SKIN HEALTH AND NATURAL PRODUCTS FOR TOPICAL AND NUTRACEUTICAL SKIN CARE

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Skin, as a protective organ of the body, is subject to many factors from within the body and from the outside environment that constantly challenge its integrity and protective functions. Diverse factors such as ultraviolet light (UV), atmospheric pollution, increased atmospheric ozone, wounds, infections, hormonal status, metabolic diseases, psychological stress and the natural process of aging have a role in causing temporary or permanent pathological changes in the skin.

Hormonal status and skin health

The outward appearance of the skin depends in large measure on the hormonal play in the body. It is recognized that the onset of menopause, characterized by a precipitous drop in levels of endogenous estrogens, correlates with the accelerated aging of skin structures and their subsequent decline in protective functions. Skin thickness and bone density are significantly decreased as early as six months after menopause begins and are increased after the same period of oral hormone replacement therapy (HRT). In addition, topical use of estrogen compounds has been found to diminish skin aging symptoms. Specifically, the functions of skin fibroblasts, cells that produce "youth fiber" collagen, and keratinocytes, cells protecting skin, are stimulated by estrogen application. Isoflavones, like soybean genistein, which are plant derived or phyto-estrogens, used in topical creams were shown to diminish skin dryness and wrinkles. Genistein used orally or applied topically may substantially inhibit skin carcinogenesis and aging induced by UV light. The mechanism of action involves the prevention of UV-exposure precipitated oxidative damage to the DNA of skin. In recent years, increasing evidence shows the health effects of genistein and other phytoestrogens in prevention and therapy of breast and prostate cancers, postmenopausal syndrome, osteoporosis, and cardiovascular disease in animals and humans. Although skin aging is certainly no indication for systemic hormone supplementation, the safe and beneficial action of nature-derived phytoestrogens, like genistein, can provide a sensible alternative or an adjunct therapy to the synthetic HRT treatment of menopause and the symptoms of skin aging.

Inflammation and skin health

With scientific advances in dermatology, skin is increasingly seen as a very complex organ regulated by an intricate system of molecules and corresponding receptors. Recently it has been found that receptors for the inflammatory enzyme cyclooxygenase 2 (COX-2) are increased in aged skin fibroblasts. The increased expression of the COX-2 enzyme is known to increase levels of inflammatory prostagalandin PGE-2, which not only corresponds to inflammation and associated diseases such as arthritis and cancer, but also to the process of skin aging. Aging skin fibroblasts, in addition to increased expression of inflammation related receptors, undergo significant degenerative changes

as compared to young and highly functional cells. Specifically, aged fibroblasts have a decreased ability for proliferation, probably due to the increased expression of the p53 gene (gene responsible for programmed cell death or apoptosis), decreased expression of the PCNA protein (a protein involved in cell repair and regenerative functions), increased expression of MMP-1 gene (gene encoding inflammatory metalloproteinase destroying skin collagen), and decreased expression of tissue inhibitor of metalloproteinase-1 (TIMP-1) and procollagen levels (less collagen production). Interestingly, the experimental use of a selective COX-2 inhibitor drug resulted in the slow down of the above described age-related changes in aging fibroblast cells.

With recent controversy surrounding the safety of pharmaceutical COX-2 inhibitors it is reassuring to note that nature has created a class of safe and effective COX-2 inhibitors found, for example, in green tea, rosemary and in turmeric. These plant derived components, used for millennia in Oriental culture as food (turmeric root in preparation of curry), are technically known as phenol compounds, useful in the prevention and intervention of inflammatory conditions. The phenolic compounds of turmeric (*Curcuma longa* fam. Zingiberaceae) are known as curcuminoids and tetrahydrocurcuminoids (THC) and have well recognized inhibitory effects on COX-2. Curcuminoids and THC have also been found to inhibit the activity of tyrosinase, an enzyme that participates in skin pigment formation or melanogenesis, thereby preventing melanin formation that often increases with aging and may predispose to skin cancer.

Inhibitory concentrations (IC50) of THC on tyrosinase

	IC50 (mcg/ml)
THC	0.0000492

IC 50 – minimum concentration of THC required for 50% inhibitory effect

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Tetrahydrocurcuminoids (THC) are color-free compounds from turmeric and therefore applicable in topical and cosmetic formulations. THC inhibits many biochemical and morphological changes in the skin, which appear to be associated with inflammation and skin tumor promotion. For example, topical application of a chemical skin irritant and carcinogen (TPA) rapidly induces skin inflammation manifested by an increase in epidermal ornithine decarboxylase activity. Based on experimental data, THC may inhibit the TPA induced inflammatory process by at least 80%. THC is also effective in the inhibition of UV-induced damage to the epidermis measured by a decrease in the extent

of sunburn lesions, the number of so-called sunburn cells in UV exposed skin, and a decrease in the number of epidermal cells with UV-damaged DNA.

Effect of Topical Application of Tetrahydrocurcumin on UVB light-induced Sunburn Lesions in SKH-1 Mice

Treatment	Days after last dose UV			
	4	5	6	7
Acetone (100ul)	++++	+++	+++	++
THC1 (10 uMol)	+	+	+	-

The area of sunburn lesion (red color) were measured: (-) no sunburn; (+) slight sunburn; (++) sunburn; (+++) strong sunburn; (++++) very strong sunburn lesion.

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Skin lipids, collagen in skin health

The outermost skin protective mechanism involves a very complex mixture of skin surface lipids (SSL), which primarily guard the skin against environmental oxidative assault. The skin content of protective lipids, which include monounsaturated and diunsaturated as well as branched monounsaturated fatty acids of triglycerides, is higher at young adults than in childhood and in old age. The protective composition of SSL is stabilized by the natural presence of squalene, vitamin E and Coenzyme Q10 (CoQ10), which compounds increase from childhood to maturity, and decrease again in old age. Vitamin E and CoQ10 are the only known lipophilic antioxidants present in SSL. These two fat-soluble compounds synergistically inhibit the UV induced depletion of squalene, cholesterol and unsaturated fatty acids from the protective lipid layer. When the skin is exposed to UV oxidation the antioxidant role of vitamin E and CoQ10 spares squalene and other components of SSL. Interestingly, exposure to UV in the absence of vitamin E and CoQ10 resulted in a 90% depletion of squalene in the SSL. One of the most important systemic and topical compounds emerging in skin care is α-Lipoic acid, which helps maintain fat soluble and water soluble antioxidants present in the skin.

α-Lipoic acid, also known as thioctic acid, functions as a co-factor for a number of key enzymes that help in the conversion of glucose, fatty acids and other energy sources into ATP acid. It has been called the "perfect" antioxidant. It is proven to quench hydroxyl radicals, hypochlorous acid and singlet oxygen as well as to chelate metal ions such as iron, copper and cadmium. α–Lipoic acid helps to recycle vitamins C and E in the body and increases the levels of CoQ (ubiquinone), which recycles vitamin E. By virtue of its high absorption and bioavailability, it rapidly reaches effective concentrations in the tissues, both in the aqueous and lipid regions of the body.

The α–Lipoic molecule consists of an 8 carbon fatty acid chain with two interlinked sulphur atoms attached. When each sulfur atom picks up hydrogen, breaking the bond between the sulfur atoms, the molecule is "reduced" to dihydrolipoic acid (DHLA), which can be readily oxidized to lipoic acid (LA). DHLA has the capacity to regenerate the endogenous antioxidants vitamin E, vitamin C and glutathione.

The regenerative effect of LA on the pool of skin antioxidants can be appreciated in view of skin exposure to free radical oxidation depleting skin antioxidants i.e. vitamin E, Coenzyme Q10 and vitamin C, while oxidizing and damaging the skin protective lipid layer. In addition, the well known application of α–Lipoic acid in prevention and

treatment of diabetes, a metabolic disease that impacts skin health and appearance (hence insipid appearance in diabetes insipidus), may further explain the role of LA in maintaining healthy skin. This role is owed in part to the mechanism preventing the diabetes-induced distortion of protein molecules of skin collagen. Skin collagen from diabetic animals shows an abnormal biochemical pattern, i.e. elevated beta component of type I collagen. These changes were significantly improved by the administration of LA, which exerted a positive influence on both quantitative and qualitative properties of skin collagen.

The biochemical and physical properties of collagen are paramount in wound healing. Skin wound healing is relatively slow in patients with diabetes due to the dysfunction of skin fibroblasts and collagen. Addition of estrified glutathione, the body's own antioxidant, to the fibroblast culture in a high glucose medium (simulating diabetic condition) improved the wound-healing properties of fibroblasts and collagen. Therefore LA, which is known to regenerate glutathione, may contribute to skin health in diabetics also by restoring physiological functions of fibroblasts and collagen in wound healing.

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Biological repair mechanisms and skin health

The mechanisms that repair skin injury from age imposed wear-and-tear (wrinkles) to wounds caused by the skin trauma, surgery or burn are still insufficiently understood. Interestingly, skin wounds in the embryonic period heal perfectly with no scar whereas wounds in adulthood heal with a scar. The developmental and age related differences of the healing process are most likely due to cellular and molecular differences between

scar-free healing in embryonic wounds and scar-forming healing in adult wounds. The lower levels of inflammatory cells combined with different quantities and composition of growth factors responsible for healing in embryo in comparison to adulthood may possibly explain the complete, scar-free healing in embryo. The embryonic wounds that heal without a scar have low levels of growth factors TGFbeta1 and TGFbeta2, low levels of platelet-derived growth factor and high levels of TGFbeta3. Other growth factors like insulin growth factors IGF-I and -II and growth hormone GH may regulate skin as a protective barrier as well as participate in wound healing. Disturbances in GH and IGF pathways are implicated in several skin pathologies, e.g., psoriasis, and skin cancer. As previously mentioned the fibroblast and fibroblasts growth factors e.g. FGF-7 and FGF-10 which are essential for wound healing also have other role and can protect epithelial cells from damaging effects induced, for example, by radiation and oxidative stress. Several genes were identified that are likely to mediate the protective effect of FGF-7 for epithelial cells *in vitro* and possibly also in injured and diseased tissues *in vivo.*

Currently available potential treatments to improve skin healing via growth factors are empirical. One such preparation with a potential in wound healing, alleviating wrinkles and supporting the growth of skin appendages such as hair follicles is coconut water. Coconut water is the liquid endosperm of *Cocos nucifera* L. The greatest amount of coconut water and the highest nutrient density is found in young, green coconuts and provides nourishment for the growth of the solid endosperm (the white coconut pulp) inside the hard shell of the fruit. The medium is rich in proteins, amino acids, sugars, vitamins, minerals and growth hormones essential to promote tissue growth. In addition, shikimic acids and quinic acids have been detected in samples of coconut water from fruits at different stages of maturity, with the maximum amounts being found in young green coconuts. The RNA-phosphorus (RNA-P) content of coconut water was found to be consistently high at all levels of fruit maturity. However the ratio of RNA-P/DNA-P is significantly higher in immature green coconuts than in the mature fruit, 342.5 vs. 13.9. The role of RNA in amino acid transport and respiratory metabolism of living cells is well known. The RNA of coconut water would therefore effectively carry out these and other functions as part of the metabolic machinery essential to the developing endosperm tissue of the coconut and therefore support the growth of other living cells as well, in tissue culture. In addition, the water from green coconuts has higher content of solids than mature coconut watre (6.5% vs. 5.4%); higher content of reducing sugars (4.4% vs. 0.2%); higher content of potassium (290mg% vs. 247mg%); higher content of iron (106mg% vs. 79mg%); lower protein levels (0.01% vs. 0.1%); lower fat content (0.01% vs. 0.1%).

Coconut water is also a rich source of cytokinins, which are a class of plant growth substances (plant hormones) active in promoting cell division. They are also involved in cell growth and differentiation and in other physiological processes. A major cytokinin found in coconut water is responsible for at least 20% of total cytokinin activity determined in coconut water for these compounds.

Water from young coconuts utilized in cosmetic applications is in the form of a freeze-dried preparation that preserves solids with their optimum biological activity. In laboratory studies,

the material was found to have zero skin irritation potential and LD_{50} values greater than 2000 mg/kg when administered through the skin.

RNA-PHOSPHORUS AND DNA-PHOSPHORUS OF THE ALCOHOL-INSOLUBLE RESIDUE FROM COCONUT WATER

	RNA-P : DNA-P	RNA-P/DNA-P
Young green	20.05 : 0.06	342.5
Mature green	32.82 : 2.45	13.9

VITAMIN, GROWTH PROMOTERS, SUGAR ALCOHOLS AND MINERAL CONTENTS IN COCONUT WATER

COMPOUND	**Mg/L**
Nicotinic acid	0.64
Pantothenic acid	0.52
Biotin	0.02
Riboflavin	0.01
Folic acid	0.003
Thiamine	Trace
Pyridoxine	Trace
Auxin	0.07
Gibberellin	Not determined
1,3-Diphenylurea	5.8
Sorbitol	15.0
M-inositol	0.01
Scyllo-inositol	0.05
	Mg/100g
Potassium	312.0
Chloride	183.0
Sodium	105.0
Phosphorus	37.0
Magnesium	30.0
Sulfur	24.0
Iron	0.10
Copper	0.04

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Skin absorption of nutrients and skin health

An emerging aspect of effective use of skin-applied nutritionals is absorption (permeation of epidermal and dermal barrier by the compound) and ultimately bioavailability of the compound to the target tissues and/or receptors. Cosmeceuticals or natural cosmetic ingredients i.e. vitamins, minerals and botanicals, some discussed above, may have different rates of absorption through the skin and bioavailability, depending on their chemical and biochemical forms. For example, free isoflavones like genistein are considered more absorbable/bioavailable than their glycosylated or bound form, genistin; organic forms of minerals, such as selenium in complex with the amino acid methionine – selenomethionine, is considered better absorbed/bioavailable than inorganic sodium selenite.

In discussing mechanisms of nutrient absorption and bioavailability a key distinction should be stressed between these two concepts. The term bioavailability should not be used interchangeably as a definition of absorption. Therefore, besides the tissue levels of a supplemented ingredient, e.g. dermal levels of genistein, the functional outcome of the supplementation should also be measured, e.g. impact of topical genistein on skin thickness in postmenopausal women. The former would measure absorption rate of genistein and the latter would measure its true bioavailability. The consensus is that both events, the measurable absorption rate and target tissue/receptor bioavailability of a nutrient ultimately contribute to the efficacy of supplemented nutrient.

As discussed above, regulation and enhancement of nutrient or drug delivery to targeted skin cells (bioavailability) has great importance in skin care and skin health. An emerging new topical bioavailability enhancer is Tetrahydropiperine (THP), a derivative of pungent alkaloid piperine from the fruits of black pepper (*Piper nigrum*, fam. Piperaceae) and long pepper (*Piper longum*, fam. Piperaceae). Alkaloid tetrahydropiperine (THP), a natural derivative of piperine, has a lower melting point (41-42C) than piperine (126-132C) and thus more amienable for use in cosmetic preparations.

Tetrahydropiperine, like its parent compound piperine, occurs naturally in black pepper (about 0.7% in black pepper oleoresin). The parent compound piperine was previously evaluated in oral administration for its potential to enhance gastrointestinal absorption of drugs and nutrients in animals and humans. Piperine enriched compounds successfully studied include drugs such as Vasicine, Pyrazinamide, Rifampicin, Isoniazid, Propranolol, Theophylline and Phenytoin, and nutrients such as fat soluble beta carotene, water soluble vitamin B6, vitamin C, Coenzyme Q10 and the mineral selenium in the form of L-selenomethionine.

A study was conducted to determine whether concentrations of THP of 0.01% and 0.1% would cause topical irritation. A patch test, using THP in a petrolatum vehicle was conducted on 50 healthy volunteers for a 48 hr reading of the results. The study supervising physician, a dermatologist, determined that there was no skin irritation with both concentrations of THP tested. This study was conducted by the US FDA accredited BioScreen Testing Inc. laboratory.

The bioenhancing potential of THP was evaluated with a topical antioxidant compound tetrahydrocurcuminoids (THC) and in experiments with the steroidal anti-inflammatory drug Betamethasone dipropionate or BMDP, and anthelmintic drugs, i.e. Fenvalerate and Albendazole.

In an *in vitro* radical scavenging test, the ability of THC to bind and inactivate the 1,1 diphenyl-2-picrylhydrazyl radical (DPPH) was measured. DPPH is considered to be an example of a very stable free radical. The control sample contained 0.01% of THC and the active samples contained 0.01% of THC with varying concentrations between 0.1% - 0.0001% of THP. Additionally, the control containing various concentrations of THP was also tested for DPPH binding.

While THP by itself did not show any significant antioxidant properties, it was shown to enhance the antioxidant properties of THC by up to 30% as compared with THC used alone. Even in its highest diluted form, 0.0001%, THP was still displaying some beneficial THC bioenhancing activity.

In experiment with the steroidal compound, the skin preparation was mounted in a Franz Diffusion Cell, which uses two compartments, the “donor” and “receptor”. The drug (100 ug/ml) was applied through the donor compartment using 0.1% THP (active sample) and no THP (control sample). Subsequently, measuring for the presence of BMDP administered with and without THP in the absorbed fluid located in the receptor compartment was done using time intervals of 5, 10, 15, 20, 30, 45 and 60 minutes. The active sample had 100 % diffusion of BMDP within the first 10 minutes. The control sample had 29% diffusion after 45 minutes and only 54% diffusion after 60 minutes.

Experiments to see how THP enhanced the permeability of anthelmintic drugs were also conducted. THP in concentrations of 0.1% to 0.5% was shown to enhance penetration of Fenvalerate (synthetic pyrethroid) through cutworms and Albendazole through earthworms.

In summary, based on the initial research using *in vitro* and *in vivo* experimental models, THP was shown to enhance the skin's natural abilities to absorb nutrients and drugs. In addition, based on 48 hour human occlusive patch testing, THP does not act as a skin irritant at a dose range considered effective in augmenting skin nutrient and drug delivery properties.

Based on preliminary experimental data THP may operate by increasing one or both of the two events: 1) membrane fluidity, and/or 2) affinity of nutrient/drug to the cell membrane. In addition, it is possible that THP, which is a lipophylic compound, may increase solubilization of the intracellular lipid moiety in the skin, thereby enhancing its permeability for the applied nutrient/drug.

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