Aus der Klinik und Poliklinik für Dermatologie, Venerologie und Allergologie
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DISSERTATION

Kinetics of carotenoid antioxidant substances in the human skin.

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2. Darvin ME, Gerzonde I, Ey S, Brandt NN, Albrecht H, Gonchukov SA, Sterry W, Lademann J. Raman Spectroscopic measurements of beta-Carotene and Lycopene in Human Skin. Proceedings of SPIE, 5474: 20-24, 2004.

3. Darvin ME, Gersonde I, Meinke M, Sterry W, Lademann J. Non-invasive in vivo determination of the carotenoids beta-carotene and lycopene concentrations in the human skin using the Raman spectroscopic method. Journal of Physics D: Applied physics. 38: 1-5, 2005.

4. Darvin ME, Gersonde I, Albrecht H, Gonchukov SA, Sterry W, Lademann J. Determination of Beta Carotene and Lycopene Concentrations in Human Skin Using Raman Spectroscopy. Laser Physics, 15(2): 295-299, 2005.

5. Lademann J, Martschick A, Jacobi U, Richter H, Darvin M, Sehouli J, Oskay-Özcelik G, Blohmer J-U, Lichtenegger W, Sterry W. Investigation of doxorubicin on the skin: A spectroscopic study to understand the pathogenesis of PPE. Supplement to Journal of Clinical Oncology, 23, 16S(1): 5093, 2005.

6. Darvin ME, Gersonde I, Meinke M, Albrecht H, Sterry W, Lademann J. Non-invasive in vivo detection of the carotenoid antioxidant substance lycopene in the human skin using the resonance Raman spectroscopy. Laser Physics Letters, 3(9): 460-463, 2006.

7. Darvin ME, Gersonde I, Albrecht H, Sterry W, Lademann J. In-vivo Raman spectroscopic analysis of the influence of UV radiation on carotenoid antioxidant substance degradation of the human skin. Laser Physics, 16(5): 833-837, 2006.

8. Darvin M, Zastrow L, Sterry W, Lademann J. Effect of supplemented and topically applied antioxidant substances on human tissue. Review. Skin Pharmacol Physiol, 19: 238-247, 2006.

9. Darwin M, Schanzer S, Teichmann A, Blume-Peitavi U, Sterry W, Lademann J. Functional Food und Bioverfügbarkeit im Zielorgan Haut. Hautarzt, 57(4): 286-290, 2006.

10. Darvin ME, Gersonde I, Albrecht H, Sterry W, Lademann J. In vivo Raman spectroscopic analysis of the influence of IR radiation on the carotenoid antioxidant substances beta-carotene and lycopene in the human skin. Formation of free radicals. Laser Physics Letters, 4(4): 318-321, 2007.

11. Darvin ME, Gersonde I, Albrecht H, Sterry W, Lademann J. Resonance Raman spectroscopy for the detection of carotenoids in foodstuffs. Influence of the nutrition on the antioxidative potential of the skin. Laser Physics Letters, 4(6): 452-457, 2007.

12. Darvin ME, Gersonde I, Albrecht H, Sterry W, Lademann J. Non-invasive *in-vivo* Raman spectroscopic measurement of the dynamics of the antioxidant substance lycopene in the human skin after a dietary supplementation. Proceedings of SPIE, 6535, 2007.

The presented results were obtained by the doctoral candidate. The co-authors mainly helped in the preparation of the measurements, with useful discussions, as well as with the preparation of the articles.

Berlin, 31.05.2007 Maxim E. Darvin

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Zusammenfassung

Das Ziel der vorliegenden Arbeit bestand in in-vivo-Untersuchungen zum antioxidativen Potenzial der menschlichen Haut unter Nutzung der Karotinoide als Markersubstanzen. Karotinoide stellen eine wichtige Klasse von Antioxidantien im menschlichen Organismus dar. Die Kinetik der Karotinoide in der menschlichen Haut unter Berücksichtigung von negativen und positiven äußeren und inneren Faktoren wurde in vivo untersucht. Diese Experimente wurden möglich, nachdem es im Rahmen dieser Doktorarbeit gelungen ist, eine laserspektroskopische Untersuchungsmethode zu entwickeln, mit der es erstmals möglich ist, die Karotinoide nichtinvasiv und online in der Haut zu bestimmen.

In in-vivo-Untersuchungen konnte nachgewiesen werden, daß die Konzentration von β-Karotin und Lycopin in der menschlichen Haut durch UV- und IR-Strahlung reduziert wird. Dabei zeigte es sich, daß die β-Karotin- und Lycopinkonzentration nicht sofort abnehmen, sondern erst mit einer Verzögerungszeit von 10 bis 120 min in Abhängigkeit von der Art der Karotinoide, den Probanden und ihrem ursprünglichen Niveau des antioxidativen Potenzials sowie von der Bestrahlungsdosis. Eine starke Korrelation konnte zwischen der individuellen Konzentration der Antioxidantien und deren Abfall nach UV- bzw. IR-Bestrahlung nachgewiesen werden. In der Literatur gab es bisher keine Hinweise darüber, daß auch Infrarotstrahlung zur Bildung von freien Radikalen in der Haut führen kann. In der vorliegenden Arbeit wurde erstmals der Beweis erbracht, daß auch durch Wärmestrahlung freie Radikale in der Haut erzeugt werden können. Hierbei dienten die Karotinoide als Markersubstanzen für das antioxidative System der Haut.

Die Ergebnisse dieser Untersuchungen sind von besonderer Bedeutung für das Verständnis von Prozessen der Interaktion von freien Radikalen mit der Haut und für die Entwicklung von neuen Schutzstrategien gegen Hautkrebs, welcher durch intensive Sonnenbestrahlung hervorgerufen wird.

Karotinoide können im menschlichen Organismus nicht oder nur unzureichend synthetisiert werden. Sie müssen daher mit der Nahrung aufgenommen werden. Der Einfluss der Nahrung, speziell von Obst und Gemüse, auf das antioxidative Potenzial der Haut konnte im Rahmen einer Studie klar nachgewiesen werden.

Eine Studie, welche über ein Jahr an Probanden durchgeführt wurde, zeigte, daß eine karotinreiche Ernährung, welche auf dem Verzehr von Obst und Gemüse basiert, den Gehalt von Karotinoiden in der Haut erhöht, während der Einfluss von Stressfaktoren wie Krankheit, Müdigkeit, Rauchen, Alkoholkonsum und Stress zu einer drastischen Abnahme der Karotinoidkonzentration in der Haut der Probanden führte. Diese Abnahme erfolgte sehr schnell, innerhalb von Minuten bzw. Stunden in Abhängigkeit von den entsprechenden Stressfaktoren und ihrer Intensität. Im Gegensatz dazu dauert es bis zu drei Tagen, bis die ursprüngliche Konzentration der Karotinoide in der Haut durch Aufnahme von Obst und Gemüse wieder hergestellt werden konnte. Während der Sommer- und Herbstmonate konnte eine saisonbedingte Erhöhung des antioxidativen Potenzials in der Haut nachgewiesen werden. Diese Erhöhung war individuell verschieden und hing mit dem erhöhten Verzehr von Obst und Gemüse während der Sommer- und Herbstmonate zusammen.

Die Untersuchungen führten dazu, daß die Kinetik der Konzentrationsänderung der Karotinoide in der Haut, welche durch negative und positive Faktoren beeinflusst wird, das erstemal im Zusammenhang beschrieben wurde. Diese neuen Ergebnisse stehen teilweise im Widerspruch zu bisher publizierten Resultaten. Das ist jedoch nicht verwunderlich, da es bisher nicht möglich war, nichtinvasiv und online das antioxidative Potenzial der Haut zu vermessen.

Ein weiterer Untersuchungsschwerpunkt im Rahmen der vorliegenden Arbeit waren Untersuchungen zum Einfluss von Antioxidantien auf die Hautalterung. So konnte gezeigt werden, dass Probanden mit einer höheren Konzentration von Antioxidantien in der Haut weniger Falten und Furchen aufweisen als Probanden mit einer geringen Karotinoidkonzentration. Es konnte klar nachgewiesen werden, daß ein hohes antioxidatives Potenzial in der Haut die Hautalterung verzögert.

In der Literatur sind Beobachtungen beschrieben, die zeigen, daß eine Mischung von topisch applizierten Antioxidantien einen höheren Schutzeffekt bewirkt als Einzelkomponenten. Diese Beobachtung wurde durch eigene Messungen unterstützt, indem nachgewiesen werden konnte, daß Karotinoide durch die Zugabe von Vitamin C und E stabilisiert werden.

Ein weiterer wesentlicher Forschungsschwerpunkt im Rahmen der Doktorarbeit war auf die Entwicklung einer Therapie zur Verhinderung der Palmar-Plantar-Erythrodysesthesia (PPE) gerichtet. Es konnte gezeigt werden, dass das Chemotherapeutikum Doxorubicin mit dem Schweiß aus der Haus austritt, hier auf der Oberfläche spreitet und wie topisch appliziert wieder in das Stratum Corneum penetriert. Die Anwendung von Creme und Lösungen, welche Antioxidantien enthalten, konnte diese Nebenwirkung drastisch reduzieren bzw. in den meisten Fällen verhindern.

Damit hat sich die Raman-spektroskopische Untersuchungsmethode zum online-in-vivo-Nachweis von Karotinoiden in der menschlichen Haut als optimale Analysemethode zur Bestimmung der Effektivität von topisch applizierten Substanzen erwiesen. Sie findet gegenwärtig in Untersuchungen zum Sonnenschutz, zur Gabe von Nahrungsmittelergänzungsstoffen und bei der Therapiekontrolle während der Chemotherapie Einsatz.

1. Abstract

The aim of the present work was an in-vivo investigation of the antioxidative protection system of the human skin, using carotenoid antioxidants as marker substances. The kinetics of carotenoid antioxidant substances in the human skin subsequent to the influence of negative and positive factors were investigated in-vivo, using a newly developed non-invasive Raman spectroscopic measuring system.

It was shown for the first time in the in-vivo measurements that the cutaneous carotenoids beta-carotene and lycopene decreased subsequent to UV and IR irradiation of the skin. It was found that the beta-carotene and lycopene concentrations in the skin do not decrease immediately after irradiation and that there is a time delay, which varies between 10 and 120 minutes, depending on the type of carotenoids, volunteers, their antioxidative levels and the doses of irradiation. Moreover, a strong correlation between the individual carotenoid antioxidant levels and the magnitude of the destruction of carotenoids after UV and IR irradiation could be observed.

The current literature gives no information about the formation of free radicals after IR irradiation of the skin. In the present work, we assume that the formation of free radicals subsequent to IR irradiation of the skin leads to the degradation of the cutaneous carotenoids beta-carotene and lycopene.

The results of this study are important for understanding the interactions of free radicals in the skin and for the development of new protection strategies against skin cancer caused by excessive sun irradiation.

Carotenoids cannot be synthesized by the human body itself, thus they should be obtained with foodstuffs or through topical application. An influence of specially selected nutrition rich in carotenoids on the carotenoid level of the skin was clearly demonstrated.

A one-year study showed that a carotenoid-rich diet, based on large amounts of fruit and vegetables, apparently increased the measured carotenoid level of the skin, while the influence of stress factors, such as fatigue, illness, smoking and alcohol consumption gave rise to a decrease in the carotenoid level of the skin of the volunteers. These decreases occurred relatively quickly, varying from minutes to hours, depending on the influencing stress factors and their intensity, while the subsequent increase (recovery) usually lasted for up to 3 days. During the summer and autumn months, a “seasonal increase” in the level of carotenoids in the skin was determined as 1.3-fold in average for all volunteers.

Thus the level of carotenoids in the skin varies individually depending on both nutrition and the influence of possible stress factors and their intensity. The kinetics of the carotenoids in the skin subsequent to the influence of negative and positive factors concerning the response time, time of degradation, magnitude of destruction and the recovery time were described and evaluated. The current opinion about the kinetics of the carotenoids in the skin is completely modified by our results presented in the present work.

Moreover, it was observed that volunteers with a higher concentration level of carotenoid lycopene in the skin had a younger-looking skin in regard to the presence of furrows and wrinkles.

The analyzed literature suggests that the protection of the skin by antioxidants seems to be much more effective by using a combination of topical and systemic application of a mixture of antioxidant substances at low concentrations.

It was shown and explained that the palmar-plantar erythrodysesthesia (PPE) syndrome, which occurs after the application of medication Doxorubicin to cancer patients, can be prevented by the topical application of the formulation containing antioxidant substances including carotenoids.

2. Introduction

The redox reactions on the cell level are an important and irreplaceable part of the cellular metabolism. Oxygen free radicals and other reactive species are constantly produced on the mitochondrial level in the living organism.
Free radicals have a powerful oxidative activity, which means that they immediately interact with their surroundings and oxidize DNA, lipids and the proteins of living cells [1-6]. As a result of such attacks, the membranes of living cells can be destroyed, which in turn gives rise to the preliminary death of cells, irreversible changes and conformations on the cell level as well as to the following disorganization in the living bio-media [5, 6]. A part of these radicals is useful for the organism, because they act as weapons against viruses and bacteria. The remaining part is harmful and should be neutralized before harmful interactions take place.
The human skin as the boundary organ between the human body and the environment is constantly under the influence of free radicals and other reactive species from the outside in and from the inside out.
For the effective protection against the negative action of the radical substances, the human skin has developed a defense system in the form of antioxidant substances such as vitamins (A, C, D and E), carotenoids (beta-carotene, lycopene, lutein/zeaxanthin), enzymes (superoxide dismutase, catalase, glutathione peroxidase) and others (flavonoids, lipoic acid, uric acid, selenium, coenzyme Q10, etc.). These antioxidant substances act as a “protection chain”, which means that different antioxidants possess a synergistic effect and protect each other from the direct destruction in the processes of neutralization of the free radicals [7, 8].
Carotenoids act effectively against the destructive action of free radicals and other reactive substances, thus quenching their oxidative activity [9], as they possess an antioxidative function. Carotenoid molecules can neutralize several attacks of free radicals and are then destroyed [1, 10, 11]. The carotenoids can therefore be rated as natural markers for the processes accompanied by the production of free radicals.
Up to now, the high-pressure liquid chromatography (HPLC) method is widely used for the determination of carotenoids in bio-media because of the absence of alternative measuring methods. The HPLC method is well suited for the determination of carotenoids in serum, but is difficult to perform on skin tissue as it requires large tissue biopsies and special preparations [12]. Moreover, HPLC is a highly invasive, in-vitro, time-consuming and relatively expensive measuring method.
The development of a non-invasive method, based on resonance Raman spectroscopy, opened up wide possibilities concerning the measurements of carotenoids in the human tissue, and especially in the human skin without the necessity of tissue biopsies.
It is well known that carotenoid levels in the skin decrease subsequent to UV radiation and increase after a supplementation rich in carotenoids. Moreover, there are some papers which showed the degradation of the carotenoids in tissue after serious diseases. All these measurements were made using HPLC and the real-time kinetics of carotenoids in the skin have therefore been unknown until now.
The knowledge of the real kinetics of antioxidant substances in the skin, subsequent to the influence of external and internal stress factors, can be important in understanding the interactions of antioxidants with free radicals and in the development of the body’s optimal defense.
As it is known that the carotenoids in the skin are constantly destroyed and re-established because of their continuous reactions with free radicals and other reactive species during life, it is logical to suppose that the kinetics of carotenoid antioxidant substances should have a quicker dynamic than was published previously [13, 14]. These substances should reflect the permanent state of the health of the living organism, thus decreasing during the influence of strong stress factors, which lead to high amounts of free radicals and increasing during the absence of stress factors as well as carotenoid rich supplementation.

3. Materials and methods

3.1. Free radicals and the antioxidants in the skin

High amounts of free radicals are produced in the skin subsequent to UV irradiation and under the negative action of environmental hazards, giving rise to inflammation processes and, as a result, to the formation of erythema on the skin. Among the variety of radicals which are produced in the skin, singlet oxygen possesses the highest oxidative activity.
Under exposure to UV light, skin sensitizer molecules such as melanin, flavin, porphyrin and others are excited from their singlet ground state to an intermediate singlet excited state. From this state, the excess energy of these molecules is relaxed to an excited triplet state. The excited sensitizer molecule in the triplet state easily interacts with molecular oxygen in the ground triplet state, which is always contained in the skin. As a result of this reaction, excited singlet oxygen is produced and the sensitizer molecule returns to the ground state [15]. More than 99% of the reactions between the sensitizer molecules in the triplet state and the oxygen molecules in the ground state give rise to the production of singlet oxygen radicals [16]. If the number of radicals formed in the tissue is significantly increased, the defense antioxidative mechanism of the body is overloaded and not able to neutralize all these reactive molecules [17]. In this case chain reactions start, which again form high amounts of free radicals, and oxidative stress occurs. Free radicals immediately react with their surroundings, thus oxidizing the DNA, lipids and the proteins of living cells [3, 6]. This gives rise to irreversible changes and conformations on the cell level, and to the strong disorganization between the cells. The accumulation of such conformational changes results in premature aging and can even lead to skin cancer [2]. Thus once formed, free radicals should immediately be neutralized by the antioxidative system of the living organism.
Carotenoids effectively neutralize the oxidative action of free radicals, thus quenching excited radical molecules. Carotenoids can neutralize several attacks of free radicals and are then destroyed [1, 10, 11].
The information about the antioxidative action of carotenoids has been published in the paper:
Darvin ME, Gerzonde I, Ey S, Brandt NN, Albrecht H, Gonchukov SA, Sterry W, Lademann J. Noninvasive Detection of beta-Carotene and Lycopene in Human Skin using Raman Spectroscopy. Laser Physics, 14(2): 231-233, 2004.

3.2. Transport of carotenoids in the tissue

The human body cannot synthesize carotenoids itself, thus they need to be obtained systemically with foodstuffs rich in these substances or through topical application.
After the supplementation, dietary carotenoids are incorporated into chylomicrons by intestinal mucosal cells, which are released into the lymph and blood systems [18]. Chylomicrons transport exogenous lipids to liver, adipose, cardiac and other tissues, where they are digested very rapidly by the lipoprotein lipase within the systemic circulation. The released carotenoids accumulate in the appropriate tissue.
Carotenoids are distributed differently among the various lipoprotein classes. Very low density lipoproteins (VLDL), high density lipoproteins (HDL) and most of all low density lipoproteins (LDL) appear to be major carriers for different carotenoids. Carotenoids are found in nearly all tissues including the skin, but the most important storage sites are the liver and adipose tissue.
Carotenoids were measured in the sweat, where they can be associated with different lipoproteins [Darvin, unpublished data]. It seems that they can be delivered with the sweat and via the lipid layers from inside the body to the skin surface, and then penetrate back into the upper skin layers, thus increasing the level of carotenoids in the skin. This hypothesis is supported by the observation of Ekanayake-Mudiyanselage [19], that lipophilic vitamin E is transported by the sweat onto the skin surface.

As the sweat can also contain reactive radical species, the alteration rate of carotenoids in the skin should be strongly pronounced for the palm and forehead, where the highest density of sweat glands was obtained, which will be shown with the results of this study.
Thus there are three main pathways for the transportation of carotenoids to the skin: by blood and lymph flows, by adipose tissue with the aid of diffusion and by the sweat. The first pathways enrich the carotenoid content of the skin from the inside, and the last pathway from the outside.

3.3. Typical kinetics of carotenoid antioxidants in the human skin

As will be shown below in the in-vivo measurements, carotenoid antioxidant substances of the skin decrease subsequent to the influence of different stress factors, such as irradiation, alcohol consumption, fatigue, illness, smoking, and increase through the special supplementation rich in these substances. The kinetics of the degradation/increase and following recovery of the carotenoid antioxidants in the skin through the influence of stress factors/supplementation are characterized by four main parameters: the response time ($T_{response}$), which is measured from the beginning of the influence of stress factor/special supplementation to the moment when the carotenoids start to decrease/increase; the time of degradation/increase ($T_{degradation}/T_{increase}$), which shows the time interval between the moment when the carotenoids start to decrease/increase up to the moment when they achieve the minimum/maximum value; the magnitude of destruction/increase ($h_{destruction}/h_{increase}$) characterizes the quantity of carotenoids, which was destroyed/increased during the time of degradation/increase; and recovery time ($T_{recovery}$), which characterizes the time needed to obtain the initial level of carotenoids in the skin after destruction/increase.
The typical kinetics of the carotenoid substances in the skin, induced by the influence of external and internal stress factors and supplementation has been introduced and described in detail by Darvin et al.:
Darvin ME, Gersonde I, Albrecht H, Sterry W, Lademann J. In vivo Raman spectroscopic analysis of the influence of IR radiation on the carotenoid antioxidant substances beta-carotene and lycopene in the human skin. Formation of free radicals. Laser Physics Letters, 4(4): 318-321, 2007.

3.4. Non-invasive resonance Raman measurements of carotenoids in the skin

For the in-vivo detection of carotenoid antioxidant substances in the human skin, the resonance Raman spectroscopy was used as a fast and non-invasive optical method.
The effect of the scattering of the light from a molecule with the change of wavelength was discovered and described by the Indian scientist Chandrasekhara Venkata Raman in 1928. Therefore, the effect of the combination scattering of the light is named Raman scattering or Raman effect.
The registered Raman bands, which are shifted in the wavelength with respect to the incident light, exactly correspond to the vibrational energy of transitions of the investigated molecule. Different molecules have different vibrational energy of transitions and, as a result, different Raman scattered wavelengths. The intensity of the Raman bands is directly proportional to the concentration of the appropriate molecules in question. Thus the Raman spectrum represents the “spectral fingerprint” of the investigated molecule.
The carotenoids beta-carotene and lycopene are Raman active molecules, i.e., a small amount of incident on the carotenoid molecule light is scattered with the change of wavelength. This weak Raman scattered light, shifted in the wavelength, corresponds to the carbon-carbon double-bond stretch vibration of the conjugated backbone of the investigated carotenoid molecules. This gives information about the concentration of carotenoid molecules in in-vivo as well as in-vitro investigations. The informative Raman signal is then filtered, collected by a lens system and

transferred to a spectrograph, where it is recorded in a spectrum and analyzed by specially developed software.
The experimental arrangement for the in-vivo measurements of carotenoid antioxidant substances in the human skin and the analytical mechanism were developed and described in detail by Darvin et al. and serve as a basis for the present investigation.
The results of the development of the Raman spectroscopic method have been published in the following papers:
Darvin ME, Gerzonde I, Ey S, Brandt NN, Albrecht H, Gonchukov SA, Sterry W, Lademann J. Raman Spectroscopic measurements of beta-Carotene and Lycopene in Human Skin. Proceedings of SPIE, 5474: 20-24, 2004.
Darvin ME, Gersonde I, Meinke M, Sterry W, Lademann J. Non-invasive in vivo determination of the carotenoids beta-carotene and lycopene concentrations in the human skin using the Raman spectroscopic method. Journal of Physics D: Applied physics. 38: 1-5, 2005.
Darvin ME, Gersonde I, Meinke M, Albrecht H, Sterry W, Lademann J. Non-invasive in vivo detection of the carotenoid antioxidant substance lycopene in the human skin using the resonance Raman spectroscopy. Laser Physics Letters, 3(9): 460-463, 2006.

3.5. Sources of irradiation

The skin erythema tester “Dr. Hönle Medizintechnik GmbH” (Germany) was used as a source of UVB radiation. The skin was irradiated with a UVB dose of approx. 30 mJ/cm^2, which was adjusted depending on the skin type of the volunteers, and was sufficient for the formation of an erythema.
The infrared lamp Philips Infrared RI 1521 was used as a source of IR radiation. The skin was irradiated with an IR light at a power density of 190 mW/cm^2 for 30 minutes.

3.6. Measurements of the skin temperature

The skin temperature was measured non-invasively with the non-contact thermometer Rytek Schlender Messtechnik.

3.7. Determination of the skin surface structure

The skin surface structure was analyzed in the non-contact mode using the 3D optical system Primos 4.0 (GFMesstechnik GmbH, Teltow, Germany), as described in detail by Jacobi et al. [21]. The roughness, which is based on the depth and the density of the furrows and wrinkles of the skin, was determined using the software Primos system. High roughness values corresponded to deep furrows and wrinkles with a high density and vice versa.

4. Results and Discussion

4.1. Distribution of carotenoids in human skin

Beta-carotene and lycopene are the main carotenoids present in human skin. Approximately 70% of the total amount of carotenoids in the skin are beta-carotene and lycopene [22]. The distribution of these carotenoids at different body sites (inner palm, forehead, flexor forearm and back) of healthy volunteers was investigated non-invasively by Raman spectroscopic measurements. It was found that the beta-carotene and lycopene distribution in the human skin strongly depends on the skin region and drastically changes inter-individually, reflecting the lifestyle of the volunteers. The beta-carotene and lycopene concentrations in the skin are lower in smokers than in non-smokers and higher in vegetarian groups [23].
The highest concentration of carotenoids was found on the forehead and the palm, which corresponds to the body sites with the highest density of sweat glands [24].
These results are supported by the hypothesis that one of the probable pathways of the transportation of carotenoids to the skin is a penetration of carotenoid-containing sweat back into the skin [Darvin, unpublished data].
These results have been published in the following papers:
Darvin ME, Gersonde I, Albrecht H, Gonchukov SA, Sterry W, Lademann J. Determination of Beta Carotene and Lycopene Concentrations in Human Skin Using Raman Spectroscopy. Laser Physics, 15(2): 295-299, 2005.
Darvin ME, Gersonde I, Meinke M, Sterry W, Lademann J. Non-invasive in vivo determination of the carotenoids beta-carotene and lycopene concentrations in the human skin using the Raman spectroscopic method. Journal of Physics D: Applied physics. 38: 1-5, 2005.

4.2. Variation of carotenoids in the human skin

The daily concentration of carotenoids in the skin of the palm of volunteers was investigated by Raman spectroscopy for a one year period. The results obtained showed individual variations in the level of carotenoid antioxidant substances in the skin of the volunteers, which strongly correlated with the specific lifestyle conditions such as dietary supplementation rich in carotenoids and the influence of possible stress factors. Carotenoid rich diets based on large amounts of fruit and vegetables, which have a large amount of carotenoids in their structure, apparently increase the measured carotenoid antioxidant level of the skin. On the other hand, the carotenoid level in the skin of the volunteers decreased subsequent to the influence of stress factors, such as exhaustion, illness, smoking and alcohol consumption. The subsequent decrease occurred relatively quickly during 24 hours, while the corresponding increase (recovery) usually lasted up to three days [25]. The results obtained showed that the level of carotenoids in the skin reflects the state of the health of volunteers. Thus the divergence of the carotenoid concentration from the average value corresponds to the special events in the lifestyle.
During the summer and autumn months, an increase in the average level of carotenoids in the skin was measured in all volunteers. The average “seasonal increase” of the carotenoid content in the skin was determined to be 1.3-fold and was statistically significant ($p=0.001$).
The seasonal variation is explained by an increased consumption of fruit and vegetables, which are naturally rich in carotenoids, in the daily ration during summer and autumn months. The seasonally increased activation of the sweat glands also contributes to the enrichment of carotenoids in the skin because of the penetration of the carotenoid-containing sweat back into the skin.
These findings will be published in the near future.

4.3. Influence of UV irradiation on the carotenoid concentrations in the skin

It is well known that the influence of high doses of UV irradiation gives rise to the production of a large amount of free radicals that are harmful for the living skin [1, 2, 17].
Using the Raman spectroscopic equipment, the kinetics of the concentration of carotenoids in human skin subsequent to irradiation with UV light was investigated in vivo. Both carotenoids beta-carotene and lycopene showed different kinetics. Lycopene degraded relatively quickly (0 - 30 min) after UV irradiation, while the response time of the beta-carotene concentration varied from 30 up to 90 min. The differences in the response time of beta-carotene and lycopene to UV irradiation are explained by the different quenching rates in the reaction of neutralization of free radicals by these carotenoids. The quenching rate is higher for lycopene than for beta-carotene, which means that lycopene reacts first to the free radicals [10, 26]. The recovery time for both carotenoids needed to obtain an initial level was approximately 2-4 days, depending individually on the volunteer.
Strong correlations between the individual antioxidant levels of volunteers and the magnitude of destruction $h_{destruction}$ of carotenoid antioxidants in the skin (R^2 = 0.93 for beta-carotene and R^2 = 0.85 for lycopene) were found. This means that in the case when volunteers had a high carotenoid level, the percentage of destruction was smaller than if the volunteers had a low level [27]. These results showed the potential injury of the living skin by high doses of UV irradiation, that gives rise to the depletion of the cutaneous antioxidative system.
These observations have been published in the paper:
Darvin ME, Gersonde I, Albrecht H, Sterry W, Lademann J. In-vivo Raman spectroscopic analysis of the influence of UV radiation on carotenoid antioxidant substance degradation of the human skin. Laser Physics, 16(5): 833-837, 2006.

4.4. Influence of IR irradiation on the carotenoid concentrations in the skin

In analogy to the UV irradiation experiments, the influence of IR irradiation on the skin was investigated [20]. IR irradiation is widely used in medicine for the acceleration of wound healing processes [28] and for the warming of muscles, thus increasing the blood and lymph flow, as a result of which the rate of metabolism and recovery increases.
It was found in the in-vivo Raman measurements that both cutaneous carotenoids decreased after IR irradiation. In contrast to the UV irradiation of the skin, the carotenoids beta-carotene and lycopene start to decrease under the IR irradiation almost immediately with a small time delay of about 10 minutes for beta-carotene.
The differences in the response time for UV and IR can be explained by the essential differences in the power densities of UV and IR irradiations (0.3 mW/cm^2 for UV irradiation and 190 mW/cm^2 for IR-irradiation) as well as exposure times (1.5 min and 30 min respectively).
The average magnitude of destruction was 27% for beta-carotene and 38% for lycopene.
The recovery time for both carotenoids was 1-2 days, depending on the volunteer.
Strong correlations were found between the individual carotenoid levels in the skin of volunteers and the magnitude of destruction of carotenoids (R^2=0.78 for beta-carotene and R^2=0.89 for lycopene) [20]. Thus, the volunteers who had a low level of carotenoid antioxidants in the skin had a higher magnitude of destruction after irradiation and vice versa.
It was shown for the first time that IR irradiation of living skin has side effects regarding a decrease in the concentration of the carotenoid antioxidants in the irradiated skin area.
The measured temperature of the skin surface increased from 32 ± 1^0C up to 41 ± 1^0C after IR irradiation of the skin. Taking into consideration the relative heat stability of carotenoids up to at least 50^{o}C, and the absence of absorption in the IR range of spectra, the most likely outcome of the decrease of carotenoids subsequent to IR irradiation of the skin is the neutralization reaction of free radicals, which can be produced in the skin subsequent to IR irradiation. The carotenoids

beta-carotene and lycopene are used as a marker of the process of free radicals formation in the skin subsequent to IR irradiation.
This is a first observation of the production of free radicals in the skin subsequent to the irradiation of the skin surface with IR light.
This data has been published in the paper:
Darvin ME, Gersonde I, Albrecht H, Sterry W, Lademann J. In vivo Raman spectroscopic analysis of the influence of IR radiation on the carotenoid antioxidant substances beta-carotene and lycopene in the human skin. Formation of free radicals. Laser Physics Letters, 4(4): 318-321, 2007.

4.5. Influence of alcohol consumption on the carotenoid concentrations in the skin

Consumption of large amounts of alcohol exerts negative influences on the human body and on the skin [29-32].
Ethanol is metabolized in a multi-step process into various metabolites, which initiate a variety of chemical reactions. Many of these reactions give rise to the production of free radicals and other reactive species, which are harmful for the human body and should be neutralized by the antioxidative system of the human body [33, 34].
It was found that the concentration of carotenoids in the skin start to decrease several minutes after the consumption of high doses of alcohol (1.6 ml ethanol/kg weight on average). The length of alcohol consumption in experiments lasted about 30 minutes. The maximum carotenoid degradation was achieved, on average, 150 minutes after the consumption and constituted 14% to 40% compared with the initial level of carotenoids in the skin, which was measured before the consumption of alcohol.
The immediate decrease of carotenoid antioxidant substances after the alcohol consumption is explained by the neutralization of the enormous quantity of free radicals and other reactive species, which are inevitably produced during the metabolism of ethanol in the organism, and which go out to the skin surface with sweat and via the lipid layers. This conclusion is supported by the in-vivo measurements of the gradual increase of the ethanol concentration on the skin surface after the alcohol supplementation [35].
The recovery time needed to achieve a baseline level of carotenoids in the skin averaged 2-3 days and depended on the individual volunteers and their diet.
Additional experiments were conducted regarding the influence of UV irradiation on the cutaneous antioxidants of volunteers, whose antioxidative potential was already decreased by the consumption of large amounts of alcohol. UV irradiation was performed when the maximal degradation of carotenoids caused by alcohol consumption was achieved.
It was shown that the time needed to obtain an erythema decreased by about 25% and the intensity of erythema on the skin, measured on day following the irradiation, increased by 15-20% for volunteers whose antioxidants had been lowered by the consumption of alcohol.
Thus, the erythema occurs faster and with greater intensity on the skin with impaired antioxidative potential.
These results showed that the negative effect of the influencing stress factor on the antioxidants of the skin, which were already impaired by another stress factor, intensified the degradation of antioxidants.
These observations will be published in the near future.

4.6. Potential methods for increasing the carotenoid level in the skin

The human body can obtain most of the antioxidant substances, including carotenoids, only with dietary supplementations rich in these substances. Fruit and vegetables contain a high amount of naturally balanced carotenoids.

It was shown that a one-time supplementation with a high dosage of foodstuffs containing high amounts of carotenoids, such as ketchup and tomato paste, increased the level of the carotenoids in the skin [36, 37]. The increase in the carotenoid level could usually be observed on the day following the supplementation. The duration of the increase varied between one and two days. The magnitude of the increase in the carotenoids in different volunteers was between 23 and 37%. The recovery time was approximately 2 days, depending on the lifestyle of the individual volunteers.
The studies demonstrate that the antioxidants which are taken up with the food partly accumulated in the skin and were stored there, thus increasing the antioxidative potential of the skin. These kinetics should be taken into consideration and can serve as a modern strategy to increase the antioxidant concentration in the skin, in addition to using topical products containing antioxidants for this purpose.
These results have been published in the following papers:
Darvin ME, Gersonde I, Albrecht H, Sterry W, Lademann J. Resonance Raman spectroscopy for the detection of carotenoids in foodstuffs. Influence of the nutrition on the antioxidative potential of the skin. Laser Physics Letters, 4(6): 452-457, 2007.
Darvin ME, Gersonde I, Albrecht H, Sterry W, Lademann J. Non-invasive in-vivo Raman spectroscopic measurement of the dynamics of the antioxidant substance lycopene in the human skin after a dietary supplementation. Proceedings of SPIE, 6535, 2007.

4.7. Medication with antioxidants

Systemic and topical application of different antioxidant substances for the medical treatment and prophylaxis of many diseases as well as the additional protection of the skin against the destructive action of free radicals and other reactive species has become very popular in recent years. Stimulated by the positive results of a fruit and vegetable diet in supporting medical treatment and in cosmetics, artificial and extracted antioxidant substances have been widely applied. In medical practice, beta-carotene, lycopene, lutein/zeaxanthin, vitamins A, C, D and E antioxidant supplements have been applied to augment the therapy of diseases such as cancer [38], coronary heart disease [39], cardiovascular disease [38, 40], arthritis [41], atherosclerosis [40], Alzheimer's disease [41], skin diseases [42], bone complications [43], asthma [44], age-related cataract and macular degeneration [45] and for photo protection [46].
A diet of fruit and vegetables as well as a herb diet, naturally rich in balanced antioxidants, such as carotenoids, vitamins and others, can exert positive influences on the support of medical therapy of many diseases and on the skin's health and appearance, according to several researchers [47-52]. Surprisingly, the systemic application of synthetic or extracted antioxidants sometimes showed positive, but sometimes also negative effects on the human organism [38, 41, 53, 54].
Literature analysis shows that the negative results obtained by the systemic application of antioxidant substances, in comparison to the positive effects, which were found for a fruit and vegetable diet, seem to be caused by the application of only a single-component antioxidant substance system at relatively high concentration during medical treatment.
The topical application of antioxidants is less critical, because of the small penetration depth of cosmetic formulations into the skin and the absence of direct contact with living cells.
The literature shows that antioxidant substances of the living organism always act as a "protection chain", i.e., different antioxidant substances possess a synergic effect and protect each other from direct destruction in the reactions of neutralization of the free radicals and other reactive species, and that there are critical concentrations where antioxidant substances can change from radical quenchers into radical producers [55].
Taking into consideration all the analyzed data, the topical and systemic application of a mixture of antioxidant substances at low concentrations seems to be more effective for the protection of

the skin. By the use of such combinations, skin health can be promoted from the outside in and from the inside out.
These results have been published in the review paper:
Darvin M, Zastrow L, Sterry W, Lademann J. Effect of supplemented and topically applied antioxidant substances on human tissue. Review. Skin Pharmacol Physiol, 19: 238-247, 2006.

4.8. PPE syndrome and antioxidants

Doxorubicin is a DNA-interacting drug and one of the most potent single chemotherapeutic agents against solid and angiomatous tumors. Doxorubicin is encapsulated in highly stable Stealth® liposomes in order to significantly reduce its toxicity. Nevertheless, severe and dose-limiting mucocutaneous reactions occur, the palmar-plantar erythrodysesthesia syndrome (PPE) being the most common one. This syndrome is mainly located on the palms and plantae, but it may also affect intertriginous sites. The cause of this syndrome is unclear. A better understanding of the pathogenesis of PPE could be the basis for the development of prevention and effective treatment.
In our study, the penetration of Doxorubicin after an intravenous injection from inside out onto the skin was studied non-invasively by laser scanning microscopy (Stratum, OptiScan Ltd., Melbourne). The fluorescence-based measurements were performed and the signal from Doxorubicin was detected at 560 nm on the skin surface.
It was found that Doxorubicin came out from the sweat glands onto the skin surface around 1 hour after an intravenous injection. From there, it was distributed homogeneously onto the skin surface and subsequently penetrated into the stratum corneum.
Intensive fluorescent signals corresponding to Doxorubicin were detected on patient's palms and plantae, where the stratum corneum is approximately 10 times thicker than on the other anatomical sites, and where the density of sweat glands is high. This thick stratum corneum represents an effective reservoir for Doxorubicin, where it can be stored for a long period of time and interact with surroundings.
Cancer treatment by Doxorubicin is partly based on the formation of free radicals, which should destroy the cancer cells. The same effect takes place on the palm and the plantae, where Doxorubicin reacts with the lipid structure of stratum corneum and then with the living cells, causing their destruction and, as a result, the possible manifestation of the PPE syndrome. Several attempts failed to stop the sweat production during the injection. Therefore, antioxidant substances including carotenoids in the form of cream were applied onto the skin surface of the palm to neutralize the produced free radicals. This effect was analyzed non-invasively using resonance Raman spectroscopy. An interaction of the free radicals with the antioxidants was observed if the fluorescent signal of Doxorubicin was detected. The PPE syndrome was not observed on the patients whose skin was pretreated with cream containing carotenoid antioxidants. Consequently, topical application of antioxidant substances seems to be an efficient way to neutralize free radicals and to prevent the PPE syndrome.
The reason why some patients developed a PPE and some did not when Doxorubicin was detected on the skin surface seems to be related to the individual level of antioxidant substances in the skin of the patients.
These results have been published in the paper:
Lademann J, Martschick A, Jacobi U, Richter H, Darvin M, Sehouli J, Oskay-Özcelik G, Blohmer J-U, Lichtenegger W, Sterry W. Investigation of doxorubicin on the skin: A spectroscopic study to understand the pathogenesis of PPE. Supplement to Journal of Clinical Oncology, 23, 16S(1): 5093, 2005.

4.9. Antioxidants protect the skin against aging

During the Raman measurements for the determination of carotenoids in the human skin, it was observed that volunteers with a higher concentration of carotenoids had a younger looking skin in regard to the presence of furrows and wrinkles in relation to their age.
In order to quantify this observation, a study was performed whereby the skin surface structure (depth and density of furrows and wrinkles) was compared to the concentration of beta-carotene and lycopene in the skin. The investigation was carried out on volunteers aged between 40 and 50 years, who had not significantly changed their lifestyle during the last three decades.
There was no statistically significant correlation for beta-carotene, while a strong correlation (R^2 = 0.79) was observed between the concentration of lycopene in the skin and the skin roughness. These results demonstrated that a correlation evidently exists between the appearance of the skin, in particular with regard to furrows and wrinkles, and the level of carotenoid lycopene in the skin.
The measured lycopene in the skin of investigated volunteers had originated from their habitual diet, but not from special food additives or cosmetic products.
The results obtained are very important and clearly support the modern conception of premature aging of the skin, suggesting that premature aging is primarily the result of the accumulation of the defects on the cell level caused by the action of free radicals. Volunteers whose antioxidative level was high had an additional protection against the negative action of free radical and, as a result, were less prone to premature aging.
These results have been published in the following papers:
Darwin M, Schanzer S, Teichmann A, Blume-Peitavi U, Sterry W, Lademann J. Functional Food und Bioverfügbarkeit im Zielorgan Haut. Hautarzt, 57(4): 286-290, 2006.
Darvin M, Gehse S, Schanzer S, Pafzelt A, Benderoth C, Sterry W, Lademann J. Cutaneous concentration of lycopene closely correlates with roughness of the skin. European Journal of Pharmaceutics and Biopharmaceutics, submitted.

5. Conclusions

The concentration of the carotenoids in the skin seems to reflect the current state of the health of volunteers and patients. The possibility to obtain in-vivo kinetics of the carotenoid antioxidants in human skin became possible with the development of a new non-invasive measuring device based on resonance Raman spectroscopy.

The results obtained showed the relatively fast kinetics of the degradation of carotenoid antioxidants in the skin after the influence of stress factors, such as irradiation, tiredness, illness and the consumption of alcohol, accompanied by the production of free radicals. The subsequent recovery is more prolonged. It is brought about by the utilization of the carotenoids, which are stored in the organism, and by the supplemented carotenoid-rich products. Moreover, the carotenoid concentrations in the skin are lower in smokers than in non-smokers and higher in vegetarians, thus reflecting the lifestyle of volunteers.

It was shown that the action of negative stress factors on the skin with impaired antioxidative potential increased the action of a stress factor, thus substantially decreasing the concentration of antioxidants in the skin and enfeebling the defense system.

The formation of free radicals in the skin subsequent to IR irradiation was demonstrated and should be taking into consideration in regard to the wide usage of IR radiation in medical practice. The application of special creams containing antioxidants, as well as increased fruit and vegetable supplementation, should be recommended.

The utilization of a carotenoid-containing cream was shown to neutralize free radicals on the skin surface of cancer patients, which arrived there with the sweat after medication with Doxorubicin. The action of the Doxorubicin-produced radicals on the skin may be the main cause of the PPE syndrome, which is still unknown.

Medication with antioxidants and utilization of antioxidants in cosmetic formulations will be developed in the near future. The utilization of a well-balanced combination of different antioxidants at low concentrations is recommended to prevent possible side effects.

Taking into consideration the strong correlation between the level of lycopene in the skin and the appearance of the skin in regard to furrows and wrinkles, as well as the important role of carotenoids as powerful antioxidants for the organism, the uptake of high amounts of fruit and vegetables, as well as topical application, are important protection strategies against the development of different cutaneous diseases and skin aging.

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Erklärung über den Eigenanteil an den Publikationen

lfd. Nr.	Publikation/ Erläuterung des Anteils von Herrn Maxim Darvin	Anteil von Herrn Darvin in %
1.	Darvin ME, Gerzonde I, Ey S, Brandt NN, Albrecht H, Gonchukov SA, Sterry W, Lademann J. Noninvasive Detection of beta-Carotene and Lycopene in Human Skin using Raman Spectroscopy. Laser Physics, 14(2): 231-233, 2004. Selbstständige Entwicklung und Erprobung der experimentellen Versuchsanordnung (Raman-spektroskopischer Aufbau durch Herrn Darvin; die Mitautoren leisteten konsultative Hilfestellung bzw. wirkten bei der Begutachtung der Ergebnisse mit. Herr Prof. Sterry und ich waren jeweils mit 10 % an der Erstellung der Publikation beteiligt.	60 %
2.	Darvin ME, Gerzonde I, Ey S, Brandt NN, Albrecht H, Gonchukov SA, Sterry W, Lademann J. Raman Spectroscopic measurements of beta-Carotene and Lycopene in Human Skin. Proceedings of SPIE, 5474: 20-24, 2004. Weiterentwicklung der Messmethode durch einen zusätzlichen Detektionskanal, der das von der Haut reflektierte Licht zur Bestimmung des Hauttyps nutzt. Selbstständiger Aufbau der Messapparatur durch Herrn Darvin sowie selbstständige Erprobung. Die Mitautoren gaben konsultative Hilfestellung bzw. waren bei der Bewertung der Messergebnisse beteiligt. Herr Prof. Sterry und ich waren jeweils mit 10 % an der Erstellung der Publikation beteiligt.	60 %
3.	Darvin ME, Gersonde I, Meinke M, Sterry W, Lademann J. Non-invasive in vivo determination of the carotenoids beta-carotene and lycopene concentrations in the human skin using the Raman spectroscopic method. Journal of Physics D: Applied physics. 38: 1-5, 2005. Nach erfolgreicher Entwicklung der Messapparatur durch Herrn Darvin erfolgte die erste Erprobung des Systems unter in-vivo-Bedingungen am Probanden. Diese Arbeiten wurden von Herrn Darvin selbstständig durchgeführt; die Mitautoren leisteten konsultative Hilfestellung bzw. waren an der Begutachtung der Ergebnisse beteiligt. Die Publikation wurde fast vollständig durch Herrn Darvin erstellt.	70 %

lfd. Nr.	Publikation/ Erläuterung des Anteils von Herrn Maxim Darvin	Anteil von Herrn Darvin in %
4.	Darvin ME, Gersonde I, Albrecht H, Gonchukov SA, Sterry W, Lademann J. Determination of Beta Carotene and Lycopene Concentrations in Human Skin Using Raman Spectroscopy. Laser Physics, 15(2): 295-299, 2005. Diese Untersuchungen zur Reproduzierbarkeit der Messungen bzw. zur Karotinoidkonzentration an unterschiedlichen Körperstellen von Probanden unter in-vivo-Bedingungen wurden von Herrn Maxim Darvin selbstständig durchgeführt. Die Mitautoren waren konsultativ unterstützend tätig und an der Bewertung der Ergebnisse beteiligt. Herr Prof. Sterry und ich waren jeweils mit 10 % an der Erstellung der Publikation beteiligt.	65 %
5.	Lademann J, Martschick A, Jacobi U, Richter H, Darvin M, Sehouli J, Oskay-Özcelik G, Blohmer J-U, Lichtenegger W, Sterry W. Investigation of doxorubicin on the skin: A spectroscopic study to understand the pathogenesis of PPE. Supplement to Journal of Clinical Oncology, 23, 16S(1): 5093, 2005. In diesem Artikel werden verschiedene Untersuchungsmethoden zur Analyse der Entstehung des Hand- und Fußsyndroms eingesetzt. Neben der Laser-Scan-Mikroskopie kommt auch die in-vivo-Raman-Spektroskopie zum Einsatz. Diese Raman-spektroskopi-schen Untersuchungen an den Probanden und Patienten wurden von Herrn Darvin selbstständig und allein durchgeführt. Der Anteil dieser Untersuchungen an dem Gesamtuntersuchungsprogramm betrug 25 %. Herr Darvin war auch bei der Erstellung der Publikation, was seinen Teil betrifft, maßgeblich beteiligt.	25 %
6.	Darvin ME, Gersonde I, Meinke M, Albrecht H, Sterry W, Lademann J. Non-invasive in vivo detection of the carotenoid antioxidant substance lycopene in the human skin using the resonance Raman spectroscopy. Laser Physics Letters, 3(9): 460-463, 2006. In dieser Arbeit wird der Einfluss von Stressfaktoren auf das antioxidative Potenzial der Haut analysiert. Die Untersuchungen wurden selbstständig von Herrn Darvin durchgeführt. Die Ko-Autoren waren konsultativ unterstützend tätig. Herr Prof. Sterry und ich waren mit jeweils 10 % an der Erstellung der Publikation beteiligt.	70 %

lfd. Nr.	Publikation/ Erläuterung des Anteils von Herrn Maxim Darvin	Anteil von Herrn Darvin in %
7.	Darvin ME, Gersonde I, Albrecht H, Sterry W, Lademann J. In-vivo Raman spectroscopic analysis of the influence of UV radiation on carotenoid antioxidant substance degradation of the human skin. Laser Physics, 16(5): 833-837, 2006. In dieser Arbeit untersuchte Herr Darvin selbstständig den Einfluss von UV-Strahlung auf das antioxidative Potenzial der Haut. Die Experimente und die Auswertung wurden selbstständig von Herrn Darvin durchgeführt. Die Ko-Autoren waren konsultativ unterstützend tätig. Herr Prof. Sterry und ich waren mit jeweils 10 % an der Erstellung der Publikation beteiligt	75 %
8.	Darvin M, Zastrow L, Sterry W, Lademann J. Effect of supplemented and topically applied antioxidant substances on human tissue. Review. Skin Pharmacol Physiol, 19: 238-247, 2006. Bei dieser Arbeit handelt es sich um einen Übersichtsartikel, der den Einfluss einer systemischen bzw. topischen Applikation von Antioxidantien auf den Gesundheitszustand des Organismus beschreibt. Hierzu wurden von Herrn Darvin hunderte von Publikationen und Studien gesichtet, verglichen und ausgewertet. Diese Analyse erfolgte hauptsächlich durch Herrn Darvin. Die Mitautoren waren bei der Bewertung der Ergebnisse konsultativ beteiligt. Die Erstellung der Publikation erfolgte ausschließlich durch Herrn Darvin.	80 %
9.	Darwin M, Schanzer S, Teichmann A, Blume-Peitavi U, Sterry W, Lademann J. Functional Food und Bioverfügbarkeit im Zielorgan Haut. Hautarzt, 57(4): 286-290, 2006. In dieser Publikation wird der Einfluss der Nahrungsaufnahme auf das antioxidative Potenzial der Haut gezeigt. Die Versuchsplanung erfolgte gemeinsam mit Frau Dr. Teichmann, Frau Prof. Blume-Peytavi und Herrn Prof. Sterry sowie mir. Frau Schanzer war messtechnisch bei der Realisierung der Untersuchung behilflich, indem sie die Hautoberflächenstruktur bestimmte. Der Hauptteil der Arbeit lag jedoch bei der Bestimmung des antioxidativen Potenzials, was ausschließlich durch Herrn Darvin durchgeführt wurde.	70 %

lfd. Nr.	Publikation/ Erläuterung des Anteils von Herrn Maxim Darvin	Anteil von Herrn Darvin in %
10.	Darvin ME, Gersonde I, Albrecht H, Zastrow L, Sterry W, Lademann J. In vivo Raman spectroscopic analysis of the influence of IR radiation on the carotenoid antioxidant substances beta-carotene and lycopene in the human skin. Formation of free radicals. Laser Physics Letters, 4(4): 318-321, 2007. In Analogie zu den Untersuchungen zum Einfluss von UV-Strahlung auf das antioxidative Potenzial führte Herr Darvin Untersuchungen zum Einfluss von Infrarotstrahlung durch. Auch hier erfolgte die Konzeption der Experimente maßgeblich durch Herrn Darvin, wobei die Mitautoren konsultativ behilflich waren. Die Durchführung der Untersuchungen lag allein bei Herrn Darvin, die Auswertung und die Publikation maßgeblich bei Herrn Darvin mit Unterstützung der Autoren.	80 %
11.	Darvin ME, Gersonde I, Albrecht H, Sterry W, Lademann J. Resonance Raman spectroscopy for the detection of carotenoids in foodstuffs. Influence of the nutrition on the antioxidative potential of the skin. Laser Physics Letters, 4(6): 452-457, 2007. Diese Untersuchungen stellen eine Erweiterung der Ergebnisse dar, welche in Publikation Nr. 10 dargestellt sind. Die Verteilung der Aufgaben und die Aktivitäten der Ko-Autoren sind analog zu Publikation 10.	80 %
12.	Darvin ME, Gersonde I, Albrecht H, Sterry W, Lademann J. Non-invasive *in-vivo* Raman spectroscopic measurement of the dynamics of the antioxidant substance lycopene in the human skin after a dietary supplementation. Proceedings of SPIE, 6535, 2007. Die Arbeitsaufgaben und die Verteilung der Anteile der Ko-Autoren an der Versuchsplanung, Durchführung und Auswertung entsprechen den Angaben in Publikation 10.	80 %

Berlin, den 31.05.2007

Maxim E. Darvin

Eidesstattliche Erklärung

Ich, Maxim E. Darvin, erkläre, dass ich die vorgelegte Dissertationsschrift mit dem Thema: “Kinetics of carotenoid antioxidant substances in the human skin”, selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutz, ohne die unzulässige Hilfe Dritter verfasst und auch in Teilen keine Kopien anderer Arbeiten dargestellt habe.

Berlin, den 31.05.2007

Maxim E. Darvin