

An Encapsulated Fruit and Vegetable Juice Concentrate Increases Skin Microcirculation in Healthy Women

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Key Words

Antioxidants · Vitamins · Microcirculation · Oxygen to see device

Abstract

Background/Aim: Microcirculation in the dermis of the skin is important for nutrient delivery to this tissue. In this study, the effects of a micronutrient concentrate (Juice Plus+[®]; ‘active group’), composed primarily of fruit and vegetable juice powder, on skin microcirculation and structure were compared to placebo. **Study Design/Methods:** This 12-week study had a monocentric, double-blind placebo and randomized controlled design with two treatment groups consisting of 26 healthy middle-aged women each. The ‘oxygen to see’ device was used to evaluate microcirculation. Skin density and thickness were measured using ultrasound. Measurements for skin hydration (Corneometer[®]), transepidermal water loss and serum analysis for carotenoids and α -tocopherol were also performed. **Results:** By 12 weeks, microcirculation of the superficial plexus increased by 39%. Furthermore, skin hydration increased by 9% while skin thickness increased by 6% and skin density by 16% in the active group. In the placebo group, microcirculation decreased,

and a slight increase in skin density was observed. **Conclusion:** Ingestion of a fruit- and vegetable-based concentrate increases microcirculation of the skin at 12 weeks of intervention and positively affects skin hydration, density and thickness.

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Introduction

Nutrition is an important factor in skin health. Studies have shown that nutritional deficiencies correlate with skin disorders and/or changes in skin physiology [1]. Next to fatty acids, micronutrients, like vitamins, carotenoids and polyphenols, are assumedly important active compounds in skin health [2]. Natural sources for these phytonutrients are fruits and vegetables. The effects are manifold, e.g. consumption of carotenoid-rich fruits and vegetables, or supplements, is known to improve skin photoprotection [3] or skin parameters such as structure or texture [4–8]. High-flavanol cocoa attenuated UV-induced erythema under experimental conditions [9]. Oxidation of proteins, DNA or lipids under UVA irradiation results in an increased cutaneous chemiluminescence

signal, which was reduced by a topical application of an oil-in-water cream containing tocopheryl acetate, ferulic acid and rutin [10].

The biochemical mechanisms for these effects are not fully elucidated. The photoprotective effect is mainly based on the ability of these molecules to quench singlet oxygen or prevent oxidative damage induced by other reactive oxygen species [3]. Micronutrients can also influence cell signaling, with direct impact on intracellular and extracellular pathways, for example triggering cell cycle, cell growth or cellular repair systems [11, 12].

Nutritional support of the skin tissue is maintained by microcirculation. The blood vessels of the skin are arranged into (a) the superficial plexus, which is located 1–2 mm under the epidermis and is formed in capillary loops, and (b) the deeper horizontal plexus [13–15]. The tips of the capillary loops are mainly responsible for nutrition and oxygen exchange, along with thermoregulation [15].

In cosmetics research, the measurement of microcirculation is used to quantify sun protection factors, skin irritation and efficiency of antiredness treatments, because blood circulation influences skin color [13]. Such methods are further used to provide information on physiology and tissue nutrition [16] or to evaluate endothelial activity, because vasomotion of the microvessels is partially regulated by endothelial activity [17]. Altered skin endothelium-dependent vasomotion has been found in several chronic conditions, such as arterial hypertension, hypercholesterolemia, chronic kidney disease and in diabetes [16, 18].

The 'oxygen to see' device allows the simultaneous measurement of blood flow and oxygen saturation (SO₂) of hemoglobin. This method is commonly used and well validated for monitoring microcirculation in diabetics, in whom changes in skin microcirculation have been found to occur years prior to microvascular symptoms in other tissues [16].

The aim of this study was to evaluate the effect of a micronutrient concentrate composed primarily of fruit and vegetable juice powder on skin microcirculation and structure, compared to placebo.

Subjects and Methods

Study Design

This was a monocentric, randomized, double-blind placebo-controlled study of 52 healthy women with normal to dry skin, aged 40–65 years, and a BMI of 18–25 who were recruited by the Institute for Experimental Dermatology (DermaTronnier®), University of Witten-Herdecke, Germany. All volunteers were screened

for general health and medical history, and only those who were nonsmokers, not pregnant or lactating and had no history of nutrient malabsorption, liver or lipid abnormalities and no history of photosensitizing disorders were eligible to enter the investigation. Use of nutritional supplements, tanning beds, sunbathing or medications that interact or interfere with the study procedures were not allowed for a month prior to and during the study.

The volunteers ingested 4 capsules of the active agent (Juice Plus+® Fruit Blend and Vegetable Blend; NSA, Collierville, Tenn., USA) or placebo daily, 2 in the morning and 2 in the evening.

The active gelatin capsule contents consisted primarily of a blended fruit and vegetable pulp and juice powder concentrate derived from acerola cherry, apple, beet, beetroot, broccoli, cabbage, carrot, cranberry, dates, garlic, kale, orange, peach, papaya, parsley, pineapple, prune, spinach, sugar beet, tomato, with *Spirulina pacifica*, *Lactobacillus acidophilus*, rice bran, oat bran and *Dunaliella salina*. These active ingredients provided β-carotene (7.5 mg), vitamin E (46 mg), vitamin C (200 mg) and folic acid (400 μg) per 4 capsules, i.e. the daily dose [19]. Small quantities of anticaking agents (calcium carbonate, magnesium oxide, magnesium stearate, cellulose) and thickeners (citrus pectin, guar gum) are added manufacturing aids.

The placebo capsules were identical in appearance and contained no antioxidants, consisting primarily of microcrystalline cellulose. All study capsules were provided by NSA. This 12-week study was carried out between October and February. Skin parameters were determined and blood samples collected at baseline (week 0) and at study weeks 6 and 12.

Written informed consent was obtained from each participant. The study design was approved by the Ethics Committee of the University of Witten-Herdecke, Germany; it was performed according to the Good Clinical Practice guidelines and the Declaration of Helsinki.

Chemicals and Reagents

Reagents were from Merck (Darmstadt, Germany) unless stated otherwise.

Skin Measurements

All skin measurements were performed on the inner forearm under consistent room temperature and humidity. No topical treatment was allowed in this area during the study.

Cutaneous Blood Flow and Oxygen Saturation of Hemoglobin

The 'oxygen to see' system (Lea Instruments, Giessen, Germany) was used to determine cutaneous blood flow. The measurement is based on the laser Doppler effect, which is triggered by movement of the erythrocytes. Results are given in arbitrary units. Capillary SO₂ was determined spectroscopically and given as percent of total hemoglobin. Both parameters were determined at 1 and 8 mm below the skin surface [16, 20] and were performed according to the Guidance of the European Expert Group on Efficacy Measurement of Cosmetics and Other Topical Products [13]. Measurement at 1 mm reflects the microcirculation of the skin, whereas at 8 mm the general blood circulation is detected. The latter was conducted as control measurement.

Ultrasound Measurements (B Scan)

Skin density and thickness (dermis and epidermis) was measured using an ultrasound device (B scan) with a frequency of 20

Table 1. Skin parameters after ingestion of a fruit- and vegetable-based concentrate (active) compared to placebo

Skin parameter	Active group			Placebo group		
	week 0	week 6	week 12	week 0	week 6	week 12
Blood flow at 1 mm, AU	6.6 ± 2.4	8.0 ± 3.7	9.2 ± 4.1*	8.4 ± 3.6	7.3 ± 3.7	5.8 ± 2.9*
SO ₂ at 1 mm, %	46.7 ± 12.1	53.7 ± 14.6*	54.3 ± 12.5*	54.3 ± 12.3	50.0 ± 13.2	48.6 ± 11.6
Blood flow at 8 mm, AU	117 ± 40.5	122 ± 51.5	136 ± 44.8	142 ± 41.9	120 ± 37.3*	106 ± 40.9*
SO ₂ at 8 mm, %	71.3 ± 14.0	70.3 ± 7.9	72.7 ± 10.3	74.0 ± 10.1	69.5 ± 9.5*	72.1 ± 8.6
Hydration, AU	35.9 ± 7.79	37.5 ± 7.65	39.3 ± 7.65*	29.8 ± 6.05	28.2 ± 6.03	30.4 ± 5.39
TEWL, g h ⁻¹ m ⁻²	8.1 ± 2.60	7.4 ± 1.66	7.6 ± 1.66	7.2 ± 1.85	7.8 ± 1.49	7.7 ± 1.86
Skin thickness, mm	1.17 ± 0.14	1.16 ± 0.28	1.24 ± 0.17*	1.16 ± 0.10	1.17 ± 0.09	1.14 ± 0.12
Skin density, mm ²	6.65 ± 0.88	7.20 ± 1.08*	7.73 ± 0.95*	6.40 ± 0.85	6.74 ± 0.75*	6.88 ± 0.86*

* p < 0.05: significantly different from week 0. Data are given as means ± SD; n = 26/group. AU = Arbitrary units.

MHz (Derma Scan C, with 2-dimensional configuration; Cortex Technology, Denmark). Density is given by pixel density in the measured area (square millimeters); thickness is measured in millimeters [21].

Skin Hydration and Transepidermal Water Loss

The determination of the skin hydration is based on capacitance measurement of a dielectric medium with a Corneometer CM 825® (Courage & Khazaka Electronics, Cologne, Germany) expressed in arbitrary units [22]. Transepidermal water loss (TEWL) was measured with a Tewameter TM 300® (Courage & Khazaka Electronics) and is expressed in grams per hour per square meter [23].

All skin measurements were performed according to the European Cosmetic Associations Guidelines [24].

Serum Analysis

Serum was prepared from whole-blood samples by centrifugation without anticoagulant and stored at -80°C until analysis. Carotenoids were determined by HPLC using a Merck-Hitachi (Darmstadt, Germany) L-7100 pump connected with a Merck-Hitachi UV/Vis detector [25]. Serum samples were extracted with n-hexane/dichloromethane (5/1 vol/vol, 0.01% butylated hydroxytoluene). The organic layer was removed and evaporated to dryness under a stream of nitrogen. The residue was dissolved in dichloromethane/mobile phase (1/10 vol/vol). Chromatograms were processed by a HPLC System Manager HSM D-7000 (Merck-Hitachi). HPLC was performed isocratically with a mobile phase containing methanol/acetonitrile/propanol (54/44/2 vol/vol/vol) and using a reversed-phase column (pKb-100, 250 × 4.6 mm; Supelco, Bellefonte, Pa., USA), protected by a guard column (4.6 × 4.6 mm²) with the same stationary phase. The flow rate was set to 1 ml min⁻¹. Carotenoids were detected at 450 nm.

Retinol and α-tocopherol were extracted and detected simultaneously, the UV/Vis detector was set at 292 nm and 325 nm [26].

The concentrations of the analytes were calculated from external calibration curves generated with original standard compounds and internal standards. Data are given in micromoles per liter.

Table 2. p values of skin parameters (table 1) for the comparison between groups using the Wilcoxon rank sum test

Skin parameter	p values	
	week 6 vs. 0	week 12 vs. 0
Blood flow at 1 mm	0.0288	<0.0001
SO ₂ at 1 mm	0.0006	0.0001
Blood flow at 8 mm	0.0182	<0.0001
SO ₂ at 8 mm	n.s.	n.s.
Hydration	n.s.	n.s.
TEWL	n.s.	n.s.
Skin thickness	n.s.	0.0143
Skin density	n.s.	0.0428

Statistics

All data are given as means ± SD. For all parameters and all time points, descriptive statistics were calculated, pre-post differences were calculated and analyzed descriptively. Within the two treatment groups, each combination of two points of time was compared using the nonparametric Wilcoxon signed-rank test. For the pre-post differences, each combination of two treatment groups was compared using the Wilcoxon rank sum test. p Values were determined at all measuring points with p < 0.05 considered statistically significant.

Results

Cutaneous Blood Flow and SO₂

Cutaneous blood flow and capillary SO₂ of hemoglobin were measured at 1 and 8 mm under the skin surface. Blood flow at 1 mm depth was increased by 21% after 6 weeks and statistically significant by 39% after 12 weeks compared to week 0 (p = 0.003; table 1, fig. 1) in the active

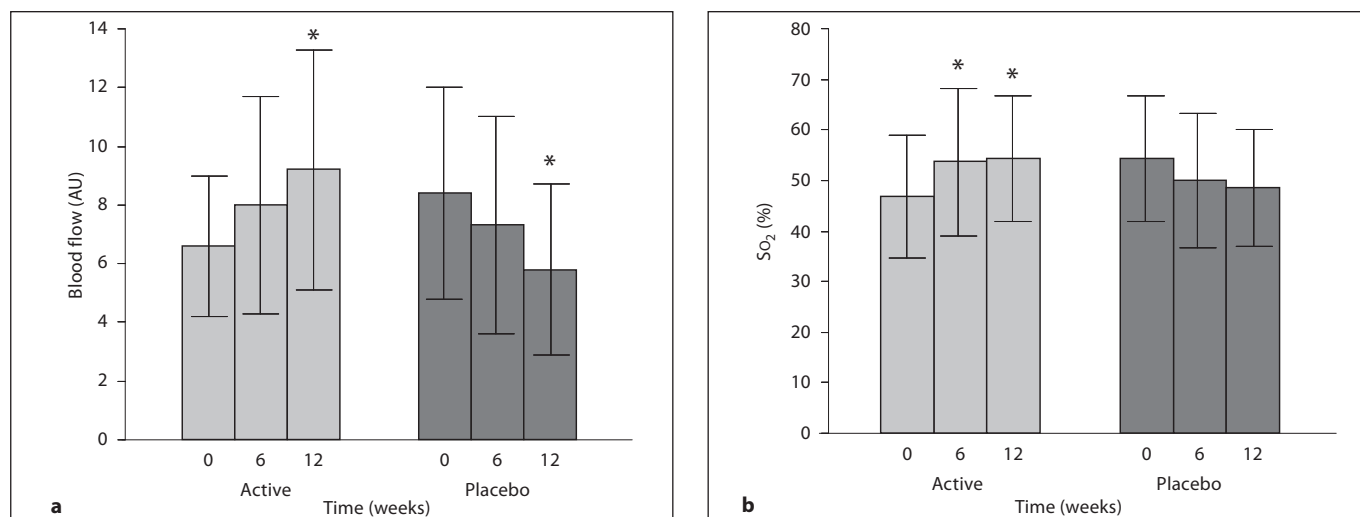


Fig. 1. Blood flow (a) in arbitrary units (AU) and SO₂ (b) at 1 mm skin depth after ingestion of the fruit- and vegetable-based concentrate (active) or placebo. Data are given as means \pm SD; n = 26/group. * p < 0.05: significantly different from week 0.

group. SO₂ also increased significantly by 15% after 6 weeks compared to week 0 in the active group (p = 0.004), while the difference between week 6 and week 12 was only 1%. In contrast, the placebo group showed a statistically significant decrease in blood flow after 12 weeks (-31%, p = 0.001) compared to week 0. For both parameters at 1 mm depth, the comparison of the pre-post differences 'week 6 - week 0' and 'week 12 - week 0' showed a statistically significant difference in favor of the active group (table 2).

At 8 mm depth, neither blood flow nor SO₂ were changed significantly in the active group (table 1). In the placebo group blood flow decreased statistically significantly by -15 and -25% after 6 weeks (p = 0.005) and 12 weeks (p < 0.0001), respectively, compared to week 0. Also the SO₂ was decreased, but only significantly after 6 weeks (-6%, p = 0.01).

The parameter blood flow showed statistically significant pre-post differences between groups in favor of the active capsules for both time differences at 1 mm (table 2). Due to the reduction of blood flow at 8 mm in the placebo group, the comparison of the pre-post-difference was statistically significant, too.

Ultrasound Measurements (B Scan)

The skin ultrasound measurement showed increased skin thickness and skin density in the active group. Skin thickness increased by 6% (p = 0.007), skin density by 16% after 12 weeks (p < 0.0001; table 1). In the placebo

group only the density of the skin increased significantly by 7% (p = 0.036). The p values for the comparison between the groups showed that the increase in thickness and density were statistically significant between weeks 12 and 0 in favor of the active group (table 2). A typical ultrasound B scan is presented in figure 2. It shows the density and thickness of the dermis before and after 12 weeks of treatment for a volunteer from the active group.

Skin Hydration and TEWL

In the active group skin hydration showed an increase of 9% after 12 weeks (p = 0.014). The placebo group showed no change.

A decrease in the TEWL was observed in the active group, with -9% after 6 weeks and -6% after 12 weeks compared to baseline; these changes were not statistically significant. The TEWL in the placebo group increased, but not statistically significantly either. The pre-post difference comparison of active versus placebo groups showed no difference in hydration and TEWL.

Serum Analysis

Carotenoid analysis showed a significant increase from baseline in β -carotene and α -tocopherol (p < 0.0001, week 6 and 12) in the active group, while no change was observed in the placebo group (table 3). Both groups showed a significant increase in β -cryptoxanthin during the study period.

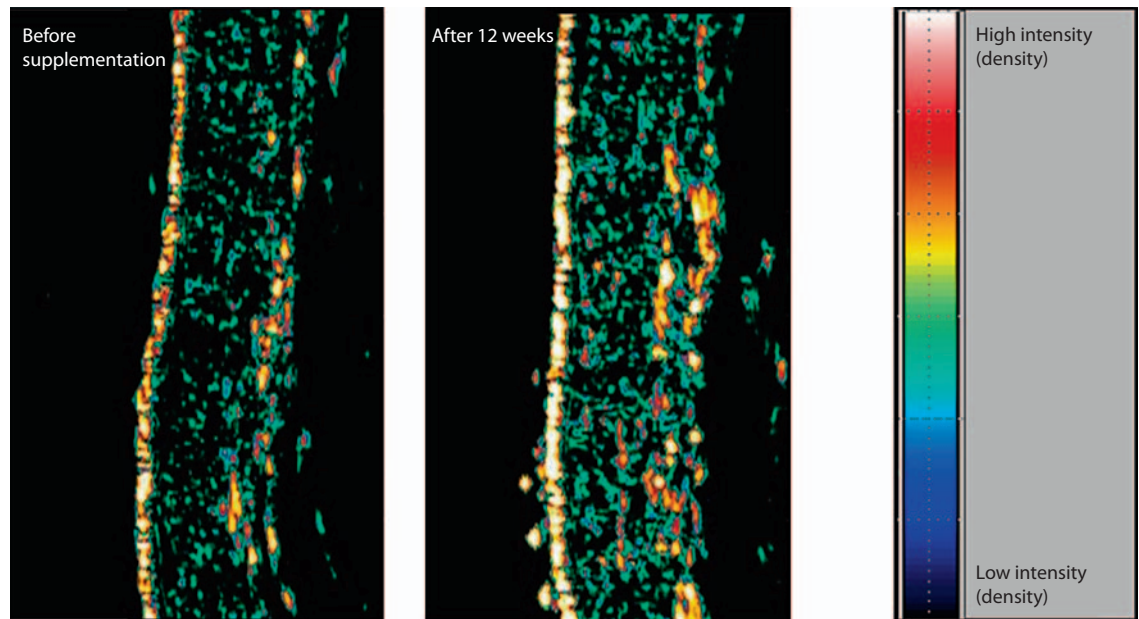


Fig. 2. A typical ultrasound B scan of the epidermis and dermis showing the density and thickness, before and after 12 weeks of treatment of the fruit- and vegetable-based concentrate (active).

Table 3. Serum levels of carotenoids, α -tocopherol and retinol ($\mu\text{mol l}^{-1}$) after ingestion of a fruit- and vegetable-based concentrate (active) compared to placebo

Substance	Active group			Placebo group		
	week 0	week 6	week 12	week 0	week 6	week 12
Lutein	0.27 ± 0.10	0.27 ± 0.08	0.27 ± 0.09	0.30 ± 0.11	0.31 ± 0.12	0.31 ± 0.11
Zeaxanthin	0.08 ± 0.03	0.09 ± 0.03	0.09 ± 0.03	0.10 ± 0.04	0.10 ± 0.04	0.10 ± 0.03
Cryptoxanthin	0.23 ± 0.11	$0.38 \pm 0.33^*$	$0.48 \pm 0.31^*$	0.27 ± 0.10	$0.40 \pm 0.21^*$	$0.40 \pm 0.23^*$
Lycopene	0.62 ± 0.30	0.59 ± 0.33	0.55 ± 0.30	0.69 ± 0.37	0.70 ± 0.35	0.65 ± 0.42
α -Carotene	0.17 ± 0.18	0.15 ± 0.12	0.15 ± 0.10	0.15 ± 0.10	0.15 ± 0.11	0.14 ± 0.10
β -Carotene	0.88 ± 0.74	$2.44 \pm 1.27^*$	$2.55 \pm 1.40^*$	0.68 ± 0.34	0.71 ± 0.41	0.65 ± 0.34
α -Tocopherol	23.4 ± 6.0	$27.4 \pm 5.6^*$	$27.5 \pm 7.3^*$	21.8 ± 5.1	20.2 ± 4.1	21.8 ± 4.7
Retinol	1.23 ± 0.27	1.23 ± 0.27	1.19 ± 0.28	1.20 ± 0.35	1.12 ± 0.35	1.12 ± 0.30

* $p < 0.05$: significantly different from week 0. Data are given as means \pm SD; $n = 26/\text{group}$. The comparison of the pre-post differences showed significant differences in favor of the active group for β -carotene ($p < 0.0001$ week 6 vs. 0 and week 12 vs. 0) and for α -tocopherol ($p < 0.0001$ week 6 vs. 0, $p = 0.0004$ week 12 vs. 0).

Discussion

Skin microcirculation is responsible for nutrition, oxygen supply and thermoregulation [14]. An optimal supply of the skin with nutrients and oxygen is necessary for skin cell metabolism, therefore supporting skin physiology, structure and health [4–8]. The question in the pres-

ent study was whether an encapsulated fruit- and vegetable-based juice powder concentrate (Juice Plus+) could modulate skin microcirculation and structure. The major micronutrients in the active capsules were β -carotene, vitamin E, vitamin C and folate. HPLC measurement showed an increase in β -carotene and vitamin E serum levels (table 3). In a study using a similar concentrate,

plasma vitamin C levels increased as well as β -carotene and vitamin E [19]. In the present study β -cryptoxanthin increased in both groups. Oranges and tangerines are natural sources of β -cryptoxanthin [27], typical fruits consumed during winter, when this study was conducted.

Microcirculation was measured with the 'oxygen to see' device. In the active group, blood flow and SO_2 increased at 1 mm skin depth. At 8 mm only minor changes were observed. These data indicate that the microcirculation of the superficial plexus was affected more than the general circulation, because the superficial vessels are surrounded by only 1–2 layers of smooth muscle cells [14]. Thinner vessels may react faster or be more sensitive to micronutrients than thicker, deeper vessels. Because of this, the skin could be supplied with oxygen and nutrients more readily, perhaps without affecting the general circulation. However, in a study with high flavanol cocoa, the deeper vessels responded with an increase in blood flow [9]. Increasing blood flow results from vasodilation of the blood vessels. Nitric oxide is one key regulator of blood vasodilation affecting the surrounding smooth muscle cells of the endothelium [21, 28]. Antioxidants, folate and a greater intake of fruit and vegetables showed beneficial effects on endothelium-dependent vasoconstriction [29, 30].

With better supply of nutrients and oxygen essential for skin metabolism, skin function, texture and structure can be improved [4–8]. Change in the skin texture is a result of loss in barrier integrity [31]. This again can result in increased TEWL and diminished skin hydration. During the study TEWL was slightly decreased and skin hy-

dration was increased; thus, there was a small but positive effect on skin barrier function of the stratum corneum.

Additionally, deeper skin levels of epidermis and dermis were affected. It is known that skin density and thickness decrease during the aging process [32]. It is unknown if this process can be counteracted by micronutrients, but intervention of 12 weeks was sufficient to improve thickness and density (table 1) in this study. A similar study, using a supplement mixture of carotenoids, vitamin E and selenium, showed an increase in these skin parameters as well [5].

In conclusion, the present study shows that intake of a fruit- and vegetable-based concentrate increases skin microcirculation and improves skin texture and structure compared to placebo.

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Disclosure Statement

The authors declare no conflict of interest.

References

- 1 Piccardi N, Manissier P: Nutrition and nutritional supplementation: impact on skin health and beauty. *Dermatoendocrinol* 2009; 1:271–274.
- 2 Boelsma E, Hendriks HF, Roza L: Nutritional skin care: health effects of micronutrients and fatty acids. *Am J Clin Nutr* 2001;73:853–864.
- 3 Sies H, Stahl W: Nutritional protection against skin damage from sunlight. *Annu Rev Nutr* 2004;24:173–200.
- 4 Udompataikul M, Sripiroj P, Palungwachira P: An oral nutraceutical containing antioxidants, minerals and glycosaminoglycans improves skin roughness and fine wrinkles. *Int J Cosmet Sci* 2009;31:427–435.
- 5 Heinrich U, Tronnier H, Stahl W, Béjot M, Maurette JM: Antioxidant supplements improve parameters related to skin structure in humans. *Skin Pharmacol Physiol* 2006;19: 224–231.
- 6 Boelsma E, van de Vijver LP, Goldbohm RA, Klöpping-Ketelaars IA, Hendriks HF, Roza L: Human skin condition and its associations with nutrient concentrations in serum and diet. *Am J Clin Nutr* 2003;77:348–355.
- 7 Darvin M, Patzelt A, Gehse S, Schanzer S, Benderoth C, Sterry W, Lademann J: Cutaneous concentration of lycopene correlates significantly with the roughness of the skin. *Eur J Pharm Biopharm* 2008;69:943–947.
- 8 Jeon HY, Kim JK, Seo DB, Cho SY, Lee SJ: Beneficial effect of dietary epigallocatechin-3-gallate on skin via enhancement of antioxidant capacity in both blood and skin. *Skin Pharmacol Physiol* 2010;23:283–289.
- 9 Heinrich U, Neukam K, Tronnier H, Sies H, Stahl W: Long-term ingestion of high flavanol cocoa provides photoprotection against UV-induced erythema and improves skin condition in women. *J Nutr* 2006;136:1565–1569.
- 10 Jain A, Rieger I, Rohr M, Schrader A: Antioxidant efficacy on human skin in vivo investigated by UVA-induced chemiluminescence decay analysis via induced chemiluminescence of human skin. *Skin Pharmacol Physiol* 2010;23:266–272.
- 11 Brigelius-Flohé R, Banning A: Sulforaphane and selenium, partners in adaptive response and prevention of cancer. *Free Radic Res* 2006;40:775–787.
- 12 Stahl W, Ale-Agha N, Polidori MC: Non-antioxidant properties of carotenoids. *Biol Chem* 2002;383:553–558.
- 13 Berardesca E, Lévêque JL, Masson P: EEMCO guidance for the measurement of skin microcirculation. *Skin Pharmacol Appl Skin Physiol* 2002;15:442–456.
- 14 Braverman IM: The cutaneous microcirculation. *J Investig Dermatol Symp Proc* 2000; 5:3–9.

- 15 Wright CI, Kroner CI, Draijer R: Non-invasive methods and stimuli for evaluating the skin's microcirculation. *J Pharmacol Toxicol Methods* 2006;54:1-25.
- 16 Forst T, Hohberg C, Tarakci E, Forst S, Kann P, Pfützner A: Reliability of lightguide spectrophotometry (O2C) for the investigation of skin tissue microvascular blood flow and tissue oxygen supply in diabetic and nondiabetic subjects. *J Diabetes Sci Technol* 2008;2:1151-1156.
- 17 Rossi M, Carpi A, Galetta F, Franzoni F, Santoro G: Skin vasomotion investigation: a useful tool for clinical evaluation of microvascular endothelial function? *Biomed Pharmacother* 2008;62:541-545.
- 18 Rossi M, Carpi A, Di Maria C, Franzoni F, Galetta F, Santoro G: Skin blood flow motion and microvascular reactivity investigation in hypercholesterolemic patients without clinically manifest arterial diseases. *Physiol Res* 2009;58:39-47.
- 19 Jin Y, Cui X, Singh UP, Chumanevich AA, Harmon B, Cavicchia P, Hofseth AB, Kotakadi V, Stroud B, Volate SR, Hurley TG, Hebert JR, Hofseth LJ: Systemic inflammatory load in humans is suppressed by consumption of two formulations of dried, encapsulated juice concentrate. *Mol Nutr Food Res* 2010;54:1506-1514.
- 20 Krug A: Mikrozirkulation und Sauerstoffversorgung des Gewebes – Methode des so genannten O2C (oxygen to see). *Phlebologie* 2006;35:300-312.
- 21 Neukam K, Stahl W, Tronnier H, Sies H, Heinrich U: Consumption of flavanol-rich cocoa acutely increases microcirculation in human skin. *Eur J Nutr* 2007;46:53-56.
- 22 Heinrich U, Koop U, Leneveu-Duchemin MC, Osterrieder K, Bielfeldt S, Chkarnat C, Degwert J, Häntschel D, Jaspers S, Nissen HP, Rohr M, Schneider G, Tronnier H: Multicentre comparison of skin hydration in terms of physical-, physiological- and product-dependent parameters by the capacitive method (Corneometer CM 825). *Int J Cosmet Sci* 2003;25:45-53.
- 23 Rodrigues LM, Pinto PC, Magro JM, Fernandes M, Alves J: Exploring the influence of skin perfusion on transepidermal water loss. *Skin Res Technol* 2004;10:257-262.
- 24 Colipa (European Cosmetic Association): Guideline for the evaluation of the efficacy of cosmetic products. 2008. <http://www.colipa.eu>.
- 25 Stahl W, Sundquist AR, Hanusch M, Schwarz W, Sies H: Separation of beta-carotene and lycopene geometrical isomers in biological samples. *Clin Chem* 1993;39:810-814.
- 26 Polidori MC, Stahl W, Eichler O, Niestroj I, Sies H: Profiles of antioxidants in human plasma. *Free Radic Biol Med* 2001;30:456-462.
- 27 Mangels AR, Holden JM, Beecher GR, Foreman MR, Lanza E: Carotenoid content of fruits and vegetables: an evaluation of analytical data. *J Am Diet Assoc* 1993;93:284-296.
- 28 Heiss C, Schroeter H, Balzer J, Kleinbongard P, Matern S, Sies H, Kelm M: Endothelial function, nitric oxide, and cocoa flavanols. *J Cardiovasc Pharmacol* 2006;47(suppl 2):128-135.
- 29 Brown AA, Hu FB: Dietary modulation of endothelial function: implications for cardiovascular disease. *Am J Clin Nutr* 2001;73:673-686.
- 30 McCall DO, McGartland CP, McKinley MC, Patterson CC, Sharpe P, McCance DR, Young IS, Woodside JV: Dietary intake of fruits and vegetables improves microvascular function in hypertensive subjects in a dose-dependent manner. *Circulation* 2009;119:2153-2160.
- 31 Callaghan TM, Wilhelm KP: A review of ageing and an examination of clinical methods in the assessment of ageing skin. 2. Clinical perspectives and clinical methods in the evaluation of ageing skin. *Int J Cosmet Sci* 2008;30:323-332.
- 32 Waller JM, Maibach HI: Age and skin structure and function, a quantitative approach. I. Blood flow, pH, thickness, and ultrasound echogenicity. *Skin Res Technol* 2005;11:221-235.