1. Product card	BeautyCare Drink
Double-action skin, hair and nails beauty supplementation drink	
Short description: Patented fish collagen peptides supplementation with added hyaluronic acid, MSM, vitamin C, biotin and zinc to stimulate natural collagen production	
Ingredients: 1. Collagen Naticol® - high-quality fish collagen peptides (vegetarian-appropriate) as compounds for natural collagen production. 2. Hyaluronic acid of bacterial origin which is vegan-appropriate (with certificate). 3. MSM - methylsulfonylmethane 4. Vitamin C. 5. Vitamin B7 – biotin. 6. Zinc.	
Problem statement: Skin ages in both men and women due to internal and external processes, which simultaneously contribute to a progressive loss of skin integrity.[1] Oxidative stress resulting from the pressure of external and internal stress agents can result in microinflammation and consequently disorganisation and destruction of collagen structure.[2] Production of collagen drops very quickly with excess sun exposure, smoking[3], excess alcohol, and lack of sleep and exercise. Some collagen products on the market use pure collagen which the body cannot absorb due to its molecular length and weight. Some add peptides. Bovine and porcine derived collagens are not suitable for vegetarians and present risks of transmitting diseases, such as BSE (mad cow disease). Those that use fish collagen peptides often mix them in shots which reduces their stability and bioavailability and also increase the risk of contamination and degradation due to oxidation and humidity. Most products do not add other ingredients to stimulate natural collagen production and integration in the skin structure. Users of collagen products typically complain of extremely bad taste of collagen supplements and of high content of sugar that is added to ameliorate the taste.	
Intended use: Supplementation of collagen compounds (peptides) and ingredients to stimulate natural collagen production (hyaluronic acid, MSM, vitamins C and B7 and zinc).	

[1] Farage, M. A., Miller, K. W., Elsner, P., Maibach, H. I., Characteristics of the Aging Skin, Advanced Wound Care, Vol. 2, Issue 1 (2013), pp. 5-10.

[2] Weishardt, New clinical study about benefits of 2.5g Naticol® for skin beauty, Innovations in Food Technology, August 2017, pp. 72-73 (72).

[3] Shuster, S., Smoking and wrinkling of the skin, Lancet, Vol. 358, Issue 9278 (2001), p. 330.

Benefits:

1. High-quality fish collagen peptides as compounds and nutrients for natural collagen production.
2. Hyaluronic acid to increase water-content of skin and facilitate collagen integration.
3. MSM to support natural collagen production and otherwise increase skin firmness.
4. Vitamin C to increase collagen formation and skin firmness and reduce oxidative stress.
5. Biotin (vitamin B_7) for maintaining healthy skin and hair.
6. Zinc as anti-inflammatory agent for healthy hair, nails & skin and reduction of blemishes.
7. Good taste without added sugar.

Main target population:

1. Adult women and men wishing to have healthy skin.
    a. Treating UV radiation (sunlight) skin deterioration.
    b. Treating age-related skin damage.
    c. Faster skin wound healing.
    d. Treating smoking-related skin deterioration.
    e. Making **skin look** younger, healthier, and shinier.
2. Adult women and men experiencing problems with receding hair or hair falling out.
    a. **Alopecia**
    b. Telogen **effluvium**
3. Adult women and men with **brittle nails**.

1. Health claims allowed by EFSA[4]

VITAMIN C:

- Vitamin C contributes to normal collagen formation for the normal function of skin.
- Vitamin C contributes to the protection of cells from oxidative stress.
- Vitamin C contributes to normal collagen formation for the normal function of bones and cartilage.

ZINC:

- Zinc contributes to normal protein synthesis.
- Zinc contributes to the maintenance of normal skin, hair and nails.
- Zinc contributes to the maintenance of normal bones.

BIOTIN:

- Biotin contributes to the maintenance of normal skin and hair.

[4] EU, Register of nutrition and health claims made on foods, V.3.6, available at: https://ec.europa.eu/food/safety/labelling_nutrition/claims/register/public (last consultation November 15, 2022).

2. Explanation of ingredients and their benefits

Collagen Naticol®

1. High-quality collagen peptides to provide nutrients for natural collagen production.

Highest-quality production standards

Collagen Naticol® is **produced by Weishardt**, the pioneer and leader in collagen production with over 150 years of tradition and experience in the field.
The raw collagen material and its amino acid composition, the enzyme (protease) mixture, and are carefully selected and the extent of hydrolysis is specially controlled to generate peptides with targeted biological activities.
Herba Medica, d.o.o. is a proud bearer of BIO/ECO, IFS, ISO 9001, SIQ, and GMP certificates which ensure highest quality standards throughout the production process.

Pure hydrolised fish collagen peptides

Collagen is a high-molecular weight protein and is not digested as such.
Collagen Naticol® are **Type I collagen peptides**, smaller molecular weight proteins, obtained through hydrolysis.[5]

Standardised to contain over 16% of essential amino acids and 50% of glycine, proline and hydroxyproline, the main peptides used by the body for natural collagen formation. This makes it **the best composition collagen on the market** specifically intended for skin health.

Type I collagen

Collagen Type I accounts for over 90% of all collagen in the human body[6] and of all 28 different collagen types, **Type I is the most abundant one in the skin**.[7]

Fish origin – better absorption & vegetarian-appropriate

Fish collagen peptides have the **highest rate of absorption** and present **no risk of transmitting diseases**, which is an issue with bovine or porcine collagens.[8]
The only type of collagen available for pescatorian (fish-eating) vegetarians.

Excellent absorption & bioavailability & fat-, gluten-, cholesterol-free

Naticol is also highly absorbed due to its low molecular weight.
It is free from fat, cholesterol, gluten and E-number components (ideal for clean label products such as BeautyCare 531).

In the form of drink powder

Hydrolisates are a product in the form of powders. Many collagen supplements are in the form of collagen shots or pre-prepared drinks (i.e. they mix the powder with liquids during the production process, which is an unnecessary and dangerous additional step). This increases the risk of contamination and degradation of peptides in the humid environment, particularly if the packaging is intended for multiple shots where the bottle is opened and closed many times.
Some supplements are in the form of powder where the user determines the quantity of each individual intake with a scoop. Again, this presents high risk of contamination and degradation of peptides.

Clinical studies confirmed effectiveness

[5] León-López, A., Morales-Peñaloza, A., Martínez-Juárez, V. M., Vargas-Torres, A., Zeugolis, D. I., Aguirre-Álvarez, G., Hydrolyzed Collagen-Sources and Applications, Molecules, Vol. 24, Issue 22 (2019), art. 4031.

Several **placebo-controlled, randomised, double-blind clinical studies proved Naticol®'s effectiveness** for skin firmness, reduction of wrinkles, elasticity and skin compelxion homogeneity.
Limited but growing literature also found evidence of collagen improving health of brittle nails and promoting hair growth.

2. Hyaluronic acid to increase water-content of skin and facilitate collagen integration

Hyaluronic acid is a natural glycosamynoglycan, i.e. a high-molecular-weight polysaccharide, which is **widely distributed in the connective tissue extracellular matrix**.
Binds 1000 times its weight in water.[9]

Plays **multifaceted role** in regulating the various biological processes such as skin repairment, diagnosis of cancer, wound healing, tissue regeneration, anti-inflammatory, and immunomodulation processes and has become an imperative nutrient for skin moisture in cosmetic and nutraceutical preparations.[10]

Placebo-controlled, randomised, double-blind studies confirmed its effectiveness in terms of wrinkle assessment, stratum corneum water content, transepidermal water loss, and elasticity.

3. MSM – methylsulfonylmethane to support natural collagen production and otherwise increase skin firmness

MSM is **a natural source of organic sulphur**, which has clinically proved antifungal, antibacterial, and keratolytic (removing of excess skin) activities.[11]
Well-investigated in animal and human clinical trials for its anti-inflammatory effects, positive effects on joint/muscle pain, oxidative stress and anti-oxidant capacity.[12]
Double-blind, placebo-controlled clinical study confirmed its effectiveness in terms of wrinkle reduction, skin texture and firmness.

4. Vitamin C to increase collagen formation and skin firmness and reduce oxidative stress

Normal skin contains high concentrations of vitamin C, which supports important and well-known skin functions, stimulating collagen synthesis and assisting in antioxidant protection against UV-induced photodamage.[13]

5. Biotin (Vitamin B_7) for maintaining healthy skin and hair

[6] Cleveland Clinic, Collagen, May 2022, available at: https://my.clevelandclinic.org/health/articles/23089-collagen (last consultation October 2022).
[7] León-López, A., Morales-Peñaloza, A., Martínez-Juárez, V. M., Vargas-Torres, A., Zeugolis, D. I., Aguirre-Álvarez, G., Hydrolyzed Collagen-Sources and Applications, Molecules, Vol. 24, Issue 22 (2019), art. 4031.
[8] Jafari, H., Lista, A., Mafosso Siekapen, M., Ghaffari-Bohlouli, P., Nie, L., Alimoradi, H., Shavandi, A., Fish Collagen: Extraction, Characterization, and Applications for Biomaterials Engineering, Polymers, Vol. 12, Issue 10, art. 2230.
[9] Walker K., Basehore B.M., Goyal A., Zito P.M., Hyaluronic Acid, StatPearls Publishing, Treasure Island, 2022.
[10] Nasir Abbas Bukhari, S., Liyana Roswandi, N., Waqas, M., Habib, H., Hussain, F., Khan, S., Sohail, M., Amlizan Ramli, N., Ei Thu, H., Hussain, Z., Hyaluronic acid, a promising skin rejuvenating biomedicine: A review of recent updates and pre-clinical and clinical investigations on cosmetic and nutricosmetic effects, International Journal of Biological Macromolecules, December 2018, pp. 1682-1695.
[11] Gupta, A. K., Nicol, K., The use of sulfur in dermatology, Journal of Drugs in Dermatology Vol. 3, Issue 4 (2004), pp. 427-31.
[12] Butawan, M., Benjamin, R. L., Bloomer, R. J., Methylsulfonylmethane: Applications and Safety of a Novel Dietary Supplement, Nutrients, Vol. 9, Issue 3 (March 2017), art. 290.
[13] Pullar, J. M., Carr, A. C., Vissers, M. C. M., The Roles of Vitamin C in Skin Health. Nutrients, Vol. 9, Issue 8 (2017), art. 866.

A water-soluble vitamin, which has received publicity for promoting the growth of hair and nails and maintaining healthy skin and has traditionally been used for treating dermatological issues.[14]

6. Zinc as anti-inflammatory agent for healthy hair, nails & skin and reduction of blemishes

Zinc has been used as **a therapeutic modality for centuries** for a number of dermatological conditions including infections (leishmaniasis, warts), inflammatory dermatoses (acne vulgaris, rosacea), pigmentary disorders (melasma), and neoplasias (basal cell carcinoma).[15] Disturbances in zinc metabolism may give rise to disorders that typically manifest themselves on the skin. Zinc supplementation is intended for its anti-inflammatory effects for healthy skin and reduction of blemishes.

Zinc deficiency is a well-documented cause for hair loss.[16] Zinc and other trace elements such as copper and selenium are required for the synthesis of thyroid hormones. Deficiency of these can result in hypothyroidism, a common and well recognized cause of diffuse hair loss.[17]

Zinc **participates in the regulation of cell proliferation** in several ways; it is essential to enzyme systems that influence cell division and proliferation[18] thus contributing to hair and nail growth.

7. Good taste without added sugar

BeautyCare Drink 531 specific combination of ingredients and a minimum amount of orange aroma results in a taste that is acceptable and considered better than any other product on the market.

Stress agents for skin, hair, and nails

UV light
UV makes collagen less soluble and causes wrinkling.[19]

Smoking
Smoking and UV light seem to induce matrix metalloproteinase MMP-1 which degrades collagen structure.[20] It affects collagen structure and/or elastin structure.[21]

Aging
With aging, oxidative stress increases.
With aging, fibroblast lose the ability to replicate. The skin is not capable of efficiently repairing damaged collagen, with a consequent loss of a functional support matrix related to the visible signs of ageing.[22]

---

[14] Bistas, K. G., Tadi, P., Biotin, StatPearls Publishing, Treasure Island, 2022.
[15] Gupta, M., Mahajan ,V. K., Mehta, K. S., Chauhan, P.S., Zinc therapy in dermatology: a review, Dermatology Research and Practice, 2014, art. 709152.
[16] Rajput, J. R., Role of Non Androgenic Factors in Hair loss and Hair Regrowth, Journal of Cosmetology and Trichology, Vol 3, Issue 1 (2017), art. 118.
[17] Betsy, A., Binitha, M., Sarita, S., Zinc deficiency associated with hypothyroidism: an overlooked cause of severe alopecia, International Journal of Trichology, Vol 5 Issue 1 (2013), pp. 40-42.
[18] MacDonald, R. S., The role of zinc in growth and cell proliferation, The Journal of Nutrition, Vol. 130 (2000).
[19] Shuster, S., Bottoms, E., Effect of ultra-violet radiation on skin collagen of intact living mice, Nature, Vol. 214, No. 5088 (1967), pp. 599-600.
[20] Lahmann, C., Bergemann, J., Harrison, G., Young, A. R., Matrix metalloproteinase-1 and skin ageing in smokers, Lancet, Vol. 357, Issue 9260 (2001), pp. 935-936.
[21] Shuster, S., Smoking and wrinkling of the skin, Lancet, Vol. 358, Issue 9278 (2002), p. 330.
[22] Reilly, D. M., Lozano, J., Skin collagen through the lifestages: importance for skin health and beauty, Plastic and Aesthetic Research, Vol. 8 (2021), art. 2.

Hormones

Menopausal hormonal changes

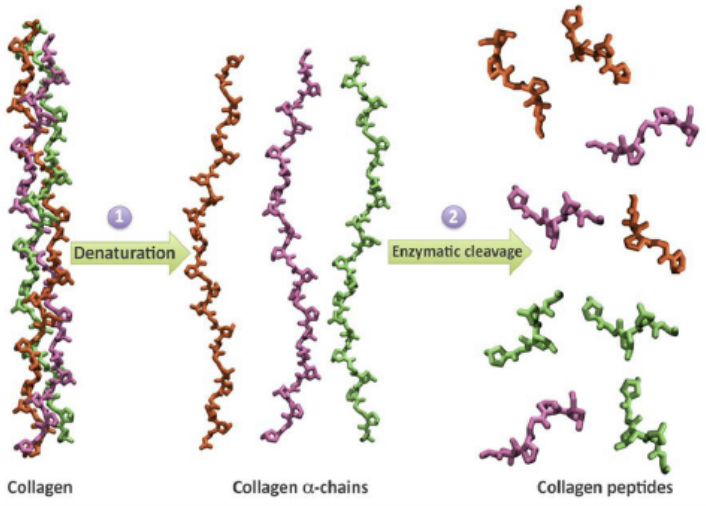
Collagen atrophy is the main factor associated with menopause-related skin aging, which contributes to reduced skin elasticity. In menopause, skin thickness and collagen content are initially reduced by 1.13% and 2.1% per year respectively.
Estrogen exerts a number of functions on connective tissue such as counteracting the degradation of collagen by matrix metalloproteinases (MMPs) induced by UV light and oxidative stress through the activation of the TGF-β1 pathway.
Post-menopausal women with less estrogen, show a decreased expression of TGF-β1 in skin fibroblasts, which may hamper the ability of fibroblasts to produce collagen, elastin and proteoglycans.[23]

Influence of testosterone

Testosterone inhibits collagen production by fibroblasts.[24]
Testosterone also inhibits hair growth.[25]

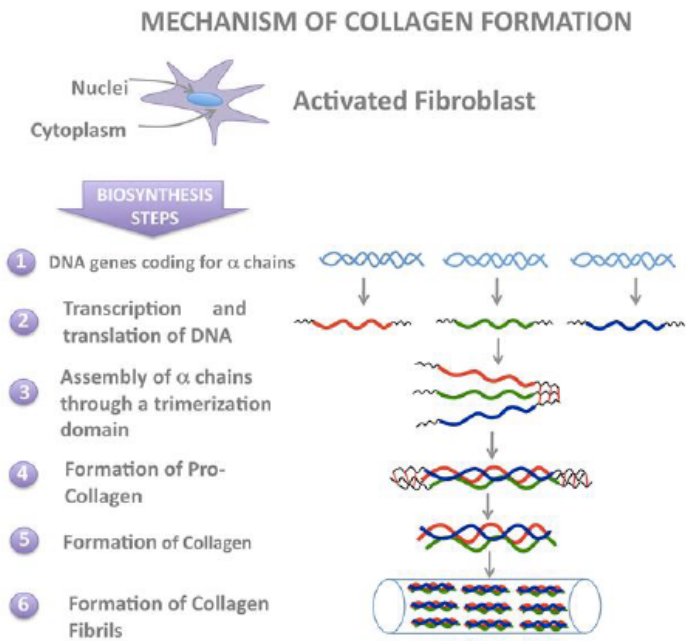
[23] Sparavigna, A., Role of the extracellular matrix in skin aging and dedicated treatment - State of the art, Plastic and Aesthetic Research, Vol. 7 (2020), art. 14.
[24] Yang, X., Wang, Y., Yan, S., Sun, L., Yang, G., Li, Y., Yu, C., Effect of testosterone on the proliferation and collagen synthesis of cardiac fibroblasts induced by angiotensin II in neonatal rat, Bioengineered, Vol. 8, Issue 1 (2017), pp. 14-20.
[25] Choi, M. H., Yoo, Y. S., Chung, B. C., Biochemical Roles of Testosterone and Epitestosterone to 5α-Reductase as Indicators of Male-Pattern Baldness, Journal of Investigative Dermatology, Vol. 116, Issue 1 (2001), pp. 57-61.

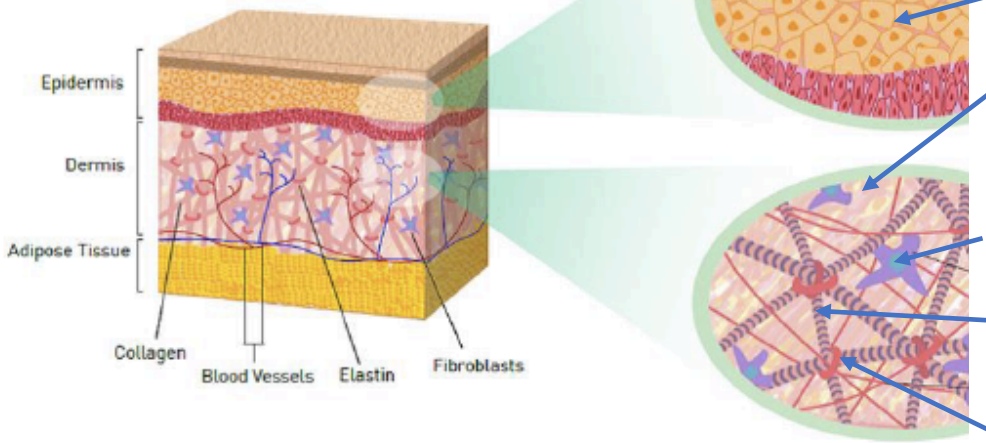
1. Graphic representation of skin structure and processes



Hydrolysis:
Industrial reduction of long collagen molecules into peptides
necessary for
natural collagen production.

Colagen synthesis by fibroblasts:
Recomposition of peptides and amino acids into collagen fibrils that compose the extracellular matrix





Keratin in stratum corneum protecting the skin

Keratinocytes producing keratin

Hyaluronic acid binding water

Fibroblasts producing collagen, elastin, keratin, and hyaluronic acid

Collagen fibers

Elastin binding collagen fibers

Fibroblast cells and the extracellular matrix (ECM)

Description of skin renewal process

- Fish collagen peptides pass through the stomach acid and are absorbed into the blood stream **in the small intestine**.
- Absorption occurs in the form of small collagen peptides and free amino acids. Naticol is standardized to contain 16% of amino acids and 50% of collagen peptides (glyceril, proline, hydroxyproline).
- Transported through blood plasma intact to the dermis, composed of:
    - Fibroblasts: cells that synthesise collagen, elastin, hyaluronic acid, and chondroitin.
    - Extra-Cellular Matrix (ECM) composed of:
        - Water
        - Proteins
            - Lifecycle:
                - Produced by fibroblasts
                - Decomposition through enzymes, such as collagenases.
            - Types:
                - 70-90% of collagen Type I
                - 10-15% of collagen Type II
                - With age, Type III content increases, Type I production is impaired.[26]
            - Functions:
                - providing the structure, elasticity, and firmness of the skin, produced by fibroblasts
                - Content of ECM
                - Structural component providing firmness (with HA)
            - Elastin
                - produced by fibroblasts from amino acids (
                - 2% of the skin content
                - provides structural stretch, recoil, and elasticity.[27]
        - Polysaccharides, such as hyaluronic acid:
            - Binds water 1000x its weight in the ECM.

[26] Lovell, C. R., Smolenski, K.A., Duance, V. C., Light, N. D., Young, S., Dyson, M., Type I and III collagen content and fibre distribution in normal human skin during ageing, The British Journal of Dermatology, Vol. 117, Issue 4 (1987), pp. 419-428.
[27] Baumann, L., Bernstein, E. F., Weiss, A. S., Bates, D., Humphrey, S., Silberberg, M., Daniels, R., Clinical Relevance of Elastin in the Structure and Function of Skin, Aesthetic Surgery Journal Open Forum, Vol. 3, Issue 3 (2021), art. ojab019.

- Offers molecular signalling pathways.
- Sustains stem cell state and cell migration pathways in case of wound healing.[28]

Description of effects on skin health of ingredients in BeautyCare Drink 531

ECM is a highly dynamic structure, constantly decomposed and remodeled, enzymatically (e.g. by collagenases) or non-enzymatically:

- Supplemented hydrolysed collagen has a **double-action mechanism in the dermis**:
  - free amino acids provide building blocks for the formation of collagen and elastin fibres;
  - collagen oligopeptides act as ligands, binding to receptors present on the fibroblasts' membrane and stimulate the production of new collagen, elastin and hyaluronic acid
- Hyaluronic acid **participates in cell mechanisms** such as differentiation, proliferation, development, and recognition on a cellular/molecular level, **and in certain physiological functions** such as lubrication, hydration balance, matrix structure, and steric interactions.[29]
  - HA oligosaccharides transported to the dermis are then **used by hyaluronan synthases** (HAS1, HAS2, HAS3) **on the membrane of fibroblasts to produce appropriate HA** with the molecular weight necessary for the tissue at hand (namely the skin).[30]
  - ABC transporter extrudes HA through the cell membrane into the extracellular matrix that composes the skin and gives it its firmness and elasticity.[31]
  - Inhibits matrix metalloproteinase 1 (MMP-1) that degrades collagen structure.[32]
- Zinc modulates the remaking of the extracellular matrix (ECM):
  - Participates in the regulation of cell proliferation in several ways, either as apoptosis inducer or reducer, depending on the cells.[33]
  - Essential for enzyme action.[34]
- MSM affects protein synthesis and oxidative stress
  - provides sulphur for collagen and keratin (hair and nail) synthesis. [35]

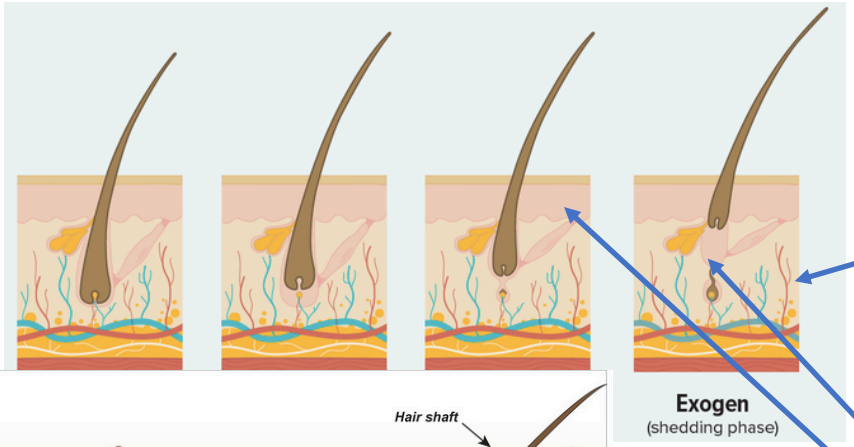
---

[28] Stern, R., Maibach, H. I., Hyaluronan in skin: aspects of aging and its pharmacologic modulation, Clinics in Dermatology, Vol. 26, Issue 2 (2008), pp. 106-122.
[29] George, E., Intra-articular hyaluronan treatment for osteoarthritis, Annal Rheum Dis. (1998) 57:637–40.
[30] Schulz, T., Schumacher, U., Prehm, P., Hyaluronan export by the ABC transporter MRP5 and its modulation by intracellular cGMP, Journal of Biological Chemistry, Vol. 282, Issue 29 (2007), pp. 20999-21004.
[31] Ibid.
[32] Shimizu, M., Yasuda, T., Nakagawa, T., Yamashita, E., Julovi, A. M., Hiramitsu, T., et al., Hyaluronan inhibits matrix metalloproteinase-1 production by rheumatoid synovial fibroblasts stimulated by proinflammatory cytokines, Journal of Rheumatology, Vol. 30, Issue 6 (2003), pp. 1164-1172.
[33] Franklin, R. B., Costello, L. C., The important role of the apoptotic effects of zinc in the development of cancers, Journal of Cell Biochemistry, Vol. 106, Issue 5 (2009), pp. 750-757.
[34] Beyersmann, D., Haase, H., Functions of zinc in signaling, proliferation and differentiation of mammalian cells, Biometals, Vol. 14, Issue 3-4 (2001), pp. 331-41.
[35] Langford, R., Hurrion, E., Dawson, P. A., Genetics and pathophysiology of mammalian sulfate biology, Journal of Genetic Genomics, Vol. 44 (2017), pp. 7-20.

    - MSM acts as antioxidant, inhibiting the degradation of collagen and extracellular matrix.[36]
- Vitamin C:
    - reduces oxidative stress that promotes glycation and lipid oxidation activities in the skin. In the process of these activities, oxidative stress is generated and some compounds in the process bind to proteins, thereby inhibiting collagen production and renewal.[37]
    - Activates the fibroblast activities, thereby increasing collagen production.[38]
- Biotin:
    - plays crucial roles in the metabolism of amino acids (structural components of collagen peptides)
    - plays a role in keratin production (in skin, hair, nails).[39]

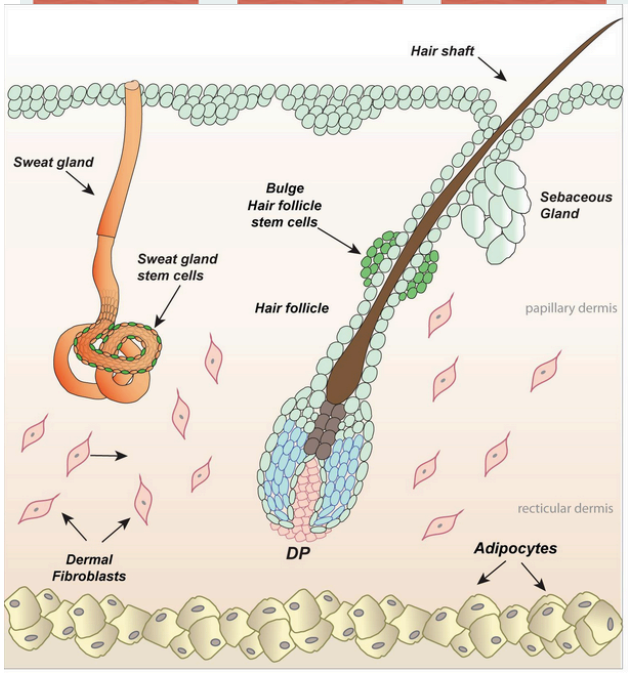
[36] Ibid.
[37] Reilly, D. M., Lozano, J., Skin collagen through the lifestages: importance for skin health and beauty, Plastic and Aesthetic Research,Vol. 8 (2021), art. 2.
[38] Munira, Sunardi Radiono Yohanes Widodo Wirohadidjojo, The effect of vitamin C on fibroblast proliferation and VEGFexpression in fibroblast culture, Journal of the Medical Sciences, Vol. 41, No. 3 (2009), art. 2964.
[39] Zempleni, J., Hassan, Y. I., Wijeratne, S. S., Biotin and biotinidase deficiency, Expert Review of Endocrinology and Metabolism, Vol. 3, Issue 6 (2008), pp. 715-724.

Hair growth initiation process and stages of hair growth

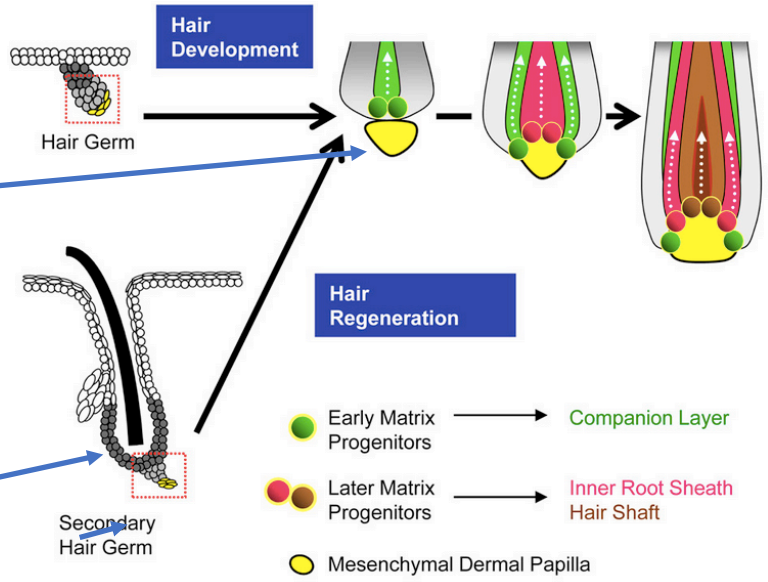


Mesenchymal cells of which Dermal papilla cells (yellow on the right) are found at the germ of the hair and fibroblasts producing collagen which composes the majority of the dermis layer (light pink on the left)

Epithelial cells (dark pink on the left) are keratinocytes (cells that produce keratin) can be found in the interfollicular part of the hair (white and grey on the right)



Hair follicle



Hair follicle

Sources[40]

[40] Right picture: Kiani, M. T., Higgins, C. A., Almquist, B. D., The Hair Follicle: An Underutilized Source of Cells and Materials for Regenerative Medicine, ACS Biomaterials Science and Enginering, Vol. 4, Issue 4 (2018), pp. 1193-1207.
Left picture: Healthline, What Are the Four Stages of Hair Growth?, Healthline online, available at: https://www.healthline.com/health/stages-of-hair-growth (last consultation October 2022).

Detailed description of hair growth stages

The hair follicle is composed of:

- epidermal (epithelial) cells surrounding the hair follicle that are keratinocytes (producing keratin for outermost layer of skin and hair), and
- dermal (mesenchymal) cells, a special type of which are dermal papilla cells (DPCs).[41]

Their interaction regulates the hair cycle stages.[42]

Hair cycle stages and the role of signalling molecules[43]

1. During the **anagen** stage, the hair follicle actively produces a keratin hair fiber (from 2 to 6 years).
    a. Dermal cells send the first signal to epidermal cells (expression of Wnts[44] and activation of β-catenin[45]).
    b. Epidermal stem cells in the bulge of hair follicle respond by starting cell differentiation, specifically the hair follicle placode growth and ingrowth of the hair into dermis (high Wnts, stabilised β-catenin, BMP inhibition, Shh expression, positive influence of FGF-1[46] and FGF-2[47]).
    c. Growth of hair (Wnt-inhibited state, BMP signalling increase, high Shh, positive influence of IGF-1 and negative influence of TGF-ß2).[48]
    d. Any event or insult that causes abrupt cessation of mitotic activity leads to weakening of the partially keratinized, proximal portion of the hair shaft, resulting in narrowing and subsequent breakage within the hair canal and even complete failure of hair formation.[49]
2. A **catagen** cycle is characterized by the end of hair growth and destructive regression (2-4 weeks).

Bottom picture: An Intrinsic Oscillation of Gene Networks Inside Hair Follicle Stem Cells: An Additional Layer That Can Modulate Hair Stem Cell Activities, Frontiers in Cell and Developmental Biology, Vol. 8 (2020), art. 595178.

[41] Houschyar, K. S., Borrelli, M. R., Tapking, C., Popp, D., Puladi, B., Ooms, M., Chelliah, M. P., Rein, S., Pförringer, D., Thor, D., Reumuth, G., Wallner, C., Branski, L. K., Siemers, F., Grieb, G., Lehnhardt, M., Yazdi, A. S., Maan, Z. N., Duscher, D., Molecular Mechanisms of Hair Growth and Regeneration: Current Understanding and Novel Paradigms, Dermatology, Vol. 236 (2020), pp. 271-280.

[42] Cotsarelis, G., Epithelial stem cells: a folliculocentric view, Journal of Investigative Dermatology, Vol. 126 (2006), pp. 1459-1468.

[43] Millar, S. E., Molecular mechanisms regulating hair follicle development, Journal of Investigative Dermatology, Vol. 18, Issue 2 (2002), pp. 216-225.

[44] Rishikaysh, P., Dev, K., Diaz, D., Qureshi, W. M., Filip, S., Mokry, J., Signaling involved in hair follicle morphogenesis and development, International Journal of Molecular Science, Vol. 15, Issue 1 (2014), pp. 1647-1670.

[45] Närhi, K., Järvinen, E., Birchmeier, W., Taketo, M. M., Mikkola, M. L., Thesleff, I., Sustained epithelial beta-catenin activity induces precocious hair development but disrupts hair follicle down-growth and hair shaft formation, Development, Vol. 135, Number 6 (2008), pp. 1019-1028.

[46] Andl, T., Reddy, S.T., Gaddapara, T., Millar, S.E., WNT signals are required for the initiation of hair follicle development, Developmental Cell, Vol. 2 (2002), pp. 643-653.

[47] Osada, A., Iwabuchi, T., Kishimoto, J., Hamazaki, T. S., Okochi, H., Long-term culture of mouse vibrissal dermal papilla cells and de novo hair follicle induction, Tissue Engineering, Vol. 13, Issue 5 (2007), pp. 975-982.

[48] Although this signaling is two-way, very complicated, and not entirely understood Yang, C. C., Cotsarelis, G., Review of hair follicle dermal cells, Journal of Dermatological Science, Vol. 57, Issue 1 (2010), pp. 2-11.

[49] Kanwar, A. J., Narang, T., Anagen effluvium, Indian Journal of Dermatology, Venereology and Leprology, Vol. 79 (2013), pp. 604-612.

a. Apoptosis (programmed cell death) of the lower part of hair follicle[50], essential for the recession of epithelial cylinder (leaving place for a new hair)[51].
b. Increase in FGF5[52] and TGFβ1 (and several other molecules) start the transition to catagen.
c. DKK-1 can also start the transition by blocking Wnt signaling (which prevents β-catenin activation and instead activates the pro-apoptotic protein Bax, inducing apoptosis in outer root sheath keratinocytes).[53]
d. Low IGF-1.[54]
e. 5alpha-dihydrotestosterone (5a-DHT), testosterone compound, inhibits hair growth.

3. **Telogen** is a resting stage, wherein the club fiber is retained (3-4 months).
    a. 2 phases:
        i. Refractory phase: hair follicle resistance to growth stimuli, increased BMP signalling
        ii. Competent phase: follicle bulge stem cells are extremely sensitive to anagen-inducing factors, BMP signalling reduced, Wnt/β-catenin signaling increases.[55]
    b. Estrogen receptor is appreciably upregulated throughout telogen.[56]
    c. Wnt-inhibited state.[57]
    d. Hair falls out due to external influences such as washing, brushing, etc.
    e. At the end, a signal is again sent from DPCs to keratinocytes and a new anagen stage begins (higher Shh).[58]
4. During the **exogen** stage, the club fiber is eventually shed, a process occurring independently of the other stages.[59]

---

[50] Millar, S. E., Molecular mechanisms regulating hair follicle development, Journal of Investigative Dermatology, Vol. 18, Issue 2 (2002), pp. 216-225.
[51] Mesa, K. R., et al., Niche-induced Cell Death and Epithelial Phagocytosis Regulate Hair Follicle Stem Cell Pool, Nature, Vol. 522, Issue 7554 (2015), pp. 94-97.
[52] Ota, Y., Saitoh, Y., Suzuki, S., Ozawa, K., Kawano, M., Imamura, T., Fibroblast Growth Factor 5 Inhibits Hair Growth by Blocking Dermal Papilla Cell Activation, Biochemical and Biophysical Research Communications, Vol. 290, Issue 1 (2002), pp. 169-176.
[53] Kwack ,M. H., Kim, M. K., Kim, J. C., Sung, Y. K., Dickkopf 1 promotes regression of hair follicles, Journal of Investigative Dermatology, Vol. 132 (2012), pp. 1554-1560.
[54] Soma, T., Tsuji, Y., Hibino, T., Involvement of transforming growth factor-beta2 in catagen induction during the human hair cycle, Journal of Investigative Dermatology, Vol. 118 (2002), pp. 993-997.
[55] Castellana, D., Paus, R., Perez-Moreno, M., Macrophages contribute to the cyclic activation of adult hair follicle stem cells, PLoS Biology, Vol. 12, Issue 12 (2014), art. e1002002.
[56] Ohnemus, U., Uenalan, M., Conrad, F., Handjiski, B., Mecklenburg, L., Nakamura, M., et al., Hair cycle control by estrogens: catagen induction via estrogen receptor (ER)-alpha is checked by ER beta signaling, Endocrinology, Vol. 146, Number 3, (2005), pp. 1214-1225.
[57] DasGupta, R., Fuchs, E., Multiple roles for activated LEF/TCF transcription complexes during hair follicle development and differentiation, Development, Vol. 126 (1999), pp. 4557-4568.
[58] Oro, A. E., Higgins, K., Hair cycle regulation of Hedgehog signal reception, Developmental Biology, Vol. 255 (2003), pp. 238-248.
[59] Kiani, M. T., Higgins, C. A., Almquist, B. D., The Hair Follicle: An Underutilized Source of Cells and Materials for Regenerative Medicine, ACS Biomaterials Science and Enginering, Vol. 4, Issue 4 (2018), pp. 1193-1207.

Description of effects on hair and nail health of ingredients in BeautyCare Drink 531

- Supplemented hydrolysed collagen
    - Provides free amino acids to fibroblasts and keratinocytes for the formation of collagen and elastin fibres, and keratin in hair and nails;
    - Collagen oligopeptides act as ligands, binding to receptors present on the fibroblasts' membrane and stimulate the production of new collagen, elastin, hyaluronic acid, and keratin.
- Hyaluronic acid **participates in cell mechanisms** such as differentiation, proliferation, development, and recognition on a cellular/molecular level, **and in certain physiological functions** such as lubrication, hydration balance, matrix structure, and steric interactions.[60]
    - Provides nutrients for natural production of hyaluronic acid compounds by keartinocytes, cells that produce hair.[61]
    - Regulates expression of IGF-1, and TGF-β that control hair growth stages.[62]
- Zinc modulates the hair growth:
    - Participates in the regulation of cell proliferation in several ways, either as apoptosis inducer or reducer, depending on the cells.[63]
    - Essential for enzyme action.[64]
    - Both pathways affect hair growth stages.
- MSM affects protein synthesis and oxidative stress
    - provides sulphur for collagen and keratin (hair and nail) synthesis. [65]
    - MSM acts as antioxidant, inhibiting the degradation of collagen and keratin and extracellular matrix.[66]
- Vitamin C:
    - reduces oxidative stress that promotes glycation and lipid oxidation activities in the skin. In the process of these activities, oxidative stress is generated and some compounds in the process bind to proteins, thereby inhibiting keratin production and renewal.[67]
    - Activates the fibroblast and keratinocyte activities, thereby increasing collagen and keratin production.[68]

---

[60] George, E., Intra-articular hyaluronan treatment for osteoarthritis, Annals of Rheumatic Diseases, Vol. 57 (1998), pp. 637-640.

[61] Malaisse, J., Bourguignon, V., De Vuyst, E., Lambert de Rouvroit, C., Nikkels, A. F., Flamion, B., Poumay, Y., Hyaluronan Metabolism in Human Keratinocytes and Atopic Dermatitis Skin Is Driven by a Balance of Hyaluronan Synthases 1 and 3, Journal of Investigative Dermatology, Vol. 134, Issue 8 (2014), pp. 2174-2182,

[62] Hou, C., Miao, Y., Wang, J., Wang, X., Chen, C., Hu, Z., Collagenase IV plays an important role in regulating hair cycle by inducing VEGF, IGF-1, and TGF-β expression, Drug Design, Development, and Therapy, Vol. 9 (2011), pp. 5373-5383.

[63] Franklin, R. B., Costello, L. C., The important role of the apoptotic effects of zinc in the development of cancers, Journal of Cell Biochemistry, Vol. 106, Issue 5 (2009), pp. 750-757.

[64] Beyersmann, D., Haase, H., Functions of zinc in signaling, proliferation and differentiation of mammalian cells, Biometals, Vol. 14, Issue 3-4 (2001), pp. 331-41.

[65] Langford, R., Hurrion, E., Dawson, P. A., Genetics and pathophysiology of mammalian sulfate biology, Journal of Genetic Genomics, Vol. 44 (2017), pp. 7-20.

[66] Ibid.

[67] Reilly, D. M., Lozano, J., Skin collagen through the lifestages: importance for skin health and beauty, Plastic and Aesthetic Research,Vol. 8 (2021), art. 2.

[68] Munira, Sunardi Radiono Yohanes Widodo Wirohadidjojo, The effect of vitamin C on fibroblast proliferation and VEGFexpression in fibroblast culture, Journal of the Medical Sciences, Vol. 41, No. 3 (2009), art. 2964.

- Biotin:
    - plays crucial roles in the metabolism of amino acids (structural components of collagen peptides)
    - plays a role in keratin production (in skin, hair, nails).[69]

Explanation of terms:

Shh (sonic hedgehog): Regulates stem cell maintenance and, at higher signaling levels, cell proliferation in adult epithelia.[70]
Shh is required after hair placode initiation to promote the proliferation of follicular epithelial cells and drives anagen regeneration[71].
Controls the hair cycle initiation in both primary and secondary hair follicles.
Wnt (Wingless-related integration site): Regulates cell growth, motility, and differentiation.
IGF-1: Insulin-like Growth Factor 1
TGF-ß2: Transforming Growth Factor ß
FGF: Fibroblast Growth Factor
BMP: Bone Morphogenetic Protein

[69] Zempleni, J., Hassan, Y. I., Wijeratne, S. S., Biotin and biotinidase deficiency, Expert Review of Endocrinology and Metabolism, Vol. 3, Issue 6 (2008), pp. 715-724.
[70] Jiang, J., Hui, C.C., Hedgehog signaling in development and cancer, Developmental Cell, Vol. 15 (2008), pp. 801-812.
[71] Oro, A. E., Higgins, K., Hair cycle regulation of Hedgehog signal reception, Developmental Biology, Vol. 255 (2003), pp. 238-248.

2. Summaries of selected clinical studies

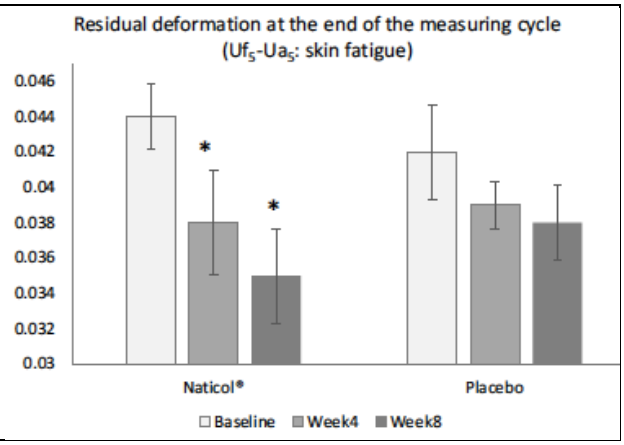
4.1 Collagen and skin

Weishardt Clinical study I

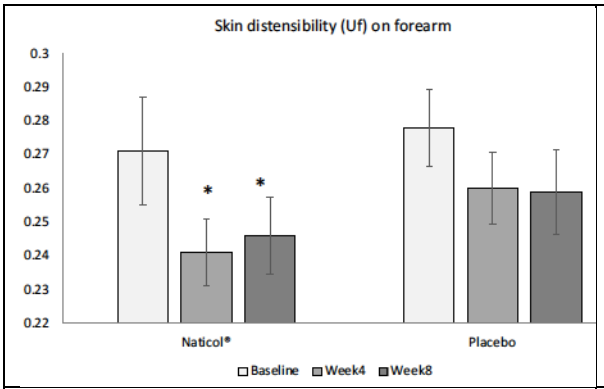
Type	In vivo
Daily intake	5g (the same dose as in BeautyCare 531)
Duration	8 weeks
Number of participants	60 women
Age	46 – 69 years
Methodology	**Double blind, randomised, against placebo**

Reference:
Duteil, L., Queille-Roussel, C., Maubert, Y., Esdaile, J., Bruno-Bonnet, C., Lacour, J.-P., Specific natural bioactive type 1 collagen peptides oral intake reverse skin aging signs in mature women, Journal of Aging Research and Lifestyle, 2016.[72]

Results
The study confirmed that a daily intake of 5 g of Naticol improves significantly skin firmness, skin elasticity and reduces wrinkles.

SKIN FIRMNESS Skin biomechanics indicated a significant improvement of skin firmness. Suction pressure is applied to the skin and the residual deformation, i.e. the deformation that stays after the test, is measured. The Naticol subjects saw a decrease in the residual deformation. In other words, their skin was more firm.	

[72] Summary in Bonnet, C., Beneficial effects of Naticol®, fish collagen peptides, on the skin ageing signs, Innovations in Food Technology, May 2017, pp. 00-00, available at: https://www.weishardt.com/wp-content/uploads/2018/02/6-Innovationsfood.com-Beneficial-effects-of-Naticol-on-the-skin-ageing-signs.pdf (last consultation October 2022).

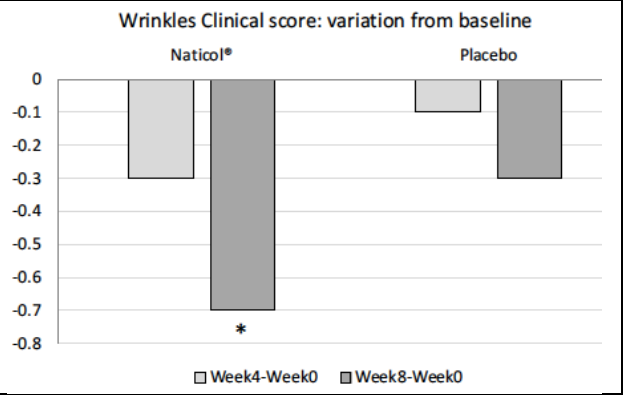


SKIN ELASTICITY

An increase of overall skin elasticity on the forearm and on the abdomen were observed.

WRINKLES

The results on biomechnaics of the skin were corroborated by the significant decrease of the crow's-feet wrinkle score that were evaluated using a Visual Analog Scale.



Weishardt Clinical study II

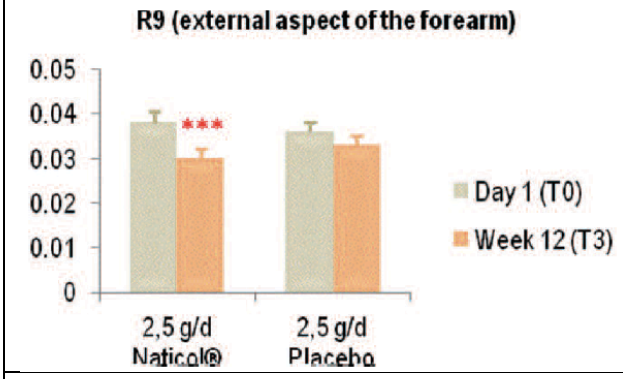
Type	In vivo
Daily intake	2,5g (half the dose in BeautyCare 531)
Duration	12 weeks
Number of participants	95 women
Age	Mean age 55,6 years
Methodology	**Double blind, randomised, against placebo**

Reference

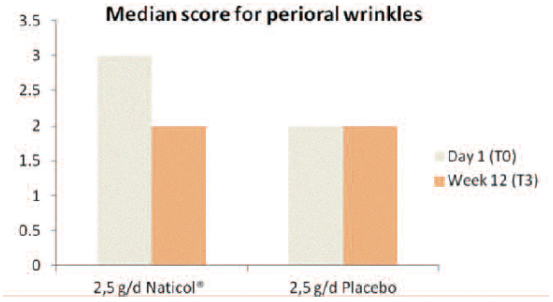
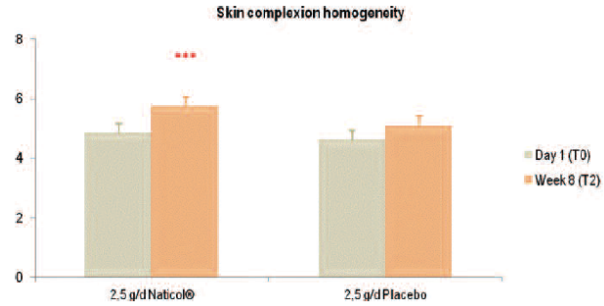
Weishardt, New clinical study about benefits of 2.5g Naticol® for skin beauty, Innovations in Food Technology, August 2017, pp. 72-73, available at: https://www.weishardt.com/wp-content/uploads/2018/02/8-Innovationsfood.com-New-clinical-study-about-benefits-of-25g-Naticol-for-skin-beauty.pdf (last consultation October, 2022).

Results

The study confirmed that a daily intake of 2,5g of Naticol reduces facial wrinkles, specifically Crow's feet and perioral wrinkles, improves significantly skin firmness, skin complexion homogeneity and skin radiance.

<table>
<tr>
<td> 
</td>
<td>SKIN FIRMNESS

Measurements of skin firmness after 8 and 12 weeks of supplementation showed a significant decrease of residual deformation on the forearm for subjects taking Naticol® compared to the Placebo group.</td>
</tr>
<tr>
<td>PERIORAL WRINKLES

Perioral wrinkles were evaluated using Bazin-Doublet’s atlas.[73] Median score for perioral wrinkles was reduced by a significant amount for the subjects taking Naticol while the placebo group showed no changes at all.</td>
<td> 
</td>
</tr>
<tr>
<td> 
</td>
<td>SKIN COMPLEXION HOMOGENEITY & SKIN RADIANCE

One of the symptoms of an aging skin are pigmented spots. Homogeneity and radiance of skin complexion were measured using a Visual Analogic Scale (VAS) with 0 signifiying a very heterogenous skin complexion and 10 a homogenous complexion. Subjects taking Naticol showed increased skin complexion homogeneity after 8 weeks, while placebo subjects did not show any significant change.</td>
</tr>
</table>

Weishardt Clinical study III

Type	In vivo
Daily intake	2,5g (half the dose in BeautyCare 531)
Duration	12 weeks
Number of participants	57 women
Age	NA (mature)
Methodology	**Double blind, randomised, against placebo**

Reference:

Duteil, L., Queille Roussel, C., Bruno-Bonnet, C., Lacour, J. P., Effect of Low Dose Type I Fish Collagen Peptides Combined or not with Silicon on Skin Aging Signs in Mature Women, Juniper Online Journal of Case Studies, Vol 6, Issue 4 (2018), art. 555692.

[73] Bazin, R., Doublet, E., Skin aging atlas, Vol. 1-5, Med’Com ed., Paris, 2007.

Results
The study confirmed that a daily intake of 2,5g of Naticol significantly improves skin firmness and skin elasticity.

Skin firmness	17% decrease in residual deformation of skin (=increase in skin firmness)
Skin elasticity	10% increase of the ratio of elastic recovery to the total skin deformation
	8% increase in the overall elasticity
Comparison between supplementation with added silicon and without it	No visible difference. Naticol in itself contributes to skin health without added silicon.

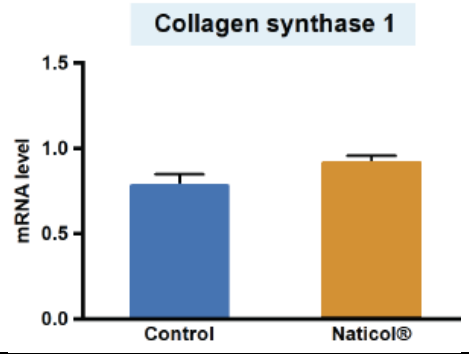
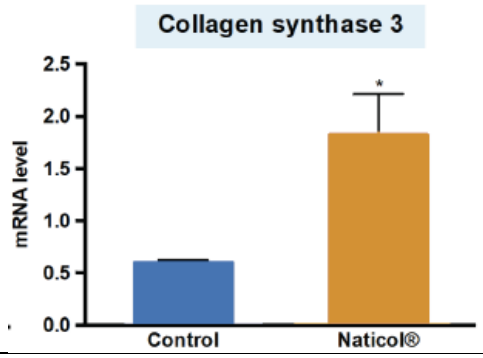
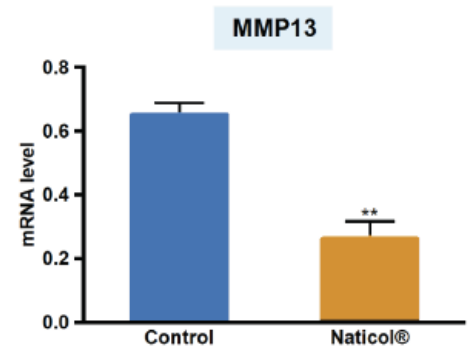
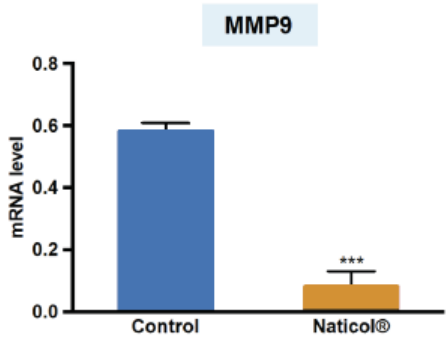
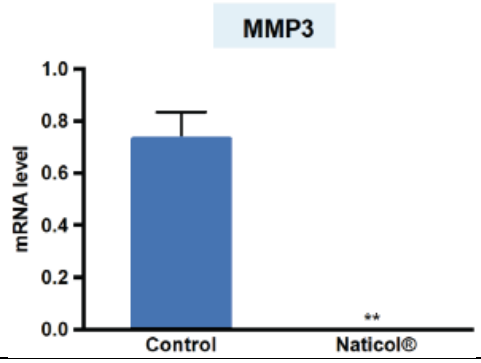
Weishardt Clinical study IV

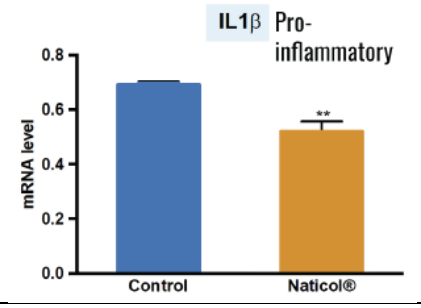
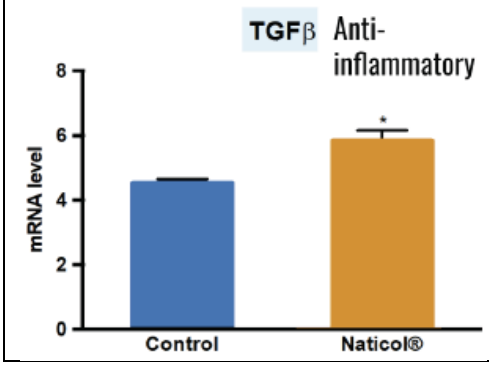
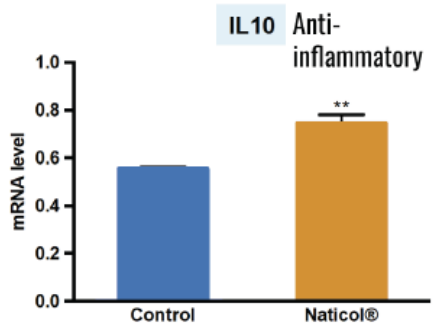
Type	In vitro
Methodology	1. Human monocyte-derived macrophages (h-MDMs; a type of white blood cells that detect and destroy bacteria and harmful organisms) and keratinocytes (HaCat; cells that produce keratine in the epidermis) were co-cultivated in transwell culture system.
	2. The cells were stimulated with LPS (100ng/ml; component of outer membrane of certain bacteria) to induce a pro-inflammatory phenotype.
	3. Cells were treated with Naticol® (1mg/ml).

Reference:
Bonnet, C., Anti-inflammatory activities of Naticol® demonstrate benefits in ageing, August 25, 2020, available at: https://innovationsfood.com/2010-2/ (last consultation October 2022). Brochure with graphs available at: https://www.weishardt.com/naticol-leaflets.

Results:
Study showed a significant increase of the collagen synthesis with Naticol®. It is due to an increase of the collagen synthase 3 and a decrease of MMP13. An increase in the level of expression of anti-inflammatory and proresolutive cytokines (TGF-β and IL-10; peptides responsible for signalling in the natural immune system) was also observed with Naticol®. In short, **Naticol stimulated formation of collagen and reduced inflammation in cells**.

<u>Increased collagen synthesis</u>

Naticol® increased collagen synthase 1 and collagen synthase 3 levels, i.e. levels of enzymes that participate in the collagen formation.	
	
<u>Reduced levels of enzymes destroying collagen structures in the skin</u>	
Naticol® decreased the levels of metalopeptidases 3, 9 and 13, enzymes that participate in the destruction of extracellular matrix containing collagen and participate in tissue remodeling.	
	
<u>Increased anti-inflammatory and decreased pro-inflammatory cytokines</u>	

Naticol® increased the levels of peptides called cytokines that have anti-inflammatory effects and decreased the levels of cytokines that have pro-inflammatory effect.	
	

3.1.2 Collagen and hair

While positive effects of fish collagen peptides on skin health are well documented, evidence of collagen's positive effects on hair health is still limited, albeit growing.[74]

Clinical study I

In vitro and in vivo (mice), fish collagen peptides significantly enhanced hair regrowth and the proliferation of human dermal papilla cells (hDPCs).

Type	In vitro & In vivo (mice)
A.1 Methodology (in vitro)	1. Human hair dermal papilla cells (majoritarily present in the hair follicle) were cultivated.
	2. The proliferation rate of hDPCs was evaluated at various concentrations of fish collagen peptides from 3,9 to 250 ppm (particles per million).
A.2 Results	**13% increase in hDPCs.** The cell proliferation rate of the group with collagen peptides at a concentration of 62.5 ppm exhibited the highest hDPCs proliferation rate with a 13% increase.

[74] Rustad, A. M., Nickles, M. A., McKenney, J. E., Bilimoria, S. N., Lio, P. A., Myths and media in oral collagen supplementation for the skin, nails, and hair: A review, Journal of Cosmetic Dermatology, Vol. 21, Issue 2 (2022), pp. 438-443.

B.1 Methodology (in vivo)	1. Mice were shaved and provided different concentrations of fish collagen peptides (500 and 1000 mg/kg).
	4. Visual and histological analyses were made.
B.2 Results	**1. Positive effect on Hair regrowth.** Supplemented mice showed visible signs of improvement in terms of hair regrowth, at the level of mice administered with finasteride, a medical drug used for hair regrowth.
	2. Positive effect on Hair Follicle Morphology. The number of hair follicles increased and the A/T ratio between anagen (A; large and thick) hair versus telogen (T; small and flat) hair significantly increased.

Reference:

Hwang, S. B., Park, H.J., Lee, B.-H., Hair-Growth-Promoting Effects of the Fish Collagen Peptide in Human Dermal Papilla Cells and C57BL/6 Mice Modulating Wnt/β-Catenin and BMP Signaling Pathways, International Journal of Molecular Sciences, Vol. 23, Issue 19 (2022), art. 11904.

Clinical study II

Type	In vitro & In vivo (mice)
A.1 Methodology (in vitro)	1. Human hair dermal papilla cells hDPCs (majoritarily present in the hair follicle) were cultivated.
	2. The proliferation rate of hDPCs was evaluated.
A.2 Results	Hair-papilla cells were found to produce considerable amounts of collagen type I and type III and fibronectin, showing correlation between hair growth and collagen.

Reference:

Katsuoka, K., Mauch, C., Schell, H., Hornstein, O. P., Krieg, T., Collagen-type synthesis in human-hair papilla cells in culture, Archives of Dermatology Research,Vol. 230, Issue 3 (1988), pp. 140-144.

Clinical study III

Type	In vivo
Daily intake	Fish collagen peptides; Vitamin C Zinc Horsetail extract (like in Beauty Care Hair & Nails Capsules 521) Flax seed extract

Duration	6 months
Number of participants	10 healthy men with diagnosed hair loss issues (alopecia)
Age	NA (adult)
Methodology	**Double blind, randomised, against placebo**
Results	**1. Increased hair count and density.** a. After 180 days, significant increases were observed for total hair count, total hair density, and terminal hair density. b. The investigator assessments revealed significant improvements in terminal and vellus hair count and terminal hair density.
	2. Positive hair pull test. Hair pull test results were significantly lower (fewer hairs removed) for study drug vs. placebo at Days 90 and 180.
	3. No adverse effects reported There were no reports of treatment-emergent adverse events.

Reference:

Ablon, G., A 6-month, randomized, double-blind, placebo-controlled study evaluating the ability of a marine complex supplement to promote hair growth in men with thinning hair, Journal of Cosmetic Dermatology, Vol. 15,Issue 4 (2016), pp. 358-366.

Clinical study IV

Type	In vivo
Daily intake	Fish collagen peptides Vitamin C Biotin Zinc Horsetail extract (like in Beauty Care Hair & Nails Capsules 521)
Duration	3 months
Number of participants	60 women of Caucasian, Hispanic and Asian origin
Age	Between 21–65 years; Mean age of 48,6 years
Methodology	**Double blind, randomised, against placebo**
Results	**1. Increase in mean number of hair.** A significant increase in the mean number of terminal hairs from 178.3 (7.8) at baseline to 235.8 (18.4)
	2. Increase in the number of terminal hair.

	The number of terminal hairs among supplemented subjects was also significantly greater than placebo-treated subjects.
	3. Visible signs of improvement. Changes in the clinical appearance of two treated subjects at baseline and after 90 days of treatment were apparent.

Reference:

Ablon, G., A 3-month, randomized, double-blind, placebo controlled study evaluating the ability of an extra-strength marine protein supplement to promote hair growth and decrease shedding in women with self-perceived thinning hair.
Dermatological Research and Practice, Vol. 2015, art. 841570.

Clinical study V

Type	In vivo
Daily intake	Fish collagen peptides Vitamin C Horsetail extract (like in Beauty Care Hair & Nails Capsules 521)
Duration	3 months
Number of participants	10 women of Caucasian and Hispanic origin
Age	Between 21-75 years; Mean age of 49,9 years
Methodology	**Double blind, randomised, against placebo**
Results	**1. Increase in mean number of hair.** The mean number of terminal hairs among supplemented subjects increased from 271,0 at baseline, increasing to 571 and 609.6 after 90 and 180 days. Placebo-treated subjects remained at the same levels.
	2. No increase in the mean number of vellus (short & thin) hair. The mean number of vellus hairs among supplement-treated subjects was 46,5 at baseline and 48,0 and 46,5 (14.4) after 90 and 180 days, respectively. The mean number of vellus hairs among placebo-treated subjects was 57,0 (32.1) at baseline and 68,0 (21.4) and 65,8 (16.6) after 90 and 180 days.

	3. Visible signs of improvement. Changes in the clinical appearance of two treated subjects at baseline and after 90 and 180 days of treatment were apparent.

Reference:
Ablon, G., A double-blind, placebo-controlled study evaluating the efficacy of an oral supplement in women with self-perceived thinning hair, Journal of Clinical Aesthetic Dermatology, Vol. 5 (2012), pp. 28-34.

1.1.2 Collagen and nails

As with hair, while positive effects of collagen on nail health are being widely promoted by sellers of nutraceutics, evidence is still limited but promising.[75]

Clinical study I

Type	In vivo
Daily intake	2,5g of porcine Type I collagen peptides (2kDa)
Duration	6 months followed by 4-week period without supplementation
Number of participants	25 healthy women with brittle nails
Age	Between 18-50 years
Methodology	**Double blind, randomised, against placebo**
Results	**1. Half less nail peeling.** After 12 weeks, the number of participants displaying “severe” or “moderate” nail peeling halved (16 to 8 out of 24), and those with the score “slight” doubled (8 to 16 out of 24). After 24 weeks, only 6 of 24 participants (25%) had the score “severe” or “moderate,” and 8% of the participants showed no symptoms. The positive results continued after the washout phase.
	2. Less longitudinal split of free edges of nails. The positive results on the longitudinal split of the free edge appeared after 24 weeks of treatment, where only 4% of participants scored “severe.” After the washout phase, none of the participants had

[75] Rustad, A. M., Nickles, M. A., McKenney, J. E., Bilimoria, S. N., Lio, P. A., Myths and media in oral collagen supplementation for the skin, nails, and hair: A review, Journal of Cosmetic Dermatology, Vol. 21, Issue 2 (2022), pp. 438-443.

	the score “severe,” and the percentage of “slight” scores increased from 17% to 38%.
	3. 54% increase in improvement of nail symptoms. Of all the 24 participants analyzed, 13 participants (54%) had fair improvement on nail symptoms at week 12 compared with baseline. At week 24, 64% achieved notably global improvement (excellent/good/fair). After the washout phase, 21 participants (88%) showed excellent/good/fair improvement.
	4. 42% reduction in frequency of broken nails. Before starting the treatment, the participants’ frequency of broken nails were on average 10 times/month. After 24 weeks, it significantly decreased to 6 times/month, a reduction of 42%. This improvement continued during the washout phase.
	5. 15% increase in nail growth. The basal nail growth rate of the participants was on average 2,65 mm/month. After 12 weeks of treatment, the growth rate improved significantly to 2,90 mm/month. These results showed an improvement in nail growth of 10% after 12 weeks of BCP intake. This increased to 12% after 24 weeks, and to 15% 4 weeks after the last intake.

Reference:
Hexsel, D., Zague, V., Schunck, M., Siega, C., Camozzato, F. O., Oesser, S., Oral supplementation with specific bioactive collagen peptides improves nail growth and reduces symptoms of brittle nails, Journal of Cosmetic Dermatology, Vol. 16 (2017), pp. 520-526.

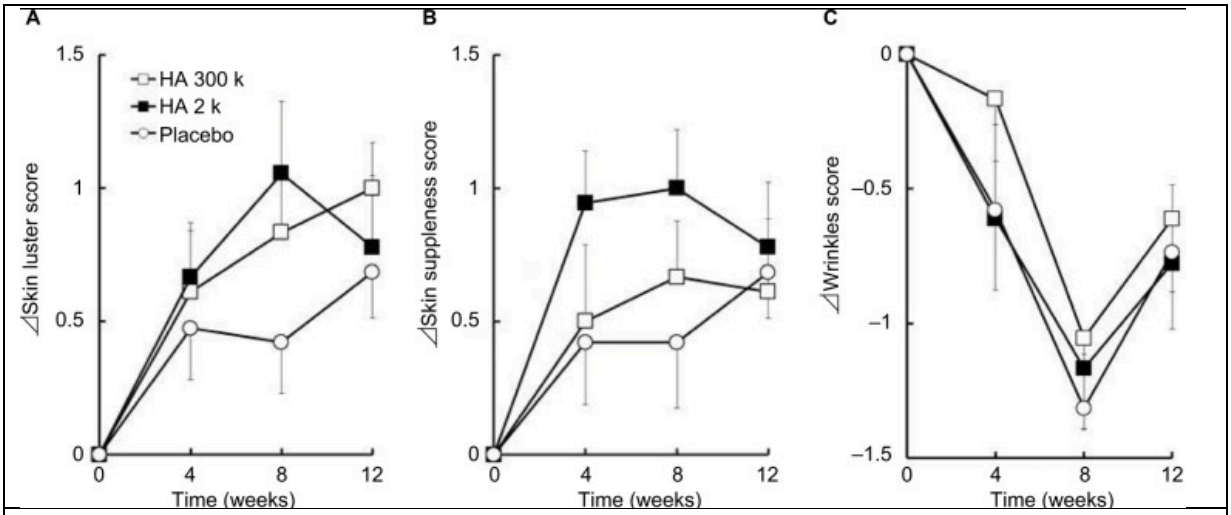
3.2 Hyaluronic acid

Hyaluronic acid is a natural glycosamynoglycan, i.e. a high-molecular-weight polysaccharide, which is widely distributed in the connective tissue extracellular matrix and that can bind 1000 times its weight

in water.[76] It plays multifaceted role in regulating the various biological processes such as skin repairment, diagnosis of cancer, wound healing, tissue regeneration, anti-inflammatory, and immunomodulation processes and has become an imperative nutrient for skin moisture in cosmetic and nutraceutical preparations.[77]
Hyaluronic acid is mostly used in topical preparations such as creams, but is increasingly used in nutraceutics, as well. In effect, utility of topical applications is limited inasmuch as skin acts as a barrier to entry.

A placebo-controlled, randomised, double-blind study was conducted over 12 weeks on 40 healthy Asian men and wommend aged 35 to 64. Skin condition was evaluated by wrinkles, stratum corneum water content, the amount of transepidermal water loss, elasticity, and through image analysis. The study showed significant improvements in skin condition in terms of wrinkle assessment, stratum corneum water content, transepidermal water loss, and elasticity.[78]

In another study, 60 Japanese male and female subjects aged 22 to 59 years with crow's feet wrinkles were randomly assigned to the hyaluronic acid oral supplement with low molecular eight (2 k) or hyaluronic acid oral supplement with high molecular weight (300 k) at 120 mg/day or the placebo group for 12 weeks. The skin wrinkles were evaluated by image analysis of skin wrinkle replicas, and their skin condition was evaluated using a questionnaire survey.[79]



DEFINITE POSITIVE EFFECTS OF HYALURONIC ORAL SUPPLEMENTATION

[76] Walker K., Basehore B.M., Goyal A., Zito P.M., Hyaluronic Acid, StatPearls Publishing, Treasure Island, 2022.
[77] Nasir Abbas Bukhari, S., Liyana Roswandi, N., Waqas, M., Habib, H., Hussain, F., Khan, S., Sohail, M., Amlizan Ramli, N., Ei Thu, H., Hussain, Z., Hyaluronic acid, a promising skin rejuvenating biomedicine: A review of recent updates and pre-clinical and clinical investigations on cosmetic and nutricosmetic effects, International Journal of Biologicla Macromolecules, December 2018, pp. 1682-1695.
[78] Hsu, T. F., Su, Z.R., Hsieh Y.H., Wang, M.F., Oe, M., Matsuoka, R., Masuda, Y., Oral Hyaluronan Relieves Wrinkles and Improves Dry Skin: A 12-Week Double-Blinded, Placebo-Controlled Study, Nutrients, Vol 13, Issue 7 (June 2021), art. 2220.
[79] Oe, M., Sakai, S., Yoshida, H., Okado, N., Kaneda, H., Masuda, Y., Urushibata, O., Oral hyaluronan relieves wrinkles: a double-blinded, placebo-controlled study over a 12-week period, Clinical, Cosmetic an Investigational Dermatology, July 2017, pp. 267-273.

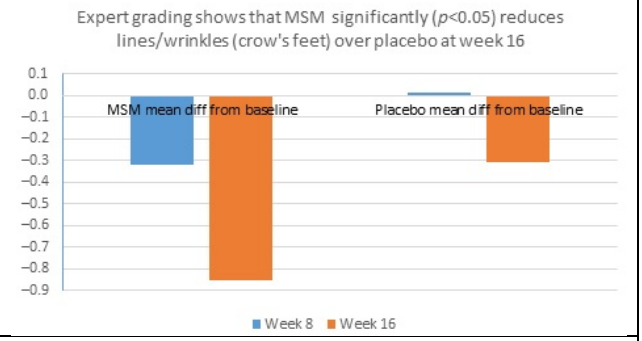
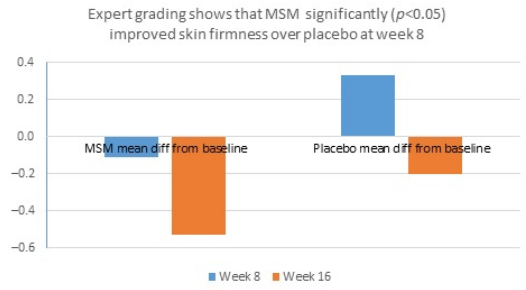
After 8 weeks of ingestion, the HA 300 k group showed significantly diminished wrinkles compared with the placebo group. Skin luster and suppleness significantly improved after 12 weeks in all groups compared with the baseline.

Hyaluronic acid in combination with hydrolised fish collagen peptides

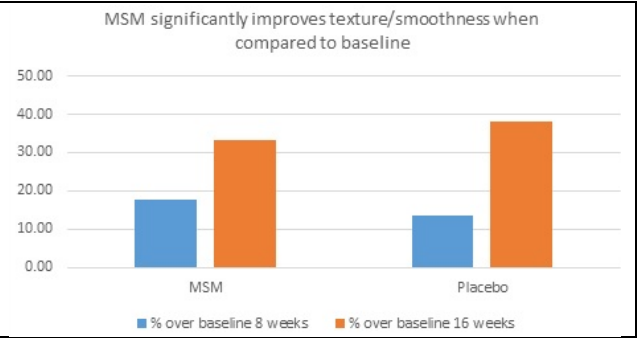
Hyaluronic acid and hydrolyzed collagen have different functions in the epidermis, but are both moisturizing ingredients that retard water evaporation from the skin and provide a large barrier to trans-epidermal water loss.
In the epidermis, hyaluronic acid forms a gel with large amounts of water, which in effect plumps up the skin by resisting the pressure from the collagen brils forming skin's matrix. Thus, the size and density of the collagen brils and hyaluronic acid in skin have a strong impact on skin's health and function. Fish collagen peptides may also have synergies with other ingredients. Studies have shown synergistic value with antioxidants like Vitamin C.[80]

3.3 MSM - Methylsulfonylmethane

MSM is a natural source of organic sulphur that has been well-investigated in animal and human clinical trials for its anti-inflammatory effects, positive effects on joint/muscle pain, oxidative stress and anti-oxidant capacity.[81] Already in 1981, Dr. Herschler was granted a United States utility patent for the use of MSM to smooth and soften skin and to strengthen nails. Modern studies proved the effects on skin health and the effect of MSM on regulation of genomic expression of key genes responsible for skin health and the prevention of aging. A 2015 double-blind, placebo-controlled clinical study involved 20 female subjects taking 3g of MSM daily over 16 weeks. Skin health was evaluated through expert grading, instrumentation, and participant self-assessment at weeks 8 and 16.[82]

MSM significantly reduces crow's feet	
	MSM significantly improves skin firmness

[80] Weishardt, Introduction to Naticol® marine collagen peptides for anti-aging and overview of clinical studies,
[81] Butawan, M., Benjamin, R. L., Bloomer, R. J., Methylsulfonylmethane: Applications and Safety of a Novel Dietary Supplement, Nutrients, Vol. 9, Issue 3 (March 2017), art. 290.
[82] Anthonavage, M., Benjamin, R. L., & Withee, E. D., Effects of oral supplementation with methylsulfonylmethane on skin health and wrinkle reduction, National Medical Journal, Vol. 7, Issue 11 (2015), pp. 1-21.

MSM significantly improves texture and smoothness of skin.	

MSM for hair growth

Analysis of hair reveals a composition of iron, oxygen, hydrogen, nitrogen and sulphur. Thus, an adequate supply of blood containing these minerals is essential for hair growth (anagen) phase. Anagen is associated with a rearrangement of skin vasculature, an increase in skin perfusion, and angiogenesis.[83]

3.4 Biotin – Vitamin B7

Biotin (B7 or vitamin H) is a water-soluble vitamin, which has received publicity for promoting the growth of hair and nails and maintaining healthy skin.[84] There is, however, limited scientific evidence to support these claims which can also be attributed to difficulties with laboratory testing of biotin effects. Taking biotin supplements can interfere with some laboratory tests resulting in false-positive and false-negative results.[85]

3.5 Zinc

Zinc has been used as a therapeutic modality for centuries for a number of dermatological conditions including infections (leishmaniasis, warts), inflammatory dermatoses (acne vulgaris, rosacea), pigmentary disorders (melasma), and neoplasias (basal cell carcinoma).[86] Disturbances in zinc metabolism may give rise to disorders that typically manifest themselves on the skin. The skin issues related to zinc deficiency can be reversed with systemic zinc repletion. Success rates for treatment with zinc vary depending on the disease, mode of application and zinc salt used.[87] Zinc is now being studied for its anti-inflammatory effects on the skin.

[83] Wall, D., Meah, N., Fagan, N., York, K., Sinclair, R., Advances in hair growth, Faculty Reviews, Vol. 11 (2022), art. 1.
[84] Bistas, K. G., Tadi, P., Biotin, StatPearls Publishing, Treasure Island, 2022.
[85] Saleem, F., Soos, M. P., Biotin Deficiency in Biotin, StatPearls Publishing, Treasure Island, 2022.
[86] Gupta, M., Mahajan ,V. K., Mehta, K. S., Chauhan, P.S., Zinc therapy in dermatology: a review, Dermatology Research and Practice, 2014, art. 709152.
[87] Nitzan, Y. B., Cohen, A.D., Zinc in skin pathology and care, Journal of Dermatological Treatment, Vol. 17, Issue 4 (2006), pp. 205-10.

Zinc deficiency is a well-documented cause for hair loss.[88] Zinc and other trace elements such as copper and selenium are required for the synthesis of thyroid hormones. Deficiency of these can result in hypothyroidism, a common and well recognized cause of diffuse hair loss.[89]

Zinc participates in the regulation of cell proliferation in several ways; it is essential to enzyme systems that influence cell division and proliferation[90] thus contributing to nail growth.

Hochman LG, Scher RK, Meyerson MS. Brittle nails: response to daily biotin supplementation. Cutis. 1993 Apr;51(4):303-5. PMID: 8477615.

[88] Rajput, J. R., Role of Non Androgenic Favtors in Hair loss and Hair Regrowth, Journal of Cosmetology & Trichology, Vol 3, Issue 1 (2017), art. 118.
[89] Betsy, A., Binitha, M., Sarita, S., Zinc deficiency associated with hypothyroidism: an overlooked cause of severe alopecia, International Journal of Trichology, Vol 5 Issue 1 (2013), pp. 40-42.
[90] MacDonald, R. S., The role of zinc in growth and cell proliferation., The Journal of Nutrition, Vol 130 (2000).