_2O_2). Huang et al. [16] demonstrated that treatment with 10-mM genistein, daidzein, or an aglycone mixture of isoflavones protected human keratinocytes from photodamage by inhibiting UVB-induced H_2O_2 production, while treatment with glycitein only provided a non-significant inhibition.

Soy isoflavones have also been reported to support skin health by additional mechanisms, which may or may not be related to their antioxidant properties. Trompezinski et al. [42] demonstrated that genistein has anti-inflammatory properties in normal human keratinocytes. Kim and colleagues [19] reported that treatment of human fibroblasts with a soy isoflavone extract consisting of predominantly genistein and daidzein significantly reduced UV-induced matrix metalloproteinase-1 (MMP-1) secretion, which is involved with collagen breakdown in the skin [18]. Both purified genistein and soy extracts have been shown to have positive effects on primary human dermal fibroblasts [41]. These investigators reported that treatment with genistein at a dose of 100 ng/ml increased collagen synthesis in human fibroblast cell cultures. Fibroblast collagen synthesis was also enhanced by treatment with a soy extract containing approximately 20% isoflavones and soy extract containing 11% isoflavones plus 14% soy saponins. Sudel et al. [41] further reported that hyaluronan and sulfated glycosaminoglycan synthesis was increased in dermal fibroblasts treated with the soy isoflavone + saponin extract, suggesting that soy's benefits for skin health may arise from more than just the isoflavones.

Other studies have also suggested that the benefits of soy for skin health do not appear to be due solely to the presence and activity of the isoflavones. Andre-Frei et al. [3] demonstrated that the application of a soy peptide to an in vitro skin model resulted in a significant increase in hyaluronic acid and a tendency for increased collagen production. Furthermore, treatment of human skin organ cultures with a soy extract (4–40 μg/ml) reduced retinoid-induced epidermal hyperplasia by up to 41% [45]. Additional results from this latter study showed that the soy extract (40 μg/ml) enhanced type I procollagen production by 50% in dermal fibroblasts.

Despite the growing evidence of potential benefits of soy isoflavones, only recently has the topical delivery and absorption of soy isoflavones been characterized [16]. These authors examined the topical delivery of genistein and daidzein along with an aglycone mixture of isoflavones in the skin of nude mice in vitro and in vivo under varying conditions. Absorption of

isoflavones prepared in an aqueous buffer was greater at pH 6 than at pH 10.8 and the absorption of genistein was greater than the uptake of daidzein in vitro. Genistein was absorbed more efficiently in an aqueous buffer than in a soybean oil solution. Similar results were observed in vivo. Interestingly, when an aglycone mixture of soy isoflavones (genistein, daidzein, and glycitein) was analyzed, the absorption of daidzein increased such that its uptake was greater than that of genistein [16].

Overall, these data suggest that various components of soy, including isoflavones, saponins, and soy peptides, may have a variety of beneficial effects on skin health. The mechanisms of these effects appear to be varied and may include antioxidant potential, suppression of inflammatory processes, production of extracellular matrix (ECM) components, and suppression of enzymes involved in ECM breakdown.

10.3.2 Topical Application

A number of studies have been conducted to determine the potential protective effects related to the topical application of soy components. The majority of these studies have focused on the potential benefits of soy against photocarcinogenesis. It has been reported that topical application of genistein inhibits both the initiation and promotion of skin tumor formation [50]. In this study, tumor incidence and multiplicity were reduced by 20% and 50%, respectively, by the daily topical application of genistein (10 μmol) for 1 week prior to 7,12-dimethylbenz[a]anthracene (DMBA) treatment. Furthermore, genistein inhibited 12-*O*-tetradecanoyl phorbol-13-acetate (TPA)-induced tumor promotion by up to 75% in mice in a dose-dependent fashion [50]. A more recent study by Wei and coworkers [51] reported that UVB radiation-induced initiation and promotion of photocarcinogenesis was inhibited by topical administration of genistein (1 and 5 μmol) in hairless mice. While the majority of research on topical application of soy isoflavones has focused on genistein, there is evidence that other soy-related isoflavones may have topical benefits for skin health. Widyarini et al. [54] demonstrated that daily topical applications of equol, a metabolite of the soy isoflavone daidzein, reduced the proportion of tumors progressing from benign papillomas to malignant squamous cell carcinomas and reduced the size of these carcinomas. This study also demonstrated that the topical application of equol (10 μmol) lengthened tumor latency and reduced tumor multiplicity.

There appear to be several different mechanism(s) responsible for the protective actions of soy on photocarcinogenesis. Wei et al. [50] reported that genistein treatment dose-dependently reduced DMBA-induced DNA adduct formation and TPA-induced hydrogen peroxide formation. It has also been demonstrated that topical application of genistein (10 μmol) suppresses UVB-induced expression of *c-fos* and *c-jun* proto-oncogenes, likely through inhibition of UVB-induced epidermal growth factor receptor phosphorylation [49]. Other mechanisms by which genistein might protect against skin cancer include inhibition of UV-induced events including suppression of pyrimidine dimer formation and 8-hydroxy-deoxyguanosine (8-OHdG) production, inhibition of PCNA expression, reduction of MAP kinase phosphorylation, and the prevention of various damages to the skin (cutaneous ulceration, apoptosis, cleavage of poly(ADP-ribose) polymerase) [40, 51]. Equol has been shown to protect against solar-simulated UV radiation (SSUV)–induced photocarcinogenesis by reducing contact hypersensitivity, inflammatory edema, DNA damage, and epidermal hyperplasia [52, 53, 55]. The upregulation of metallothionein expression by equol may be partly responsible for the suppression of SSUV-induced epidermal hyperplasia and contact hypersensitivity [55].

In addition to potential protection against skin photocarcinogenesis, soy isoflavones have been reported to have beneficial effects on photoaging. Wei et al. [51] demonstrated that topical application of 5-μmol genistein to mouse skin 60 min prior to exposure to UVB radiation blocked UVB-induced sunburn. Furthermore, these authors reported that application of genistein both before and after chronic exposure to UVB radiation reduced photodamage. Topical application of genistein (5 μmol) also suppressed UVB-induced erythema in human subjects. The protective effect of genistein against sunburn was greater when it was applied prior to UVB exposure since both erythema and discomfort were alleviated, while application after UVB exposure improved discomfort only [51]. Topical application of genistein provided similar

benefits to hairless mice treated with 2 minimal erythema dose of UVB radiation [8]. In this study, treatment with 5-μmol genistein reduced UVB-induced sunburned cells (44% and 20% by pre- and post-treatment, respectively), reduced leukocyte numbers (8% and 11% by pre- and post-treatment, respectively), and restored levels of the intracellular adhesion molecule E-cadherin (within 19% and 25% of unexposed levels by pre- and post-treatment, respectively). Topical application of individual isoflavones onto the skin of weanling piglets has also been shown to protect against photodamage. Treatment with 0.5% solutions of genistein substantially reduced erythema at 2, 3, 4, and 5 MEDs, while a 0.5% solution of daidzein had the same benefits at 2, 3, and 4 MEDs [23]. This study also reported that 0.5% solutions of genistein and daidzein suppressed sunburn cell formation compared to controls. Additionally, topical application of equol has also been shown to reduce SSUV-induced epidermal hyperplasia, mast cell numbers, elastosis, collagen degradation, and glycosaminoglycan deposition in mice [32].

These skin health benefits are apparently not limited to just isoflavones derived from soy. Topical application of a 2% soy extract has been shown to significantly increase the number of dermal papillae in human skin, suggesting a rejuvenation of the dermal-epidermal junction [41]. More recently, a standardized soy extract containing non-denatured soybean trypsin inhibitor (STI) and non-denatured Bowman-Birk inhibitor (BBI), two soy peptides with protease activities, has been shown to improve skin texture and tone, reduce fine lines, and improve overall appearance as early as 2 weeks after topical application to human facial skin [22].

10.3.3 Dietary Consumption

Despite the mounting in vitro and topical evidence elucidating the potential benefits of soy for skin care, little research has been done to examine the dietary benefits of soy for skin health. Kim et al. [19] orally administered a soy extract to mice (500 mg/kg of body weight per day) for 4 weeks and exposed them to UV radiation three times per week. Skin roughness was significantly improved by the administration of the soy extract, while transepidermal water loss, fine wrinkles, and UV radiation-induced thickening of the epidermis were non-significantly reduced [19]. A separate study demonstrated that adding genistein to the drinking water for 27 weeks (starting 2 weeks prior to chronic UVB exposure) resulted in a reduction of mouse skin photocarcinogenesis [51].

We recently conducted a pilot study examining the effects of dietary soy consumption in postmenopausal women 50–65 years of age with mild to moderate photoaging [13]. In this study, participants consumed one soy protein shake (20 g soy protein with ~160 mg total glycoside isoflavones) daily for 6 months. Study subjects were examined by a board-certified dermatologist to assess the health and appearance of their skin, hair, and nails at baseline, and at 3 and 6 months. Our study demonstrated that the addition of soy to the diet on a daily basis might support a number of skin benefits (Table 10.1). Improvements from baseline were significantly greater in the soy group compared to the control

Table 10.1 Change from baseline in facial skin severity scores[a] for skin appearance parameters in postmenopausal women consuming soy protein shakes or their regular dietary pattern for 6 months

Skin parameter	3 months		6 months	
	Control	Soy	Control	Soy
Roughness	−0.30 ± 0.23	−0.88 ± 0.26	−0.50 ± 0.30	−1.00 ± 0.26
Wrinkling	−0.15 ± 0.11	−0.25 ± 0.11	−0.20 ± 0.17	−0.73 ± 0.15[b]
Flaking	−0.20 ± 0.24	−0.81 ± 0.25[b]	−0.65 ± 0.30	−1.07 ± 0.23
Discoloration	0.05 ± 0.05	−0.19 ± 0.10[b]	−0.15 ± 0.21	−0.40 ± 0.13[b]
Overall appearance	−0.20 ± 0.12	−0.56 ± 0.13[b]	−0.35 ± 0.23	−1.27 ± 0.15[b]

[a]Data is shown as mean ± sem.
[b]Represents significant change from baseline score; $p < 0.05$.

group (no dietary intervention) for facial skin flaking, discoloration, and overall appearance after 3 months. Similarly, improvements were observed after 6 months for wrinkling, discoloration, and overall appearance of facial skin [13].

In a subsequent study [6] we hypothesized that supplementation of dietary soy to overweight, premenopausal women as part of a meal replacement diet plan would improve the health and appearance of the skin by normalizing skin pigmentation and reducing wrinkles. When comparing change from baseline between treatment groups, a statistically significant difference in skin wrinkling was observed at 3 months with the soy group exhibiting a greater decrease in wrinkling than the control (milk protein) group. No other treatment differences were observed at either 3 or 6 months.

In contrast, numerous benefits were observed when the data were examined within individual treatment groups and compared to baseline values. Dietary supplementation with either soy or milk protein for 3 months resulted in substantial improvements in skin roughness and desquamation; however, skin discoloration was significantly improved only in the soy protein group. Skin desquamation, roughness, and discoloration were each significantly improved by dietary supplementation with both soy and milk protein shakes after 6 months (Table 10.2). In contrast, only soy protein supplementation significantly improved skin wrinkling and erythema after 6 months compared to baseline. These data suggest that both soy and milk proteins have multiple benefits for facial skin appearance; however, only dietary soy provided benefits for erythema and wrinkling [6].

10.4 Soy for Hair Care

In addition to the benefits of soy and/or soy isoflavones for skin care, recent research has suggested that soy may be beneficial for hair health as well. McElwee et al. [27] demonstrated that dietary consumption of soy oil reduced the incidence of alopecia areata. In this study, the incidence of alopecia areata in soy oil–treated mice was 86%, 39%, and 18% in mice receiving 1%, 5%, and 20% soy oil, respectively. These authors also reported that only four of ten mice injected with genistein developed alopecia areata compared to nine of ten control mice [27]. In neonatal rats, soymetide-4, an immunostimulating peptide from soy protein, inhibited chemotherapy-induced alopecia [43]. It has been suggested that the soymetide-4 mechanism of action may be through prostaglandin E_2 suppression of hair follicle apoptosis [44]. Co-administration of soy isoflavones with capsaicin has been shown to have hair health benefits in both mice and human subjects [15]. In mice, subcutaneous treatment with a combination of isoflavones and capsaicin for 4 weeks promoted hair re-growth to a greater extent than capsaicin alone. Oral administration of capsaicin plus soy isoflavones for 5 months promoted hair growth in 64.5% of human subjects with alopecia compared to only 11.8% of subjects consuming the placebo treatment. While the mechanism by which these benefits were achieved remains somewhat unclear, treatment with capsaicin plus isoflavones increased dermal and serum insulin-like growth factor-1 (IGF-1) levels in mice and human subjects, respectively, suggesting that these benefits were potentially due to the increases in IGF-1 [15].

Table 10.2 Facial skin severity scores[a] for skin appearance parameters in premenopausal women consuming milk protein or soy protein shakes for 6 months

	Milk protein			Soy protein		
	Baseline	3 months	6 months	Baseline	3 months	6 months
Erythema	2.20 ± 0.34	2.00 ± 0.32	2.00 ± 0.29	2.40 ± 0.32	1.75 ± 0.30	1.40 ± 0.27[b]
Desquamation	2.35 ± 0.20	1.46 ± 0.27[b]	1.56 ± 0.24[b]	2.70 ± 0.19	1.94 ± 0.17[b]	1.20 ± 0.20[b]
Roughness	2.85 ± 0.21	1.85 ± 0.25[b]	1.78 ± 0.22[b]	3.45 ± 0.15	2.25 ± 0.21[b]	1.53 ± 0.24[b]
Wrinkling	2.25 ± 0.14	2.46 ± 0.14	2.00 ± 0.24	2.75 ± 0.12	2.44 ± 0.13	1.80 ± 0.22[b]
Discoloration	3.20 ± 0.20	2.69 ± 0.21	2.00 ± 0.29[b]	3.30 ± 0.13	2.63 ± 0.18[b]	2.00 ± 0.24[b]

[a]Data is shown as mean ± sem. Severity scores used were: 0 (none), 1 (slight), 2 (mild), 3 (moderate), and 4 (severe).
[b]Represents significant change from baseline score; $p < 0.05$.

Soy has also been reported to have some hair-related cosmetic benefits. In mice, topical application of STI and BBI as well as application of soymilk containing these protease inhibitors delayed hair growth and reduced hair shaft length, hair follicle size, hair shaft thickness, hair bulb diameter, and hair pigmentation [35]. These investigators also demonstrated similar results in human subjects in whom topical application of soymilk containing STI and BBI to facial and leg skin reduced the length and thickness of hair shafts and reduced hair growth [35].

Draelos and colleagues [13] reported that hair appearance in postmenopausal women was improved after 3 and 6 months of regular soy consumption. Compared to the control group (subjects maintaining their normal dietary pattern), improvements from baseline values after 3 months were significantly greater in the soy group for hair roughness, manageability, and overall appearance. The soy group also saw marked improvements in hair roughness, dullness, and overall appearance from baseline compared to the control group after 6 months. In premenopausal women, dietary soy consumption for 6 months significantly reduced hair roughness and manageability scores [6]. These data suggest that dietary supplementation with soy protein may have beneficial effects on hair appearance in women, though postmenopausal women appear to receive greater benefits than premenopausal women.

10.5 Soy for Nail Health

To our knowledge, very few studies have examined the effect of dietary soy consumption on fingernail health and appearance. A preliminary study in postmenopausal women suggests that consumption of soy protein shakes for 6 months may lead to an improvement in fingernail health [13]. These investigators reported that compared to a non-intervention control group, improvements from baseline were observed for nail roughness, ridging, flaking, splitting, and overall appearance in women consuming soy protein. Additionally, parameters of fingernail appearance (roughness, ridging, flaking, and splitting) were all significantly improved after 3 months of soy consumption compared to baseline in premenopausal women [6]. While improvements were observed in the control (milk protein) group at 3 months in this study, these did not reach statistical significance. In contrast, both the soy protein and milk protein groups exhibited significant improvements in each parameter of nail appearance after 6 months compared to baseline. These data suggest that while dietary supplementation with both soy protein and milk protein can support improved fingernail health, improvements may appear earlier with soy protein supplementation.

10.6 Summary and Conclusion

It is becoming clearer that topical application of personal care products is only one way to protect our appearance and health as we age. While much of the research on soy and skin care to date is based on topical application of soy components, an emerging body of evidence supports the potential benefits of dietary soy consumption for skin health and appearance. The majority of this emerging research has been conducted in animal models; however, the data supports the potential dermatological health benefits of soy and various individual soy components. Our pilot studies [6, 13] are some of the first to demonstrate the potential benefits of dietary soy consumption for skin, hair, and nail appearance in women. Overall, these data indicate that dietary soy (protein and/or isoflavones) consumption can improve the appearance of the skin, hair, and nails and that dietary soy consumption may complement topical application of soy-containing products to provide a full inside–outside approach to personal care.

Take-Home Pearls

- Regular dietary consumption of soy may support skin health by protecting the skin from photodamage.
- Adding soy to the diet on a regular basis may support healthier appearing, more manageable hair.
- Fingernail health as assessed by ridging, flaking, splitting, and roughness may be improved by the addition of soy to the diet.

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Photoprotection by Nicotinamide

11

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Core Messages

- Nicotinamide, the amide form of vitamin B3, is particularly found in foods such as yeast, lean meats and fish, nuts and legumes.
- Nicotinamide is currently used for a variety of dermatological applications, with little or no toxicity even at high doses.
- Nicotinamide reduces ultraviolet (UV)-induced immunosuppression and photocarcinogenesis in mice.
- Both topical and oral nicotinamide protect against the immune suppressive effects of UV radiation in humans, but do not affect the erythemal (sunburn) response to UV exposure.
- Nicotinamide is likely to exert its photoprotective effects *via* regulation of cellular energy metabolism.

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11.1 Nicotinamide Plays a Key Role in Cellular Energy Metabolism

Nicotinamide is the primary precursor of nicotinamide adenine dinucleotide (NAD) [1], which is required for the manufacture of adenosine triphosphate (ATP) in the citric acid cycle (Fig. 11.1). Ultraviolet (UV) irradiation causes both DNA damage and depletion of cellular energy (NAD) [2], which is required for efficient DNA repair. Following UV irradiation, cellular NAD content is an important determinant of cell survival [2]. Human skin cells with reduced levels of NAD have a lower survival rate and higher genomic instability following UV exposure [2], whereas increased intracellular NAD is associated with enhanced protection against photo-oxidative stress [3]. As the precursor of NAD, nicotinamide would be expected to enhance DNA repair in UV-irradiated cells. At low concentrations (less than 3 mM), nicotinamide does enhance repair in UV-irradiated, repair-proficient cell lines, although with higher nicotinamide concentrations (5 mM), DNA repair returned to control levels [4].

NAD is also the sole substrate of nuclear poly-ADP ribose polymerase (PARP). PARP is a multifunctional enzyme with roles in DNA repair, genomic stability, and regulation of p53 gene expression [5]. PARP-deficient cells exposed to UV radiation have delayed DNA base-excision repair and higher rates of cell death [6, 7], whilst PARP-deficient mice exhibit genomic instability even in the absence of noxious stimuli [6]. DNA damage signals PARP binding to both single- and double-stranded DNA breaks. Upon binding PARP catalyses the cleavage of NAD^+ into nicotinamide and ADP ribose, which are used to synthesize branched nucleic acid-like polymers (poly-ADP ribose) [6]. At a transcription level PARP regulates the expression of a

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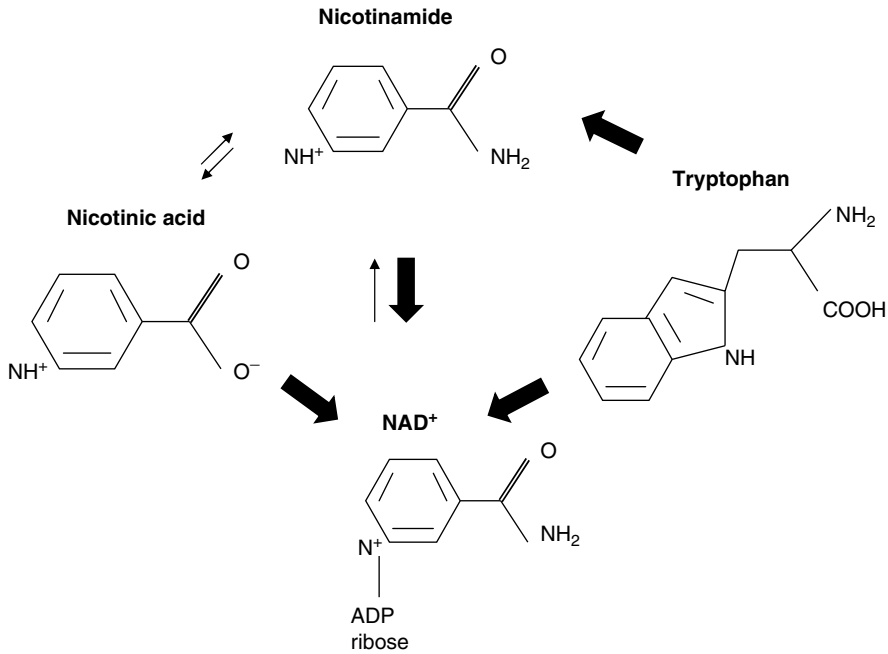

Fig. 11.1 Nicotinamide may be derived directly from food sources, or from the conversion of dietary tryptophan. Nicotinamide, tryptophan, and nicotinic acid are metabolised via distinct pathways for production of nicotinamide adenine dinucleotide (NAD+), essential in adenosine triphosphate (ATP) production and cellular energy metabolism (Adapted from [78]).

variety of proteins including inducible nitric oxide synthase (iNOS), intercellular adhesion molecule 1 (ICAM-1), major histocompatibility complex class II (MHC-II) and NF-κB mediated transcription, as well as playing a role in regulating replication, differentiation, and telomerase activity [8].

UV-induced DNA damage is a known trigger for UV-induced immunosuppression [9] and also stimulates PARP activity, utilizing NAD. Overactivation of PARP can, however, lead to NAD and ATP depletion, glycolytic blockade, and energy failure, which can cause cell death [5, 10]. Nicotinamide acts on this pathway by increasing cellular NAD and hence energy levels, and also by directly inhibiting PARP [8]. Hence nicotinamide may enhance postirradiation DNA repair by providing cells with adequate energy levels and preventing PARP overactivation.

11.2 Sources of Nicotinamide

Nicotinamide is a white, water or glycerine-soluble powder [11], which is commercially available as single ingredient and multivitamin tablets. The recommended daily adult intake of nicotinamide is ~15 mg [12] and typical supplement doses range from 20 to 500 mg daily. Niacin, or nicotinic acid, is a common dietary source of nicotinamide, to which it is converted in vivo [13] (Figure 11.1).

Foods rich in niacin and nicotinamide include meats, legumes, nuts, grains and cereals, coffee and tea [14]. Many breads, cereals, and dairy products are niacin fortified [15]. Nicotinamide is also synthesised endogenously in the liver from the amino acid tryptophan, which comprises ~1–2% of the protein in a range of foods such as eggs, dairy products, fish, meat, and soybeans [1]. This conversion requires the presence of vitamin B2 and B6, and can produce half the required daily amounts of niacin [15].

11.2.1 Nicotinamide Deficiency

Nicotinamide deficiency, or pellagra, may result from poor nutrition, malabsorption syndromes, abnormalities in tryptophan metabolism [16], or medications such as isoniazid which competitively inhibit niacin activity or tryptophan conversion to niacin [15]. Pellagra (*Italian:* "rough skin") is characterized by diarrhoea, dementia, and a striking photosensitive dermatitis and can be fatal if severe and untreated [15]. Treatment of pellagra usually requires 300–500 mg of nicotinamide daily (i.e., ~5–8 mg/kg/day). Relative nicotinamide deficiency is also frequently observed in patients with HIV infection, likely as a result of increased tryptophan oxidation [17]. These patients often exhibit unusual photosensitivity dermatoses [18].

11.3 Pharmacology

11.3.1 Pharmacokinetics

Nicotinamide is readily absorbed by the gastrointestinal tract, and then enters erythrocytes by facilitated transport [19]. Serum concentrations usually peak within an hour of oral ingestion [20] but with large inter-subject variations in absolute peak plasma concentrations and the time taken to peak concentrations [21–25]. Nicotinamide is then widely distributed in all tissues with a metabolic half-life of ~45 min, before undergoing hepatic metabolism [20] and renal excretion [11]. The half-life in skin is as yet unknown. Approximately, 10% of topically applied nicotinamide can be systemically absorbed through human skin [11], depending on the vehicle used.

11.3.2 Adverse Effects

Nicotinamide is well tolerated in doses up to ~80 mg/kg/day [26]. Higher doses have been associated with reports of nausea and vomiting, headache, fatigue, and dizziness [22, 24, 27–29] but with no clear relationship between ingested dose, peak blood level, or body weight, and adverse effects [27, 30]. The vasodilatation and flushing seen with nicotinic acid is not observed with nicotinamide [31], which has no effect on blood pressure, pulse rate, or body temperature [23]. Transient abnormal liver function tests and reversible renal failure have been reported when nicotinamide was used in combination with other drugs such as tetracycline, but not when nicotinamide was given as a sole agent [29, 32, 33]. Nicotinamide is an inhibitor of human P450 enzymes, which are responsible for the metabolism of more than 80% of pharmaceuticals [34], but the clinical significance of this potential for drug interaction is unclear. Nicotinamide crosses the placenta [12], and is not teratogenic in mice [11], but the teratogenic effects of high-dose nicotinamide supplementation in humans are not known.

Nicotinamide is currently found at concentrations varying from 0.001% to ~3% in a range of cosmetic, hair, and skin care preparations. Studies in both rodents and humans have found that topical nicotinamide is neither an irritant nor a sensitiser at tested concentrations of up to 10% [35].

11.4 Nicotinamide in Dermatology

Oral nicotinamide is used in combination with tetracyclines as an adjunctive, steroid-sparing treatment for autoimmune blistering disorders such as bullous pemphigoid and, less commonly, pemphigus [29, 32, 33, 36–40]. The exact mechanism of action of nicotinamide in these conditions is unclear; however, it has been postulated that nicotinamide inhibits polymorphonuclear cell and eosinophil chemotaxis and also blocks IgE-mediated mast cell degranulation and histamine release [41]. Successful systemic therapy of necrobiosis lipoidica has also been reported [42], while topical nicotinamide has shown efficacy in the treatment of acne [43], rosacea [44], and hyperpigmentation [45].

By increasing stratum corneum glycosylceramide and sphingomyelin synthesis, nicotinamide was found to improve epidermal barrier function [46], reducing transepidermal water loss by 27% compared to vehicle [46]. In a left–right comparison study of 2% nicotinamide cream and petrolatum, nicotinamide significantly decreased transepidermal water loss and increased stratum corneum hydration after 4 and 8 weeks of treatment in 28 patients with atopic dermatitis [47].

11.5 The Induction of Skin Immunity Is a Dynamic Process

The induction of an effective immune response is a highly dynamic process with high energy requirements (recently reviewed by Halliday and Rana [48]). Nicotinamide is necessary for ATP production [1]. Although the exact requirement for nicotinamide in immunity has not been adequately determined, these cellular processes could not occur without ATP. First, contact with antigen must result in the activation of the small number of antigen-specific lymphocytes present in the body of an unimmunised (immunologically naïve) individual. Many of these activated lymphocytes develop into long-lived memory lymphocytes so that upon second contact with the same antigen, considerably larger numbers of antigen-specific lymphocytes are present. Secondary immunity then occurs more rapidly and vigorously. In both cases, cellular stress and inflammation cause keratinocytes to produce a large array of cytokines and chemokines to signal that the skin is under immune attack. This activates dendritic cells and sets up an environment conducive to immunity.

Local dendritic antigen-presenting cells, such as Langerhans cells (LC) in the epidermis and dermal dendritic cells (DDC) then become activated. LC need to produce proteolytic enzymes to enable them to break down and pass through the basement membrane, so that both the LC and DDC can migrate through the dermis, enter dermal lymphatics, and travel to the draining lymph nodes. During this migration they functionally mature in order to optimally activate T lymphocytes, and produce cytokines, adhesion molecules, and co-stimulatory molecules that are essential for this process. Meanwhile, dynamic changes occur in the lymph node, which undergoes "shut down" whereby cells may still enter the lymph node but their egress is inhibited.

T-cell activation is then a dynamic process with massive amounts of cell division occurring over a period of only several days. The activated T cells then need to migrate from the lymph node into the circulation from where they migrate across endothelial cells into the dermis. Only then are they able to orchestrate destruction of the antigen (Fig. 11.2). This dynamic process, involving activation of multiple cell types, massive amounts of cell division, implementation of new functional pathways, and protein production for signalling and new cellular functions, in addition to migration, cannot occur without an abundance of cellular chemical energy in the form of ATP. This in itself without the additional stress of UV radiation places substantial pressure on reserves of cofactors for ATP production, such as nicotinamide.

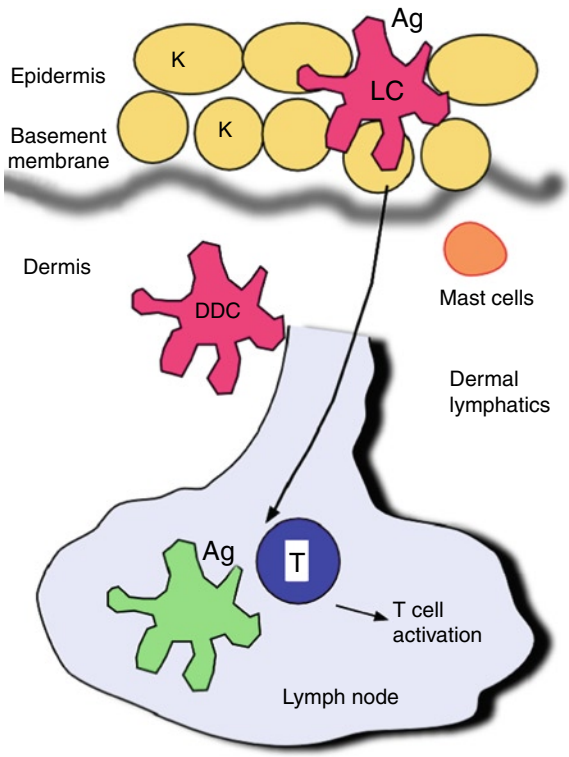

Fig. 11.2 The induction of skin immunity is a dynamic process with a high requirement for cellular energy. Antigen (Ag) applied to the skin to be taken up by dendritic antigen presenting cells, including Langerhans cells (LC) in the epidermis and dermal dendritic cells (DDC). Keratinocytes (K) also respond to stress caused by the antigen, producing a range of cytokines to induce immunity. LC then break down and cross the basement membrane, and with DDC migrate along dermal fibres into the lymph node where they present the antigen to T cells (T), leading to their activation. Activated T cells then migrate into the skin to orchestrate destruction of the antigen.

11.5.1 Considerable Cellular Energy is Required to Combat Ultraviolet Radiation-Induced Cutaneous Stress

Ultraviolet irradiation of the skin causes considerable oxidative stress, including production of reactive oxygen and nitrogen species which damage membrane lipids, proteins, and DNA [49]. Inflammation, gene mutation, and immune suppression in response to UV-induced oxidative damage contribute to photocarcinogenesis [49]. This oxidative damage depletes LC from the skin and creates oxidative products than can cause immunosuppression [50]. Nitric oxide appears to be a mediator of UV-induced immunosuppression in humans [51]. The skin has an active antioxidant defence system, including quenchers such as vitamin E and enzymes that inactivate these damaging molecules. Oxidative damage interferes with mitochondrial energy production in the skin [52], indicating that UV-induced oxidative damage decreases the energy available to the skin.

Another major pathway by which UV radiation suppresses immunity is via the formation of DNA photolesions, which presumably impair cellular function. This genetic damage has the potential to lead to mutations in dividing cells, which can occur if there is insertion of an incorrect nucleotide opposite to the photodamaged DNA in the newly synthesised DNA strand. Cells have a complex array of enzymes that repair this genetic damage to prevent

mutations, and enhancement of photolesion repair prevents UV immunosuppression in animal models and in humans [9, 53]. Cyclobutane pyrimidine dimer formation is a molecular trigger for solar-simulated (ss) UV radiation-induced suppression of memory immunity in humans [53]. Repair of photodamaged DNA requires large amounts of cellular energy. The DNA needs to be unwound with its compact chromatin structure remodelled to enable access of repair enzymes to the damaged DNA. This remodelling of chromatin utilises large amounts of ATP [54]. Other enzymes in the DNA repair process are also ATP-dependent [55].

Thus, exposure to UV radiation puts the skin in a state of energy stress. It needs to utilise large amounts of ATP to repair photodamaged DNA and probably also respond to damage in other macromolecules or replace damaged proteins or lipids with newly synthesised molecules. However, energy production is compromised due to oxidative damage inhibiting mitochondrial function. This would limit the amount of ATP available for the activation of effective immunity, which as discussed above is an energy hungry process. It therefore appears likely that restricted access to ATP following UV radiation may prevent the immune system from operating optimally, contributing to immunosuppression. In this energy-deprived state following UV exposure, the number of effector and memory T cells activated by antigen is low, while regulatory B and T lymphocytes instead predominate [56, 57].

11.6 Nicotinamide and Photocarcinogenesis

In mice, nicotinamide is an effective inhibitor of photocarcinogenesis. BALB/c mice were treated twice weekly with topical nicotinamide (~2.5%) or its vehicle, and then irradiated for 17 weeks with UVB radiation with lotions applied immediately after each UV exposure. The incidence of skin tumours was 75% in vehicle-treated animals, but only ~43% in those treated with nicotinamide [58]. The average number of tumours was also reduced from 0.95 per mouse to 0.50 [58].

Oral niacin, which is converted to nicotinamide [13], has also been shown to reduce UV-induced carcinogenesis in mice supplemented with dietary niacin 3 weeks prior to irradiation and then UVB-irradiated over 22 weeks [59]. Sixty-eight percent of the irradiated control group had skin tumours, whereas dietary supplementation with 0.1%, 0.5% and 1% niacin significantly reduced the incidence to 60%, 48%, and 28%, respectively. The number of tumours per mouse was also dose-dependently reduced by niacin supplementation [59]. In the same study, oral niacin was shown to increase skin NAD levels, with NAD content approaching saturation at 0.5–1% niacin supplementation [59] In contrast, mice not receiving niacin showed reduced NAD levels after UV irradiation. Topical nicotinamide also has anticarcinogenic effects in mice with chemically initiated (tetradecanoylphorbol-13-acetate) and phorbol-ester promoted skin tumours [60]; these effects were found to be independent of nicotinamide-induced inhibition of PARP.

11.7 Nicotinamide and Photoimmunosuppression

11.7.1 Ultraviolet Radiation-Induced Immunosuppression

Cutaneous immune responses can be suppressed by even very low, suberythemal UV doses equivalent to less than 6 min of noon summer sunlight [61]. This UV-induced immunosuppression plays a central role in the development of skin cancers by impairing anti-tumour immunity [49]. The immune-suppressive effects of UVB have been recognised for a number of decades [62], but there is increasing evidence that long-wave UVA radiation, which is far more abundant in sunlight than UVB, is also a large contributor to the immune-suppressive effectiveness of sunlight [63, 64].

Various models of skin immunity have been used to demonstrate UV-induced immunosuppression in vivo. Ultraviolet irradiation can impair both induction of new immune responses (primary sensitisation) and elicitation (reactivation) of memory immune responses to epicutaneously applied antigens (contact hypersensitivity; CHS) or intradermally delivered antigens (DTH) [65, 66]. UV radiation can cause both local immunosuppression (reduced immunity at irradiated sites) and also systemic immunosuppression (reduced

immune responses at distant, unirradiated sites) [49]. In mice, 2.5% topical nicotinamide [58] and oral niacin [67] both have been shown to protect CHS responses against UVB irradiation, with topical nicotinamide additionally protecting against UV-induced immunosuppression in a separate passive transfer assay, whereby splenocytes from irradiated animals enhanced the growth of antigenic tumours in unirradiated, recipient mice [68]. Using the Mantoux model of DTH in healthy human volunteers, we have recently shown that nicotinamide is also immune protective in humans [25, 69].

Mantoux testing with tuberculin purified protein derivative (PPD) is used in clinical practice to assess tuberculosis immunity and exposure status. Mantoux positive subjects, who have been either exposed to tuberculosis or vaccinated with Bacille Calmette-Guerin (BCG), develop localised induration and erythema 48–72 h after intradermal injection of PPD. The diameter of induration can then be measured by marking the outer aspects of the Mantoux response with a ballpoint pen [70]. Mantoux-induced erythema, which can be measured with a reflectance spectrometer, provides a more sensitive measure of Mantoux intensity which correlates well with the diameter of induration [61]. This can be used as a measure of the magnitude of the immune response for intradermally injected PPD. The Mantoux model can be used to measure UV immunosuppression by comparing Mantoux-induced erythema and induration at irradiated and unirradiated sites on the same volunteer. Mantoux reactions are readily suppressed in a dose-responsive manner by low, suberythemal doses of ssUV (UVB+UVA)[71] and this can be used as a measure of UV-induced immunosuppression.

11.7.2 Topical Nicotinamide Protects Against UV-Induced Immunosuppression in Humans

Healthy, Mantoux-positive volunteers were UV-irradiated on their backs, with 5% nicotinamide or vehicle applied to different sites in a randomised, double-blinded manner [69]. Mantoux testing at irradiated and adjacent unirradiated sites enabled the measurement of UV-induced immunosuppression with and without nicotinamide. In 20 volunteers, 5% nicotinamide or its 1:2:1 water, alcohol, and propylene glycol base lotion was applied 15 min before each of 3 daily exposures to ~0.7, 1.5, or 2.2 J/cm^2 ssUV for 3 consecutive days. These UV doses were equivalent to less than ~8 min of Sydney spring sun exposure [61]. In skin treated with vehicle, Mantoux-induced immunity was suppressed by 19% and 28% respectively with the two highest, but still largely sub-erythemal UV doses. In skin treated with nicotinamide, significant immunosuppression no longer occurred. This protective effect of 5% nicotinamide applied before UV exposure was confirmed by repeated measures ANOVA. In another 20 volunteers, the same protocol was repeated but with lotions only applied immediately after each irradiation, and nicotinamide again conferred significant immune protection (Fig. 11.3). Hence nicotinamide did not provide immune protection via a sunscreening effect, as confirmed by its lack of effect on minimal erythema dose (MED; sunburn threshold) in another group of volunteers, and its negligible absorption in the UV range when measured with spectrophotometery [69].

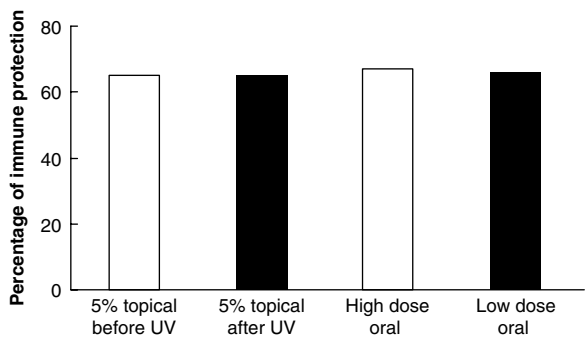

Fig. 11.3 Topical and oral nicotinamide provided consistent levels of immune protection against solar-simulated (ss) UV in healthy, Mantoux-positive volunteers. The relative immune effects of nicotinamide versus placebo in 4 different dose-responsive studies are summarised by showing relative immune protection against 2 J/cm^2 ssUV (a dose common to all studies) delivered to the lower back daily for 3 consecutive days. Topical 5% nicotinamide reduced the observed immunosuppression by 65% compared to base lotion, when applied either immediately before or immediately after irradiation (each n = 20; adapted from [69]). Oral nicotinamide reduced immunosuppression by 67% and 66% respectively when given for 1 week at 1,500 or 500 mg daily (n = 30 and 31; adapted from [25]). In all studies, nicotinamide afforded significant immune protection across the range of UV doses used (repeated measures ANOVA; each study used three graded UV doses [25, 69]).

Recently, we also determined the effect of topical (5%) nicotinamide on immunosuppression by single exposures to narrowband UVB (310 nm) or UVA (385 nm) radiation in Mantoux-positive volunteers, and found that nicotinamide protects against both wavebands, as well as reducing ssUV-induced immunosuppression at concentrations as low as 0.2% [72].

11.7.3 Oral Nicotinamide Protects Against UV-Induced Immunosuppression in Humans

Oral nicotinamide is also immune protective in humans. Using a randomised, placebo-controlled study with a cross-over design, nicotinamide significantly reduced ssUV-induced suppression of DTH responses [25]. Thirty healthy Mantoux-positive volunteers were given nicotinamide 500 mg 3 times daily or placebo for 7 days, during which time they were irradiated on separate areas of the lower back with 1, 2, and 4 J/cm^2 ssUV daily for 3 days. The 3 doses of ssUV used (1, 2, and 4 J/cm^2) were equivalent to 20%, 39%, and 78%, respectively, of the MED of these volunteers. Mantoux tests were performed at irradiated and adjacent unirradiated sites immediately after the final UV exposure, and then Mantoux-induced immunity was measured 72 h later. After a 4-week washout period with no capsules and no irradiation, volunteers took the opposite tablet before having identical irradiation and Mantoux testing on the opposite side of the back. Each volunteer's MED was also measured during both the nicotinamide and placebo arms of the study. While nicotinamide had no effect on either MED or the intensity of Mantoux responses in the absence of UV, it significantly reduced immunosuppression, by ~60%, at all UV doses (Fig. 11.3). This study was then repeated in another 31 volunteers, this time using a lower nicotinamide dose of 500 mg daily. Again, this dose had no effect on MED, but conferred a similar level of immune protection as the higher nicotinamide dose [25]. Although even doses of 1,500 mg daily were well tolerated, with no adverse effects, the lower daily dose may be more appropriate as a daily UV-protective supplement.

11.8 Nicotinamide and Photoageing

Nicotinamide is found in a wide range of cosmetics and facial moisturisers, and some studies suggest that nicotinamide may be effective against photoageing. A left–right randomised, double-blinded, vehicle-controlled study of 50 women found reduced facial hyperpigmentation, yellowness (sallowing), and fine wrinkles with 5% nicotinamide applied twice daily over 12 weeks [73], while a more recent split-face study in 30 Japanese women found significant improvement in periorbital wrinkling with 4% nicotinamide compared to the base cream [74]. Nicotinamide inhibits melanosome transfer in vitro, and reduced facial hyperpigmentation in 18 volunteers using 5% nicotinamide for 4 weeks [75].

11.9 Mechanisms of Photoprotection

The exact mechanisms of nicotinamide's photoprotective effects in vivo in humans are still unclear, but are likely to involve its role in cellular energy metabolism and high-energy cellular processes such as DNA repair. We performed microarray analysis of human skin treated with topical nicotinamide or vehicle [69]. In this study, six healthy volunteers were irradiated with a single suberythemal dose of ssUV to the lower back. Discrete UV and non-UV exposed areas were then treated with 5% nicotinamide or vehicle and small biopsies were taken 24 h later. Gene set enrichment analysis (GSEA) identified differentially regulated gene sets; there was UV-induced downregulation of genes involved in energy metabolism and anti-apoptotic pathways in skin treated with vehicle, but this was normalised in skin treated with 5% nicotinamide. Nicotinamide also modulates the production of various immunoregulatory cytokines, including IL-1β, IL-6, IL-8, TNF [76], and IFN-γ [77], by mechanisms apparently independent of its inhibitory effects on PARP.

11.10 Conclusions

Nicotinamide is an essential vitamin which plays an integral role in cellular energy metabolism and is thus crucial in many cellular processes such as responses to UV-induced stress and cutaneous immunity, which is

highly energy-dependent. Nicotinamide is safe and inexpensive and provides a high level of photoprotection to skin immune responses, and is therefore a promising agent for skin cancer chemoprevention.

Take Home Pearls

- Nicotinamide protects against photocarcinogenesis and photoimmunosuppression in mice.
- Both topical and oral nicotinamide protect against the immune-suppressive but not the erythemal effects of UV radiation in humans.
- Nicotinamide has shown some effectiveness against visible photodamage, including fine wrinkling, facial sallowness, and hyperpigmentation.
- Nicotinamide is a safe and inexpensive compound which could be added to sunscreens or after-sun lotions, to improve protection from UV-induced immunosuppression.
- Low-dose, daily oral nicotinamide holds promise as a means of reducing skin cancer incidence, especially in high-risk individuals, but would not be recommended until the safety and efficacy of oral nicotinamide for this application have been confirmed by clinical trials.

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Probiotics and Regulation of Immune Responses

12

Josette Péguet-Navarro

Core Message

› We usually live In harmony with millions of bacteria in our gut that provide a protective barrier against pathogens and actively participate to the regulation of immune responses and establishment of oral tolerance. Probiotics are live bacteria exhibiting health-promoting effects. Among the numerous purported health benefits attributed to probiotics their capacity to improve some skin diseases such as atopic dermatitis is now supported by increasing number of clinical experiments, Recently, we showed that oral supplementation with *Lactobacillus Johnsonni* in healthy volunteers can accelerate the recovery of cutaneous immune homeostasis following UV exposure and may thus represent a new strategy for photoprotection.

12.1 Introduction

The recognition of probiotics dates back to 1907 when Elie Metchnikoff associated the longevity of certain populations to their practice of eating fermented dairy products. This Nobel Prize-winner assumed that the bacteria contained in these products would be responsible for the observed effect. Currently, probiotics are defined as live microbial food supplements which, when administered in adequate amounts, confer a health benefit on the host. The most frequently used probiotics belong to lactic bacteria, especially Lactobacillus and Bifidobacterium species, which transform carbohydrates into lactic acid by fermentation. Some enterococci and yeasts are also used as probiotics (Table 12.1). These microorganisms can be found in both supplement form and as components of fermented food such as yogurts or other dairy products. The majority of these microorganisms are part of the normal commensal intestinal flora. However, for the same species, the effects are strictly dependent on the strain.

In this chapter, we made a short recall on the importance of the intestinal microbial flora, especially in the development of the immune system and on the mechanisms that allows us to tolerate these foreign components. The field of probiotic application is very large and the chapter is focused to their regulating purpose at the skin level.

12.2 Mammals Depend on Intestinal Microflora to Promote the Development of Efficient Immune System

The mammalian gut is one of the most densely populated ecosystems on Earth. The bacterial load borders 10^{14} organisms, i.e., 10–100 times the number of cells that composed the human body. For a given individual,

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Table 12.1 The principal microorganisms considered as probiotics

Lactobacillus	Bifidobacterium	Others
L. Acidophilus	B. Adolescentis	E. Coli nissle
L. Casei	B. bifidum	Enterococcus faecalis
L. crispatus	B. Breve	Saccharomyces boulardii
L. fermentum	B. Lactis	Saccharomyces cerevisia
L. Gasseri	B. longum	
L. Johnsonnii	B. Infantis	
L. Paracasei		
L. Plantarum		
L. Reuteri		
L. rhamnosus		

more than 400 different species colonize the digestive tract, the majority being anaerobic and localized in the colon, as assessed by cultivation-based studies. However, the development of molecular methods revealed that the diversity of the fecal gut microbiota may be considerably higher than anticipated. Indeed, it appears that about one half of the observed bacterial species are not cultivable.

The normal intestinal microflora is acquired during the first years of life and, for a given individual, remains remarkably stable in time. In contrast, it displays a great variation from one individual to another. The residing microorganisms are associated with the flora in transit, coming from ingested food.

The gut microbiota represents a complex ecosystem that is very important to preserve [1]. Indeed, the bacterial residents protect us against the invasion by pathogenic bacteria. They ensure the degradation and digestion of some indigestible carbohydrates and the production of vitamins and growth promoters essential to the host intestinal cells. Moreover, the host–microbe interaction is critical for normal development of the intestinal and systemic immune system [2]. Mice bred in germ-free conditions have a strong reduction in the number of $CD4^+$ and $CD8^+$ T lymphocytes in the spleen, a quasi-absence of Immunoglobulin A (IgA) in the Peyer's patches and are more susceptible to infection than conventionally colonized animals [23]. These anomalies can be corrected in a few weeks if one allows the colonization of the intestine by bacteria, for example, by placing in the cage a non-sterile animal.

12.3 The Immune System: Innate and Acquired Immunity

The role of the immune system is to preserve self-integrity and to fight against infections. For this purpose, it takes advantage of two distinct mechanisms: the innate immunity and the adaptive immunity.

Innate or natural immunity constitutes the first line of defense against the infections and even the most primitive animals have this system of defense. Innate immunity involves nonspecific mechanisms such as epithelia that constitute natural barriers. The principal effector cells are the phagocytic cells, mostly macrophages and polynuclear cells, which ingest and destroy the foreign elements. Innate immunity also involves the complement system and the production of multiple soluble factors, of which some cytokines and antimicrobial peptides like défensins.

Innate immune receptors can recognize a limited number of molecules, some of which are evolutionarily conserved and shared by many infectious agents. For example, the toll-like receptors (TLR) localized at the membrane surface or in endosomes, identify many bacterial, viral, or parasitic compounds such as lipopolysaccharides, peptidoglycans, or double-strand RNA. TLR are present on many cellular types and especially dendritic cells (DC), which constitute the most efficient antigen-presenting cells [6]. DC are present in the majority of lymphoid or not lymphoid tissues. They are essential in the induction of immune responses and they ensure the bond between innate and acquired immunity [19]. Indeed, TLR ligation causes phenotypic and functional activation of DC. This activation is essential to the induction of an effective primary immune response and in particular to the differentiation of $CD4^+$ helper T lymphocytes, (Th).

Acquired or adaptive immunity represents the specific line of defense against a pathogen. It requires both antigen-presenting cells and lymphocytes that display specific surface receptors. It is now recognized that the polarization of effector T cells relies critically upon DC. According to several parameters including the engaged TLR, phenotypic state of activation and

cytokines produced, DC will direct the differentiation of $CD4^+$ T lymphocytes toward the Th1, Th2, Th17, or even T regulatory pathways [4]. Th1 cells are characterized by a strong production of interferon-γ and control cell-mediated immune functions such as the activation of macrophages while the secretion of interleukin-4, IL-5, and IL-13 by Th2 cells leads to the stimulation of humoral immunity by aiding B cell activation and class switching. IL-17-secreting T cells are thought to play a major role in inflammation and autoimmunity. In some circumstances, especially in the presence of IL-10, T cells can differentiate into regulatory T cells, which can inhibit both Th1, Th2 pathways [21].

12.4 The Interaction of Commensal Bacteria with the Host Leads to a State of Tolerance

The gut harbors abundant immune system, including Peyer'patches, mesenteric lymph nodes, and numerous myeloid and lymphoid cells in the lamina propria. The challenge for these immune components is to tolerate food antigens and commensal microflora, while being able to respond to pathogens. Several mechanisms contribute to maintain gut homeostasis [14, 25]. The intestinal mucosa, reinforced by mucus and antibacterial peptides, constitutes a rather efficient natural barrier. In addition, IgA produced at high level in the digestive tract, cover the bacteria, and limit their penetration.

Nevertheless, the gut epithelial barrier does not completely prevent luminal antigens from entering the tissues. Indeed, a few gut bacteria can be detected in the mesenteric lymph nodes draining the gut of healthy animals. Bacteria can be internalized by enterocytes and M cells, which can transfer intact bacteria to the neighboring lymphoid tissue [15]. Moreover, DC can send processes between gut epithelial cells without altering tight junction integrity and sample commensal or pathogenic microorganisms. The majority of the bacteria that enter the gut mucosa are quickly eliminated by macrophages. However, some of them can survive several days in DC and trigger immune response.

In healthy individuals, the cross-talk between the gut immune system and bacteria is tightly regulated to avoid excessive immune response and chronic inflammation while ensuring the elimination of potentially dangerous pathogenic bacteria. The mechanisms involved in this process are far from being completely understood. It has been shown that the gut immune system contains high levels of the suppressive cytokines IL-10 and transforming growth factor β, which can play a role in the maintenance of immune homeostasis. Moreover, the presence of regulatory T cells has been reported in the intestinal mucosa. Thus, under normal conditions, the host tolerance toward commensal bacteria results from an active process, which allows both to reinforce the barrier through IgA production and limit excessive T-cell responses.

Accordingly, perturbations of this equilibrium can cause many disorders. Indeed, bacteria differ in their capacity to stimulate inflammation. For example, the harmful role of the endogenous flora is highly suspected in primary inflammatory bowel diseases such as Crohn disease, characterized by chronic inflammation of the intestinal tract, excessive activation of Th1 T cells, and large production of inflammatory cytokines. This pathology has been related to genetic factors [20] but also to an imbalance in the normal commensal microbiota, known as dysbiosis. Comparison of clone libraries reveals the depletion of some commensal bacteria in the microbiotas of patients with Crohn disease, as compared with controls [7].

In such pathological conditions, it appears therefore logical to intervene by modifying the enteric bacteria and using probiotics. Increasing data now suggest that some probiotics can alter the intestinal microflora and modulate the host immune system.

12.5 Probiotics: A Large Field of Application but yet Misunderstood Mechanisms

Recent research has reported a beneficial effect of probiotics in many diseases. As expected, the best evidence of their efficacy was brought in acute or chronic affections of the gastrointestinal tract. Probiotics are resistant to gastric acid and bile salts and can survive and compete with pathogenic microbes for nutrients. They can therefore assist the recovery of healthy microflora and reduce infections. Thus, long-term intake of probiotics improved the eradication of *Helicobacter Pylori* by reducing gastric inflammation [13]. Feeding with the probiotics *L. Reuteri or*

L. rhamnosus GG can shorten the incidence and the duration of diarrheas due to rotavirus [5]. The beneficial effects of probiotics have also been reported in inflammatory bowel diseases, although larger controlled trials are necessary to confirm the results [22].

There are several putative mechanisms by which probiotics can prevent gastrointestinal disorders. The capacity of the microorganisms to adhere to the intestinal epithelial cells and to cross the intestinal barrier undoubtedly plays an important part. Probiotics are known to produce antimicrobial compounds such as lactic acid, able to inhibit the growth of some pathogenic bacteria. Moreover, a large body of data now suggests that probiotics can modify the immune response of the host. Some probiotics were shown to strengthen the mucosal barrier by stimulating local IgA production, thus leading to a mucosa-stabilizing effect. Probiotics can interact with immune cells by modulating the secretion of antiinflammatory cytokines, which would result in a reduction of inflammation [18]. However, the active component(s) of the bacteria is largely unknown (bacterial wall, DNA, or soluble factors?). Moreover, one must keep in mind that the effect of probiotics on the immune response is difficult to generalize. Distinct probiotic strains may generate divergent immune responses, which, in turn, depend on the host's immune status.

Due to their regulating effect on the immune system, the beneficial effect of probiotics has been tested in many pathologies including autoimmune and cancer diseases. In this chapter, we focused on the effects of probiotics on skin immune responses.

12.6 Regulating Activity of Probiotics in Atopic Dermatitis

Atopic dermatitis (AD) is a chronic inflammatory skin disease mediated by T lymphocytes and characterized by hyper-reactivity toward environmental antigens. It is a genetic disease associated to a deterioration of the skin barrier. Induction of AD involves skin DC, which present the allergens and induce a typical Th2 response.

According to the hygiene hypothesis, insufficient or aberrant exposition to environmental microbes is one of the causes of the development of allergy. Indeed, improved hygiene, vaccination, antimicrobial medication, and consumption of almost sterile food have reduced our exposure to microbes. The absence of such an appropriate exposure may pose a problem for the development of child's immune system. In fetuses and neonates, the immune response is exclusively skewed to Th2 and pathogen stimuli are thought to play a key role in the tuning of the immune system toward a more finely balanced Th1 and Th2 immune response.

The use of probiotic in the treatment of the AD is not fortuitous. Indeed epidemiologic studies have shown a qualitative modification of the intestinal flora in allergic children when compared to matched controls. In particular, a clear reduction in lactobacilli, bifidobacteria, and enterococci was observed together with an increase in potentially pathogenic bacteria like *Clostridia* and *Staphylococcus aureus*. In addition, it is known for a long time that breast-fed infants have a lower incidence of allergies, which correlates with higher level of bifidobacteria in the mother's milk.

The first clinical trial using probiotics was published by the group of Kalliomaki [10]. In this double-blind, placebo-controlled, clinical trial *Lactobacillus Rhamnosus* was given (10^{10} cfu/day) to pregnant women for 2–4 weeks before delivery. The inclusion criterion was a family history of atopic disease in first-degree relatives or partner. After birth, the treatment was continued for 6 months in the nursing mothers or in newborns. Under these conditions, the frequency of atopic eczema at age 2 years was decreased by half in the probiotic group (15/64 versus 31/68, $p < 0.08$). This effect was confirmed at age 4 years since eczema was detected in only 14 out of 53 as compared to 25 out of 54 untreated children [11]. These first results were thus very encouraging. However, current data are much more moderate regarding the beneficial effect of probiotics in this pathology. Especially there seems to be a strong difference in the efficacy of probiotics, according to their preventive or curative use. Lee et al. recently summarized the results of clinical trials carried out between 1997 and 2007 [12]. Data from six prevention studies, i.e., probiotic administration to the mother or newborn babies, relate to nearly 1,500 children and three out of five trials utilized *L. rhamnosus*. Globally, the results support a beneficial effect of probiotics in the prevention of AD. In contrast, results were far less convincing for probiotic efficacy in AD treatment [12].

12.7 Oral Probiotic Bacteria Can Reduce Contact Hypersensitivity in the Mouse

Contact hypersensitivity (CHS) is one of the most common skin diseases. It is mediated by $CD8^+$ T cells and characterized by a delayed inflammatory response to environmental haptens, which cross the skin barrier and bind proteins to be immunogenic. A recent study in the mouse showed that daily oral treatment with *Lactobacillus casei* can reduce CHS to dinitro chloro benzene, provided the mice were fed at least 14 days before sensitization [3]. This inhibition was related to a reduced number of $CD8^+$ effector T lymphocytes and required the presence of $CD4^+$ regulating T lymphocytes able to limit the proliferation of the effector cells.

This study was the first demonstration that oral consumption of probiotics can modulate inflammatory response at the skin level.

12.8 Oral Probiotic Bacteria Facilitate the Recovery of Cutaneous Immune Homeostasis After UV Exposure

It has long been known that, in addition to being carcinogenic via DNA damage and mutations, solar UV radiation induces local and systemic immune suppression, which represents a major risk for induction and development of skin cancers in sun-exposed areas. The process is related to multiple mechanisms, including the production of many cytokines and direct impairment of skin DC through induction of apoptosis and impairment of antigen-presenting function [24].

Lactobacillus Johnsonii NCC 533 (La1) has been isolated from healthy adult microbiota and shown to have strong anti-pathogenic activity against a wide variety of entero-pathogens. A study published in 2006 by Nestle and L'Oreal Laboratories showed that oral administration of La1 into mice prevents the UV-induced depletion of epidermal Langerhans cells while limiting IL-10 production in the skin [9].

In collaboration with Nestle and L'Oreal Laboratories we have recently analyzed whether ingestion of the probiotic (La1) can counterbalance the UV-induced immunosuppressive effect in humans [17]. For this purpose, a randomized, double-blind, placebo-controlled clinical trial was carried out. The study involved 54 male volunteers, of phototype II/III and aged from 20 to 40 years (Table 12.2). Except the probiotic, the subjects did not consume fermented dairy products throughout the study. Experimental design was the following: after a washout period of 6 weeks, volunteers were randomly divided into two groups of 27 individuals, receiving daily oral supplementation with either *L. johnsonii* La1 or placebo (maltodextrin) for 66 days. On day 56 of supplementation, subjects were exposed to UV (1.5 minimal erythematous dose, MED) twice within 10 h, on the right buttock. Suction blister roofs and biopsies were collected from the right and left buttocks before treatment and at day 1, 4 and 10 post-irradiation (Table 12.2). Immunohistological study was carried out on the biopsies while epidermal cells were isolated from the roofs and used in mixed epidermal cell lymphocyte reaction (MECLR) to assess their allostimulatory capacity.

Results showed that La1 uptake was well tolerated and did not modify erythema upon UV exposure [17]. La1 did not prevent the well-known decrease in epidermal cell allostimulatory function on day 1 post-irradiation. In the placebo group, this decrease persists at day four post-irradiation, in correlation with significant decrease in $CD1a^+$ DC within irradiated epidermis. The important result is that La1 intake facilitates an earlier recovery of epidermal cell allostimulatory function, a process that correlates with recovery of basal $CD1a^+$ cell staining within the irradiated epidermis. The origin of these $CD1a^+$ cells remains an open question. They are most probably $CD1a^+$ repopulating cells, derived from precursors.

This study was the first to demonstrate that oral probiotic intake can facilitate the recovery of skin immune homeostasis following UV exposure. Whether La1 bacteria is able to modulate the level of some cytokines or chemokines that favor the homing of skin LC precursors requires further investigations, however.

12.9 Conclusions

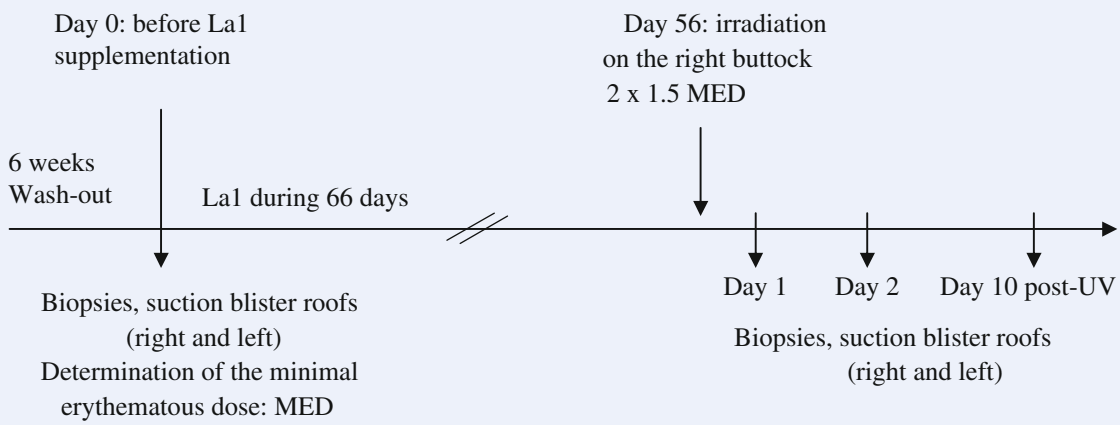
In conclusion, the improved understanding of the role of the normal gut microbiota has made the manipulation of the gut ecosystem a valid and realistic therapeutic tool. The commensal microorganisms represent essential

Table 12.2 Oral probiotic bacteria and skin immune status following UV exposure: human volunteers and experimental design

Human volunteers:

- Fifty four male Caucasian healthy volunteers, skin-type II/III
- Aged 20 to 40 years
- Low consumers of fermented milk products
- Not allowed to consume any products containing live bacteria throughout the study
- Double blind, placebo-controlled study

Experimental design:



partners to the host from birth to death. They stimulate immune system development and can resist the colonization of exogenous pathogens. Therefore, disruption of this equilibrium can have disastrous consequences for the organism. Probiotics can assist in the recovery of normal gut microflora. They are ingredients active on immunity and, as such, they profit from considerable market prospects, either in pharmacologic, food or in cosmetic industry.

The interest of probiotics has been largely demonstrated in intestinal pathologies. Very interestingly, however, a growing number of data now suggest that oral probiotics may have systemic effects, especially in the skin. Probiotics most likely function by influencing local and systemic immune system but the precise mechanisms are still to be clarified.

Given the multitude of bacteria present in the digestive tract, it seems unlikely that ingestion of only one probiotic can have major therapeutic effect. Currently, a detailed attention was paid to prebiotics, defined as indigestible substances that activate the growth of host probiotic bacteria. Prebiotics are found in many foods and can also be isolated from plants (chicory root) or synthesized. Current research also aims to combine prebiotics and probiotics (an association called synbiotics) to obtain a maximum effect. Caution must be given to these combinations, however, given that the effect of probiotics is highly dependent on the strain used.

Finally, probiotics represent a huge field of investigation and a better understanding of their mechanisms of action is essential for controlling their safety use and therapeutic effectiveness. Another interesting issue will be to apply the principle of probiotics to any environment where a microbiota exists and especially the skin, whose microbiota is likely involved in competitive exclusion of environmental pathogens [8, 16].

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Prebiotic Cosmetics

13

Rainer Simmering and Roland Breves

Core Messages

- The microbiota of the human skin is characterized by a complex and filigrane network of interactions between the microbes and the skin surface cells.
- Most of the resident microbes on healthy skin can be regarded as being harmless or even beneficial to skin.
- The topical application of prebiotic and probiotic actives turns out to become a new and promising approach in therapy and cosmetics.
- In cosmetics, prebiotic actives with the ability to rebalance the skin microflora may be used. Probiotic approaches mainly consist of applying inactivated microbial biomass rather than live bacteria.
- Stimulating the expression of the skin's own antimicrobial peptides provides another way of improving the skin microbiota.
- First examples are given for the treatment of mild to moderate microbial disorders on the skin of face and body, on the scalp, and in the oral cavity.

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13.1 Introduction: Human Microflora Is a Constituent of Healthy Skin

The human body is colonized with a vast amount of bacteria. The number of bacterial cells living in and on the human body exceeds the amount of endogenous cells by a factor of 10. Most of these bacteria live in the digestive tract, reaching up to 10^{12} cells/g feces in the colon. But also the oral cavity harbors high amounts of microbes typically reaching 10^9 or 10^{10} cells/cm^2 tongue surface [34]. Less bacterial growth is detected on the human skin. However, Bojar and Holland [6] have found up to 10^8 cells/cm^2 on the cheek surface.

Composition, density, and complexity of the human microbiota differ depending on the site of the body and on factors like temperature, humidity, and sebum content. Here, we will give a short overview on the microflora of the human skin and oral cavity, as well as of the human skin defense system.

13.1.1 Skin Microflora

Holland and Bojar [38] divided the members of the human skin microflora into three groups: transients, which are intermittently found on the skin; temporary residents, which can maintain growth rates to remain on the skin for a short period of time; and residents, which permanently inhabit the skin. The members of these three groups differ from site to site and belong to a variety of genera. Whereas until the new millennium culture-based techniques were used to assess the composition of the microflora (for reviews see [21, 51, 67]), nowadays molecular techniques using the 16S rRNA-Gene as the

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target are state-of-the-art to investigate the human microbiota [2, 19, 23].

On the forehead mainly propionibacteria and staphylococci were found with cell-counts of more than 10^6 cells/cm^2 [51], whereas *Staphylococcus epidermidis* and *Propionibacterium acnes* were the most frequent species. But also other bacterial species belonging to the genera *Acinetobacter*, *Corynebacterium, Stenotrophomonas, Bacillus*, and *Pseudomonas* were found, as well as yet-uncultivable bacteria [2, 16].

Under the armpit total viable cell counts of 10^6 cells/cm^2 can be detected. The axilla supports a dense bacterial population, which is known to be dominated by the two genera *Staphylococcus* and *Corynebacteria* [50], though also propionibacteria play an important role. However, again by using molecular techniques the picture was broadened and further important bacterial species were detected. It was shown that the armpit microflora of men is also dominated by species of the genus *Anaerococcus*. Furthermore, it turned out that besides *S. epidermidis*, *S. hominis* is also a central member of the axilla microbiota [75].

The armpit microflora is of particular interest for cosmetic products, since it is responsible for body malodor. The axilla of humans contains a dense arrangement of apocrine sweat glands and sweat as it is secreted in the axilla is odorless. But it was already shown in 1953 [70] that by the action of skin bacteria a typical axilla odor can be released from non-smelling molecules present in apocrine secretions. Hereby again *Staphylococcus* and *Corynebacterium* species are the key player in that process.

In contrast to the armpit, most other sites of the skin are in a relative dry condition. Here the bacterial population is less dense. Some work was done to investigate the hand microflora [19, 49, 60]. Average cell counts are about 10^5 cells/cm^2 [49]. Due to the nature of the hand, the amount of transient bacteria is higher than on other sites less exposed to the environment. Therefore, the bacterial composition is more diverse and more gram-negative bacteria are found. Pancholi et al. [60] have collected samples from human subjects and their surrounding environment and found a high similarity between the detected strains from each environment. This was in particular true for gram-negative bacteria. Using a pyrosequencing method, Fierer et al. [19] investigated the diversity of the palm microbiota of 51 human subjects. A typical hand surface harbored >150 unique species-level bacterial phylotypes, and they have identified a total of 4,742 unique phylotypes comprising also yet-uncultivable bacteria. Although there was a core set of bacterial taxa commonly found on the palm surface, they observed pronounced intra- and interpersonal variation in bacterial community composition: hands from the same individual shared only 17% of their phylotypes, with different individuals sharing only 13%. Women had significantly higher diversity than men, and community composition was significantly affected by handedness, time since last hand washing, and an individual's sex. However, again *Staphylococcus* and *Propionibacterium* ssp. were major inhabitants of the hand-microflora, next to bacteria belonging to the genera *Streptococcus, Corynebacterium*, *Lactobacillus*, and *Burkholderia*. These findings are in good accordance to results gained in our group (unpublished data).

A similar study conducted by Gao et al. [23] investigating the microflora of the forearm also resulted in the first four above-mentioned bacterial genera as the major components. In addition, *Acinteobacter* and *Finegoldia* ssp. were also detected in this site. In contrast, Grice et al. [26] investigated the skin microbiota of the inner elbow and found a microflora mainly composed of proteobacteria.

Despite the fact that the new molecular methods have broadened our knowledge about skin microflora composition, the role of the skin microflora is yet not fully understood and little work was done on this topic.

For sure, skin is, unlike any other organ, exposed to external impacts and thus needs potent protective mechanisms. Bacterial colonization is one of these mechanisms because bacterial species belonging to the residential flora inhibit the growth of pathogens. The bacterial production of acids might, besides endogenous factors, contribute to the "acid mantle" of the stratum corneum. In addition, the production of specific antibacterial products (e.g., lantibiotics; [3]) by the commensal flora is another way to control the bacterial status of pathogens on the skin [13]. Preliminary data from the same authors suggested that *S. epidermidis* plays an additional protective role by influencing the innate immune response of keratinocytes through Toll-like receptor signaling.

13.1.2 Oral Microflora

In the human oral cavity, more than 500 bacterial and fungal/yeast species have been identified. The microorganisms interact in a complex manner with the host and with each other [45, 61], resulting in the formation

of microbial biofilms (dental and subgingival plaque) and tongue surface debris. These microbial consortia are leading to dental caries, periodontal disease, and oral malodor. Similar to the skin, also the composition and density of the bacterial load depends on the ecological niche in the mouth.

On the surface of the teeth aerobic and anaerobic bacteria can be found. Species of the genera *Streptococcus* are supposed to be the first colonizers on the pellicle. These are often followed by *Actinomyces*, *Veillonella*, and *Fusobacterium* species, leading to a biofilm also called dental plaque [29, 41].

Subgingival plaque is considered the principle etiological factor in the onset and progression of periodontitis [56, 61]. Mainly anaerobic bacterial species like *Actinobacillus actinomycetemcomitans*, *Micromonas micros*, *Prevotella intermedia*, *Porphyromonas gingivalis*, and *Tannerella forsythensis* are major players in the formation of periodontitis [76, 79], and these species have also been linked to the progression of the disease [30].

In contrast to densely packed dental plaque, the tongue biofilm is thought to be less dense, allowing most of the bacterial cells ready access to nutrients via the salivary fluid that bathes the surface at high dilution rate. A few of the tongue bacteria generate highly malodorous compounds (VSC, polyamines, indoles) by degrading food and endogenous proteins leading to oral malodor and halitosis. *Eubacterium, Atopobium*, *Veillonella*, and *Fusobacterium* species are most prominent in human subjects with halitosis [31, 77].

13.1.3 The Skin's Innate Defense System

Despite high numbers of bacteria residing on the skin it is kept intact although being composed of biological, biodegradable material. An important feature of skin and mucosa is, in contrast to abiotic surfaces colonized by bacteria, the ability to (re)generate cells – or to create a response reaction which provides a protection against infection and degradation. This innate immune system has to be considered as an additional factor influencing the microbial equilibrium on the skin.

An important part of the antimicrobial defense system of the human skin constitutes of small cationic peptides, i.e., human β-defensins hBD-1, hBD-2, hBD-3, hBD-4 [24, 33, 69], of the human cathelicidin LL-37 [22], of antimicrobial enzymes like lysozyme and RNAse 7 [32], and of several other molecules exhibiting antimicrobial potential (for a survey see [9]).

Cathelicidin LL-37 in its active form is derived from the precursor protein hCAP18 by cleavage by serine proteases. It consists of 37 amino acids, exhibits an α-helical form, and is active against bacteria, fungi, and viruses [8].

The β-defensins consist of small peptides (4–5 kDa) with a characteristic set of three disulfide bonds. In general, hBD-1 is regarded as being constitutively expressed in the epithelium while hBD-2 and hBD-3 are induced by inflammation. In acne lesions a strong induction of hBD-2 was observed in highly inflammated pustules while hBD-1 was only moderately expressed with the strongest signal in the papules [64]. In contrast hBD-2 seems not to be upregulated in atopic dermatitis [58] while both hBD-2 and hBD-3 are expressed in inflamed psoriatic lesions [33, 55]. In keratinocytes exposed to *Staphylococcus aureus* the expression of hBD-2 was strongly induced while hBD3 and LL-37 showed only moderate and hBD-1 virtual no induction [54].

Malassezia furfur was shown to induce the expression of hBD-2 via protein kinase C, but not hBD-1 in keratinocytes [17]. RNAse 7 was found to be induced in keratinocytes by contact with heat-inactivated cells of bacterial pathogens like *Pseudomonas aeruginosa, S. aureus, E. coli*, *and S. pyogenes* [32].

These antimicrobial peptides represent one element, among others, of the innate immune system. Thus, their role is by far not only restricted on the direct inactivation of microbes but on the stimulation of further cellular reactions. As an example, a complex cascade involving IL-6, IL-10, and other cytokines is activated by cathelicidin LL-37 via cell-surface receptors (for an overview see [68]).

The composition of the skin and oral microflora depends as outlined above on several factors that can easily disturb the bacterial equilibrium. It is, however, important that the members of the protecting flora are kept in a suitable balance. One route to reach this balance could be using the prebiotic concept. The concept of prebiotics was introduced by Gibson and Roberfroid in 1995 [25], who defined prebiotic actives as "non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one, or a limited number of, bacteria in the colon." This concept was adapted in 2004 to cosmetic products, by introducing the prebiotic coefficient [4]. Carolan et al. also act on the suggestion of this concept [12].

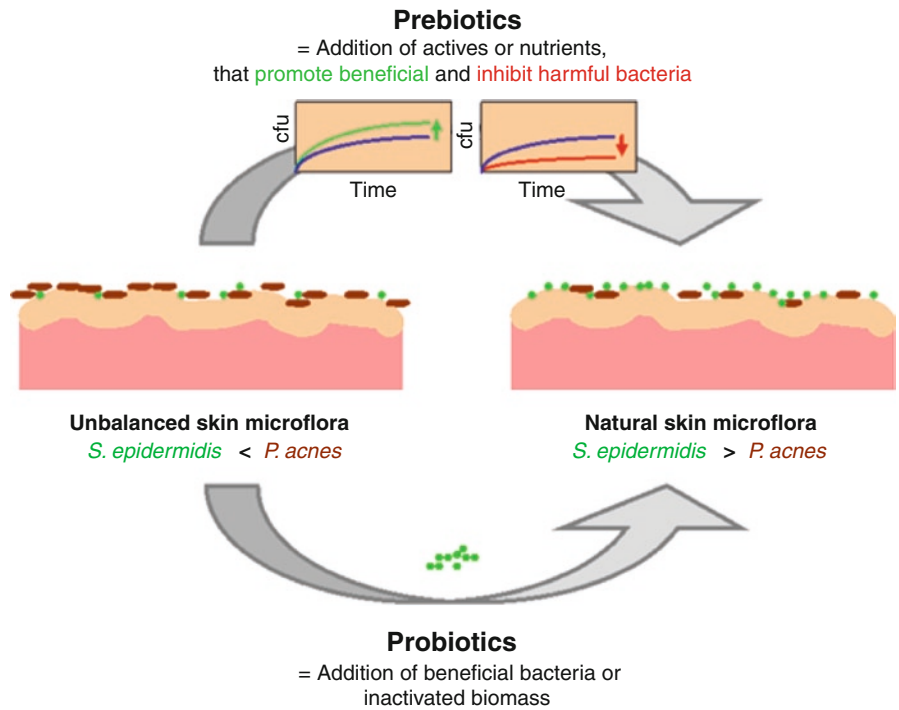

Fig. 13.1 Schematic illustration of prebiotic and probiotic cosmetics (Modified after [46])

Applying beneficial bacteria as probiotic agents directly to the skin represents another way of achieving a rebalanced microbiota situation. While this in principal can be done in live form especially in cosmetics the dosage of the beneficial microbes as inactivated biomass preparations is predominant. This will lead us finally to the approach of stimulating the skin's immune defence by microbial preparations or other suitable actives. The two principle routes of prebiotic and probiotic skin microflora manipulation are illustrated in Fig. 13.1.

13.2 Application of Nutrients/Actives to Balance the Skin Microflora

There is an increasing amount of prebiotic products that were developed in recent years. Different plant extracts were investigated on their effect on *P. acnes,* involved in acne generation, as well as on the commensal bacterium *S. epidermidis* [5]. It was possible to identify plant extracts that demonstrate inhibitory effects on *P. acnes* and stimulating effects on *S. epidermidis*. In particular, a mixture of pine and black currant was very effective, whereas black currant extract alone did not work as well. These effects were of particular interest since *P. acnes* is seen as to be a major reason for the development of inflamed skin conditions. Therefore, a reduction of this bacterial species will help to provide a good solution for inflamed and acne prone skin.

In a human study, the efficacy of the above-mentioned substances in cosmetic formulations was proven. It was observed that twice daily application of a cosmetic product containing 0.5% of selected plant extracts of pine, black currant, and ginseng to human skin for a total of 3 weeks was effective in inhibiting the growth of *P. acnes*, whereas coagulase negative staphylococci (CNS), to which *S. epidermidis* belongs to, was not affected. The fraction of *P. acnes* on the total skin microflora was reduced and the fraction of CNS was increased [5].

In a clinical study a combination containing a wash gel, a toner, and a skin fluid was tested on 30 volunteers with a mild form of impure skin. All formulas contained in total 1% of the above-mentioned prebiotic actives. A significant improvement for papules, pustules, comedones, and sebum production was measurable [40].

As a consequence, the first prebiotic cosmetic product has entered the market in 2005. These products contained plant extracts like pine/blackcurrant and ginseng, which are able to equilibrate the human skin microflora as described above.

Another approach to balance the skin microflora was used for people suffering from atopic dermatitis (AD) and dry skin, respectively. Although genetic factors determine the predisposition to AD also environmental factors influence the severity of that disease. Furthermore, it is known that the gram-positive microorganism *S. aureus* produces a number of toxins and enzymes that often seriously worsens the state of the skin [44]. It was reported that *S. aureus* was detected and the microflora was unbalanced on not only severely diseased, but also dry-type skin of patients with atopic dermatitis [1, 42, 57, 78]. Masaoka et al. [52] have described the use of farnesol and xylitol to balance the skin microflora of patients with atopic dermatitis. They were able to remove and prevent the adhesion of biofilm-producing species *S. aureus* on the skin. Furthermore, they found out that the commensal bacterial species *S. epidermidis* was less affected by these substances. In a further study the same group has performed a randomized, double-blind, placebo-controlled right-and-left comparison study [53]. The arms of 17 patients with dry-type AD were applied with a skin-care cream including/or not including a 0.02% farnesol and 5% xylitol combination for 1 week. It was shown that the percentage of *S. aureus* of the total viable bacteria was significantly decreased after 1 week at sites to which the cream had been applied, compared with before application and with placebo sites. On the other hand, similar to the studies described above [5], the number of coagulase-negative staphylococci was not affected or slightly increased after 7 days. The authors concluded that their study provided evidence supporting the idea that creams containing farnesol and xylitol is a useful skin-care agent for atopic dry skin colonized by *S. aureus* [53].

Also "classical" prebiotics like oligofructose or glucans are supposed to have a selective activity on skin bacteria. It was described that glucooligosaccharides show a prebiotic effect on intestinal bacteria [71]. A raw material supposed to be used in skin care was also described that contains inulin, a prebiotic compound, known to be effective in the gut [65]. It was shown that the product was able to favor the growth of *S. epidermidis*, in comparison to *S. aureus* and *E. coli* [12].

13.3 Microbial Products

The term "probiotic" in its original meaning is defined by the WHO and FAO as "live organisms which, when administered in adequate amounts, confer a health benefit to the host." Very often it is used in a broader sense covering not only viable microorganisms but also certain types of microbial preparations as supernatants, extracts, and cell lysates. Especially for the use in cosmetics, the application of live bacteria would require huge efforts to introduce modified procedures for handling during the production, storage, and delivery of the products. Cosmetic products are normally not provided in cooled ambience in stores and are not stored in refrigerators in consumer households, respectively.

This will explain why up to today, with the exception of a few preparations for oral care products, no cosmetic product containing live microbes is currently on the market.

Nevertheless, several attempts are already made to establish inactivated microbial preparations for the topical application on the skin, which are discussed briefly below.

Ouwehand et al. [59] proposed propionibacteria for cosmetic use. Food grade strains were chosen as candidates because cutaneous isolates might be correlated with skin infections. The antimicrobial activity against the skin pathogens *M. furfur*, *C. albicans*, and *S. aureus*, presumably due to the secretion of organic acids and the interference with the attachment of these target germs to keratin was demonstrated.

A rather therapeutic approach was followed in a pilot study with inactivated *Lactobacillus acidophilus* which was used as a treatment against mild to moderate vernal keratoconjunctivitis, an allergic eye disease. After 2–4 weeks a reduction of symptoms as well as of the molecular markers ICAM-1 and TLR-4, proteins of the innate immune system, was observed [39].

Preparations of different lactic acid bacteria, i.e., *L. paracasei*, *L. brevis*, or *L. fermentum*, were proposed as ingredients for skin-care products. Cultures of lactobacilli were investigated in vitro and in vivo on the skin of individual volunteers for their probiotic potential and were found to promote *S. epidermidis* and to block *S. aureus*, *E. coli*, or *M. luteus* [48].

Further the use of lactobacilli (i.e., *L. plantarum*, *L. crispatus*, or *L. acidophilus*) for the reduction of axillary malodor was claimed in another patent application [47]. Here, cultured lactobacilli were proposed

as actives for deodorants based on an in vitro assay for the reduction of the formation of 3-methyl-2-hexenoic acid by *Corynebacterium jeikeium* from odorless axillary sweat.

After disintegration of probiotic bifidobacteria in a milk-based nutrient a cosmetic ingredient was obtained which was shown to increase the amount of lysosomes after undernourishment and to reduce the IL-10 production in vitro. An in vivo study further revealed a significant reduction of skin irritation, i.e., skin redness, after exposure to UV light of volunteers treated with a 5% O/W cream [36].

While most of these examples deal with the application of food grade, mostly lactic acid bacteria, recently a different approach was used. *Vitreoscilla filiformis*, an isolate from a thermal spring which traditionally has been used in the treatment of skin diseases, was presented as a beneficial organism. A cream containing 5% of bacterial cell lysates was used in an in vivo study with volunteers with mild to moderate atopic dermatitis and was found to improve the skin conditions within 4 weeks [28]. The microflora profiles were monitored in parallel. Due to rather low initial counts of *S. aureus*, a microorganism which often is correlated with more severe cases of atopic dermatitis, the reduction rates during the study were not significant. Interestingly, the occurrence of *S. epidermis* was not affected.

In a second study with volunteers suffering from moderate scalp seborrheic dermatitis a 4 weeks daily application of a 5% lotion of *V. filiformis* preparation showed a significant reduction in the clinical score as well as in the self-evaluated pruritus [27]. Therefore, *V. filiformis* was proposed as an active ingredient for an anti-dandruff shampoo. The active used in these two studies did not exhibit antimicrobial activity neither against *S. aureus* nor against *M. furfur*. In conclusion, the beneficial effect on the skin and its microflora derived disorders is obvious although the distinct mechanism is not revealed yet.

Much more explorative approaches dealing with genetically modified bacteria, again mostly lactic acid bacteria, might be thinkable. Examples for oral care are discussed below [37]. Since tools for the safe modification of lactic acid bacteria are available nowadays, the technical feasibility of a topical usage might be given. Steidler and Vandenbroucke [72] gave an overview on the current state of the technology. A first clinical trial, involving thymidin dependent IL-10 delivering lactic acid bacteria, was successfully conducted [7]. This might open the opportunity that the use of genetically modified microbes for therapeutic purposes might become accepted in the society in the nearer future. It has to be stated that, based on the experiences in the discussion on GMOs in food, severe doubts remain about the acceptance for cosmetic applications.

13.4 Probiotic Oral Care Compounds

Next to the investigations of prebiotic and probiotic cosmetics for skin applications, even more effort was made to examine the effect of pro- and prebiotics for the oral cavity. The oral cavity is, like to the gut, colonized by a huge amount of different micro-organisms, which often have a detrimental effect on the oral health status. Therefore, balancing the oral bacterial status by pro- and/or prebiotics might be of great potential.

Several studies have been performed using live bacteria to modulate the oral microbiota. Comelli et al. [14] aimed at the selection of bacterial strains for the prevention of dental caries. They have examined more than 20 microorganisms, used in dairy products, out of which they have identified 2 *Streptococcus thermophilus* and 2 *Lactococcus lactis* strains that were able to adhere to saliva-coated hydroxyapatite beads to the same extent as *Streptococcus sobrinus*. They even incorporated two of them into a biofilm model modulating the dental plaque, in which they affected the growth of the present oral bacteria.

In another study, the effect of consuming a yogurt containing bifidobacteria on the salivary levels of mutans streptococci and lactobacilli was examined [11]. A double-blind, randomized crossover study was performed with 21 healthy individuals. Compared to the controls, a significant reduction of salivary mutans streptococci was observed after consumption of the probiotic yogurt. A similar trend was seen for lactobacilli, but this decrease failed to reach statistical significance.

Similar effects were also shown by Koll et al. [43]. They characterized oral lactobacilli for their potential probiotic properties for oral health. Most of the strains tested suppressed the growth of the oral pathogens *Aggregatibacter actinomycetemcomitans, P. gingivalis*, *P. intermedia*, and *Streptococcus mutans* in vitro.

A further example of *Lactobacillus salivarius* W24 with the ability to modulate oral microbiota was just recently described [63].

Another interesting probiotic strain is *Streptococcus salivarius* K12, which is also commercially available in lyophilized form. This species was shown to be effective for oral health in a number of studies [10, 15, 73]. Its effects are proposed to be due to the organism's ability to affect the host immune system. That is in particular eliciting no proinflammatory response, but stimulating an anti-inflammatory response, and modulating genes associated with adhesion to the epithelial layer and homeostasis. *S. salivarius* K12 might thereby ensure that it is tolerated by the host and maintained on the epithelial surface while actively protecting the host from inflammation and apoptosis induced by pathogens [15].

Another explanation how probiotics might modulate the oral microbiota might be the modulation of pellicle proteins. It was shown that probiotic bacteria bound to saliva-coated hydroxyapatite reduced the adhesion of *S. mutans* and to lesser extent also the one of *S. gordonii.* The salivary pellicle protein composition was modified and the modifications in the pellicle affected the adherence of *S. mutans* but not of *S. gordonii*. Two of the proteins missing from the pellicles made of saliva treated with the probiotic bacteria were identified as salivary agglutinin gp340 and salivary peroxidase [35].

In contrast to the probiotic strains mentioned above, which were isolated from natural habitats, Hillman and colleagues described a genetically modified probiotic strain [37]. An effector strain has been constructed for use in the replacement therapy of dental caries. Recombinant DNA methods were used to produce a lactate dehydrogenase deficient *S. mutans* strain, resulting in hardly any detectable lactic acid production during growth on a variety of carbon sources. Furthermore, it produced significantly less total acid due to its increased production of ethanol and acetoin. The new strain was significantly less cariogenic than the control in both gnotobiotic- and conventional-rodent models, but it colonized the teeth of conventional rats in the same amount as the control.

A very good overview of the actual status of oral probiotics can be found in the review of Teughels et al. [74].

13.5 Stimulating Skins Own Defense

The effects on the skin caused by prebiotic and probiotic actives presumably are not restricted to a direct promotion or restriction of microbes on the skin and oral cavity but must be seen in the triangle formed by the active, the microbe, and the epithelial cells. Especially the probiotic applications using microbial preparations as described above probably involve the participation of the skins innate immune system. Thus, the idea to stimulate the skin defense directly by actives and therapeutic agents appears to be very attractive [20]. Several examples are already being discussed.

Donnarumma et al. [18] have shown that an extract from avocado was able to influence the adherence of *M. furfur* to keratinocytes and to induce the production of the human β-defensin 2 (hBD-2). The extract mainly consisted of two rare sugars mannoheptulose and perseitol. The activity of these substances might be due to a structural resemblance to constituents of the yeast cell wall. The mode of binding to receptors and the influence on the cytokine expression was discussed.

An even more complex reaction was induced by the probiotic strain *S. salivarius* K12 which was shown to stimulate an anti-inflammatory response [15] (see above).

The main task in further development will be to stimulate the antimicrobial defense system without causing an overreaction leading to allergic reactions, severe inflammation, or even sepsis [20]. This duty was addressed in a screening system based on keratinocytes in which the induction of hBD-2 and hBD-3 by a series of natural products extracts was investigated [62]. Nine extracts were found to be positive without inducing pro-inflammatory cytokines such as IL- 8, IL-1α, or MIP-3α. Thus, these extracts like Arnica, Betel, Black elder, and Mugwort were discussed as being suitable for cosmetic or therapeutic applications.

Interestingly, the expression of the antimicrobial peptide cathelicidin LL-37 is regulated by a vitamin D3 responding element in the promoter region. The oral supplementation with vitamin D3 has been discussed as beneficial in atopic dermatitis as well as the direct topic dosage although the latter is being hampered by skin irritation effects found in experiments with mice [68].

Nevertheless, the induction or even direct application of human antimicrobial peptides has to face the risk of raising resistances. Especially *S. aureus* strains turned out to develop resistance mechanisms against these molecules. Forty-four clinical isolates of *S. aureus* among them being 22 methicillin resistant strains (MRSA) were tested for their susceptibility to

antimicrobial peptides [54]. hBD-3 and LL-37 were more effective than hBD-1 and hBD-2. More than 50% of the MRSA isolates tested showed considerable resistance against the molecules while the methicillin-sensitive strains appeared to be more susceptible.

On the other hand, since the antimicrobial peptides represent only one element out of the several that constitute the innate defense of skin, the probability for the pathogen to circumvent this system might be relatively low [20].

13.6 Conclusions

Driven by the advances made in prebiotic and probiotic food supplements the awareness for beneficial effects of microbes was boosted. This holds true not only for the intestinal tract but also for other parts of the human body such as face and body skin, the armpit, or the oral cavity. Nowadays even for a well-known pathogen such as *P. aeruginosa* a beneficial impact on the skin microflora is being discussed [13].

We have illustrated several attempts to regain a well-balanced skin microflora by using prebiotic compounds or preparations derived from beneficial microbes. Starting from a rather therapeutical background we now see positive examples entering the world of cosmetics [5, 36, 47, 48]. Serious efforts are made to substantiate "cosmetic" product claims with significant results.

While in therapeutics the beneficial effects of a product are well defined, this sometimes turns out to be more difficult for cosmetic products that are intended to treat milder forms of skin disorders, i.e., sensitive or dry skin instead of atopic dermatitis.

The translation of scientific results into product claims with sufficient attractiveness to the consumer is a challenging task. Experiences about how pre- and probiotic food was communicated to the consumer during the market entrance of such products certainly might be helpful for that.

Nevertheless, effects based on scientific evidence are a prerequisite for successful products. Here we still see several questions to be answered in more detail:

- What are the molecular effects of prebiotic and probiotic active on the skin and on the skin microflora? A deeper understanding of the interactions of active, microbe, and skin cells is strongly required.
- What are the beneficial effects of the resident microflora on the molecular level and how will that finally be perceived by the individual human person?
- How to deal with regulatory issues? When beneficial effects can be attributed to strains of the resident microflora a rethinking about safety level classifications might be necessary. *S. epidermidis* which is a major constituent of the human skin microflora is classified as a safety level 2 organism due to its role in nosocomial infections. Formal concern might arise about a product "promoting *S. epidermidis* on the skin." Factors have been identified which differentiate pathogenic from commensal isolates. The pathogenic character of *S. epidermidis* presumably is caused by the selection in the specific ecological niches of the hospital environment [80]. Recently, a study clearly demonstrated that mutants of *S. epidermidis* with a deletion in the icaABCD operon outcompete wild type strains on the skin of volunteers within 1–10 days [66]. Thus, due to the fitness cost for the biofilm and pathogenicity factor icaABCD, the non-pathogenic cells will mainly contribute to the commensal population of *S. epidermidis*. This type of scientific arguments might help to conduct a differentiated and unagitated discussion.

Finding answers to such questions is important since consumer safety is an indispensable prerequisite of all products launched by seriously acting companies.

We conclude that fulfilling the huge demand for further scientific research activities in this field combining molecular microbiology and dermatology will foster this fascinating technology.

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Micronutrients for Hair and Nails

14

E. Haneke and Robert Baran

Core message

› As the appearance of hair and nails is a major concern for woman worldwide, we have tried to collect the most reliable therapeutic sources with a particular interest for micronutrients. The latter is a term used to include trace elements found in minerals, vitamins, amino acids, and herbs. Some of them may be used in both hair and nails, for example, biotin or cystine. There is a general tendency to reverse the conclusion from some deficiency states of certain substances leading to poor hair and nail growth and/or quality that their administration would improve hair and nails even without a proven lack. But, generally speaking, there is a lack of scientific evidence that the nutrients have a real effect on hair and nail quality. Consequently, despite some anecdotal reports relating that some substances are able to improve hair and/or nails changes, in clinical practice, the results obtained are still disappointing when we are confronted to problems.

As the appearance of hair and nails is a major concern for woman worldwide, we have tried to collect the most reliable therapeutic sources with a particular interest for micronutrients. The latter is a term used to include trace elements found in minerals, vitamins, amino acids, and herbs. Some of them may be used in both hair and nails, for example, biotin or cystine. But, generally speaking, there is a lack of scientific evidence that the nutrients have a real effect on hair and nail quality. Consequently, despite some anecdotal reports relating that some substances are able to improve hair and/or nails changes, in clinical practice, the results obtained are still disappointing when we are confronted to problems.

There is no doubt that the skin as the body's largest organ needs a balanced diet to be able to fulfill all its many functions. However, concerning hair and nails, there is an amazing discrepancy between the innumerable claims and assumptions that healthy or organic food, vitamins, calcium, iron, zinc, selenium, phosphorus, essential elements, sulfur-containing amino acids, unsaturated fatty acids, proteins, herbs, and other food additives would ensure full and glossy hair and strong beautiful nails and the sparsety of controlled studies in the scientific literature. Food stables, health shops, drugstores, internet shops, lay healers, even physicians, etc. offer so many miracle cures for hair and nail problems that it is impossible to know all the currently available substances and agents, not to say anything about their purported effects. We will therefore try to restrict ourselves to those micronutrients that have at least a certain scientific basis.

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14.1 Hair and Nails: Anatomy and Physiology (Figs. 14.1 and 14.2)

Hair and nails are unique structures made up of sulfur-rich keratin fibers that are embedded in a sulfur-rich

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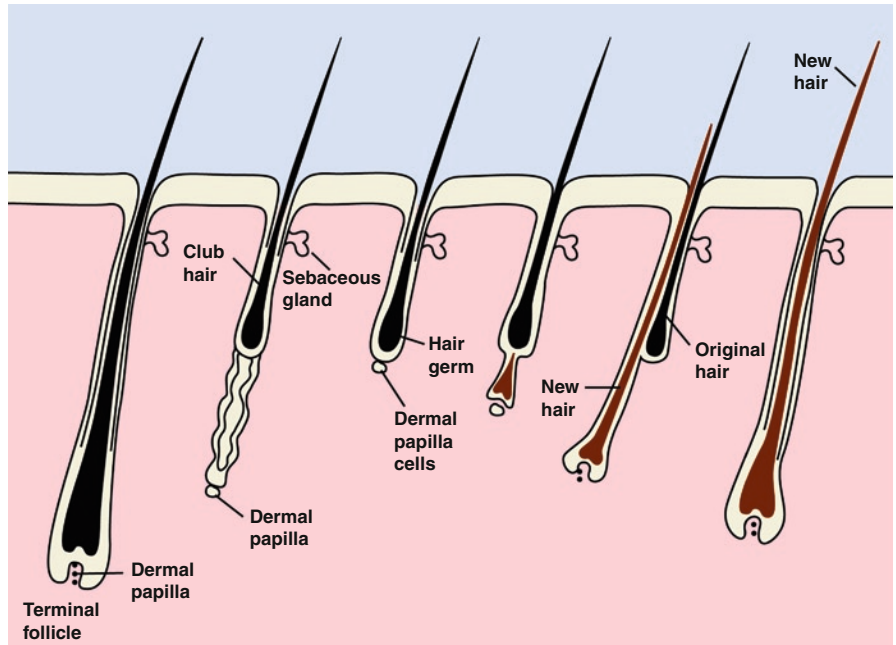
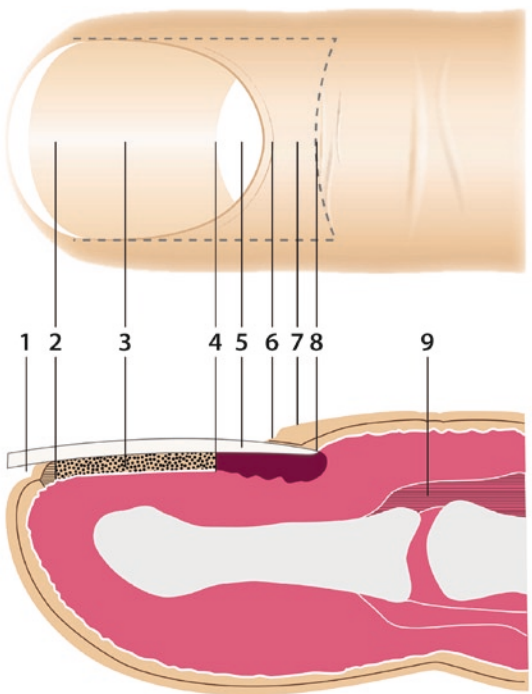


Fig. 14.1 Normal hair growth (After F. Camacho et al.)



1 – Distal groove
2 – Hyponychium
3 – Nail bed
4 – Distal border of the Matrix
5 – Lunula
6 – Cuticle
7 – Proximal nail fold
8 – Proximal edge of the nail plate
9 – Extensor tendon

Fig. 14.2 Nail anatomy

amorphous matrix. Their chemical composition is strictly regulated by a variety of genes and does not – or almost not – depend on the exogenous intake of its basic elements and amino acids. They are produced by the hair follicle and the nail apparatus, respectively. Whereas the hair is a round to oval structure and has a particular pattern of cuticle cells and cortex and medulla, the nail is a plate-like structure lacking any type of specialized superficial cell layer although its uppermost layer is denser than the middle and deeper ones and is responsible for the nail sheen. Hair and nails grow more or less life-long, but the hair has, in contrast to the continuously growing nail, a cyclical growth pattern that is hormone-dependent. Nail growth is controlled by a variety of cell–cell, cell–matrix, and cell–tissue interactions as well as by signaling factors, many of which are not yet clearly defined. It also depends on age, blood supply, intensity of mobilization, dominance of the respective hand, a variety of diseases and drugs, as well as hereditary factors, temperature, altitude, etc. [19].

Some hormones, in particular androgens, influence the hair follicle activity whereas this is not known for the nails. Hair bulbs contain a variable number of usually very active melanocytes, depending of course on the normal hair pigmentation, whereas the nail plate is not pigmented in light-skinned persons though may

take on some melanin pigmentation during the course of life in dark-skinned individuals. Graying of the hair is common in all skin types and is due to more or less complete loss of melanin. A comparative phenomenon does not occur in the nails. Scalp hair follicles have a proliferation rate that is about two to three times higher than that of nails [29]. Human scalp hair grows about 13 mm per month, the nail of the middle finger of the dominant hand about 3 mm; toenails grow only about one third the rate. The function of scalp hair, often called the pride of a woman, is in our society mainly psychosocial whereas the nails' functions are protection of the finger and toe tips, defense, scratching, and enhancement of the many sensory functions of the finger pulps.

The knowledge about the importance of micronutrients derives from experiments where particular deficiency states were produced and treatment with normal diet or food supplements could alleviate or cure the symptoms. However, hair and nails have not many different ways to react to deficiencies and their alterations to micronutrient deficiencies are therefore relatively uniform: the hair follicles may react with hair loss, thinning of the hair diameter, finally with alopecia whereas the nail may grow slower and become brittle (Fig 14.3).

Fig. 14.3 Brittle nails (onychoschizia)

Since the morphological modifications overlap and many deficiency states have no typical or diagnostic laboratory abnormalities they are often treated uncritically with multivitamin and multielement supplements.

14.2 Nutrition in General

Hair is composed of 98% protein, so healthy hair requires an adequate daily source of protein, from either animal protein or nonanimal source. This is the basic important requirement for healthy hair.

Frank malnutrition, either by lack of adequate food intake, malabsorption, metabolic diseases, or grossly abnormal eating behavior leads to sparse hair and nail growth disturbance [26, 45, 65]. This is seen in certain malabsorption syndromes, serious malnutrition, and kwashiorkor, after bariatric surgery like the short bowel syndrome, in anorexia nervosa and bulimia and other conditions leading to cachexia [31, 59]. Some hair and nail conditions could be traced to specific deficiencies, such as zinc deficiency in persons on parenteral nutrition or "astronaut food" for intractable Crohn's disease, after gastrectomy for gastric cancer or in alcohol-induced insufficient zinc resorption [28] not to mention the poor quality of the extremely thin hair and of the nails in acrodermatitis enteropathica. It is also well known that overt iron deficiency causes anemia, hair and nail growth disturbances including koilonychia, as well as it induces burning of mucosal membranes and increases the tendency to develop hypopharynx carcinoma. Vitamin C deficiency leads to a variety of diseases, the most severe of which is scurvy, but it also interferes with hair and nail growth. Risk persons for micronutrient deficiency are elderly persons, those with eating disorders, and those on diets lacking important nutritional factors. A general undernourishment should prompt the intake of multivitamin and multimineral preparations. A low-energy nutrition below 1,500 kcal is usually also associated with an insufficient micronutrient intake. This inference is particularly evident in anorexia nervosa and bulimia where hair loss resulting in alopecia as well as brittle nails is almost the rule. Commonly the faster growing hair reacts earlier with alopecia or hair loss than the slower growing nails.

On the other hand, people immigrating from the poorest countries who never had the opportunity to watch the quality of their food as long as it was just enough not to starve often have silky soft skin, thick hair, and excellent nails.

A recent study found that nitrogen and sulfur contents in the nails are not related with dietary intake. Although females have a higher sulfur content in their nails than males their nitrogen content reflects the amount of amino acids is lower. The nitrogen content decreases with age [17].

There are very slight differences between the nutrients used for treating hair or nails. Trace elements and minerals, vitamins, amino acids, and herbs are more often prescribed for treating hair than nails.

14.3 Diets

Obesity is one of the most frequently seen symptoms of our society. The result is the emergence of thousands of diets for weight loss. If a diet should really lead to the loss of excess adipose tissue, it must be calorie-deficient. Here the diets advertised vividly in the media, yellow press, journals, and health books will not be evaluated; instead, some remarks will be given concerning some widely used diets.

Weight Watchers: This diet considers the carbohydrate, fat, and bulky substance content of the food and contains sufficient fruits, vegetables, and antioxidants. It is balanced and well adapted for long-term use. It will most probably not cause hair and nail disorders.

Low fat 30: The main point is the reduction of the proportion of fat to less than 30% of the total energy intake. There is a high carbohydrate intake without consideration of the glycemic index. A sufficient intake of antioxidants and unsaturated fatty acids is not ensured and may lead to micronutrient deficiency in the long run. Phrynoderma-like changes were observed in people with insufficient intake of polyunsaturated fatty acids.

Mediterranean diet: This is a well-balanced diet considered for long-time use with sufficient intake of antioxidants, vitamins, and unsaturated fatty acids.

Montignac diet: Well-balanced diet with sufficient micronutrients.

Separation diet: This is an unscientific diet that separates carbohydrates, fats, and proteins, has a very low milk proportion, and the intake of antioxidants is usually too low.

Protein diets: Proteins are the main energy carriers, the proportion of animal derived food is too high and that of unsaturated fatty acids too low.

Atkins diet: High intake of fat and proteins, insufficient vegetable and fruit consumption with lack of plant-derived vitamins, and unsaturated fatty acids.

The potential hair and nail signs will be described below.

14.4 Vitamins

The role of vitamins for beautiful hair and good nails is usually overestimated. Of the many vitamins, some may be beneficial, some are probably inert, and some may even deteriorate the quality of hair and nails.

14.4.1 Vitamin A

Vitamin A, often designated as the skin vitamin, is essential for soft skin. Hypovitaminosis A, probably in conjunction with other deficiency states, leads to phrynoderma, a condition that bears some similarity with pityriasis rubra pilaris. However, hypervitaminosis A is not only a potentially life-threatening condition due to intracranial hypertension but is also hepatotoxic. When extremely high doses of vitamin A were given to cancer patients, they experienced loss of hair, thin hair, and brittle nails. Since vitamin A deficiency is no longer observed in people with normal nutrition the addition of vitamin A to preparations intended to improve hair and nails is not justified.

14.4.2 Vitamin B_1: Thiamine

Thiamine deficiency leads to beriberi, one feature of which is also thin hair. Recently, also particular nail alterations were described under extreme malnutrition. They consisted of nail layering and extremely painful intra-nail hemorrhage. These changes were confirmed to be due to thiamine deficiency though a deficiency of niacin or riboflavin might also have played a role. Both skin and nail alterations responded dramatically to

thiamine injections and improvement of nutrition [41]. Thiamine is also a component of a hair and nail remedy sold in many European countries (see below).

14.4.3 Vitamin B_3: Niacinamide

Niacin and niacinamide, respectively, make up the vitamin B_3 complex. Niacinamide (aka nicotinamide) and niacin (aka nicotinic acid) are heterocyclic aromatic compounds that function in cosmetics primarily as hair and skin conditioning agents. Niacinamide is used in around 30 cosmetic formulations including shampoos, hair tonics, skin moisturizers, and cleansing formulations. Niacin is used in a few similar product types. The concentration of use of niacinamide varies from as low as 0.0001% in night preparations to as high as 3% in body and hand creams, lotions, powders, and sprays. Niacin concentrations of use range from 0.01% in body and hand creams, lotions, powders and sprays to 0.1% in paste masks (mud packs). Both ingredients are accepted for use in cosmetics in Japan and the European Union. Both are Generally Recognized As Safe (GRAS) direct food additives and nutrient and/or dietary supplements. Niacinamide may be used in clinical treatment of hypercholesteremia and niacin in prevention of pellagra and treatment of certain psychological disorders [15]. Despite their well-known function in redox enzymes their action on hair and nails is not exactly understood. In patients suffering from chronic preterminal renal insufficiency and undergoing hemodialysis, niacin deficiency is assumed to cause onycholysis [36]. In pellagra, a general hair and nail growth disturbance is seen. No specific data were reported on the effect of niacin substitution on hair and nail growth and quality in normal persons. Topical application of niacin derivatives, on the contrary, was shown to improve hair growth in female pattern hair loss [18]. Whether this is due to the local hyperemic effect of these nicotinic acid esters or on a vitamin B_3 effect thus warranting oral niacin supplementation for better hair growth is not clear.

14.4.4 Vitamin B_5: Pantothenic Acid

Panthenol, the alcohol of pantothenic acid, is assumed to be a humectant and to improve the strength and flexibility of hair and the nails. When applied externally, the panthenol concentration in the nail plate and nail bed increases thus improving the hydration of the nail [34]. Panthenol is also the major constituent of several oral preparations claiming to enhance hair and nail growth [68] and also Si-Nails capsules® contain 6 mg vitamin B_5 in addition to vitamins B_2 (1.2 mg), B_6 (2 mg), and H (150 µg biotin) as well as extract of horsetail (equisetum arvense) (corresponding to 12 mg silicon), methionine, cystine, and zinc. However, excess vitamin B_5 caused enlargement of the testis, diarrhea, and hair damage in Wistar rats [63].

14.4.5 Vitamin B_6: Pyridoxal Phosphate

Pyridoxal phosphate is the active form of vitamin B_6. It is involved in many reactions, including decarboxylation and transamination. It has been shown to inhibit DNA polymerases and several steroid receptors and may prove useful as an adjunct in cancer chemotherapy.

Vitamin B_6 administered parenterally for a period of several weeks induced improvement in the hair condition in a number of women and reduced hair loss, especially in telogen effluvium whereas oral calcium pantothenate in feminine diffuse alopecia did not show this positive effect [11]. In homocystinuria, vitamin B_6 is able to recolor the hair of the patients [47, 62]. As mentioned above, pyridoxine is part of several over-the-counter preparations sold for hair and nails.

14.4.6 Vitamin B_{12}: Cyanocobalamin

Up to now, no positive effect of vitamin B_{12} on hair and nail quality of well-nourished subjects has been demonstrated [60].

14.4.7 Vitamins B Plus Amino Acids

Producer-initiated studies with a preparation containing thiamine mononitrate (vitamin B_1) 60 mg, calcium-D-pantothenate (vitamin B_5) 60 mg, medicinal yeast 100 mg, L-cystine 20 mg, keratin 20 mg, and p-aminobenzoic acid 20 mg per capsule (PANTOVIGAR®)

claimed a reduction of hair loss and improvement of hair quality, which was significantly better than the treatment with a combination of calcium-D-pantothenate 60 mg and L-cystine 220 mg [12, 52]. It was also effective in spontaneous hair loss as measured with trichogrammes [33]. The hair quality of ultraviolet-damaged and undamaged hair improved.

14.4.8 Vitamin C: Ascorbic Acid

Vitamin C is one of the most widely known vitamins and is believed to exert a positive effect on an almost limitless number of processes, ranging from the proven effect on collagen synthesis to radical scavenging to improvement of immune functions and protection against cancer. Its daily need is about 90 mg for men and 75 mg for women, but persons with cancer and other consuming diseases, diabetes mellitus as well as smokers need higher doses. Severe vitamin C deficiency results in scurvy, which is however, rarely seen in developed countries. Symptoms develop when the serum level is below 0.15 mg/100 ml. Apart from the typical symptoms of weakness, joint pain, gum bleeding, easy bruising, petechiae and delayed wound healing, areas of thinned hair on the scalp, and corkscrew hairs emerging from purpuric follicular hyperkeratotic areas are characteristic. However, even though vitamin C addition significantly enhanced the growth of human hair papilla cells in culture there is no scientific basis for a general recommendation of vitamin C supplementation in individuals with poor hair and/or nail growth except when a vitamin C deficiency has convincingly been proven [8, 60, 74]. Very high bolus doses of vitamin C were shown to counteract the vasoconstrictor effect of smoking a single cigarette [75]. However, whether this may have a beneficial effect on the nails or hair in chronic tobacco abuse is not clear.

14.4.9 Vitamin D

The processes of epidermal growth and keratinization are highly dependent on hormonal control like vitamin D_3, calcium homeostasis, retinoids, and growth hormones. Skin and hair follicles contain the nuclear vitamin D receptor (VDR) for 1,25-dihydroxyvitamin D_3, the active hormone. VDR also binds omega3/omega6 polyunsaturated fatty acids. Activation of VDR by polyunsaturated fatty acids and curcumin may elicit unique, $1,25(OH)_2D_3$-independent signaling pathways to orchestrate the bioeffects of these lipids in intestine, bone, skin/hair follicle, and other VDR-containing tissues [35]. The gene for atrichia has been linked to the same as for vitamin D resistant rickets. In skin, VDR expression in keratinocytes is essential in a ligand-independent manner for the maintenance of the normal hair cycle. Therefore, VDR but not vitamin D deficiency results in alopecia. Despite these apparent junctions, vitamin D supplementation has not proven to be beneficial for hair or nail quality.

14.4.10 Vitamin E: α-Tocopherol

Vitamin E is the classical lipophilic antioxidant. Together with vitamin C, the hydrophilic antioxidant, it protects against the noxious influence of reactive oxygen species, e.g., against lipid peroxidation. It is a component of many food additive preparations. Whether or not it has a positive effect on hair density and quality or on nails remains to be shown. Vitamin E in moderate to high doses – both topical and systemic – has been described to be beneficial in the yellow nail syndrome [3, 4, 44] especially in combination with fluconazole 300 mg once a week. Vitamin E is also said to have a positive effect on brittle nails in individuals with eating disorders [66]. There are no reports about vitamin E in persons with hair problems [51].

14.4.11 Vitamin H: Biotin

Biotin was called the hair and nail vitamin (Fig. 14.4). It is a cofactor of several enzymes that are important for carboxylation and epidermal differentiation. It has been used to treat lameness in animals and to improve the quality of hooves. Biotin has been shown to be a beneficial therapy. The daily requirement of biotin is

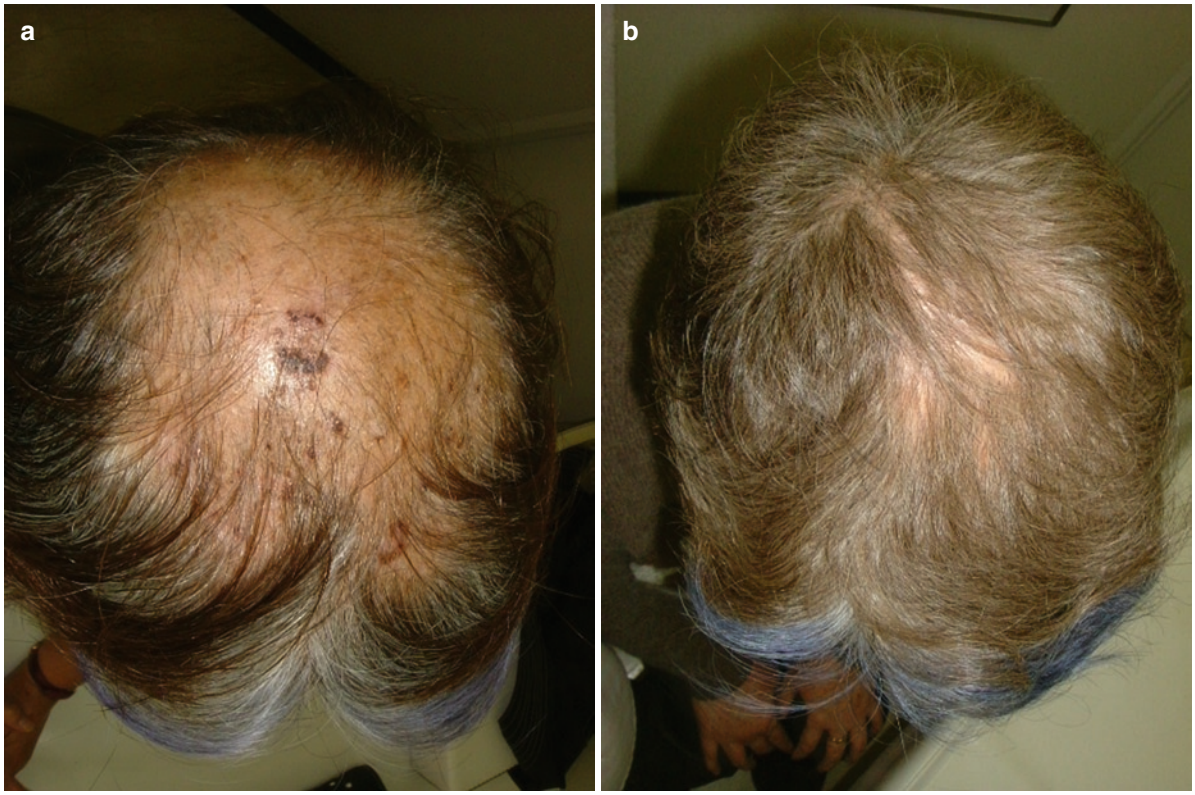


Fig. 14.4 Biotin treatment (courtesy of H. Arai) (**a**) before treatment (**b**) after 2 years

unknown since it is produced in large quantities by intestinal bacteria. In man, daily biotin in a dose above 2.5 mg was shown to improve brittle fingernails and onychoschizia. In one study, biotin 2.5 mg/day for 6–15 months improved brittle nails; nail thickness improved by 25%, and lamellar splitting improved in all patients. In another study, biotin 2.5 mg/day for 1.5–7 months resulted in clinical improvement in 67% of the patients. However, both studies were carried out in small groups of patients without a control group [9, 14, 32]. Biotin also improves hair density (Fig. 14.4). In our experience, the daily dose should not be lower than 5 mg [30]. Apparently, when given long enough, it can also restore hair color.

Increase of linear nail growth has been reported with local application of hydroxypropyl chitosan in a double-blind study [5].

14.5 Miscellaneous

14.5.1 Essential Fatty Acids

Essential fatty acids are polyunsaturated fatty acids (PUFA) (*n*-3 and omega-6). If there is a lack of PUFA a variety of skin lesions can develop that may mimic phrynoderma and can also cause hair loss and brittle nails. Although it is generally beneficial to guarantee a minimum daily intake of PUFA it is not clear whether this is important for hair and nail health in otherwise well-nourished people. However, PUFA addition significantly improves the hair coat in dogs [37].

14.5.2 Starflower Seed Oil

Starflower (borage, Borrago officinalis) seed oil is the richest in ***gamma linolenic acid of all plant products,

followed by black currant seed oil and evening primrose (Oenothera biennis) oil. Borage oil also contains valuable minerals said to be needed for proper cardiovascular function and healthy skin and nails. The recommended daily allowance of borage oil is 1,300 mg. It was recently reported to improve brittle nails [19] but has also been claimed to cure scalp and hair disorders. High doses of borage oil can be toxic due to their pyrrolizidine alkaloids [76].

14.5.3 Melatonin

Melatonin is the hormone produced by the pineal gland, but recently it was also shown that both the skin and the hair follicle are not only the target of melatonin but also produce melatonin themselves. Melatonin is a powerful antioxidant and oxygen radical scavenger, it has a regulative action on photoperiod-associated changes, regulates chronobiological and reproductive systems, coat phenotype in animals, and mammary gland functions. In addition, it is important for DNA repair via immunomodulation, body weight control and wound healing, and the modulation of secondary endocrine signaling such as prolactin release and estrogen receptor-mediated signaling [23].

Its action on the human hair follicle is a prolongation of the anagen phase, particularly in women with diffuse and androgenetic alopecia [21, 22]. Oral supplementation with melatonin, phytosterols, and isoflavones may be helpful in the treatment of female androgenetic alopecia. In high concentrations, however, melatonin inhibits human hair follicle proliferation [20]. Topical application of a 0.1% melatonin solution on the scalp once daily over 6 months significantly increased the anagen hair rate in alopecic women as compared to placebo-treated patients. Until now, there is no scientific basis for oral melatonin supplementation to improve hair growth although a topical formulation containing melatonin, biotin, and Gingko biloba extract has recently come on the market (Asatex®) for the treatment of hair loss and thin hair.

14.5.4 Cysteine and Cystine

The sulfur-containing amino acids cysteine and cystine have long been thought to be able to improve hair and nail growth because keratin is a sulfur-rich structure. Though sulfur itself does not improve nail growth it was found that cystine may have a positive effect on the growth of hyponychium cells. Cystine was also claimed to be incorporated into growing hair and nails [48]. However, this has never been confirmed in humans. Cysteine was found to be slightly diminished in the tricho-onychotic ectodermal dysplasia, which is characterized by brittle hair and nails [25]. A low cystine content of 4.6% was found in Tay's syndrome as compared to 8.4% in normal hair [49]. Some preparations mentioned earlier contain also cystine [68].

Some preparations also contain pulverized keratin. A positive effect has not been proven.

14.5.5 Taurine

Taurine (2-aminoethyl sulphonic acid) is a β-amino acid present in many animal tissues. It can be synthesized by the human body but only to a certain degree and has therefore to be delivered with food, such as meat, fish, and milk. Apart from many other functions, it is an important factor for retina, nervous system, and kidney functions. It was shown to improve hair growth in culture and is marketed, together with polyphenols of green tea and grape seed extracts, for improvement of thin hair (Innéov Hair density®, Kerastase Densitive®). Taurine 150 mg was said to improve hair density [13]. Another new preparation (Innéov Lab) containing taurine (75 mg), polyunsaturated fatty acids (omega-3 plus omega-6 195 mg), vitamins C (15 mg) and E (2.5 mg), lycopene (0.5 mg), plant polyphenols (140 mg), and zinc (7.5 mg) was recently tested for the treatment of hair loss. Compared to placebo, hair loss was considerably reduced and hair density and quality increased [10].

14.5.6 Gelatin

Gelatin is produced by acid hydrolysis of collagen, the main sources of which are dermis, tendons, and bones. More than half a century ago, it was suggested that large amounts of gelatin [73] increase the cystine content of nails [55], which may reflect keratin formation and cross-linking. Gelatin contains 84–86% of protein and 2–4% of mineral salts. It is rich in glycine (24%), proline (14%), and hydroxyproline (10%), but poor in

essential and particularly in sulfur-containing amino acids: cystine < 0.1%, methionine < 1%. However, hair and nails do not contain collagen or gelatin. A pharmacodynamic effect of those collagen amino acids on hair and nails is not proven. Even though it is still recommended for brittle nails and hair [50, 73], there are no data to confirm these speculations.

14.5.7 Iron

Low iron store represents a risk factor for hair loss in non-menopausal women.

Iron is an essential nutrient necessary for oxygen metabolism and mitochondrial function. It exhibits a fundamental importance as a trace metal in the normal growth and functional maturation of the skin and in the health of hair and nails [38].

The major hair and nail signs of iron deficiency are hair loss as well as koilonychia, ridging, and brittle nails. If iron anemia is present it should be treated with iron supplementation. However, treatment without anemia is still controversial. Iron overload is a risk and has to be avoided [71]. Almost 50 years ago, the importance of iron supplements in nonanemic iron-deficient women with hair loss was demonstrated. This is best measured with serum ferritin concentrations, as they are a factor in female hair loss. However, what level of serum ferritin to employ in subjects with increased hair shedding remains yet to be definitively established but 70 μg/l, with a normal erythrocyte sedimentation rate (<10 mm/h), is recommended [58]. Anyway, low iron stores are a risk factor for excessive hair loss in non-menopausal women [16].

The major cause of hair loss in women before the age of 50 is nutritional, with 30% affected. Increased and persistent hair shedding (chronic telogen effluvium) and reduced hair volume are said to be the principal changes occurring. The main cause appears to be depleted iron stores, compromised by a suboptimal intake of the essential amino acid l-lysine. Correction of these imbalances stops the excessive hair loss and returns the hair back to its former glory. However, it can take many months to redress the situation [56, 57].

14.5.8 Silicon

Silicon (Si) is assumed to be important for the synthesis of connective tissue and bone. Nutritional Si deficiency impairs the synthesis of these tissue components and of glycosaminoglycans. Si is also thought to improve the synthesis of sulfur-rich proteins. Between 1 and 10 ppm of Si are found in hair and nails [2, 64]. A structural role of Si in the cross-linking of glycosaminoglycans in connective tissue has also been suggested [61]. Hence, it is hypothesized that treatment with Si might also improve the keratin structure in hair and nails.

Soluble Si is present as orthosilicic acid (OSA) in beverages and water, but the concentrations are very low as OSA polymerizes at concentrations higher than 10^{-4} in neutral pH. OSA is bioavailable whereas its polymerized forms are not. However, dietary Si polymers are hydrolyzed in the intestines and absorbed. Two uncontrolled studies using colloidal silicic acid for combined oral and topical treatment claimed an improvement of both brittle hair and fragile nails in about 50% of the treated patients [39, 40]. A more recent double-blind placebo-controlled study using choline-stabilized OSA 50 mg/day, equivalent to 10 mg of Si, over a period of 20 weeks showed significantly lower scores for hair and nail brittleness in the OSA-treated group as compared to the placebo group [6]. A scanning electron microscopy microanalysis study recently found Si in 5% of female patients with telogen effluvium (one patient) as compared to 35% (seven women) in the control group. This study also found a higher calcium content in the control group as compared to the telogen effluvium group [67]. The authors speculate that Si may be important for hair trophism. As Si is found in the normal nutrition such as cereals, bread, bananas, beer, and coffee it is not clear whether or not Si supplementation will really help in persons with effluvium and/or brittle nails. Silica is nevertheless often recommended to individuals with hair loss.

14.5.9 Rhodanide

Rhodanide is claimed to play an essential role in the hair cells. As the molecule contains sulfur, carbon, and nitrogen it is claimed to be the most important energy transfer molecule and helps cells division and mediates important metabolic processes. No single scientific report is available concerning rhodanides and hair growth.

14.5.10 Fluor and Fluorides

Fluor is the most important element for hardness of dental enamel. This may be the reason why some unsubstantiated reports in the lay press claim that adding fluoride to the food would also increase the hardness of the nails. However, even though the level of fluorides in the nails reflects fluor saturation there is hitherto no proof that this is true as there is no study available on the effect of fluoride addition and nail quality [42, 46].

14.5.11 Calcium

Calcium is useless and overdoses of vitamin A can deteriorate the nails.

Calcium is apparently not responsible for hair and nail hardness as the nail is relatively poor in calcium [24]. However, a study using daily calcium 1.0 g versus evening primrose oil 4.0 g, calcium 1.0 and 440 mg marine fish oil (Efacal) over 1 year improved the nail quality in both pre- and postmenopausal women [7, 53].

14.5.12 Zinc

Zinc deficiency leads to poor hair and nail growth as is evidenced in acrodermatitis enteropathica and acquired zinc deficiency syndromes [7]. Toe and finger nail zinc concentrations vary with dietary zinc intake, even in a healthy population with presumably little zinc deficiency [27]. Whether or not zinc in normally nourished persons can improve the quality, density, and growth rate of hair and nails is doubtful.

14.5.13 Selenium

Selenium occurs as inorganic selenite or selenate and in organic forms in plants and other organisms used for food. The human selenoproteome consists of 25 selenoproteins. The main groups are glutathione peroxidases 1–5, iodothyronine deiodinases 1–3, thioredoxin reductases, selenoprotein P (SelP), and other proteins mostly with unknown function. In selenoproteins, selenium occurs as selenocysteine. Excess selenium can produce selenosis in humans affecting liver, skin, nails, and hair. Recommended intake and upper tolerable level are 40–55 and 300 μg/day [1, 43].

14.5.14 Thyroid Hormones

Thyroid function is essential for many metabolic processes. Whereas hyperthyroidosis leads to hair loss and brittle nails hypothyroidosis causes fine thin hair and thin fragile nails. The influence of weight-loss pills illegally containing thyroid hormones on hair and nails has not yet been investigated, but it has to be assumed that it is similar to that of an endogenous hyperthyroidosis.

14.5.15 Honey and Gelée Royale

Folk medicine claims that honey and particularly lyophilized gelée royale stimulate hair and nail growth. No scientific studies are available.

14.6 Herbal Medicine

The number of herbal medicines that are advertised for hair growth is tremendous. According to the producers and sellers, the function and effects of the herbs vary. Hence, it is said to be important to understand what goes into the product when considering which remedy is best for a given case. For easy comparison and evaluation, they suggest seeking out the credible ones and highlighting those ingredients that are beneficial to hair loss conditions. The main advantage that these products are claimed to have over drugs is that they address the problems effectively with no side effect.

Many herbal remedies for hair loss contain one or more ingredients. Though they come in various forms (pills, tablets, tonic) these herbal hair loss solutions are said to be created as a feasible and safe option for men and women to tackle their hair loss problem. However,

both their efficacy and their safety remain a very delicate issue as many products have never been scientifically investigated. On the other hand, heavy metals are part of Ayurvedic medicine and were repeatedly described to produce serious intoxications. Also herbal supplements were described to contain high amounts of arsenic and cause chronic arsenicism with progressive alopecia.

14.6.1 Soy

Soybean proteins have been used in East Asia for centuries. Women working in soy-processing factories were known for their silky and light skin, which was thought to be due to the high amount of soy phytoestrogens. Also the hair quality was believed to be improved by soy. This has recently been supported by another mechanism of soy ingredients, an anti-alopecia activity of the immunostimulatory soy peptide soymetide-4. This effect is probably due to PGE2: prostaglandin E_2, which is produced after activation of COX by soymetide-4 and might suppress apoptosis of hair matrix cells and etoposide-induced alopecia by activating NF-kappaB [72].

14.6.2 Mulberry

Mulberry Pills®™ are claimed to target and affect the scalp skin, thereby balancing the metabolism of hair follicles, activating cells of the hair papilla, improving blood circulation, increasing nutrition supply, recovering autoimmune regulation, relieving dysfunction of hair follicles and restoring the function of hair follicles, preventing hair loss, and in the end make the hair regrow [77].

14.6.3 Gingko biloba

Gingko biloba is a large tree, originally from East Asia, which is now found in many parks in the temperate zones around the world. The extract of Ginkgo biloba is a very popular plant remedy that is thought to help with many problems, among them improving the circulation of blood to the brain and skin. Therefore, it is frequently used as a memory and concentration enhancer. The majority of herbalists who prescribe this for loss of hair do so believing that the increase of blood to the brain and skin delivers more nutrients to the hair follicles and so promotes hair regrowth. No clinical studies on its efficacy in treating hair loss have been conducted yet. Nonetheless, ginkgo is used as an active ingredient in some commercial hair loss remedies. Given its many suspected side effects, ginkgo should be used with caution [77].

14.6.4 Green Tea (Camellia sinensis)

Green tea is another popular herbal remedy. It is made from the dried leaves of the tea plant whereas black tea is made from dried tea plant leaves that have gone through a fermentation process. It is believed that the enzyme 5-alpha-reductase is inhibited by the catechins found in the green tea. Green tea has been credited with providing a wide variety of health benefits, many of which have not been validated by scientific evidence (e.g., its potential for treating male pattern baldness). However, some herbalists claim that the risk of male pattern type baldness will be reduced if several cups of green tea are consumed or it is taken in capsule form on a daily basis. It is tasty, it might be healthy, too, but it should not be expected to grow new hair [77].

14.6.5 He Shou Wu or Fo-Ti (Polygonum multiflorum)

He Shou Wu is an ancient Chinese herb that has been used for centuries for hair loss and baldness. It is available in both tea and capsule form and is one of the main ingredients found in many commercial remedies for the treatment of hair loss. In a recent study published by American Botanical Council (read article), the authors note that this Chinese herb shows promise as a hair and color restorative and is capable of inducing terminal hair to grow instead of vellus hair (the fine baby hair growth associated with use of minoxidil).

14.6.6 Pygeum (Pygeum africanum)

Pygeum africanum is a large evergreen tree found in central and southern Africa. The extracts from the pygeum bark contain several compounds thought to be helpful in prostate health and have been used for decades to treat benign prostatic hyperplasia (BPH). Like saw palmetto and nettle root, pygeum is also believed to inhibit the enzyme 5-alpha reductase, which converts testosterone to the follicle-harming dihydrotestosterone (DHT). Despite the lack of clinical evidence of any positive impact on male pattern baldness, pygeum can be found in a number of natural hair loss remedies [78].

14.6.7 Saw Palmetto (Serenoa repens)

Saw palmetto is an extract made from the fruits of a small palm tree (Serenoa repens), which is endemic to the southeastern USA. It is the primary active ingredient in almost every natural hair loss remedy, including Provillus, Procerin, Advecia, Avacor, Revivogen, Scalp Med, and many others. Saw palmetto has long been used to treat benign prostatic hyperplasia (BPH). It is rich in fatty acids and phytosterols and it is often claimed to be able to block dihydrotestosterone (DHT). There are plenty of research papers claiming saw palmetto is beneficial in treating BPH but only one piece of research proved that it can reduce the level of DHT in the prostate. However, the latest research contradicts this claim by showing that saw palmetto may not have any effect on the plasma concentration of DHT. In addition, one recent clinical study claims that saw palmetto does not shrink enlarged prostates either. Despite the fact that saw palmetto has been used to treat BPH for decades there is no conclusive proof that it is effective.

Saw palmetto has never been clinically tested as a hair loss treatment and its efficacy and mechanism of action are not known. In addition, it is suspected its side effects might be more severe than those of finasteride but they have been poorly documented to date. Saw palmetto cannot be recommended as a hair loss remedy [78].

14.6.8 Stinging Nettle (Urtica diocia)

Stinging nettle is a perennial plant and common weed that grows in temperate climates throughout the world. It is among the most common herbs used in traditional folk medicine. Both the leaves and the root are used in various cures. The nettle root is often found in natural medicines to relieve the symptoms of BPH. It is therefore assumed it could prevent the conversion of testosterone to DHT but no clinical studies have been conducted yet on the use of nettle in treating DHT-related hair loss. Although no one is certain whether it really helps against baldness, nettles are frequently used as one of the active ingredients in many commercial hair loss remedies [78]. Stinging nettle blocks the conversion of testosterone into dihydrotestosterone (DHT), which is the main cause of hair loss in men. It can be bought in either pill or capsule form and is said to be more effective when used in combination with saw palmetto or pygeum.

14.6.9 Dong Quai (Chinese angelica)

Like He Shou Wu, Dong Quai is a traditional Chinese herb that is used to stop hair loss and even regrow hair. Dong Quai contains phytoestrogens that reduce the formation of DHT.

14.6.10 Panax ginseng

Used in Asia for thousands of years, ginseng's purported benefits include improving vascular circulation and regulating cellular metabolism. Used in shampoo or hair tonic, Ginseng is said to help to nourish and strengthen hair. Panax ginseng was shown to prevent irradiation-induced programmed cell death in hair follicles, which might be a rationale for its use in hair loss [69].

14.6.11 Eleuthero Root

Eleuthero root, also called Siberian ginseng and a distant relative of Asian ginseng, is a popular folk medicine in Russia and China. Eleuthero is a herbal antioxidant and it is believed to be anti-inflammatory and to be able to increase endurance, improve memory, boost the immune system, and help protect cells from damage due to environmental conditions. Eleuthero is often used as one of the substances in natural hair loss cures,

although there is no clinical proof of its positive effect on human hair [78].

14.6.12 Pumpkin Seed Oil

Pumpkin seed oil is pressed from pumpkin seeds and is rich in iron, zinc, and essential fatty acids. It has been used for centuries as a folk remedy for prostate problems, particularly for benign prostate hypertrophy. Therefore, pumpkin seed oil is believed to be a natural DHT blocker. However, no clinical study exists regarding its potency to block DHT or its effectiveness in treating baldness [78]. It is healthy and tasty.

14.6.13 Rosemary

Rosemary was used as a hair growth stimulant. It is also considered as an effective conditioner for greasy hair, a general tonic imparting body and sheen to hair, and an antidandruff ingredient when combined with sago.

14.6.14 Colloidal Oatmeal

Colloidal oatmeal is effective in protecting and repairing skin and hair damaged from UV radiation, smoke, bacteria and free radicals.

14.6.15 Arnica

Arnica has been evaluated as a treatment for hair loss as a result of androgenetic alopecia, stress and psychological causes.

14.6.16 Oral Supplementation with Melatonin

Oral supplementation with melatonin, phytosterols, and isoflavones may be helpful in the treatment of female androgenetic alopecia [70].

14.6.17 Ageratina Pichinchensis Extract

This Mexican traditional medicine works on onychomycosis associated with Trichophyton rubrum. A comparative study with Ciclopirox has not demonstrated differences between treatments [54].

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Part III

How to Use Functional Food in Clinical Dermatology

Legal Aspects: How Do Food Supplements Differ from Drugs, Medical Devices, and Cosmetic Products?

15

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Core Messages

- Drugs, medical devices, food supplements, and cosmetics are all defined by regulation but differ in terms of degree of stringency and harmonization.
- By regulation, nutraceutical and functional foods do not exist. Rather, such nutritional products that provide a benefit for consumers are considered foods or food supplements.
- While harmonization in food supplement legislation is ongoing, it is still largely not harmonized, resulting in inconsistent formulations, claims, and regulatory classifications in different countries in the European Union (EU).
- Health claims and maximum levels are the two key areas of harmonization currently underway that will make a significant difference in easing consumer confusion about food supplement products.
- Medicinal products are stringently regulated with full harmonization of regulations across the EU, including harmonized approval processes that can be managed centrally or at the Member State level.
- Similarly, medical devices and cosmetics have clear regulations that are harmonized across the EU with a "mutual recognition" principle followed by EU Member States.

Abbreviations

AESGP	Association of the European Self-medication Industry
BGH	Bundesgerichtshof (German Federal Higher Court)
CECP	Committee of Expert on Cosmetic Products
CHMP	Committee on Human Medicinal Products
EAS	European Advisory Services
EC	European Commission
EFSA	European Food Safety Authority
EMEA	European Medicines Evaluation Agency
ERNA	European Responsible Nutrition Alliance
EU	European Union
GCP	Good Clinical Practice
GMOS	Genetically Modified Organism
ILSI	International Life Sciences Institute
INCI	International Nomenclature of Cosmetic Ingredients

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MEDDEV	Medical Device
MRP	Mutual Recognition Procedure
OTC	Over The Counter
QUID	Quantitative Ingredient Declaration
SCC-NFP	Scientific Committee on Cosmetic Products & Non Food Products
SCCP	Scientific Committee on Cosmetic Products
UV	UltraViolet

15.1 Introduction

About 2500 years ago, Hippocrates, the father of modern medicine, shaped the relationship between the use of appropriate foods for health and their therapeutic potential and quoted "…let food be the medicine…."

In our days, scientists are continuing to discover and demonstrate the beneficial role of proper diet and nutrition. This has led to an increase in the range of nutraceutical, nutrition, and functional food products on offer around the world. As a consequence, consumers as well started to make increasing reference to "nutraceuticals" regarding prescription drugs as often being unnecessary, too expensive, unsafe, and of dubious benefit once all the risks are considered. The downside to this trend is the assumption by the general public that because nutraceuticals are derived from natural sources, these products are safe to consume without consideration of dosage.

However, in contrast to the stringently regulated and harmonized medicinal, medical device, and cosmetic markets, even today the food supplement market, while having many European and national pieces of legislation, is perceived as not regulated and is clearly not harmonized. Because of the perception of having insufficient regulation, the food supplement industry is facing several challenges. Quality challenges are particularly challenging given that the composition and contents of active constituents in natural plants vary depending on season, climate, etc. The manufacturing processes may influence the presence of contaminants in the samples. The same product might be marketed under different regulations and in different regulatory categories in various countries, leading to confusion for the consumers. Thus, implementation of harmonized regulations, appropriate to assure safety and efficacy in order to protect consumers as well as free market movement, is crucial.

The objective of this chapter is to show how the legal aspects of micronutrients sold as food supplements differ from those of medicinal, medical device, and cosmetic products. For this reason, the legal situation of each product category is outlined, borderline situations are discussed, and main differences highlighted.

15.2 Micronutrients/Food Supplements

15.2.1 Definition

Food is essential to human life. During his or her lifetime, an individual consumes, on average, 30 t of food in almost endless dietary varieties. However, digestion reduces all the foods into the same basic nutrients [1]. The nutrients account for more than 99.9% of the food content. The main classes of nutrients are carbohydrates, proteins, fats, vitamins, and minerals. The former three constituents of food are called macronutrients and the latter are called micronutrients [20].

Micronutrients are essential for human physiology. Different adverse effects may result from micronutrient intakes that are too low (deficiency diseases) or too high (toxicity).

The expression "food supplements" is used often to describe products containing micronutrients and intended to supplement the normal daily diet.

According to the European Directive 2002/46/EC, food supplements are defined as follows:

> "Food supplements" means foodstuffs the purpose of which is to supplement the normal diet and which are concentrated sources of nutrients or other substances with a nutritional or physiological effect, alone or in combination, marketed in dose form, namely forms such as capsules, pastilles, tablets, pills, and other similar forms, sachets of powder, ampoules of liquids, drop dispensing bottles, and other similar forms of liquids and powders designed to be taken in measured small unit quantities;

Whereas "nutrients" means the following substances:

(i). vitamins
(ii). minerals

A number of elements from that definition are important to determine the types of products that fall under the scope.

First, the definition makes obvious that food supplements being *foodstuffs* are regulated as well by the "General Food Law Regulation" or as it is officially called Regulation (EC) No. 178/2002, which contains the foundation of European Food Law. This regulation defines "food" (or "foodstuff") as "any substance or product, whether processed, partially processed or unprocessed, intended to be, or reasonably expected to be ingested by humans….shall not include…medicinal products within the meaning of Council Directives 65/65/EEC and 92/73/EEC, cosmetics within the meaning of directive 76/768/EC…." The fact that this definition excludes medicinal products means that the decisive step to judge whether medicinal law or food law applies to a specific product will have to be sought in medicinal law [5].

A second element is that food supplements may contain substances with a *nutritional or physiological effect without being medicinal*. Recent European Commission (EC) Court of Justice rulings, especially Case C-319/05, support this premise that a physiological effect is not specific to medicinal products, but is also a criterion in food law. Therefore, it is not sufficient that a product has properties beneficial to health in general, but it must have the function of treating or preventing a disease to be considered medicinal. Thus, if a substance or a concentration of it can be demonstrated to modify the functions, or to restore or correct the functions, the substance or the product containing it may be considered as medicinal [19].

The third element is that a product which is to be considered as a food supplement must be in a *concentrated form*, presented to be taken in measured small unit quantities.

Also an important aspect to the challenges that exist for the food supplement industry is the confusion of terminology. The terms "nutraceutical" and "functional food" are not defined by regulation and are therefore not recognized by regulators. The terms defined above are, from a regulatory standpoint, the only valid categories for food-type products that deliver a nutritional benefit to consumers.

15.2.2 Legal Aspects

Following growing concern about the type and quality of food supplements available in the EU, the EC published the European Food Supplement Directive which concerns food supplements marketed as foodstuffs and presented as such for the purpose of supplementing the human diet [11]. This directive came into force in 2005 in order to control the number and quality of permitted food supplements through the creation of a "positive list" of approved vitamins and minerals and sources of those ingredients.

15.2.2.1 Harmonized Within the European Union

Additional to the definition of food supplements and the positive list of ingredients, food labeling and general advertising principles are highly regulated in the EU [2].

The positive list of the EU Directive on Food Supplements includes 13 vitamins with 32 allowable sources and 15 minerals with 80 allowable sources. Manufacturers and suppliers whose products have not been evalu ated and are not on the positive list had time until the end of 2009 to apply for derogation to the competent authorities in the Member States. This approach has alone led to 421 applications in the UK [16].

Several directives are setting the frame for the labeling, presentation, and advertising of foodstuffs, the indications or marks identifying the lot to which a foodstuff belongs, the derogation from the compulsory Quantitative Ingredient Declaration (QUID), labeling of ingredients derived from genetically modified organisms (GMOS), and nutrition and health claims made on food. The labeling provisions for food supplements of Directive 2002/46/EC have to be met by all food supplements, irrespective of whether their ingredients are regulated on a national or on a Europe-wide basis [1-13].

Claims are regulated by Regulation 1924/2006 on Nutrition and Health Claims made on Foods. The scope of the regulation covers all claims made in commercial communications, whether in the labeling, presentation, or advertising of foods. The overall principle of the Regulation is that claims may only be made on foods with a positive nutrient profile and if they are authorized. Three types of claims are distinguished by the regulation:

- *Nutrition Claims* describe the foods with regard to the nutrients or energy they contain or provide.

Permitted nutrition claims and their conditions of use are listed in the Annex of the Regulation.

- *Health Claims* are claims stating, suggesting, or implying that a relationship exists between a food category, a food, or one of its constituents and health. They are subdivided into the following categories:
  - Claims based on generally accepted scientific evidence. They may only by made if they are included in a positive list, which is being compiled based on lists submitted by the Member States. The submissions are been evaluated by the European Food Safety Authority (EFSA) and will be put into a regulatory framework
  - Claims relating to children's health and development. They may also be made after submission of a dossier, EFSA evaluation and authorization.
- Disease risk reduction claims in regard to a significant reduction of a risk factor in the development of a human disease. They may only be made if they have been authorized after submission of a dossier substantiating the claimed effect that is evaluated by EFSA.

If claims are authorized based on significant, "new" proprietary studies, a 5-year data protection period can be granted. This is, of course, very interesting for the nutritional industry and has the potential to stimulate innovation. As this regulation is new, though, what constitutes "new" data is subject to interpretation.

Public advertising is possible for food supplements under the above-mentioned claim and labeling provisions. Furthermore, some more specific prohibitions exist, e.g., they are not allowed to claim that adequate amounts of nutrients cannot be obtained from a balanced diet [1].

The responsibility for the safety of food supplements under the directive lies with EFSA, which is as well tasked with determining the safe maximum and minimum levels of vitamins and minerals [30]. Maximum levels are defined as the maximum level of a nutrient allowed to be in a food or food supplement. These maximum levels are, as noted in Directive 2002/46, to be established based on upper safe levels and normal intake and are designed to ensure that products can be used safely by the consumer [20]. EFSA has completed a scientific review of 29 vitamins and minerals and established upper safety levels for 16 of these. For the remaining 13, no safety concern was determined even at very high levels so no upper safety limit was established. These data, along with normal dietary intake data, are being used by the EC and other scientific organizations to determine appropriate maximum levels for foods and food supplements. At present, the EC is concentrating on defining maximum levels and the difficulty is finding the right model to use in order to ascertain which levels to choose and why. The European Responsible Nutrition Alliance (ERNA) has developed a risk management model as an example of how such maximum levels can be set [31]. The International Life Sciences Institute (ILSI) developed a similar model for determining the maximum amounts of vitamins and minerals for addition to regular foods [5]. The EC is currently favorable toward the ERNA model, which is as well favourable to the industry. A draft proposal on maximum levels is expected from the EC probably in 2010 after which adoption and implementation will occur.

Food supplements production is regulated by Regulation 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs [1]. Furthermore, food supplements have to comply with the traceability requirements as laid down by Regulation 178/2002 of the European Parliament and of the Council of 28 January 2002.

15.2.2.2 Responsibilities of the Member States

Although EFSA is responsible for the safety of food supplements currently, until the EC has harmonized minimum, maximum, and tolerance levels, this responsibility still lies under the responsibility of the Member States. The same is the case as well for the use of so-called other substances, the classification of products, the need or not of a marketing authorization by a competent authority, and the market distribution [2].

Food supplements may only contain the vitamin and mineral sources mentioned in the Directive. Under the current regulatory situation, each country has individual maximum levels for these ingredients, leading to potentially 27 different groups of levels. Thus, free-market movement of food supplements is almost impossible within the EU. A first step toward the solution of this issue will be the decision of the EC about the most suitable approach to setting maximum levels.

Other substances, as defined in Directive 2002/46/EC on Food Supplements, are ingredients other than vitamins and minerals with a nutritional or physiological effect which may be used in food supplements. Their use is not yet harmonized within the EU; therefore, Members States are following their own rules [2].

With the responsibility for maximum levels and ingredient harmonization still with the Member States, it is possible that the same product is classified as food supplement in one Member State and as medicinal product in another one. This, of course, creates confusion for consumers, regulators, and manufacturers. Harmonization, therefore, is considered a key to minimizing the challenges and improving the perception of the food supplement industry.

A food supplement ingredient may also be classified as a novel food if it was not marketed for human consumption to a significant extent in the EU before 15 May 1997. Novel foods are covered by Regulation 258/97 on novel foods and novel food ingredients. They require preapproval and have to undergo a stringent scientific safety evaluation prior to authorization.

There are two other EU directives related to the safety of food and herbal medicines which influence the discussion around the EU Directive for Food Supplements. More specifically, there are the directives related to traditional herbal medicines [14], and the conduct of clinical trials for medicinal products for human use [9]. In spite of these, the use of herbal substances in food supplements is regulated on national level.

Marketing authorization for new products by a competent authority lies within the responsibility of the Member States and is varying country by country. In some countries notification only is needed, in other countries approval is required, while other countries do not require any notification.

Distribution of food supplements is as well determined by national law and is normally "general sale," although a few countries restrict food supplements to pharmacy-only distribution [1].

15.2.3 Conclusion

Directive 2002/46/EC on food supplements, represents only *a first step* in the harmonization process as it focuses only on vitamins and minerals. Full implementation of this directive is critical as it would harmonize several key aspects of the food and food supplement categories. Although the number of directives and regulations is important, the legislation is very complex and there is no regulatory framework for "nutraceuticals" or "functional foods" [5].

Ingredients further to vitamins and minerals are regulated by national law, country by country [17]. For example, in some EU countries, botanical products are sold as foods, or incorporated in functional/fortified foods or as food supplements, meaning that no medicinal claims are made, whereas in other EU countries these same preparations are seen as herbal medicines registered by full or simplified registration procedures. In some countries even, the medicinal product status is automatically linked to pharmacy-only status [19].

Besides the classification, already the harmonization of the maximum allowed levels for food supplements would be a big step toward free-market movement for these products, leading to more clarity as well for the consumer.

15.3 Medicinal Product/Drug

15.3.1 Definition

According to the new European Directive 2004/27/EC [15], an amendment to the Directive 2001/83/EEC [10], a medicinal product is defined as:

- "any substance or combination of substances presented as having properties for treating or preventing disease in human beings; or
- any substance or combination of substances which may be used in or administered to human beings either with a view of restoring, correcting or modifying physiological functions by exerting a pharmacological, immunological or metabolic action, or for making a medical diagnosis"

15.3.2 Legal Aspects

The primary aim of the European pharmaceutical law is to safeguard public health, while encouraging the development of the pharmaceutical industry and the creation of a single market for pharmaceuticals in the EU [29].

In order to complement national procedures and to offer a centralized, pan-European approval regime, the European Agency for the Evaluation of Medicinal Products, otherwise known as the European Medicines Evaluation Agency (EMEA) based in London, was created in 1995 [28]. The EMEA is using a two-sided regulatory approach, with a centralized procedure managed by it and a decentralized procedure managed by Member States [3].

In the case of the centralized procedure (CP), which is compulsory only for biotechnology and other high-technology products, for orphan drugs, for new actives treating HIV/AIDS, cancer, diabetes, or neurodegenerative disorders, one national agency undertakes the scientific evaluation, and the assessment process is overseen by the agency's Committee on Human Medicinal Products (CHMP). The centralized procedure leads to a single marketing authorization that is valid across the EU.

The second set of procedures is focused at the national level, with two options for approval – the decentralized procedure (DCP) and mutual recognition procedure (MRP). Both involve the use of a "reference member state" (RMS) and other concerned Member States (CMS). The difference is that, in the MRP, the RMS first reviews the application and approves it. The MRP then starts after this approval where the CMSs review the application and "mutually recognize" the approval of the RMS. The DCP, on the other hand, does not start with an approval by the RMS. Rather, the RMS and CMSs all receive the application at the same time and review it concurrently. The RMS serves as the "coordinator" for the procedure and works with the CMSs to gain agreement and manage issues. Both the MRP and DCP result in an approval in more than one European country, but not necessarily all European countries, as is the case with the CP.

The preexisting national procedure remains for applicants targeting approval in only single countries [28].

Furthermore, conditional marketing authorizations (to be granted on yearly basis and revised annually) are now also possible for drugs developed for chronically or seriously debilitating diseases or life-threatening diseases, orphan drugs, and use in emergency situations in response to public health threats. Authorizations are subject to a mandatory 5-year final rigorous reassessment.

Medicinal products are regulated in Europe by the Directive 2001/83/EEC [10], amended by the new European Directive 2004/27/EC [15]. Requirements for testing of medicinal products are described in these directives and in the corresponding Notes for Guidance published by the EMEA. European law governs various aspects of pharmaceuticals in the EU:[26].

- Marketing authorization
- Manufacturing authorization, importations
  - Labeling of packaging and package leaflet
  - Classification for the supply (prescription or not, renewable or not, etc.)
  - Wholesale distribution
  - Advertising
  - Analytical, pharmaco-toxicological and clinical standards, and protocols in respect of the testing of proprietary medicinal products
  - Pharmacovigilance
- Implementation of GCP in the conduct of clinical trials
- Principles and guidelines of GMP
- Intellectual property
- Coloring matters
- Orphan medicinal products

Pharmacovigilance is established within the Member States and the EU, but it needs strengthening. Nevertheless, very clear pharmacovigilance requirements exist for marketing authorization applications themselves.

Distribution of medicinal products is regulated at national level, with some Member States applying a pharmacy only status and other applying a more diversified approach [1].

Finally Member States maintain autonomy on matters dealing with pricing and reimbursement issues. The scope of the community law has been limited in this domain to agreed principles of transparency and fairness [29].

15.3.3 Conclusion

In Europe, a harmonized legislative framework has been developed for medicinal products. The new system entered into force in 1995 with the creation of EMEA and by offering two separate regulatory procedures – the centralized and the decentralized one. The

preexisting national procedure remained into force for companies interested in one single market.

The new system, in theory, has important consequences for the Member States, consumers, and manufacturers, such as: (1) time and effort saving for the Member States regarding the evaluation of new drug applications; (2) more consistent and quicker availability of medicines in EU countries; (3) establishing of a homogeneous regulatory policy throughout the EU [24]. Time will tell if the theory of these advantages will result in a true benefit for European consumers.

15.4 Medical Devices

15.4.1 Definition

The EU directives define a medical device as follows [8]:

Medical device means any instrument, apparatus, appliance, software, material, or other article, whether used alone or in combination, including the software intended by its manufacturer to be used specifically for diagnostic and/or therapeutic purposes and necessary for its proper application, intended by the manufacturer to be used for human beings for the purpose of:

- Diagnosis, prevention, monitoring, treatment, or alleviation of disease
- Diagnosis, monitoring treatment, alleviation of, or compensation for an injury of handicap
- Investigation, replacement, or modification of the anatomy or of a physiological process
- Control of conception

and which *does not* achieve its principal intended action in or on the human body by pharmacological, immunological, or metabolic means, but which may be assisted in its function by such means.

The principal intended action as indicated and declared by the manufacturer is crucial for determining the class of the medical device. For medical devices, the intended action must be fulfilled by physical or mechanical means. Where there is significant doubt about the classification, it is usual to follow the regulatory sche me which gives the higher level of health protection [8, 22].

Despite this harmonized legal basis, differing national interpretations in the various European countries leading to different possible classifications for one and the same product are possible. For example a product which is according to the German Medicinal Products Act [18] clearly a medicinal could be classified based on the Austrian Dug Law (Medicinal Products Act 1983) as a medical device. However, a product considered to be a medical device cannot simultaneously be registered as a medicinal [22].

15.4.2 Legal Aspects

Medical devices are regulated in Europe by the Medical Device Directives of the EU. These regulations define the requirements for medical device design, development, manufacture, production release, and risk assessment in order to ensure that products reaching the public are safe and effective.

In order to bring a medical device on to the market, the manufacturer has to demonstrate that the product is safe for use and effective for its intended purpose. All serious adverse incidents need to be reported to the National Authorities. Post-market surveillance measures as well as the development of a vigilance system are required. Furthermore, the directives establish a framework for the control of clinical trials which is rather similar to the one for pharmaceuticals [8, 21].

Medical devices are classified based on the level of risk to the patient and user. The level of risk associated with the device is defined based on the type and duration of contact with the patient, the technology internal to the device, the mode of action of the device, and the severity of the consequences of failure of the device. Medical devices are grouped in four risk classes with class I containing those with the lowest risk [8].

Healthcare products which contain substances intended to supplement or to support the primary effect of the product, and which have an effect upon the human body, and which, used separately, can be considered to be medicinal products pursuant to Directive 2001/83/EC [10], are classified as medical devices. However, the active ingredient being released from the medical device must be documented according to the medicinal product legislation. If the purpose of a medical device is merely supported by an active pharmaceutical ingredient, the product is regulated as a Class III medical device.

Assessment of the devices is conducted by an authorized third party, the so-called Notified or Conformity Body. Only manufacturers of Class I medical devices that neither are sterilized nor provide a measuring function, and custom-made devices or systems, are exempt from audit by certified body [8]. Thus, for medical devices, the Member States' involvement is limited to the appointment and monitoring of Notified Bodies and laboratories and the monitoring of the clinical trial, the market, the manufacturers, and the operators.

Conformity is denoted by affixing *CE* (*C*onformité *E*uropéenne) mark to the device, which signifies compliance with the Directive [3].

Certification is conducted for medical devices once only in a specific country for the whole European market. This approach could be seen as well as an automatic mutual recognition. Thus, the single market approach is even more evident for devices than for pharmaceuticals. However, there is a safeguard clause, which Member States can invoke if evidence of a major public health concern is identified [21]. Furthermore, enforcement and implementation of the directives is only decentralized, with Member States responsible for their transposition. There is no European Medical Device Agency which would be similar to the prototype European Agency for the Evaluation of Medicinal Products (EMEA).

Problems in relation to the uniform interpretation and application of the Medical Device Legislation are discussed during meetings of the so-called medical devices experts group. This group is comprised of experts from national governments, representatives of industrial federations, healthcare professionals, and European standardization bodies and Notified Bodies. For more specific questions, several working groups have been established. In support of the uniform application of the directives, a set of guidance documents, the so-called MEDDEV-documents have been elaborated in consultation with the parties' concerned [4].

15.4.3 Conclusion

In contrast to the approval procedure established for drugs, the medical device directive requires a Conformity Assessment Procedure to be performed by the manufacturer himself and/or a so-called Notified or Conformity Body, depending on device classification [8]. Devices are regulated as engineering products and this reflects the potential shorter development times for products, the shorter product life span, and the difficulties in undertaking major clinical studies in some areas before marketing [21].

The post-marketing surveillance approach used for medical devices has many similarities to the one being in place for medicines but is more complicated due to the wider range of reporters existing for medical devices.

The borderline between medical devices and medicinal products can be a difficult one. The way by which the principal intended action of a product is to be achieved is the limiting factor for its classification as medicine or medical device.

15.5 Cosmetic Products

15.5.1 Definition

In the EU and according to Council Directive 76/768/EEC [6], a cosmetic product is defined as following:

> A "cosmetic product" shall mean any substance or preparation intended to be placed in contact with the various external parts of the human body (epidermis, hair system, nails, lips, and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance and/or correcting body odors and/or protecting them or keeping them in good condition.

As with the other product categories discussed above, classification issues may be faced as well with cosmetic products. In general, claims and ingredients determine whether a product is cosmetic, medical device, or medicinal.

A product can only be classified as a cosmetic when it fulfills all obligations of the national or EU regulations with reference to function (definition of directive 76/768/EEC) presentation (claims and advertising), method of application (galenical form), and composition [23].

15.5.2 Legal Aspects

In the EU, cosmetics are regulated under the auspices of the EC Enterprise and Industry Directorate General. The

EU cosmetic regulation has been introduced with rules set out by the Council Directive 76/768/EEC [6]. The so-called Cosmetic Products Directive has been adopted in 1976 in order to ensure the free circulation of cosmetic products in the whole EU market and to ensure the safety of cosmetic products brought to market in the EU.

Since its adoption, the Cosmetics Directive has been amended by the European legislators (the European Parliament and the Council) seven times in order to reflect new trends and challenges concerning cosmetic products [12]. For example, the "sixth amendment" led to the adoption of the inventory of ingredients used in cosmetic products and introduced the principle of marketing ban in relation to tests on animals. The "seventh amendment" provided inter alias for more detailed provisions on the phasing out of animal testing and introduced the "period-after-opening labeling [12]." Apart from these so-called "amendments," the Commission has adopted more than 30 "adaptations" in order to adapt to technical progress the provisions in the annexes to the Cosmetics Directive. The latest publication related to the cosmetics directive was published on February 5, 2008 and is intended to strengthen product safety, bring new adverse event reporting requirements into effect and cut bureaucracy.

In the EU, no general registration of cosmetic products is required. Only a notification to the Member States where a product is first imported to the EU is needed and in some Member States, notification to the various Poison Control Centres is expected.

However, with the publication of proposed revisions to the cosmetic directive, this procedure could be replaced with a single submission to the EC prior to placing a product on the market. Further, this single submission would allow for one legal text to be valid in all Member States. This is designed to simplify actions needed by the manufacturer by requiring one single submission which is applicable for all Member States.

The European cosmetic legislation – previously varying from country to country – is now harmonized by the use of the dossier system. The Dossier is a compilation of documents designed to verify a product's safety and must include a Safety Assessment. The safety of a cosmetic product must be assessed by taking into consideration the general toxicological profile of the ingredients, their chemical structure and their level of exposure [7]. It is not submitted formally prior to marketing, but must be available on request by the corresponding health authority. The Safety Assessment is a core document in a cosmetic products file.

Further to safety, the Dossier must include data to support the effect claimed for the cosmetic product, where justified by the nature of the effect or product [7]. This requirement has incited the search for improved methods to evaluate treatment effects e.g. non-invasive bioengineering methods [25].

15.5.2.1 Raw Materials

In the EU the Scientific Committee on Cosmetic Products and Non-food products (SCC-NFP), now known as the Scientific Committee on Cosmetic Products (SCCP), considers raw materials and specific technical issues. The SCCP reviews ingredient files and issues recommendations to the EC which then are routed through the political system and often are promulgated into European law. No formal raw material approval process exists except for preservatives, colorants, and ultraviolet (UV) filters. These items are noted both on positive lists and restricted lists. Other preservatives, colorants, and UV filters are listed on negative or prohibited listing, along with other miscellaneous ingredients that have potential safety issues.

Regarding all other potential cosmetic ingredients, since the majority of them are used as well for many other purposes, they fall under the scope of more than one EU Directive [27].

Part of the effort to harmonize the cosmetic law not only in Europe but worldwide, is the advent of the International Nomenclature of Cosmetic Ingredients (INCI). This labeling system supports the simplification of raw material labeling and usage and is mandatory within the EU. Europe requires on the label of a cosmetic product a descending metric declaration of the quantity of all the ingredients contained.

15.5.3 Conclusion

Within the EU, cosmetic law has been harmonized for several years. The Cosmetic Products Directive was adopted in 1976 ensuring free circulation of cosmetic products in the whole EU market and safety of the cosmetic products placed in the market. However, classification issues may be faced as with the other product categories, especially between cosmetics and medicines or cosmetics and medical devices.

In the EU no general registration for cosmetic products is required at the current time, however, as noted; this may change in the near future. Manufacturers prepare the so-called cosmetic dossiers which document the safety and efficacy of the corresponding products. The safety relates to composition, packaging and information, and it falls totally under the responsibility of the producer who takes upon himself the marketing liability.

15.6 Classification of Borderline Products

Classification of borderline products might be problematic not only between medical devices and medicinal products, but also between a medicinal and a food supplement or a medicinal and a cosmetic or a food supplement and a medical device, etc., as already indicated. For example, there are products containing glucosamine or chondroitin sulfate being marketed as foodstuffs, although products with similar composition in terms of the active ingredient are being registered as medicinal in various EU Member States. The main difference between these products is obviously not the ingredients, but rather the dosage and the claims. For example, the glucosamine/chondroitin food supplements make claims related to maintaining the health of joints and generally have lower doses compared to the drug products. The drug products, on the other hand, make claims related to reducing the pain and stiffness associated with osteoarthritis and generally have doses higher than those found in food supplements.

Difficulties are arising already in the interpretation of the definition of the various products. For example, European Legislation and national laws failed until now to include a valid legal definition of the term "pharmacological action" [22]. The German Federal Higher Court (Bundesgerichtshof, BGH) included a definition of pharmacological action in its decision on food supplements containing L-carnitine. The BGH concluded in that case that a pharmacological action can reasonably be assumed if "the action of a product exceeds the effects physiologically resulting from food intake in the human body" [22]. But, as this definition applies only in the differentiation between food supplement and medical product it is not of any help in the case of medical devices and medicinal products. Thus, borderline situations need to be analyzed and solved case by case. Even good knowledge and understanding of the regulatory frame is not a guarantee for proper product classification. The situation is especially difficult in the case of food supplements were the legal framework is not yet fully harmonized throughout the EU.

At present, EU regulations leave to the Member States the decision about product classification. In the long term, these classifications will tend to converge toward a more common view, but there is no sign of such a process taking place at present [23].

15.7 How Do Food Supplements Differ from Drugs, Medical Devices, and Cosmetics?

In contrast to the European Regulations for drugs, medical devices and cosmetics which are harmonized enabling the single-market approach, in the case of food supplements each country varies in the extent and process of regulatory control. The food supplement market is not yet well consolidated and the industry is facing several challenges. It is almost impossible to market the same product composition in all EU countries. Furthermore, the actual needs of the expanded EU are actually not yet fulfilled as the various directives and regulations are focusing only on vitamins and minerals. The other ingredients are regulated by national law, country by country.

Besides the lack of harmonization, further differences become apparent when looking more in detail into the nature and regulatory framework of the various product categories (Tables 15.1 and 15.2).

Product development for food supplements is mainly based on new combinations of long-known vitamins and minerals or other nutrients. Long, expensive, and basic research as it is the case for drugs is usually not needed. New extensive clinical data are not required in order to launch a product, as far as the composing ingredients are included in the positive list of the Directive or accepted by National Regulations, being within the nationally set minimum/maximum or tolerance levels, and making claims that fall within the new claims legislation.

Labeling and claims for food supplements are highly regulated. Authorized claims included in positive lists can freely be used for the corresponding

Table 15.1 Differences in the nature of nutritional products, cosmetic products, medical devices and drugs [3]

	Food supplements	Cosmetic products	Medical devices[a]	Medicinal products[a]
Typical composition/ innovation	Traditionally based on vitamins and minerals Continuous quasi-innovation in order to satisfy consumer expectations	Traditionally based on galenics and trendy "actives"	Traditionally based on mechanical, electrical, and materials engineering	Traditionally based on pharmacology and chemistry and contain well established or new ingredients
Typical development	Development based on literature knowledge about vitamins and minerals.	Product development by trial on complete formulas. "Active substances" knowledge based most on in-vitro data.	Products engineered to perform certain functions based on performance and safety; the therapeutic effect be patient -triggered or automatically adapted to the patient condition	Product development by trial on active substances selected on the basis of safety and efficacy
Typical mechanism of site of action	Nutritional and physiological mode of action.	In contact with external parts of the body or teeth and mucous membrane	Physical/mechanical mode of action	Pharmacologically active; effective when absorbed into the human body
Legislation	EU and national legislation	EU Legislation	EU Legislation	EU legislation
Registration	No EU registration, national authorization depending on country	No registration needed at the current time; however future may require a single submission to the EC, notification in the first EU Member State to be marketed	CE marking ensures product conformity to essential requirements. Notified Bodies appointed by the governments to certify the conformity assessment procedures	Central registration by EMEA and/or the regulatory authority of the Member States
Dossier, approval	Dossier type depending on national regulation	Dossier containing safety assessment and claim substantiation data	Assessment, controls and dossier requirements increase proportional to the risk/ classification	Drug dossier covering full technical, safety, & efficacy data. All products are subject to similar approval
Typical development time	Short development and market authorization procedure	Very short development and market authorization procedure	Short to medium development and market authorization procedure, depending on classification	Can be long and expensive development and market authorization procedure
Product lifecycle	Short product life cycle due to continuous incremental improvements; often user-related/driven; short amortization period	Short product life cycle due to strong competition and high unsatisfied consumer demands	Short to medium product life cycle due to continuous incremental improvements; often user-related/driven; short amortization period	Extensive product life cycle with prescription only often moving to OTC allowing for long amortization period

[a] Partly from [3]

Table 15.2 Market authorization for nutritional products, cosmetic products, medical devices, and medicinal products: A comparison [3]

	Food supplements	Cosmetic products	Medical devices[b]	Medicinal products[b]
Risk analysis	Tolerance based on literature data and nationally set minimum/maximum/ tolerance levels	Tolerance (part of the efficacy and safety evaluation)	– Awareness of all potential risks associated with the use of a medical device – Estimation of risks and consequences in relation to the intended purpose – Conduction according to pertinent standards	– Tolerance (part of the efficacy and safety evaluation) – Risk benefit analysis – does benefit outweight risk?
Quality	– Regulation on food hygiene – Standards of food industry	– Quality of the raw materials – Quality and stability testing of the final product	– Demonstrate conformity with: European Standards or – An organized quality assurance standard at the manufacturing site	– Analysis of raw materials according to Pharmacopoea – Conformity with GMP – Quality assurance standard of pharmaceutical companies
Safety	Mainly literature data unless "novel food" where significant safety and toxicological data are required	– Literature data and tolerance tests. – No animal tests on final products.	– Verification of biocompatibility – Verification of biological safety through laboratory tests and/or pre-clinical trials in animals	– Verification of biocompatibility – Therapeutic action and secondary effects – Pre-clinical studies required for new substances
Claim	Claims related to maintaining the health of a system in the body or, in some cases, disease risk reduction claims	Claims related to improving the appearance or smell/ fragrance of the body	Claims related to diagnosing, treating, or preventing a disease	Claims related to diagnosing, treating, or preventing a disease
Efficacy	– Literature data on ingredients – Rarely clinical trials – Nutrition, health, disease risk reduction claims	– Literature data on ingredients – In-vitro testing – Non-invasive bioengineering methods – Cosmetic performance trials	– Clinical tests of performance and safety according to pertinent standards or scientific literature – Search for undesirable effects	– Phase I: dose finding – Phase II: pharmacological safety – Phase III: clinical efficacy
Marketing authorisation	Not needed except in some countries Distribution nationally regulated, normally "general sale"	– Not needed except notification in some countries – Free distribution in EU	– Audit by Notified Body (except class I) – Certification by NB – Free distribution within EU	– EMEA and/or regulatory authority of the Member State – Distribution nationally regulated, usually Pharmacy
After sales	Market surveillance	Market surveillance	– Marketing – Materio-vigilance – Audit by Notified Body – Renewal of market authorization	– Phase IV: post-marketing clinical trials – Pharmacovigilance – Pharmacological inspection – Renewal of AMM

[b]Partly from [3]

ingredients/ products without further substantiation. In case claims are authorized based on significant, "new" proprietary studies, a 5-year data protection period can be granted [2]. This option is unique for food supplements providing some similarities to patent protection for medicines.

Food supplements do not require registration in all Member States unless required by national law, contrary to what is required for medicines. Thus, overall, development time and costs for food supplements can be much lower than for medicines and comparable to those for cosmetics. However, while cosmetics and medical devices can freely be distributed at least within the EU after having achieved marketing authorization, this is not necessarily the case for food supplements. Because of the national variation of the maximum levels, the same composition can be considered in some countries as a medicine and in other as a food, obviously hindering free market movement.

Finally, the delineation between food supplements, medicinal products, medical devices, and cosmetic products largely depend on the intended primary purpose. Products should be interpreted and categorized in the way most likely to protect public health. Although the regulatory system for each product type is different, the shadow of the pharmaceutical regime is long and affecting the regulatory activities in the other product categories. Thus, further harmonization is very welcome to assure safety and efficacy in order to protect consumers as well as free market movement.

Legislation is still in the harmonization process making free good movement and innovation more complicated than with other product categories. Innovation in this category generally comes in the form of new claims related to maintaining the health of a body system from traditional ingredients and with new galenic forms.

- The success of drug products often depends on patent protection as development is often quite long and costly. New chemical entities, which require a comprehensive evaluation of safety and efficacy, are often the basis of successful innovation in this category. The fully harmonized approval process might be stringent but at the same time enabling the global medicinal market.
- The clearly regulated medical devices are typically based on mechanical and/or electrical apparatus designed to have a mechanical or physical impact on the body. Innovation is often faster than with drug products as the methods for testing medical devices are often simpler.
- The harmonized but less stringent regulatory frame in the cosmetic category enables innovation which is generally based on "novel" or trendy actives and new galenic forms. Speed to market is quick as safety and efficacy are relatively easier to establish.

Take Home Pearls

- Determining the most appropriate category for a specific product is a case-by-case exercise requiring a thorough evaluation of desired claims, key ingredients and product/brand lifecycle management. A balance between innovation, impactful claims and speed to market must be sought to ensure commercial success.
- Food supplements are traditionally based on vitamins and minerals with claims based on existing literature supporting the ingredients.

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How to Prove Safety and Efficacy in Nutrition-Based Intervention Studies for Human Skin*

16

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Core Messages

- Nutritional Components (NC) are often underestimated by dermatologists who neglect them and patients who do not hesitate to use them without thinking of other parallel medication or consumption. This phenomenon has already led to some incidents, such as chronic hypervitaminosis A following retinoid therapy for aging skin and acne. Consequently, the development of new nutritional components now necessitates safety assessments in order to respond to legal requirements but also to improve the knowledge and confidence of professionals and consumers. These studies have to be associated first with bioavailability studies and *in vitro* studies to improve the understanding of their mechanisms of action, then with clinical studies to demonstrate their efficacy.
- The objective of this article was to underline the necessity to observe, in the implementation of NC tests, the same rigour as in drug assessments: double blind, with homogeneous control and active groups, in suitable volunteers.
- When additional data on the efficacy of NC are collected and safety is better documented, NC will be considered with less mistrust by consumers and dermatologists who will in turn improve the follow up of safety assessments and thus decrease the risks.

16.1 Introduction

Nutritional Components (NC) are often underestimated by dermatologists who neglect them and patients who do not hesitate to use them without thinking of other parallel medication or consumption. This phenomenon has already led to some incidents, such as chronic hypervitaminosis A following retinoid therapy for aging skin and acne [1]. A summary of adverse events in connection with dietary supplements was published in the *Lancet* in 2003, to alert scientists and medical staff about this issue [2]: the authors analyzed the reports from 11 poison control centers in the USA, who received up to 2,332 telephone calls in 1998 about ingestions of dietary supplements (within these complaints, 784 concerned patients who had symptoms). Brannon et al. [3] have recently underlined the number of pending questions about Vitamin D and health

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*One does not expect nutritional complements to be as effective as therapeutics, but only to be useful

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which persist in spite of the considerable number of articles published about this compound. This issue seems to be due to a lack of information in published studies (such as diet, body mass index, age, pubertal stage, diseases, season, compliance, and physical activity).

All these new questions and problems have led to a proactive approach by regulatory authorities with regard to their safety assessment: A European directive n°2002/46 was published in 2002, transposed in 2006 (decree n° 2006–352) into the French legislation, and it finally provides the definition of a nutritional supplement. The new directive clarifies the limits between the nutriment, which contributes to the wellness of healthy people by its nutritional and physiological effects, and the drug which allows the prevention or cure of human diseases by its pharmacological effects. To distinguish both types of products with accuracy, specific requirements have been introduced to improve the consumers' awareness of the claims and labeling of nutritional compounds. The directive also sets common safety limits. A positive list of authorized vitamins and mineral substances has been published: It specifies purity criteria as well as maximal limitation in terms of dosage on the one hand, taking into account scientific assessments of risks and different levels of exigency regarding the safety of particular groups of consumers (children, pregnant women, seniors, …), and vitamins and mineral substances coming from other sources on the other hand. The daily dose has to be mentioned and, whenever required, a warning against overdose risks.

Consequently, the development of new NC now not only necessitates safety assessments in order to respond to legal requirements but also to improve the knowledge and confidence of professionals and consumers. These studies have to be associated first with bioavailability studies and in vitro studies to improve the understanding of their mechanisms of action, and then with clinical studies to demonstrate their efficacy. In view of the low doses of nutriments or vitamins in these products, the bioavailability and the efficacy are much less significant than with other types of products (drugs, cosmetics, …). Dr Jean-Michel LE CERF has declared: "One does not expect nutritional complements to be as effective as therapeutics, but only to be useful." The implementation of such studies should therefore be very rigorous and take into account many parameters, in particular with regard to the volunteers inclusion criteria, the environmental conditions control, and the sensitivity of the analytical methods.

16.2 Safety Assessment

The safety of new ingredients (novel foods) which are not specified in the positive lists must be tested by the manufacturers themselves. For the registration of a novel food in the positive list of the regular authorities, a toxicological file has to be provided, which includes six sections: acute toxicity, chronical toxicity, results from mutagenesis and cancerogenesis studies, from teratogenesis studies, research of allergy potential and/or potential effects on immune functions, and data collected from human studies.

The first step in any safety assessment implies the research of information about the origin, production, composition and analysis, nutritional characteristics, and history of previous human exposure to the product. The information collected may sometimes be sufficient to establish safety. It is essential to take into account the biological risks, the conservation of the product, as well as potential associated nutritional allergic reactions. It is therefore necessary to examine the composition of the product, its chemical or biological qualities, to collect all the nonclinical data available from toxicological studies and the clinical data obtained about the same product or corresponding to previously known medical effects.

Assessing safety implies the consideration of several concepts: the hazard (it is important to check the harmlessness of a nutritional component) and the risk (probability of this hazard to arise and the matter of its consequences) [4]. Four different stages are necessary for the evaluation: the identification of the hazard, its characterization, the assessment of its exposure, and the characterization of the risk.

16.2.1 Acute Oral Toxicity

The aim of toxicological studies is to assess the doses inducing no adverse effects [3–6]. Acute oral toxicity is determined by assessing the harmful effect which occurs in a short period of time and results from the oral administration of one or several doses of a substance during 24 h. The dose is the administered quantity, and is expressed as the weight of the substance per unit. Several doses are tested and the intoxication signs as well as the lethal dose (LD) are registered. The LD50 is thus determined and is the value obtained

from statistical analyses as the dose leading to the death of 50% of the animals tested (over a 14-day period of time). This dose determines the classification of the product according to its toxicity level (extremely toxic (<1 mg/kg), very toxic, moderately toxic, slightly toxic (>15,000 mg/kg)).

This process has to be completed by subchronical oral toxicity tests, in order to study the potentiality of a product for inducing harmful effects in animals following repeated administration of the compound.

16.2.2 Subchronical Oral Toxicity

Several doses are tested in groups of animals, for 90 days, then the animals are studied each day (in terms of growth, behavior, mortality, and their blood and urine are analyzed) to detect any sign of toxicity.

16.2.3 Genotoxicity

Risk assessment involves the study of genotoxicity, which is defined by investigations on:

- Mutagenesis (process of genetic information mutation transmissible to daughter cells)
- Cancerogenesis (mutagenesis process with the addition of malignancy capacity):
  - Cells have become immortal.
  - They cease to respond to the tissue regulation factors.
  - They are able to colonize other tissues.
- Cancerogenesis always starts with the mutagenesis process. Since the occurrence of the malignancy process is usually unknown, any mutagenic substance is considered as potentially cancerogenic according to the precaution principle.

16.2.4 Teratogenesis

Process in which the embryo development is disturbed, resulting in embryo or fetus malformations.

The application of these studies to humans presupposes the establishment of an Authorized Daily Dose, which is calculated from the No Observable Adverse Effect Level (NOAEL) obtained from the most sensitive animal species divided by an empirical factor 100:

- Ten to take into account the difference of sensitivity between species and the synergy or antagonism between products
- Ten to take into account individual variations: physiological state, nutritional state, health

Once the safety of the NC has been studied, their interest in terms of biological properties or prevention capacity has to be validated by epidemiological data as well as bioavailability studies of their actives.

16.3 Bioavailability Assessment

NC have to transit by the gastrointestinal tract, cross the intestinal barrier to reach the blood circulation, and go to the skin via the microcirculation. Skin is a vascularized organ (thanks to the hypodermis, dermis, dermal papillae, hair follicles, sebaceous and sweat glands) which makes it a "reservoir" rich in nutriments and micronutriments for the whole body. Interestingly these bioactive compounds can be metabolized and then distributed into the whole tissue, potentially in an active form. It is therefore necessary to investigate the bioavailability of ingredients which claim effects on the skin, with special attention to their distribution and mechanisms of transport.

Recently, several studies on the skin bioavailability of major ingredients have been published [7–12]. Vitamin E, carotenoids, polyphenols, vitamin C, Zinc, selenium, linolieic acid have been described as substances having a direct action on the skin or acting as a second messenger. Many studies have shown that it is the association with a fatty compound for lipophilic substances (lactolycopene) [8] or with a milk matrix which facilitates the intestinal absorption of nutriments [9]. The control of absorption mechanisms has considerably improved and offers the opportunity to choose among the actives but also among their forms. Manach et al. have recently published on several polyphenols and their bioavailability [10]. Wolf [11] demonstrated in 2006 that an excess of alpha-tocopherol intake can reduce the bioavailability of gamma-tocopherol, which has beneficial properties as an anti-inflammatory, anti-atherogenic and anticancer agent.

The International Life Sciences Institute has developed over the past years a framework to evaluate the human relevance of mechanisms of action in animals, which is now widely adopted and used by government agencies and international organizations.

In dermal absorption studies, it is important to take into account some sources of uncertainty which may be associated in particular with comparisons of in vivo and in vitro data: differences in solvents used, dose levels, and duration of exposure [12].

Ultimately the thorough apprehension of NC involves a double approach: a fundamental one on the target tissue and a clinical one to assess its efficacy [13].

16.4 In Vitro Efficacy Assessment

The fundamental approach is based on the knowledge of the skin physiology and the bioavailability of the ingredients. It allows to determine the relevant parameters and to select the actives and their association correctly. The comprehensive knowledge of cellular and tissular mechanisms is essential to define accurately the delay for any response/effect.

Many studies have already been published on in vitro models, in particular 3D models [13–16].

In all these investigations, the ingredients were used at their circulating concentration and structural form usually found after ingestion (determined from bioavailability studies).

Thanks to new omic techniques (genomic, transcriptional profiling, proteomic, and metabolomic) these types of investigation can go further [13, 17–20]. Research of this kind in the nutritional field is at a very early stage yet, but the first results are promising. With the help of such studies in tissues such as the skin, the stratum corneum, and the sebum, it is possible to detect quickly the efficacy of a NC and to define which clinical effect may be expected.

Histological analyses can also provide useful complementary evidence of the biological effects of NC [18].

With NC, the studied effects are exclusively biological contrary to tests on cosmetics in which they can be physical (i.e., solar filters). These effects can be obtained only after repeated administrations, as opposed to drugs which are therapeutics; thanks to their pharmacological action. These effects will furthermore occur in the whole tissue.

16.5 In Vivo Efficacy Assessment

It is important to keep in mind that the nutritional supplement will induce biological effects preceding clinical effects. In vivo studies should be carried out over a relatively long time (above 3 months), and conducted in a randomized, placebo-controlled manner in a sufficient number of volunteers to observe significant results [21]. The placebo effect is usually important in this kind of study and sometimes prevents drawing conclusions on the efficacy of the investigated compound.

Efficacy assessments performed on NC imply the implementation of studies in parallel groups, as opposed to cosmetics which only act on a selected area and thus allow the comparison of several products in each volunteer. The choice of the placebo is critical because it has to be neutral while having the same matrix as the active, to obtain the same presentation. Ideally there should be three groups of subjects: an untreated one, a group with placebo, and a third group with the active, because the placebo effect is often present in this type of studies. However, for financial reasons, this is often impossible.

An alternative study design which helps to limit the number of volunteers and thereby study costs is that of a cross-over study, which in our hands has proven to be very well suited to demonstrate the efficacy of flavonoids and carotenoids in photoprotection of human skin [22].

Compared with the effects of drugs, the modifications induced by the NC are light, the experiments are conducted in healthy volunteers, and the daily doses correspond to average nutritional amounts. The quantity reaching the target area will thus be small or very small.

On account of these parameters (healthy volunteers, low doses, …), the selection of subjects is of major importance: they have to be recruited so as to form very homogeneous groups. It is therefore necessary to consider the clinical signs that will be studied, the volunteers' sex, their hormonal status, their nutritional habits, their life style (smoking, stress, sport, solar exposure), the environment (season, UV, pollution, climate, …) [13].

Ignoring these data while selecting the volunteers could result in hiding the efficacy of the product. As in any other clinical study, the primary criterion and the secondary criteria must primarily be defined precisely. The rationale of the protocol is of major importance in

Fig. 16.1 Because of the slight effects induced by the NC, the association of techniques (self-assessment (**a**), clinical scoring (**b**) and biometrological assessments (**c**)) improves the assessments reliability and understanding. Moreover, they have to be performed in standardized conditions (after acclimatization of the volunteer in an air-conditioned room with artificial lighting, a rest of at least 15 min, and on skin areas where neither water nor products have been applied for several hours)

order to give the possibility to explain the results and to observe the coherence between the skin physiology, the mechanisms of action of the NC, and the observed/measured effects.

As usual in cutaneous clinical studies, three levels of investigations can be associated to objectivate the clinical efficacy of NC (Fig. 16.1):

1. *Self-assessment* is important as volunteers are able to judge the state of their skin and to detect an improvement in some parameters.
2. *Clinical scoring* performed by a dermatologist remains the most usual assessment. Scoring can be descriptive (absence, mild, mild/moderate, moderate/severe, severe), analog (using a horizontal line – usually 10 cm long – on which the investigator places a mark corresponding to the estimated value of the parameter), or performed with photographic scales [23–26].
3. *Instrumental measurements* can complete objective data but must be selected and interpreted from the knowledge of the physiological mechanisms involved in the skin and the expected action of the NC. Significant results have already been obtained on skin aging, assessed from microrelief studies [13, 27–30], involving measures of the skin density by ultrasound [27, 31–33], the skin barrier function by transepidermal water loss measurement [34, 35], and the skin hydrophilicity by contact angle measurement [28].
4. Assessment of molecular parameters is another important approach to determine the efficiency of a nutritional supplement in a human intervention study. The advantage is twofold: (a) molecular changes can be detected much earlier during the intervention, e.g., after of 2 weeks as compared with 3 months and (b) they provide mechanistic information about the mode of action of a given supplement or micronutrient. In combination with instrumental measurements, molecular analysis is in fact the gold standard for claim proof when it comes to functional food for skin. A disadvantage, that can, however, be

Table 16.1 Summary of the most common activities and claims of NC [21]. The most pertinent studies concern research on antioxidant intake. It is possible to create biological and clinical effects on the skin sometimes thanks to UV action, and to observe and quantify the efficacy of this type of products [27, 35–51]

Main activities	Main claims	References
Antioxidant/anti-radicals activity	Skin aging	[1, 13, 18, 29–33, 37, 38]
	Photoprotection	[31, 34, 39, 40]
	Skin hydration	[28, 35, 41, 42]
	Improvement of skin barrier function	[16, 35–37]
	Weakness of hairs and nails / Hair loss	[15, 17, 43]
	Prevention of skin cancer	[44]
	Alternative treatment to the symptoms and pathological effects of menopause	[45]
Pro-oestrogenic action	Alternative treatment to the symptoms and pathological effects of menopause	[46]
Modulation of the immune system in particular on cytokines modulation	Atopic dermatitis, eczema	[47–49]

resolved by the investigator, is the requirement of bioptic material. In fact, several studies have been carried out successfully to determine the efficacy of nutritional supplementation with flavonoids, carotenoids, and probiotics on UV-induced gene expression in human skin [22, 36].

Many works have been published in recent years and have demonstrated the activity of NC in many applications (Table 16.1).

These assessments may be associated with biochemical and/or biological assessments to check the bioavailability of the components or their metabolites [26] and to explain the mechanisms responsible for the clinical changes: if significant and positive results are found, all these data confirm the claims unquestionably. Biochemical investigations, such as epidermal lipids synthesis, for example [27], will also evaluate the efficacy of the treatment on the skin physiology.

16.6 Conclusion

The objective of this article was to underline the necessity to observe, in the implementation of NC tests, the same rigor as in drug assessments: double blind, with homogeneous control and active groups, in suitable volunteers. Furthermore the tested dosage must be the same as in the product final form. Only statistically significant results should be taken into account and their interpretation ought to be rigorous and not excessive. To claim an effect, at least one in vivo clinical study must have been carried out, and the claim has to be in conformity with the investigations.

Nevertheless, the assessment of a new NC requires several studies over a period of at least about 3 years. The most suitable procedure is the combination of safety studies, bioavailability studies, and in vitro studies to predict the efficacy of the NC, then in vivo studies to check it (Table 16.2). Moreover all these constraints entail high costs.

The remaining doubts today regarding the efficacy of NC come from divergent or missing data about efficacy and safety. These divergences are linked to differences in experimental conditions (skin condition, dosage, duration, combination of ingredients, and so on).

When additional data on the efficacy of NC are collected and safety is better documented, NC will be considered with less mistrust by consumers and dermatologists who will in turn improve the follow up of safety assessments and thus decrease the risks.

Table 16.2 4-Step method to assess a nutritional formula [13]

1. Safety studies	1. Acute toxicity test 2. Chronical toxicity test 3. Mutagenicity and cancerogenicity tests 4. Teratogenicity tests 5. Research for allergic potential and/or immune functions effects 6. Data collected in humans
2. Bioavailability studies	Study of the pharmacokinetics of active components
3. In vitro efficacy assessment	• Study of the anti-radical activities • Stimulation of skin cells proliferation, etc.
4. In vivo efficacy assessment	• Self-assessment of volunteers • Assessment of objective clinical criteria including photographs • Measurement of clinical or biometrological skin parameters such as skin hydration, density, elasticity, roughness, etc. • Assessment of molecular parameters in human skin, e.g., gene expression by TACmen-PCR

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Beauty from Inside: Nutrition-Based Strategies in Cosmetic Dermatology

17

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Core Messages

- "Skin is the mirror of the soul", as well as the first barrier between the whole body and its environment. These essential aesthetic and functional roles depend on skin structure and functionalities and namely on its integrity and ability of renewal. The patients' lifestyle, and namely nutrition, are of most importance. Indeed skin requires appropriate nutrients, both in terms of quality and quantity. The food has to answer the needs in macronutrients:
  - Proteins, which play key roles in structural support, enzymatic catalysis, immunity..., and aminoacids, among which essential aminoacids: L-lysine and leucine
  - Lipids and essential fatty acids in optimal proportions: 5 ω6 fatty acids (linoleic acid family) for 1 ω3 fatty acid (linolenic acid family), involved as well in skin structure and barrier function as in the regulation of the inflammatory process
  - Carbohydrates, but not in excessive amounts
- The supply in micronutrients should include vitamins and oligoelements, which are of most importance for skin and hair renewal and antioxidant defences.
- The recommended daily water intake is 1.5l. A regular consumption of fermented milky products, providing probiotics, is also likely to improve the natural skin barrier function and antagonize its inflammatory alterations.
- However food supply in nutrients sometimes appears insufficient (teenagers, stressed people, sportsmen, elderly) and food supplements can be useful for patients with dermatological and cosmetic concerns. An appropriate supplementation can be helpful in preventing sunburns, limitating skin aging and some dermatoses and favouring hair and nail strength and beauty.
- As a consequence dietary advice and the prescription of food supplements could be a part of a comprehensive approach to treating patients with cosmetic concern.

"Skin is the mirror of the soul," as well as the first barrier between the whole body and its environment. These essential aesthetic and functional roles depend on skin structure and functionalities and namely on its integrity and ability of renewal. However, skin is constantly submitted to mechanical, physical, chemical, and oxidative attacks resulting from the breath, metabolism, or external factors such as ultraviolet exposure. In order to respond to these attacks and preserve its optimal cosmetic attractiveness and functionalities, the patients' lifestyle, including behavior and nutrition, is of most importance [56]. Indeed skin requires appropriate nutrients, both in terms of quality and quantity. Nutrients are supplied by food and delivered to the skin through the vascular system. The hypodermis and the dermis

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directly receive the nutrients, whereas the epidermis receives the nutrients diffusing from the dermis.

The needs in nutrients for a healthy skin are comparable to those of the body. The food has to answer the energy needs by insuring a contribution being enough in macronutrients (carbohydrates, proteins, and lipids) while supplying micronutrients essential in the cutaneous metabolism, in particular vitamins and oligoelements which play a major role in cell proliferation and as antioxidants.

The main cosmetic orientations of nutrition are directed toward skin hydration, anti-aging strategy [46, 50, 56, 64], prevention of solar exposure [13] and hair beauty.

17.1 Cosmetic Role of Food Nutrients

17.1.1 Supply in Macronutrients

17.1.1.1 Proteins

Proteins play crucial roles in almost every biological process and are responsible for a variety of physiological functions, including structural support, enzymatic catalysis, binding, transport and storage of molecules, and immunity. They are also essential for skin renewal and wound healing.

Proteins are made of assemblies of aminoacids among which eight are essential aminoacids that cannot be synthesized by the organism and have to be supplied through food. A balance between proteins of animal origin and vegetable is advised to cover all the needs in essential amino acids.

With regards to the particular needs of skin in terms of proteins/aminoacid supply, collagens represent about 70% of skin proteins and are composed of repeats of three aminoacids, one of which being glycine, and are often rich in hydroxyproline and hydroxylysine derived from proline and lysine. Glycine and proline can be synthesized de novo, whereas lysine is an essential aminoacid [19]. Deficiencies in L-lysine are associated with hair loss [55].

Elastin is also composed of aminoacids including the essential aminoacid leucine.

So skin appearance and mechanical properties (elasticity) strongly depend on food supply in aminoacids.

17.1.1.2 Lipids and Essential Fatty Acids

Lipids are essential components of cell membranes and their amount and organization in the intercellular domains of the *stratum corneum* are also of great importance in the skin barrier function [10,33]. The main cutaneous lipids are ceramides, cholesterol and free fatty acids. They are synthetized from intermediary products of the metabolism or from essential fatty acids and are secreted by Odland's lamellar bodies [20]. Essential fatty acids are also key factors in the control of the inflammation, in the regulation of the immune system and the microcirculation via the synthesis of eicosanoïdes. Deficiencies or imbalance in free unsaturated fatty acid were shown to be involved in different dermatosis including psoriasis and acne. Dietary deficiency in linoleic acid and 18-carbon polyunsaturated fatty acid results in characteristic scaly skin disorder and excessive epidermal water loss [67].

Since the human body is unable to synthesize essential fatty acids, they have to be provided by food intake. Optimal proportions should be 5 ω6 fatty acids (linoleic acid family) for 1 ω3 fatty acid (linolenic acid family). Principal sources of ω6 fatty acids are: vegetable oils of first cold pressure (seeds of grape, sunflower, germ of wheat, corn, walnut, soya) and meats. ω3 fatty acids are present in fat fishes (salmon, halibut, mackerel, anchovies, sardines) [67], in walnuts, in green vegetables (lamb's lettuce, cabbage, lettuce), and in oils of linen and colza.

Payin attention to the balance of the nutritional contributions in fatty acids will facilitate the protection of skin beauty.

17.1.1.3 Carbohydrates

Carbohydrates play both a structural and immunological role. They are involved in the synthesis of skin constituting glycosaminoglycanes as well as immunoglobulins. They are also essential components of nucleic acids.

Carbohydrates can result from endogenous origin through neoglucogenesis or from exogenous supply by food. The last ones should favor complex carbohydrates. However, carbohydrate intake should not be excessive, otherwise skin disorders such as acne may occur [59]. Moreover Cosgrove et al. have shown that a 50 g increase in carbohydrate intake increased the likelihood of a wrinkled appearance and skin atrophy.

17.1.2 Supply in Micronutrients

Micronutrients provided by food contribute to the body's natural defences at three levels by supporting physical barriers (skin/mucosa), cellular immunity, and antibody production [36]. They thus appear to be essential to maintain skin equilibrium. They include vitamins, oligoelements, and other nutrients such as flavonoids. Deficiencies are associated with skin disorders [52].

Some of these nutrients are involved in skin renewal. Others participate in the protection of skin against oxidative stress resulting from cellular respiration, oxidative metabolism, and external attacks (UV radiations, pollution, …). Skin is naturally equipped with antioxidant defenses [43] from enzymatic (catalase, glutathione peroxidase, superoxide dismutase, …) and non-enzymatic nature (glutathione, vitamins, …). Internal mechanisms of restoration of these molecules exist. However, these defenses also rely on sufficient external contribution in order to be restored.

17.1.2.1 Vitamins

Most of the vitamins have to be taken in by food. The organism is unable to synthesize vitamins, except for a few of them including vitamin D (vitamin D3 is produced in skin exposed to sunlight) and vitamin K. Moreover protective skin care measures against sun exposure may result in vitamin D deficiencies [41]. In a recent review, Moyad concludes that increasing the recommended daily allowance of this vitamin to 800–1,000 IU per day may be beneficial in most age groups. Vitamin D, found in food of animal origin and vegetable oils, exerts antiproliferative and immunoregulative properties [50].

Some vitamins, such as B9 vitamin, play a role in cutaneous cell proliferation and in skin renewal. Vitamins B2 and B5 are cofactors of macronutrients metabolim. Deficiencies of vitamins from the B group are often associated with cutaneomucous signs such as dermatitis and depigmentation [46].

Vitamin A appears to determine sebum content and skin surface pH [7]. Indeed Boelsma et al. have shown that an increment of 100 μg dietary vitamin A was associated with a significant increase in surface pH in women. On a cellular level, retinoids are involved in the expression of proteins such as keratins, collagen, collagenase, transglutaminase, and laminin [60], and retinoids affect the differentiation and proliferation of both epidermal and mesenchymal cell types [38].

Vitamin A, also called retinol, is a liposoluble vitamin which is involved in keratinisation, in the regulation of sebaceous gland activity and immunomodulation [42]. Vitamin A is of particular abundance in oils of fish (halibut, cod, tuna) and in the liver of animals (turkey, beef, chicken, calf).

Vitamins C (ascorbic acid) and E (α-tocopherol) are major antioxidants and can serve as direct free radical scavengers. Vitamins C and E are efficient in the protection against UVB. They reduce the sunburn reaction and prevent the formation of thymine dimers, thus preventing DNA damage [48]. Vitamin C is responsible for the regeneration of vitamin E.

Vitamin C is also involved in the synthesis of collagen [12] and takes part in the maturation of procollagen to collagen [38]. Indeed the deficiency in vitamin C leads to an accumulation of procollagen and to an inhibition of collagen synthesis, which is reversed by the addition of ascorbic acid to the diet.

In order to achieve sufficient amounts of vitamin C, the dietary intake should include fruits and vegetables (citrus fruits, kiwi, tomatoes, pepper). Vitamin E is a liposoluble vitamin that can be found in butter, vegetable oils (sunflower, soybean, wheat germ), and eggs.

17.1.2.2 Oligoelements

Oligoelements are essential for the optimal functioning of enzymatic antioxidants. Selenium is the cofactor of glutathione peroxidase, thioredoxin reductase, and selenoprotein P (responsible for the detoxification of peroxinitrites), while copper, manganese, and zinc are involved in superoxide dismutase activity. The activity of the antioxidant enzymes directly depends on the availability of trace elements in food. A copper overdrawn regime, e.g., decreases the activity Cu-Zn-superoxyde dismutase, a deficit in manganese leads to a decrease of the activity Mn-superoxyde dismutase, and a deficit in selenium to a decrease of the activity glutathione peroxydase.

Excess of oligoelements, however, can provoke paradoxical, i.e., toxic and pro-oxidative effects; therefore, a fine balance is essential for health [14]. Accordingly excessive intake of selenium can produce selenosis in humans affecting liver, skin, and also nails and hair [63]. Recommended intake and upper tolerable levels

are 40–55 and 300 μg/day [1]. Similarly, iron is involved in the production of free radicals through the Fenton reaction; iron deficiency, however, is associated with hair loss [55].

Zinc deficiency is associated with periorificial rash and sometimes teenage acne [16], or rough and dry skin, and also with hair loss [52]. Indeed zinc is required by the immune system and plays an anti-inflammatory role [51]. Zinc is the cofactor of hormone synthesizing enzymes, such as D5 reductase (testosterone metabolism) or D9 desaturase (prostaglandins metabolism). Zinc also has positive effects in cutaneous wound healing and exerts direct antioxidative effects by inhibiting the Fenton reaction responsible for the production of hydroxyl radicals. It is also involved in the synthesis of antioxidant mflothioneins. Zinc is mainly present in meat, eggs, milk, and seafood. However, deficiencies can be observed in pregnant women or old people [22].

17.1.2.3 Other Micronutrients

Cartenoids, which are found naturally in the skin, show photoprotective effects and are thus effective as preventive anti-aging micronutrients [44]. Moreover β-carotene may have a direct photoprotective effect because of its physical ability to absorb light [6]. However, intake should be moderated since prooxidant effects were shown with high β-carotene concentrations [45].

17.1.3 Supply in Water

Skin hydration depends on the quality of both the *stratum corneum* and the hydrolipidic film of surface. Water represents a major component of the dermal fundamental substance and comes from the plasma. Part of this water can diffuse to the *stratum corneum* where it is retained namely by natural moisturizing factors. The distribution of water depends strictly on aquaporines, in particular, on the aquaporine 3. The presence of an adequate amount of water in the *stratum corneum* is important for a general appearance of a soft and smooth skin [7]. However, the preservation of the water reserve depends on the volemy, thus, to the hydration. To insure a good hydration of the body and also of the skin, the recommended daily water intake is 1.5 l.

Fluid intakes were also shown to be inversely associated with the surface pH in men, but not in women [7].

17.1.4 Probiotics

Probiotics are living microorganisms found in fermented milk which exert direct effects on the intestine by improving the digestive function and the intestinal microbial balance [3]. They include *Lactobacillus* (*L. casei, L. rhamnosus, L. Johnonii*) and *Bifidobacterium* species (*B. breve, B. longum, B. bifidum*), which belong to the lactic acid bacteria group, as well as *Enterococcus*, *Escherichia coli*, *Propionibacterium*, *Bacillus,* and some yeast [40]. Probiotics, by improving gut barrier function, restoring a healthier gut microecology, stimulating the host immune system, and antagonizing the inflammatory alterations [15,47], allow a significant improvement of atopic dermatitis symptoms [28, 11]. A regular consumption of fermented dairy products is also likely to improve the natural skin barrier function, as shown by the decrease of trans-epidermal water loss [32], and improved its cosmetic appearance.

17.2 Relevance of Nutritional Supplements

Food supply in nutrients sometimes appears insufficient (teenagers, stressed people, sportsmen, elderly) and food supplements can be useful for patients with dermatological and cosmetic concerns [21]. Indeed many common micronutrient deficiencies cause mitochondrial decay with oxidant leakage leading to accelerated aging and also skin aging [2]. Active molecules with different cellular and molecular targets are available. These bioactive molecules or "actives" are characterized by their capacity to actively modulate biological processes which take place in human skin, e.g., by stimulating beneficial properties or by interfering with signaling pathways which are known to lead to skin damage.

17.2.1 Nutritional Photoprotection

Various studies [61,62] demonstrate that it is possible to decrease the risk of sunburn by eating food rich in

carotenoids, tocopherols, vitamin C, and omega 3 long enough before sun exposure (8–10 weeks). These nutrients directly absorb or disperse light by interfering with UV-induced signaling cascades and exert antioxidation effects and thereby limit UV-induced skin damage and contribute to fighting against UV rays and preserving the skin. The long-term (3 months) oral administration of a combination of vitamin C and vitamin E was also shown to reduce the sunburn reaction to UVB irradiation [48]. Another study by Poli [49] demonstrated that a diet high in flavonoids (apples, oranges and green tea), even over a short period time (11 days), can provide anti-UVA protection. In a meta-analysis, Kopcke and Krutmann [31] showed that taking β-carotene is effective in preventing sunburn, but also that one must begin taking supplements at least 10 weeks prior to sun exposure in order for it to be effective.

17.2.2 Prevention of Skin Aging

Actinic skin wrinkling appears to be correlated with food and nutrient intake [54]. Indeed Purba et al. [54] observed less actinic skin damage in subjects with a higher intake of vegetables, olive oil, and fish, whereas more actinic damage was seen with higher intake of dairy foods, butter, margarine, and sugar products. Vitamin C, retinol, and intake of minerals such as calcium, phosphorus, magnesium, iron, and zinc also appeared to be protective against cutaneous actinic damage. Moreover higher vitamin C intake as well as higher linoleic acid intake is associated with a lower likelihood of senile dryness. Higher vitamin C intake is associated with a lower likelihood of wrinkled appearance [18]. Higher linoleic acid intake is related to less skin atrophy. Lower intake of fats and carbohydrates are associated with better skin-aging appearance [18]. Oral intake of soy isoflavone aglycone (40 mg/day) was also shown to be effective in middle-aged women [29]. Oral intake of carotenoids such as lutein and zeaxanthin, acting as antioxidative agents, protect skin from actinic aging [44].

Silica from Blue Lagoon stimulate keratinocyte functions by inducing cytokine, in particular IL-1 production [35], keratinocyte differentiation and fibroblast collagen synthesis [24]. Food supplements containing a combination of marine protein and lipids, together with polyunsaturated fatty acids of omega-3 type, tocopherols, and plant flavonoids, were also shown to protect skin from the effects of aging and to support its repair process [5].

The administration of synergistic combinations of antioxidants such as vitamins C and E, carotenoids, and oligoelements is of particular interest in postmenopausal women, in particular if they do not consume a healthy diet that includes five daily rations of fresh fruits and vegetables [39].

17.2.3 Skin Moisturization

Primavera and Berardesca [53] have demonstrated in a placebo-controlled clinical study (2005) that skin parameters were improved, in terms of skin moisturization, surface roughness, and wrinkle depth, in 32 women treated for 40 days with food supplements containing plant ceramides, fish cartilage amino acids, and essential fatty acids. Borage oil, taken during 2 months, also decreased skin dryness and subsequent pruritus in elderly people [9].

Some carotenoids may influence skin hydration. Indeed Boelsma et al. [7] have shown that an increase of the serum β-cryptoxanthin was associated with an increase in skin hydration.

17.2.4 Skin Barrier Function

Food nutrients can enhance skin barrier function. The regular consumption of dairy products containing borage oil (gamma-linoleic acid), green tea extracts (catechin), vitamin E, and probiotics (*Lactobacillus casei*) caused a significant decrease of TEWL [64]. Moreover the dairy matrix improves the biodisponibility of the nutrients.

17.2.5 Diet and Prevention of Acne Lesions

Acne lesions can be reduced by following simple dietary advices, in particular by reducing sugar intake. A controlled study published in 2007 [58] in patients with acne confirmed that a diet low in simple carbohydrates

can reduce the number of acne lesions. Previous studies [17,34] already indicated the possible link between food and acne. These epidemiologic studies were carried out in primitive populations demonstrated that these populations had little or no acne compared with the 70–90% of teenagers in Western countries who have it.

The group that followed a low-carbohydrate diet showed fewer acne lesions and also weight loss. In fact, a diet low in carbohydrates decreases hyperinsulinism and could as a result reduce androgenicity (decrease in dehydroepiandrosterone-sulfate concentrations and the bio-availability of testosterone) and insulin-like growth factor-1 (IGF-1).

17.2.6 Nutritional Factors and Hair Beauty

Nutritional factors are likely to condition hair beauty. Indeed a supply deficient in proteins, and in sulphurated amino acids in particular, has repercussions on the metabolism of the hairy follicle and thus on the life of the hair [8]. Moreover, deficiencies in pantothenic acid, riboflavin, and biotin were shown to be responsible for hair loss [55]. Iron stores, reflected by serum ferritin concentration, also appear to determine hair loss when insufficient [30, 65]; in this context, the importance of iron supplements has been demonstrated for a long time in non-anemic iron-deficient women with hair loss [26]. L-lysine, appears to act as a key amino-acid for hair, maybe via the increase of iron and Zn uptake [55].

Silicon administered as choline-stabilized orthosilicic acid was also shown to improve hair tensile strength, including elasticity and break load, and to result in thicker hair [66].

Supplements, however, should be used carefully, because excessive intake of micronutrients may also cause hair loss. Accordingly, a link was established between excessive vitamin A intake and hair loss [37].

17.2.7 Nutritional Factors and Nail

Nail health and appearance are a matter of concern for subjects with cosmetic requirements. Complaints of brittle or soft nails are frequent, particularly among women [25]. Of course proper care is essential in order to preserve nail health [57], but adequate supplements of micronutrients can also be useful. Brittle nail syndrome appears to abate with supplementation with a 2.5 mg dose of biotin daily [23] or a 10 mg dose of silicon daily [57]. Supplementation with calcium (1 g/day during 12 months) was also shown to improve nail quality [4]. The regular consumption of carotenoids, from food or supplements, also appears to be effective in chronic onycholysis [27].

17.3 Concluding Remarks

It is without doubt that nutrition affects the beauty of skin. Both macronutrients including essential fatty acids and micronutrients such as vitamins A, C, E, and oligoelements are of importance in order to preserve skin functionalities and cosmetic attractiveness. A diversified and well-balanced diet definitely helps to preserve the healthy appearance of our skin. As a consequence dietary advice and the prescription of food supplements could be a part of a comprehensive approach to treating patients with cosmetic concerns.

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Appendix

Examples of Available Products, Producers, and Sources for Further Information

Alessandra Marini and Thomas Jaenicke

The list includes some of the large number of suppliers worldwide. The authors would like to point that all the information given in this chapter has been provided by the industry. In other words, inclusion or exclusion of a specific company from this chapter might result from ignorance of this company by the authors or from lack of response of the company to the authors' request for information. Also, we do not take responsibility for the validity of the information provided by the companies listed here.

Producer	Products
Ardea Beverage Co. 527 Marquette Ave., Ste. 600, Minneapolis, MN 55402-1313, USA Web: www.nutrisoda.com	*Nutrisoda Drink*
Nutrisoda is a drink with antioxidants for a healthy glow. It contains pomegranate and blackberry with L-lysine, L-proline, L-arginine, alpha lipoic acids, vitamins A, B5, B6, E and D, selenium, green tea, and folic acid.	
BeautyNutraceuticals NZ P.O. Box 9288 Newmarket Auckland, New Zealand Web: www.beautynutraceuticals.com	*Cellular-Detox*™ *SuperAntioxidant*™ *Smooth Skin*™
All BeautyNutraceuticals™ products are specially designed as beauty dietary supplements to help enhance and maintain beauty. Cellular-Detox™ is specially designed for detoxification occurring during rebuilding. SuperAntioxidant™ is designed to help maintain youth and beauty by prolonging the life of the cell through its super antioxidant capabilities and the stimulation of key antioxidant enzymes glutathione peroxidase and superoxide dismutase. Smooth Skin™ helps to maintain a constant level of lipids in the upper layer of the epidermis and the integrity of the skin cell walls.	
BORBA C/O Serec of California 15351 E. Stafford St. City of Industry, CA 91744-4421 Web: www.borba.com	*Firming Aqua-less Crystalline drink* *Age Defying Aqua-Less Crystalline* *Gummi Bear Boosters*
Borba offers several anti-aging nutraceutical products which improve general appearance and provide antioxidant protection. The Firming Aqua-less Crystalline drink contains a bio-vitamin complex and a blend of nutrients intended to promote the natural support system of the skin, helping to nourish and tone the skin. The Age Defying Aqua-Less Crystalline promises to help soften the appearance of fine lines and wrinkles and to renew the natural glow of the skin. Borba's Gummi Bear Boosters are gummi bear candies that contain as antioxidants green tea extract and grape seed extract. Gummi Bear Boosters are supposed to help "increase the potential to absorb skin caring ingredients into the epidermis."	

(*continued*)

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Producer	Products
Coca Cola Tokyo Web: www.cocacola.co.jp	*Yokuasa Purun Drink*
Yokuasa Purun promises to promote beauty. It is a milk-based drink fortified with cysteine, hyaluronic acid, ceramide, vitamin C, and biotin.	
Danone 17, Boulevard Haussmann 75009 Partis, France Web: www.danone.com	*Essensis Yoghurt*
Essensis help maintain a beautiful skin. It contains a combination of omega 6 from starflower oil, green tea, vitamin E, and probiotics.	
Dr. Brandt Skin Care Cosmetic Dermatology, Inc. 8798 NW 15th street Miami, FL 33172 Web: www.drbrandtskincare.com	*Dr. Brandt Water Boosters*
Dr. Brandt Water Boosters come as concentrated drops that contain green tea (powerful antioxidant), grape seed extract (protects collagen and elastin), lo han (reduces cravings for sugar), and white tea (anti-oxidant). The promise is to repair skin and slow future signs of aging from the inside out.	
Dr. Murad 20540 Belshaw Avenue Carson, CA 90746 Web: www.murad.com	*Wet Suit Cell Hydrating Dietary Supplement* *Sleep Reform Dietary Supplement* *Youth Builder Dietary Supplement* *Firm and Tone Dietary Supplement*
Dr. Murad offers a selection of nutraceutical products to improve the general appearance of the skin. Wet Suit Cell Hydrating Dietary Supplement contains phosphatidylcholine, vitamin E, zinc, copper, manganese, and CoQ10. It fortifies cell membranes to help skin maintain a healthy moisture balance. Sleep Reform Dietary Supplement contains melatonin, glucosamine, and B-vitamins and helps to repair the skin during sleep. Youth Builder Dietary Supplement contains glucosamine, grape seed extract amino acid complex, and vitamins A, C, and E. It promises to reduce lines and wrinkles. Firm and Tone Dietary Supplement Pack contains multivitamins, essential fatty acids, minerals, and plant extracts and promises to firm up skin and improve disappearance of stretch marks.	
H.-G. Berner GmbH & Co. KG Hasenholz 10 24161 Altenholz, Germany Web: www.cellagon.de	*Cellagon felice Drink*
Cellagon felice contains grape seed extract OPCs (to protect collagen and elastin), phospholipides (to support membranes of the cells), Noni juice (rich in polysaccharides that promote healthy tissue), aloe vera, L-carnitine, spirula algae, and vitamins.	
IMEDEEN Denmark Ferrosan A/S Sydmarken 5 2860 Soeborg, Danmark Web: www.imedeen.dk	*Imedeen Derma One™* *Imedeen Time Perfection™* *Imedeen Prime Renewal™*
All IMEDEEN® skincare tablets are formulated to improve skin quality and moisture balance all over – face and body. IMEDEEN Derma One skincare tablets combat loss of radiance and hydration associated with the first signs of ageing. IMEDEEN Time Perfection tablets are claimed to combat visible signs of skin aging by reducing fine lines and wrinkles. IMEDEEN Prime Renewal tablets address the specific skincare needs of women post menopause with innovative natural-based ingredients.	
INNEOV- L'ORÉAL International 41, Rue Martre 92217 Clichy Cedex, France Web: www.loreal.com	*Innéov Firmness™* *Innéov Anticellulite ™* *Innéov Solaire™* *Innéov Hair Mass™* *Innéov Dry Skin™*

Innéov nutritional produces supplements for skin and hair. Innéov claims their "beauty pills" act at the very heart of the metabolism to deliver global results on the whole face and body. Inneov Dry Skin supplement, for example, helps to balance and moisturize skin from within with a combination of omega 3, omega 6, lacto-lycopene, and vitamins C and E. Inneov Firmness contains lacto-lycopene (stimulates cellular renewal), vitamin C (promotes synthesis of collagen and elastin fibers) and soy isoflavones (support cellular renewal, protect collagen). Inneov Anticellulite comes in tablets or sachets of powder to be diluted in water. Calcium, green tea, pine-extract, and marine glucosamine fight cellulite and are believed to strengthen skin tissue and help burn fat. Innéov Hair Mass contains a combination of taurine, zinc, and catechins and is claimed to be the first nutritional supplement designed to "energize" and strengthen hair, promote its growth and slow down hair loss.

L'ORÉAL International *Kérastase Nutrients Densitive*
41, Rue Martre
92217 Clichy Cedex, France
Web: www.loreal.com

Kérastase Nutrients Densitive is a hair re-densifying food supplement for daily use. It is claimed that hair feels stronger, denser, healthier, and thicker because of the formula of taurine, green tea extract, grape seed polyphenols, and zinc.

Eurogran *Le Royal ChocoDark*
Web: www.eurogran.com

Le Royal ChocoDark is a smooth, rich, dark chocolate drink from a select blend of cacao beans. Dark chocolate has been considered a healthy indulgence, thanks to its high levels of the antioxidants polyphenols and flavanols.

LaneLabs USA *Toki*
3 North Street
Waldwick, NJ 07463, USA
Web: www. lanelabs.com

Toki is the collagen you drink. It promises to reduce the appearance of age spots and wrinkles. Toki contains active collagen, calcium, and a mucopolysoccharide complex. These nutrients have been combined with amino acids from Hijiki seaweed.

Mars *Dove Chocolate*
800 High Street
Hackettstown, NJ 07840 USA
Web: www.dovechocolate.com

Dove chocolate helps promote beautiful skin with cocoa flavanols and a range of vitamins.

Maswell Brands *Sipping Beauty Tea*
915 E Squantum
Quincy, MA 02171, USA
Web: www.maswelbrands.com

Sipping Beauty Tea offers a range of different organic tea. It promises to fight the effects of aging. The sipping tea works to keep damage away and weary skin on delay.

Mediniche *Impruv®*
MediNiche, Inc.
PMB 300, 167 Lamp & Lantern Village
Chesterfield, MO 63017
Web: www.mediniche.com

Impruv, a dietary supplement developed specifically to help maintain healthy skin, is rich in fat-soluble vitamins that protect skin cell membranes, and water-soluble vitamins that help protect the inside of the skin's cells and their DNA from harm. Impruv contains a balanced blend of antioxidants and dermal-specific micronutrients that work at the cellular level

Nestlé S.A. *Glowelle*
1800 Vevey, Switzerland
Web: www.glowelle.com

Glowelle combines vitamins, phyto-nutrients, botanical,s and fruit extracts such as beta-carotene, vitamins C and E, lycopene, lutein, CoQ10 and selenium, pine bark, pomegranate, and green tea extracts. It promises to protect, nourish, and hydrate skin from within.

(*continued*)

Producer	Products
Nestlé Brazil Web: www.nestle.com.br	*Molico Beauty Yoghurt*
In Brazil, Nestle has introduced Molico Beauty, a 0% fat yoghurt containing aloe vera, an ingredient associated with health and beauty issues. The product is also claimed to be a good source of vitamins A, D, and W and contains a small amount of oat bran for fibre.	
The Nut Company Web: www.thenutcompany.com	*Beauty Mix (nut & fruit mix)*
Beauty mix is a good source of vitamin B2 and E, which helps to enhance your skins natural glow.	
Nutri Ltd. Meridian House Botany Business Park Macclesfield Road Whaley Bridge High Peak Web: www.nutri-online1.co.uk	*Eskimo-3*
Containing optimal concentrations of the essential fatty acids EPA and DHA, Eskimo-3 is a stable fish-oil formula, and one of the most scientifically researched of all supplements.	
Oenobiol – Laboratoire 59 bd Exelmans 75016 PARIS Web: www.oenobiol.co.uk	*Oenobiol Anti-Wrinkles Q10 Capsule*
Oenobiol prevent and reduce wrinkles. It combines actilycopene, lutein, and selenium.	
Pharma Nord ApS Sadelmagervej 30-32 DK-7100 Vejle, Denmark Web: www.pharmanord.com	*Evelle*
Evelle combines pycnogenol, vitamins C and E, carotenoids, selenium, zinc, amino acids and glycosaminoglycans, and blueberry extract. It promises to prevent wrinkles.	
Santica Research Labs, LLC 1141 South Rogers Circle, Suite No.5 Boca Raton, FL 33487 Web: celluscience.com	*CelluScience*
CelluScience promises to fight cellulite by promoting healthy cell metabolism, maintaining micro-circulation in the skin, and delivering potent antioxidants. Contains fish oil, borage oil, grape seed extract, ginkgo biloba extract, gotu kola extract, yellow sweet clover extract, butcher's broom extract, olive extract, etc.	
Soft Gel 6982 Bandini Blvd. Los Angeles, CA 90040, USA Web: www.soft-gel.com	*Injuv®*
Injuv® is a dietary supplement containing 9% low molecular weight (50,000–200,000 Da) hyaluronic acid for oral consumption. Hyaluronic acid is believed to ward off the aging process by helping the tissues of the body to retain moisture, keeping joints lubricated, protecting the retina, and keeping skin smooth and elastic..	
SPI Swiss Pharmaceutical Industries SA Rue de la Treille 4 CH-2001 Neuchâtel Switzerland Web: www.swipi.com	*Estime®*
Estime® is a unique internal skin rejuvenation formula that supports the natural regenerative properties of the skin.	

Producer	Products
Sustainable Youth™ Technologies 303 Fifth Avenue Suite 1007 New York, NY 10016 Web: www.sustainableyouthtechnologies.com	*Elastifirm Supplement*
Elastifirm Supplement improves skin elasticity and firmness. It contains alasta complex, defatted rice bran, hypromellose, di-calcium phosphate, silicon dioxide, and magnesium stearate.	
VitaMedica 1140 Highland Avenue, CA 90266 Web: www.vitamedica.com	*VitaMedica's Anti-Aging Formula* *VitaMedica's Dry Skin Formula*
VitaMedica's Anti-Aging Formula contains vitamins A and E to support skin health, the full B-complex to support healthy hair, skin, and nails, flax seed oil, an excellent source of omega-3s for skin lubrication and moisturization, and vitamin C plus potent antioxidants to neutralize free radicals. VitaMedica's Dry Skin Formula is designed for individuals with skin that lacks hydration and/or lubrication. It contains flax seed oil, super EPA/DHA fish oil to promote favorable prostaglandins, hormone-like compounds that play a key role in reducing dermal inflammation, and hyaluronic acid to maintain water balance in the dermis and provide support for other dermal elements like collagen and elastin.	
Votre Vu, LLC Customer Care 549 Heartland Drive, Unit F Sugar Grove, IL 60554 Web: www.votrevu.com	*SnapDragon drink*
SnapDragon drink promises to help maintaining beautiful skin with a radiant glow. The drink is composed of fruits, botanicals (mango, pomegranate, and acai berry juices), teas (green, white, and gorgeous red), vitamins plus collagen, aloe vera, foti, ginkgo biloba, and baobab fiber.	
Wimm-Bill-Dann 16 Yauzsky Boulevard Moscow 109028, Russia Web: www.wbd.com	*Neo-Beauty drink*
Neo-Beauty drink is designed to improve skin, nails, and hair. The exclusive 3D Regeneo formula contains aloe vera, antioxidants, minerals and vitamins.	

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