Aus der Klinik für Dermatologie, Venerologie und Allergologie
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

The influence of chemotherapy and lifestyle on the antioxidative status of human skin

zur Erlangung des akademischen Grades
Doctor medicinae (Dr. med.)

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von

Bich Na Lee

aus Berlin

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For my parents

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Abstract (English)

Background: Antioxidants are a protective and defensive mechanism against free radicals, also known as reactive oxygen species. As free radicals and oxidative stress are involved in many pathologic phenomena, such as premature skin aging, immunosuppression and cardiovascular diseases, the question arose, how the external and internal factors, especially nutrition or stress, can influence the antioxidant status. In addition, the differences in antioxidant status between two countries, South Korea and Germany, were investigated to determine the cultural influence.

Material and Methods: Using a miniaturized mobile measuring device based on reflectance spectroscopy, the cutaneous carotenoid concentrations of healthy volunteers were investigated. The volunteers were of different age, social and cultural groups. In addition, questionnaires were applied to assess the nutritional behavior and stress circumstances as well as smoking and alcohol consumption habits. Furthermore, the antioxidant status of cancer patients was measured to determine the possible pathomechanism of palmoplantar erythrodysesthesia (PPE), a frequent adverse effect of chemotherapeutic agents.

Results: The results of these investigations were published in four scientific papers. Both healthy nutrition with antioxidant-rich food and stress management are crucial for the higher cutaneous carotenoid concentration. As antioxidant status results from multifactorial elements, the lifestyle of volunteers could be reflected in the antioxidant status. The studies highlighted the importance of stress management and avoiding stress. The measurements with the reflectance spectroscopy based mobile device could inspire the volunteers to have healthy nutrition and to avoid stress. The cutaneous carotenoid concentration of cancer patients after the systemic administration of chemotherapeutic agents decreased. The reduction could be shown in all investigated drugs.

Conclusion: The measuring device with its non-invasiveness, handiness and mobility is practical to assess the antioxidant status of individuals quickly. The application of this device in public places such as schools and companies can promote healthy lifestyle effectively. For cancer patients receiving chemotherapy, an antioxidant-containing ointment can be a preventive and therapeutic option for PPE.

Abstract (German)

<u>Hintergrund:</u> Antioxidantien sind Schutzmechanismen gegen freie Radikale, die auch als reaktive Sauerstoffspezies bekannt sind. Da freie Radikale und oxidative Stress mit vielen pathologischen Ereignissen wie vorzeitige Hautalterung, Immunsuppression und kardiovaskuläre Erkrankungen assoziiert sind, stellte sich die Frage, wie externe und interne Faktoren, besonders Ernährung oder Stress, das antioxidative Potential (AOP) beeinflussen. Außerdem wurden Unterschiede des AOPs zwischen zwei Ländern, Südkorea und Deutschland, untersucht, um den kulturellen Einfluss auf die kutanen Antioxidantien herauszufinden.

<u>Material und Methoden:</u> Mittels eines miniaturisierten und mobilen Messgeräts, das auf Reflexionsspektroskopie basiert ist, wurden die Carotenoidkonzentrationen in der Haut bei den gesunden Probanden gemessen. Die Probanden waren von ihrem Alter und sozialer und kultureller Herkunft unterschiedlich. Zudem wurden die Ernährungsgewohnheiten und stressige Ereignisse sowie Rauchen und Alkoholkonsum mithilfe vom Fragebogen erfasst. Darüber hinaus wurde das AOP der Krebspatienten bestimmt, um den Pathomechanismus der Palmoplantaren Erythrodysästhsie, einer häufigen Nebenwirkung von Chemotherapeutika, näher zu verstehen.

<u>Ergebnisse:</u> Die Ergebnisse der Studien wurden in vier wissenschaftlichen Publikationen veröffentlicht. Antioxidantienreiche gesunde Ernährung und Stressmanagement sind wichtig für die hohe Carotenoidkonzentraion in der Haut. Da multiple Faktoren für das AOP verantwortlich sind, konnten sich die Lebensgewohnheiten der Probanden im AOP widerspiegeln. Die Studien hoben die Bedeutsamkeit der Stressbewältigung und der Vermeidung von Stress hervor. Die Messung mit dem mobilen, auf Reflexionsspektroskopie basierten Messgerät konnte die Probanden anregen, sich gesund zu ernähren und Stress zu vermeiden. Die kutanen Carotenoidkonzentrationen der Krebspatienten nahmen nach der systemischen Gabe von Chemotherapeutika ab. Der Rückgang des Messwertes konnte in allen untersuchten Chemotherapeutika gezeigt werden.

<u>Schlussfolgerung:</u> Das nichtinvasive, praktische und mobile Messgerät ist zur Bestimmung von AOP schnell und einfach einsetzbar. Die Anwendung des Messgeräts in öffentlichen Einrichtungen wie Schulen und Firmen kann den gesunden Lebensstil effektiv fördern. Für Krebspatienten, die Chemotherapie bekommen, kann eine antioxidantienhaltige Salbe eine Möglichkeit zur Prävention und Therapie für die Palmoplantare Erythrodysästhesie sein.

1. Introduction

1.1 Antioxidants

In the last few decades, public awareness of health increased excessively. It led to a growing interest in antioxidants and their role in health. Antioxidants have been especially of particular interest to researchers. A large number of free radicals resulting from external environmental or internal factors involve developments of various pathological conditions and diseases, e.g. premature skin aging, cancer, immunosuppression and cardiovascular disease by destroying cells and cell compartments[1]. Antioxidants, as a defense mechanism, neutralize free radicals, also known as reactive oxygen species (ROS). Antioxidants include both enzymatic, such as superoxide dismutase, glutathione peroxidase and catalase, and non-enzymatic substances, such as carotenoids, vitamins (A, C, D and E), flavonoids and other substances[2]. They all have together synergistic effects by acting as a protective chain. Most of the antioxidants cannot be synthesized by the human organism in sufficient amounts or fully[3]. Therefore, it has to be taken from the nutrition that is rich in antioxidants such as fruits and vegetables. Since human skin is the biggest organ of the human body and is in direct contact to the environment, the protective effect of antioxidants in the skin is crucial. While the intake of the antioxidant-rich nutrition increases the antioxidant level in the skin, the external environmental and behavioral factors, e.g., UV-radiation of the sun, smoking and alcohol consumption, as well as internal factors like stress, sleep deprivation and illness, could have a negative effect on the antioxidant potential in the skin[4]. In human skin, carotenoids play an important role in fighting against free radicals[5]. Additionally, they represent marker substances for the whole antioxidative status of human skin[6]. A previous study could demonstrate that cutaneous carotenoids can serve as a biomarker for the complete antioxidant status in the human body[7]. Consequently, this biomarker could be applied to investigate the changes of antioxidant level and to help understand further about multifactorial influences on antioxidant potential in the body.

1.2 Aims of studies

The following studies were carried out to investigate the influence of lifestyle such as nutritional behavior and stress circumstances as well as to explore the changes in the antioxidant potential of cancer patients after exposure to the certain chemotherapeutics.

Through daily assessment of cutaneous carotenoids among teenagers attending high school (Publication 1) and middle-aged women (Publication 2), the trend of antioxidant potential depending on their eating habits and stress condition was investigated. The differences in the cutaneous carotenoid concentration between South Korea and Germany, were examined (Publication 3) to determine the discrepancy of the antioxidant levels and the effects of nutritional behavior and individual lifestyle based on different cultural backgrounds. In this binational study, Korean volunteers living in Germany were also investigated to detect the adaptation of the lifestyle and its effect on the cutaneous carotenoid concentration.

Not only were the healthy patients without any severe disease the subject of the study but also cancer patients who were treated with chemotherapeutic agents (Publication 4). The pathomechanism of palmoplantar erythrodysesthesia (PPE), a frequent dermal side effect of chemotherapeutic agents, has remained mostly unclear for the causing drugs. PPE, also known as hand-foot-syndrome, is not life threatening, but decreases the quality of life for affected patients. To understand the pathomechanism of PPE for prevention and therapy, the changes of the cutaneous carotenoid concentration prior to and after the treatment with systemic chemotherapeutic agents were assessed.

2. Methodology

2.1 Measuring instrument / Reflectance spectroscopy

The measurements in all studies were performed with a noninvasive portable measuring Biozoom® sensor which is based on the principle of reflectance spectroscopy. Earlier methods for the determination of the antioxidant level were problematic in the invasiveness, as they needed either blood or skin samples for their measurements[8,9]. Besides their invasiveness, these measurements were also expensive and time-consuming.

As mentioned above, carotenoids can serve as marker substances for the whole antioxidant level. In addition, there was a correlation between the concentration of cutaneous carotenoids and the concentration of serum carotenoids[10]. The new measuring device used in our research applies a light emitting diode (LED) at the wavelength of 465±25 nm which is the maximum absorption range of carotenoids. The Biozoom® sensor carries an optical window emitting a ray of light for measuring. This channel window is brought into tight contact with skin, which enables it to exclude all the unnecessary lights from the outside. The signal reflected from the tissue goes back into this window for the analysis. The results were displayed in the linear scale from 1 to 12 in arbitrary units. The measurements were performed on the thenar muscle of both right and left hands. Each measurement took about 60 to 90 seconds. The detailed description about the measuring device can be found in a study of Darvin et al.[6].

2.2 Study designs and subjects

The study subjects in all four studies were recruited on volunteer basis. The studies included in this dissertation present various study designs and groups of study subjects of different age, social backgrounds and even cultural heritages. The details of the four publications are described in the following:

Publication 1:

"Spectroscopic analysis of the influence of nutrition, lifestyle enhancement and biofeedback on the cutaneous carotenoid concentration of adolescents"

Yu RX, Köcher W, Darvin ME, Büttner M, Jung S, Lee BN, Klotter C, Hurrelmann K, Meinke MC, Lademann J. J Biophotonics 2014;7:926-37.

The antioxidant level of 50 high school students between 17 and 20 years was investigated in Germany. The study consisted of three phases which were a static phase, an intervention phase and a follow-up. In the static phase, the students were informed about the research. Subsequently, the carotenoid concentrations of the students were measured. The measured values were not disclosed to the students in this phase. In the intervention phase, the students were instructed about healthy nutrition and lifestyle and asked to work on their lifestyle according to the lecture. The measured values were revealed to each student as a biofeedback. Five months later, the follow-up was performed without any previous notice in order to review the sustainability of the effects achieved in the intervention phase.

The measurements were carried out twice a week with a phase of 4 weeks. Before each measurement, the questionnaires about lifestyle, eating habits and stress factors such as smoking, illness or specific stress events were filled in by students.

Publication 2:

"Cutaneous carotenoids: the mirror of lifestyle?"

Lademann J, Köcher W, Yu RX, Meinke MC, Lee BN, Jung S, Sterry W, Darvin ME. Skin Pharmacol Physiol 2014;27:201.

In this study, the cutaneous carotenoids values of four female German residents, aged 42 to 45, were observed in a period of 13 days. The assessment of the carotenoid concentration was carried out every day and they saw their values every evening. They were asked to keep a journal about their diet and any psychological or physical stress factors in this period.

Publication 3:

"Antioxidants in Asian-Korean and Caucasian Skin: The influence of Nutrition and Stress"

Jung S, Darvin ME, Chung HS, Jung B, Lee SH, Lenz K, Chung WS, Yu RX, Patzelt A, Lee BN, Sterry W, Lademann J. Skin Pharmacol Physiol 2014;27:293-302.

In this binational study, a total of 714 healthy volunteers with different ethnic and cultural backgrounds were recruited to explore whether nutrition- and culture-related lifestyle was reflected in their antioxidant values. There were three main groups of volunteers: South Korean residents, Korean immigrants in Germany and German volunteers in Germany.

The Korean cuisine has been widely regarded as healthy because of its large amount of raw fruits and uncooked vegetables. Compared to the Korean cuisine, the German cuisine includes dairy products and boiled dishes such as potatoes and noodles. Vegetables are usually served boiled or fried. Besides the differences of the cuisine in both countries, the different culture-related stress perceptions were a crucial point in this study. Since Korean society puts enormous pressure on individual success based on hard work, academic achievements and a high position in the job, the question arose, if this pressure was reflected in Koreans' cutaneous carotenoids concentration in spite of their healthy nutrition by traditional Korean food.

Publication 4:

"The influence of chemotherapy on the antioxidant status of the human skin"

Lee BN, Jung S, Darvin ME, Eucker J, Kühnhardt D, Sehouli J, Patzelt A, Fuss H, Yu RX, Lademann J. Anticancer Res 2016;36:4089-93.

In this study, 42 cancer patients who received chemotherapy were investigated to determine the change of cutaneous carotenoid concentration after exposure to chemotherapeutic agents. The carotenoid levels in the skin of the patients were measured prior to and after the systemic administration of chemotherapeutic agents. The cytotoxic drugs investigated in this study were paclitaxel, docetaxel and 5-fluorouracil (5-FU). The patients included in this research suffered from one of the following cancers: primary or metastatic breast or ovarian cancer, oral, nasopharyngeal, pancreatic or colorectal cancer, or neuroendocrine tumor. They were only included if they did not show any sign of pathological skin lesion nor initial symptoms of PPE such as swelling, erythema, dysesthesia or paresthesia on their palms and soles.

2.3 Statistical analysis

For data acquisition and statistical analysis, IBM SPSS version 19.0.0 and 22 for Windows, Microsoft Office Excel 2007 and Microsoft Excel 2008 for Macintosh were used. Student's t test, analysis of variance (ANOVA), analysis of covariance (ANCOVA), post hoc Scheffé's test, post hoc Tamhane's T2 test, Mann-Whitney test and Wilcoxon signed-rank test were applied for appropriate calculation and analysis.

3. Results

3.1 Summary of publication 1:

"Spectroscopic analysis of the influence of nutrition, lifestyle enhancement and biofeedback on the cutaneous carotenoid concentration of adolescents"

Yu RX, Köcher W, Darvin ME, Büttner M, Jung S, Lee BN, Klotter C, Hurrelmann K, Meinke MC, Lademann J. J Biophotonics 2014;7:926-37.

The high school students between the age of 17 and 20 were noninvasively measured for their cutaneous carotenoid concentration in three phases. The students were highly motivated and participated in all measurements, when they were present at school. In phase 1, the steady phase, an average attendance of the whole 50 students were 88%. The questionnaires besides measurements were mostly properly completed. The questions about alcohol consumption were often answered incompletely. The measurements in the steady phase showed the correlation between the volunteers' lifestyles and the cutaneous carotenoid concentrations. It became evident that healthy nutrition, non-smoking behavior and good health condition lead to a high carotenoid concentration. The questionnaires that accompanied the measurements clearly showed that the combination of diverse healthy lifestyle habits and circumstances such as non-smoking, eating fruits and vegetables and the absence of illness has a positive effect on the cutaneous antioxidant concentration. It was also found that a balanced psychosocial status is associated with high carotenoid concentration in the skin. It could be observed how the subjective view on the quality of life influences the cutaneous carotenoid concentration. In the intervention phase, 30 students had increased values of carotenoids. Mainly the volunteers with a low baseline in phase 1 attained the most significant increase in their carotenoid concentration in phase 2. In phase 2, 22% of smokers did not smoke at least in this phase, and the number of cigarettes the smokers consumed was lower.

In the third phase, the follow-up after five months without any notice, a sustainability of the achieved effects on healthier lifestyle and the carotenoid concentration could be found. Even fewer students smoked in the third phase than in the intervention phase.

3.2 Summary of publication 2:

"Cutaneous carotenoids: the mirror of lifestyle?"

Lademann J, Köcher W, Yu RX, Meinke MC, Lee BN, Jung S, Sterry W, Darvin ME. Skin Pharmacol Physiol 2014;27:201.

The daily measurements of a period of 13 days with four female volunteers showed that the cutaneous carotenoid concentration could be a reflection of a lifestyle. Through the observation of changes in the volunteers' carotenoids value, it became clear that the daily nutrition, illness and stress level are associated with the cutaneous carotenoid concentration. The measurements of four volunteers showed a gradual increase of carotenoid levels after the intake of healthy meals, while the stressful circumstances and illness caused an immediate decline in their cutaneous carotenoid concentration. In spite of their healthy diet, the negative effect of stressors was reflected in their carotenoid levels. In contrast with a previous study in which massive amount of alcohol caused a sudden decrease in antioxidant concentration[11], the volunteers in this study had constant antioxidant levels after alcohol consumption. It could be assumed that a reasonable amount of alcohol did not affect the carotenoid concentration excessively.

3.3 Summary of publication 3:

"Antioxidants in Asian-Korean and Caucasian Skin: The influence of Nutrition and Stress"

Jung S, Darvin ME, Chung HS, Jung B, Lee SH, Lenz K, Chung WS, Yu RX, Patzelt A, Lee BN, Sterry W, Lademann J. Skin Pharmacol Physiol 2014;27:293-302.

The examination of the cutaneous carotenoid concentration in the groups of 279 South Korean residents, 332 Germans and 103 Korean immigrant volunteers in Germany could show that the group of South Korean residents have a significantly higher carotenoid mean concentration than both the group of German volunteers and the group of Korean immigrants in Germany. In the group of Korean immigrants, the first generation of Koreans who are over the age of 50 showed a significantly higher carotenoid mean concentration than the younger generations of Koreans who were mostly born or grew up in Germany.

The questionnaires about their nutrition and stress in the life of volunteers could demonstrate the differences of daily diet in South Korea and Germany. While 100% of Korean immigrants in Germany consumed vegetables and fruits every day, the frequency in intake of vegetables and fruits varied greatly in the group of German volunteers. Among Korean immigrants, the volunteers who consumed a mixture of Korean and Western food had a decreased antioxidant level compared to the other volunteers who had only traditional Korean food in their meals.

The influence of stress in their cutaneous carotenoid concentration could also be found. Korean residents in South Korea had lower level of antioxidants despite their daily consumption of vegetables and fruits highly rich in antioxidants. The gender-adjusted analysis with ANCOVA could show the significant effect of stress depending on age.

3.4 Summary of publication 4:

"The influence of chemotherapy on the antioxidant status of the human skin"

Lee BN, Jung S, Darvin ME, Eucker J, Kühnhardt D, Sehouli J, Patzelt A, Fuss H, Yu RX, Lademann J. Anticancer Res 2016;36:4089-93.

The investigation of changes in the cutaneous carotenoid concentration subsequent to chemotherapy was performed in 42 cancer patients. The measurement prior to and after the systemic administration of chemotherapeutic agents showed a significant reduction in the carotenoid levels subsequent to chemotherapy. All chemotherapeutic agents included in this study, which were paclitaxel, docetaxel and 5-FU, respectively, caused a significant decrease of the antioxidant status.

As the volunteers suffered from cancer which can possibly cause psychological and physical stress, the cutaneous carotenoid concentrations of the cancer patients were expected excessively lower than shown in this study. It varied considerably between patients.

4. Discussion

<u>Publication 1</u>

The study was conducted to investigate whether lifestyle habits of adolescents have an influence on the cutaneous carotenoid concentrations and how the instant biofeedback influences the lifestyle of teenagers.

This study showed that eating habits and stressful events have an effect on the cutaneous carotenoid concentrations. Higher cutaneous concentrations was associated with non-smoking behavior, healthy nutrition and little stress. The carotenoid concentrations of subjects investigated

in this study were higher than those of the average adult population.
In spite of the significant results, the study may not be fully generalized due to the small number of subjects. Moreover, it should be taken into account that the nutrition and stress assessments through questionnaire are subjective evaluations of the volunteers. It is also possible that the effect of intervention in phase 2 was underestimated, as the students had many incidents of illness and more exams associated with higher stress level. The high baseline values and the change of lifestyle made already in the first phase by some students could have contributed to smaller increase in the intervention phase as well.
A biofeedback system on cutaneous carotenoids and the noninvasive measurement at easily accessible body sites, such as hands, demonstrated a possible health prevention program which can help teenagers to form a healthy lifestyle.

Publication 2

The carotenoid concentrations of the volunteers clearly reflected their lifestyle. As the volunteers constantly consumed fruits and vegetables, the gradual increase of their carotenoid values could be observed. While the intake of healthy nutrition led to a slow increase in the carotenoid concentrations, the stressful events caused a sudden decrease in the carotenoid values. Despite healthy eating habits of the volunteers, the stressors had a negative effect on their antioxidant status. It revealed that the prevention and stress management are as important as healthy nutritional behavior for a high antioxidant level.

Publication 3

By comparing populations from two different countries, South Korea and Germany, the study showed that eating habits containing a large amount of vegetables and fruits and the cuisine play a pivotal role in high carotenoid values. Depending on age groups, the carotenoid concentration of Korean residents was associated with their stress level. The age group from 13 to 17 years showed a strongly reduced antioxidant status. This is possibly due to the massive competition in the Korean educational system. It causes extremely high social and psychological stress for teenagers in South Korea. Besides the competitive educational system, there is huge social and economic pressure in Korean society. Rigid hierarchy, collectivism and longer work hours result in the stress environment in Korea. The high level of stress exposure was reflected in the carotenoid concentration of Korean residents.
Though the clear effect of nutrition and stressful events on the antioxidant status, it should be taken into account that Koreans avoid direct sun exposure, since white and clear skin is highly

appreciated in Korea. This behavior can enhance the accumulation of antioxidants additionally.

<u>Publication 4</u>

This study revealed another negative factor to the cutaneous carotenoid concentration. As the main cytotoxic effects of the chemotherapeutic agents included in this study are not based on the radical formation, this result points out that oxidative stress might play an important role in the pathomechanism of PPE induced by these agents. Thus, the topical application of an antioxidant-containing ointment could be preventive and therapeutic for PPE associated with paclitaxel, docetaxel and 5-FU.

Since there are many factors which can affect the antioxidant status of individuals such as psychological stress, it is possible that the clinical setting for chemotherapy and the therapy itself caused the psychological distress to the volunteers.

The studies described in this dissertation elucidated that the antioxidant status in the skin can be assessed quickly and easily by using the noninvasive, mobile device based on reflectance spectroscopy. The application of the device in public places like schools and companies can promote a healthy lifestyle with healthy nutrition and stress management, which would be an effective way of prevention. Because of its handiness and non-invasiveness, the device could also be used effectively in clinical research for other skin diseases.

5. Bibliography

1. Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A. Biomarkers of oxidative damage in human disease. Clin Chem 2006;52:601-23.

2. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007;39:44-84.

3. Lademann J, Meinke MC, Sterry W, Darvin ME. Carotenoids in human skin. Exp Dermatol 2011;20:377-82.

4. Darvin ME, Patzelt A, Knorr F, Blume-Peytavi U, Sterry W, Lademann J. One-year study on the variation of carotenoid antioxidant substances in living human skin: influence of dietary supplementation and stress factors. J Biomed Opt 2008;13:044028.

5. Stahl W, Sies H. Antioxidant activity of carotenoids. Mol Aspects Med 2003;24:345-51.

6. Darvin ME, Sandhagen C, Koecher W, Sterry W, Lademann J, Meinke MC. Comparison

of two methods for noninvasive determination of carotenoids in human and animal skin: Raman spectroscopy versus reflection spectroscopy. J Biophotonics 2012;5:550-8.

7. Haag SF, Taskoparan B, Darvin ME, Groth N, Lademann J, Sterry W, Meinke MC. Determination of the antioxidative capacity of the skin in vivo using resonance Raman and electron paramagnetic resonance spectroscopy. Exp Dermatol 2011;20:483-7.

8. Talwar D, Ha TK, Cooney J, Brownlee C, O'Reilly DS. A routine method for the simultaneous measurement of retinol, alpha-tocopherol and five carotenoids in human plasma by reverse phase HPLC. Clin Chim Acta 1998;270:85-100.

9. Scarmo S, Cartmel B, Lin H, Leffell DJ, Welch E, Bhosale P, Bernstein PS, Mayne ST. Significant correlations of dermal total carotenoids and dermal lycopene with their respective plasma levels in healthy adults. Arch Biochem Biophys 2010;504:34-9.

10. Meinke MC, Darvin ME, Vollert H, Lademann J. Bioavailability of natural carotenoids in human skin compared to blood. Eur J Pharm Biopharm 2010;76:269-74.

11. Darvin ME, Sterry W, Lademann J, Patzelt A. Alcohol consumption decreases the protection efficiency of the antioxidant network and increases the risk of sunburn in human skin. Skin Pharmacol Physiol 2013;26:45-51.

Affidavit

I, Bich Na Lee, certify under penalty of perjury by my own signature that I have submitted the thesis on the topic "The influence of chemotherapy and lifestyle on the antioxidative status of human skin". I wrote this thesis independently and without assistance from third parties, I used no other aids than the listed sources and resources.

All points based literally or in spirit on publications or presentations of other authors are, as such, in proper citations (see "uniform requirements for manuscripts (URM)" the ICMJE www.icmje.org) indicated. The sections on methodology (in particular practical work, laboratory requirements, statistical processing) and results (in particular images, graphics and tables) correspond to the URM (s.o) and are answered by me. My contributions in the selected publications for this dissertation correspond to those that are specified in the following joint declaration with the responsible person and supervisor. All publications resulting from this thesis and which I am author of correspond to the URM (see above) and I am solely responsible.

The importance of this affidavit and the criminal consequences of a false affidavit (section 156, 161 of the Criminal Code) are known to me and I understand the rights and responsibilities stated therein.

Date Signature

<u>Declaration of any eventual publications</u>

Bich Na Lee had the following share in the following publications:

<u>Publication 1:</u> Yu RX, Köcher W, Darvin ME, Büttner M, Jung S, **Lee BN**, Klotter C, Hurrelmann K, Meinke MC, Lademann J. Spectroscopic analysis of the influence of nutrition, lifestyle enhancement and biofeedback on the cutaneous carotenoid concentration of adolescents. J Biophotonics 2014;7:926-37.

Contribution in detail: 20%.

The doctoral candidate contributed to this publication in several ways, including developing study design, data interpretation and revision of the manuscript.

Publication 2: Lademann J, Köcher W, Yu RX, Meinke MC, **Lee BN**, Jung S, Sterry W, Darvin ME. Cutaneous carotenoids: the mirror of lifestyle? Skin Pharmacol Physiol 2014;27:201.
Contribution in detail: 20%.
The doctoral candidate collected data, performed data interpretation and revised the manuscript.

Publication 3: Jung S, Darvin ME, Chung HS, Jung B, Lee SH, Lenz K, Chung WS, Yu RX, Patzelt A, **Lee BN**, Sterry W, Lademann J. Antioxidants in Asian-Korean and Caucasian Skin: The influence of Nutrition and Stress. Skin Pharmacol Physiol 2014;27:293-302.
Contribution in detail: 20%.
The doctoral candidate partly performed experiments, worked on analysis and interpretation of data and revised the manuscript.

Publication 4: **Lee BN**, Jung S, Darvin ME, Eucker J, Kühnhardt D, Sehouli J, Patzelt A, Fuss H, Yu RX, Lademann J. The influence of chemotherapy on the antioxidant status of the human skin. Anticancer Res 2016;36:4089-93.
Contribution in detail: 80%.
The doctoral candidate carried out all experiments. These include the development of study design, the recruitment of study subjects, performing the measurements and statistical analysis, data interpretation, preparation of the figures, drafting and revising the manuscript.

Signature, date and stamp of the supervising University teacher

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Signature of the doctoral candidate

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Selected publications

Publication 1: Yu RX, Köcher W, Darvin ME, Büttner M, Jung S, **Lee BN**, Klotter C, Hurrelmann K, Meinke MC, Lademann J. Spectroscopic analysis of the influence of nutrition, lifestyle enhancement and biofeedback on the cutaneous carotenoid concentration of adolescents. J Biophotonics 2014;7:926-37.

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Influence of Chemotherapy on the Antioxidant Status of Human Skin

BICH NA LEE[1], SORA JUNG[1], MAXIM E. DARVIN[1], JAN EUCKER[2], DAGMAR KÜHNHARDT[2], JALID SEHOULI[3], RADOSLAV CHEKEROV[3], ALEXA PATZELT[1], HARALD FUSS[4], RUO-XI YU[1] and JÜRGEN LADEMANN[1]

[1]Center of Experimental and Applied Cutaneous Physiology, Department of Dermatology, Venereology and Allergology, and Departments of [2]Hematology and Oncology, and [3]Gynecology, Charité – University of Medicine Berlin, Berlin, Germany; [4]Department of Hematology, Oncology and Palliative Care, Helios Hospital, Bad Saarow, Germany

Abstract. *Background: Palmoplantar erythrodysesthesia is a frequent dermal side-effect during chemotherapy. Previous investigations showed radical formation subsequent to doxorubicin infusion and preventative and therapeutic effects of an antioxidant-containing ointment. Patients and Methods: Using a non-invasive vivomeasuring system (Biozoom®; Biozoom Services GmbH, Kassel, Germany) changes in the antioxidant status (as measured by relative carotenoid concentration) of the skin prior to and after intravenous administration of paclitaxel, docetaxel and 5-fluorouracil were investigated in 42 patients with cancer. Results: A significant decrease of antioxidant concentration subsequent to intravenous administration was found for all investigated chemotherapeutic agents. The mean concentration of carotenoids decreased from 3.59±1.26 arbitrary units (a.u.) to 3.41±1.28 a.u. (p<0.001) after paclitaxel administration, from 6.33±2.43 to 5.63±2.29 a.u. after docetaxel (p=0.027) and from 4.26±1.81 to 3.98±1.53 a.u. (p=0.042) after 5-fluorouracil infusion. Conclusion: Oxidative stress might play a significant role in the pathomechanism of palmoplantar erythrodysesthesia associated with paclitaxel, docetaxel and 5-fluorouracil. Therefore, an antioxidant-containing ointment might serve as preventative and therapeutic option.*

Developed in the early 20th century, chemotherapy is an effective way to fight cancer cells using a variety of potent chemotherapeutic agents (1). These treatments have specific effects on arresting the progression of tumor cells by acting in various stages of the cell cycle (2, 3). Antimetabolites, such as 5-fluorouracil (5-FU), hamper the synthesis of DNA or RNA either by inhibiting the production of normal purine or pyrimidine precursors, or by interfering with them (3). Anthracyclines, such as doxorubicin, not only intercalate into DNA, but also produce free radicals causing single-strand breaks in DNA (3).

Although anticancer therapy is targeted to eliminate cancer cells, there exists a variety of side-effects affecting healthy cells of other organs. A frequent dermal side-effect due to chemotherapeutic treatment is hand–foot syndrome, also known as palmar-plantar erythrodysesthesia (PPE). This is a common dose-limiting adverse effect under treatment with several agents, such as pegylated liposomal doxorubicin (PLD), 5-FU, capecitabine, docetaxel, or tyrosine-kinase inhibitors such as sorafenib and sunitinib (4-6). The initial symptoms are swelling or erythema in the palmar and interdigital areas of the hands and soles of the feet, accompanied by dysesthesia and paresthesia, which can progress to burning pain with dryness, rhagades, desquamation, ulceration, edema and rash (5). PPE is rarely life-threatening, but it can significantly impair the quality of life of patients, leading to the need for dose reduction or interruption of therapy (6). Depending on the severity, there are three grades of PPE according to the National Cancer Institute (Table I).

The exact pathomechanisms of PPE are unclear for most chemotherapeutic agents and treatment options are very limited. Martschick *et al.* showed that systemically applied PLD, one of the most widely used antitumor agents, is secreted with sweat onto the skin surface, spreading over the skin and penetrating into the stratum corneum as if topically applied (7). Using *in vivo*laser scanning microscopy, PLD was detected on the skin surface about an hour after

Correspondence to: Sora Jung, MD, Center of Experimental and Applied Cutaneous Physiology, Department of Dermatology, Venereology and Allergology, Charité – Universitätsmedizin Berlin, Germany.

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Table I. *Classification of palmar-plantar erythrodysesthesia (25).*

Grade	Definition
1	Minimal skin changes (*e.g.* erythema, swelling, or hypokeratosis) without pain
2	Skin changes (*e.g.* peeling, blisters, bleeding, edema, or hyperkeratosis) with pain; limiting instrumental ADL*
3	Severe skin changes (*e.g.* peeling, blisters, bleeding, edema, or hyperkeratosis) with pain; limiting self care ADL**

ADL: Activities of daily living; *Instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, *etc*.; **Self care ADL refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not being bedridden.

intravenous infusion. Doxorubicin exerts its cytotoxic effect primarily through interactions with DNA. In addition, doxorubicin interacts with molecular oxygen, producing reactive oxygen species (ROS) and causes single-strand breaks in DNA (3). It seems that the effects of PLD, both on the tumor and on the skin, are similar, explaining a possible mechanism of doxorubicin-induced PPE.

Free radicals can damage cells and cell compartments (8). The fact that PPE occurs mainly on the palms and soles is due to the anatomical difference between these areas and other skin sites, namely the increased thickness of the stratum corneum and the high density of eccrine sweat glands. It was discussed that the secretion of doxorubicin with sweat after intravenous administration subsequently induced the formation of free radicals in the epidermal skin layers, reducing the antioxidant potential of the skin. Antioxidant substances such as carotenoids (beta-carotene, lycopene, and lutein/zeaxanthin), vitamins (A, C, D, and E), enzymes (superoxide dismutase, catalase, and glutathione peroxidase), as well as various other substances (flavonoids, lipoid acid, uric acid, selenium, coenzyme Q10), are very efficient in neutralizing free radicals and especially ROS (9-11). Based on these mechanisms, a preventative and therapeutic strategy for PPE has been developed using topical application of antioxidant-containing ointment. In previous investigations, it was demonstrated that the regular topical application of this antioxidant-containing ointment had efficient preventative and even therapeutic effects on doxorubicin-induced PPE (12, 13).

In order to enhance the understanding of PPE pathogenesis and preventative options, the present study investigated the change in antioxidant status of the skin after systemic administration of paclitaxel, docetaxel, or 5-FU. We hypothesized that a decline in the antioxidant status after systemic administration of these chemotherapeutic agents would indicate a similar pathomechanism to that of doxorubicin-induced PPE.

Materials and Methods

Patients. Patients were recruited at the Department of Hematology and Oncology and the Department of Gynecology of the Charité University of Medicine Berlin. The included patients suffered from primary and metastatic breast or ovarian cancer, oral, nasopharyngeal, pancreatic or colorectal cancer, or neuroendocrine tumors. Prior to the beginning of the investigation, the study had been approved by the Ethics Committee (approval number EA1/235/14) of the Charité University of Medicine Berlin. The investigations were conducted in accordance with the ethical guidelines of the Declaration of Helsinki. All participants gave their informed written consent. Volunteers were only included if they showed neither any pathological skin lesion nor initial symptoms of PPE, such as swelling, erythema, dysesthesia or paresthesia on their hands and feet at the time of the measurements.

Chemotherapeutic agents. The chemotherapeutics investigated in this study included docetaxel, paclitaxel and 5-FU. The chemotherapeutic agents investigated in our study can be classified into different groups regarding their mechanism of action. In previous studies, we observed that doxorubicin leads to radical formation and thus to a decline in the antioxidant status of the skin (7, 14). The majority of patients in our study received paclitaxel, which has high activity against ovarian and breast cancer (3). By binding to tubulin molecules, it stabilizes these excessively so that depolymerization in mitosis does not occur, which leads to cell death. Docetaxel is a taxane having a similar mechanism to paclitaxel, but with slightly different side-effects (2, 3). The third chemotherapeutic agent applied in our investigation was 5-FU, a pyrimidine analog. It impedes the conversion of deoxyuridylic acid to thymidylic acid. Without thymidine, DNA synthesis decreases, which leads to imbalanced cell growth and cell death.

Each treatment was systemically applied by intravenous infusion. Paclitaxel was administered at a dose of 60-90 mg/m^2 weekly or 175 mg/m^2 every 3 weeks, while the patients treated with docetaxel had their infusion at a dose of 30-35 mg/m^2 weekly or 75-100 mg/m^2 every 3 weeks. 5-FU was administered at a dose of 2,000 mg/m^2 weekly or 2,400 mg/m^2 every 2 weeks.

Study design. The concentration of carotenoids, serving as markers of the overall antioxidant status of the skin, was non-invasively measured immediately before and directly after termination of intravenous administration of chemotherapy using a miniaturized measuring system (Biozoom® sensor; Biozoom Services GmbH, Kassel, Germanya; see below). Measurement was made in each patient once before and once after chemotherapy administration during the scheduled treatment period. Regarding paclitaxel and docetaxel infusions, the time period between the first and the second measurement was 1.5 to 2 h. Depending on the individual therapy regimen for each patient, 5-FU was administered over a period of 22 to 46 h. Therefore, measurements of the antioxidant status in

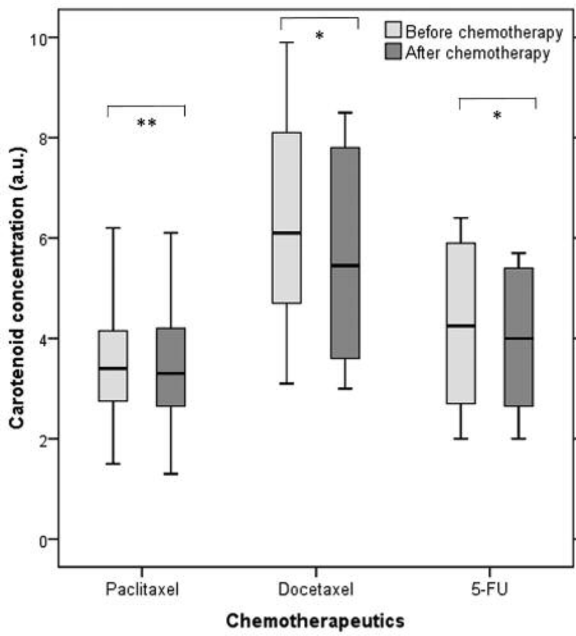

Figure 1. *Boxplot of carotenoid concentrations according to chemotherapeutic agent. Whiskers represent the minimum and the maximum concentrations within the 1.5-fold interquartile range. *p<0.05; **p<0.01.*

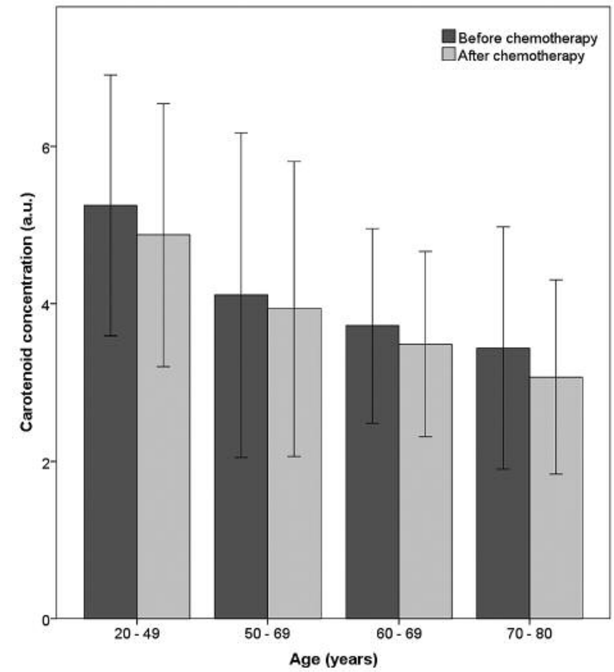

Figure 2. *The chart shows the mean carotenoid concentrations before and after chemotherapy administration in different age groups. Whiskers represent the standard deviation.*

these patients were conducted before infusion and 22 to 46 h afterwards.

Overall, the investigations were conducted over the course of 24 months.

Noninvasive determination of antioxidants in the skin. In previous studies, it could be demonstrated that carotenoids can be measured as a marker substances for the whole antioxidant status of the human skin (15). Using a Biozoom® sensor, whose working principle is based on reflection spectroscopy, the concentration of carotenoids in human skin was determined *in vivo*and noninvasively (16, 17). The measuring device has been described in detail previously (15). Briefly, the device is a mobile measuring system with a light emitting diode (LED) operating at a wavelength of 465±25 nm as a source of excitation. This wavelength was chosen due to the absorption maximum of carotenoids in this range. The bottom of the skin scanner contains a measuring head carrying an optical window. The LED emits a ray of light through this channel window which is brought into contact with the skin. The design of the channel window requires tight contact between the skin and the measuring head to exclude the undesirable influence of light from the outside. This optical window not only sends light out to excite the tissue below the surface of the skin, but also receives the light reflected back from the tissue. The signal reflected from the human skin was analyzed by a miniature replicated holographic grating spectrometer (15, 18). Measured carotenoid concentrations were displayed on a linear scale from 1 to 12 in arbitrary units (a.u.).

The measurement of carotenoids was performed twice on the thenar eminence of each patient's palms: once prior to the systemic application of chemotherapeutic agent and once after termination of the intravenous drug administration. Each measurement took about 60 seconds. For every measurement, both left and right palms were measured twice each to determine the mean carotenoid concentration. Along with the measurements, the patients were asked about the occurrence of dermatological side-effects under treatment with the chemotherapeutic agents. Their skin status, including skin lesions and conditions such as dryness, wounds or ulcerations, was also examined prior to making the measurements.

Statistical analysis. The statistical analysis was conducted using SPSS version 22 for Windows (IBM Corporation, Armonk, NY, USA). The Kolmogorov–Smirnov test was applied to test for normal distribution of continuous data. All described data were analyzed using nonparametric testing. The Wilcoxon signed-rank test was used to analyze differences in the antioxidant status before and after treatment. *p*-Values of less than 0.05 were considered to indicate statistical significance.

Results

A total of 42 patients, aged from 27 to 77 years, were enrolled, including 28 patients treated with 60-175 mg/m^2 paclitaxel, six treated with 30-100 mg/m^2 docetaxel, and eight treated with 2000-2400 mg/m^2 5-FU.

Influence of chemotherapeutic agents on carotenoid concentration. The carotenoid concentration before the application of the chemotherapeutic agents showed a high variance, ranging between 1.5 and 9.9 a.u. The mean carotenoid concentration prior to the infusion of the chemotherapy in all patients was measured at 4.08±1.77 a.u., while it decreased to 3.81±1.63 a.u. after infusion; this difference was found to be statistically highly significant (p=0.000017).

Influence of the different chemotherapeutic agents. All chemotherapeutic agents significantly reduced the antioxidant status.

The mean concentration of carotenoids before the application of paclitaxel was 3.59±1.26 a.u. After infusion, the mean concentration was significantly reduced to 3.41±1.28 a.u. (p=0.000231). Patients who received docetaxel showed a significant decline of their antioxidant status, from a mean value of 6.33±2.43 to 5.63±2.29 a.u. (p=0.027). 5-FU also significantly reduced the carotenoid concentration: the mean antioxidant level decreased from 4.26±1.81 to 3.98±1.53 a.u. (p=0.042). Figure 1 presents carotenoid concentration depending on the chemotherapeutic drug.

Further observations. Prior to the study it was expected that patients with cancer in general would have a notably lower antioxidant concentration than shown in this study, as they suffer from a disease that can cause psychological and physical distress. Surprisingly, the concentration of carotenoids varied considerably between individual patients and showed a high overall variance. The patients who had higher antioxidant levels reported that they tried to eat and live healthily, avoiding stress exposure and consuming high amounts of antioxidant-rich fruit and vegetables.

Although no significant differences were found between different age groups of patients, Figure 2 shows that the group of younger patients had a tendency to have a higher mean level of carotenoids. Furthermore, there was no significant difference found in the antioxidant status depending on the type of cancer nor on gender.

Discussion

Influence of the antioxidant status. The antioxidant status of the human skin correlates with antioxidant concentrations measured in the blood circulation (19-21). Systemically applied substances can reach the papillary dermis by blood circulation and can be secreted on the skin surface *via* the sweat glands. This can account for the significant influence of intravenously administered chemotherapeutics on the antioxidant status of the skin. Furthermore, there is an individual variance due to lifestyle factors, living environments, as well as medication and disease. All influencing factors form an equilibrium between antioxidants and free radicals in the human body. Many antioxidants cannot be synthesized by humans, but have to be taken-up in healthy nutrition, especially fruits and vegetables. Therefore, the antioxidant status of individuals depends on many factors, among them a healthy diet (22). However, not only nutritional habits but also smoking and alcohol consumption influence the antioxidant status (21). Previous investigations showed that low carotenoid values correlate with physical stress factors, such as illness and sleep deprivation, as well as psychological stress and excessive physical activity, which can cause their rapid decline (23). Since the measurements of this study were conducted before and directly after chemotherapy administration, it is possible that psychological distress due to the clinical setting and medication process enhanced the decrease of carotenoids.

PPE association with chemotherapeutic agents. There are many case reports and studies with patients affected by PPE as a side-effect of chemotherapeutic agents such as doxorubicin, paclitaxel, docetaxel and 5-FU (4, 24). Despite the differences in the investigated chemotherapeutic drugs and doxorubicin in their mechanisms, the carotenoid concentration in the skin declined significantly after administration of all investigated agents. Skin toxicity can effect dose density, with treatment delay, dose reduction and discontinuation, and influence the quality of life of the patients. These can negatively influence the clinical outcome and tumor control rate.

The cytotoxic effect of doxorubicin is based on free radical processes, which are also assumed to cause the inflammatory and necrotic skin lesions of PPE. This study revealed subsequent radical formation after infusion of the PPE-associated chemotherapeutics paclitaxel, docetaxel and 5-FU. This finding is interesting, since the main cytotoxic effects of these chemotherapeutic agents are not attributed to radical formation. Although the measured decline in carotenoids after infusion of the investigated chemotherapeutics, especially paclitaxel, was relatively small, a cumulative toxic effect can be presumed after several infusions of chemotherapy. On the other hand, the small effect after paclitaxel infusion correlates with the rare occurrence of PPE with this drug. Whether there is a concentration-dependent or a time-dependent development of PPE lesions after a critical antioxidant decline due to chemotherapeutic treatment remains to be investigated.

The purpose of the present study was to identify a possible mechanism of PPE induction by cytotoxic drugs reported to cause PPE. Since all the chemotherapeutic agents administered in this study showed subsequent antioxidant decay in the skin, it can be assumed that oxidative stress might play a significant role in the pathomechanism of PPE induced by these agents. Therefore, the topical application of an antioxidant-containing ointment could also be effective in the prevention and treatment of PPE associated with paclitaxel, docetaxel and 5-FU, as was shown for doxorubicin-induced PPE (12, 13).

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References

1 Chu CS and Rubin SC: Basic Principles of Chemotherapy. *In*: Clinical Gynecologic Oncology. Di Saia PJ and Creasman WT (eds.). St. Louis: Mosby, pp. 515-538, 2012.
2 Chu E and Sartorelli AC: Cancer Chemotherapy. In: Basic & Clinical Pharmacology. Katzung BG and Trevor AJ (eds.). New York: McGraw-Hill Medical, pp. 949-975, 2012.
3 Chapter 39: Anticancer Drugs. *In*: Lippincott's Illustrated Reviews: Pharmacology. Finkel R, Cubeddu LX, Clark MA (eds.). Philadelphia, PA.: Kluwer, Lippincott, Williams and Wilkins, pp. 457-488, 2009.
4 Hueso L, Sanmartín O, Nagore E, Botella-Estrada R, Requena C, Llombart B, Serra-Guillén C, Alfaro-Rubio A and Guillén C: Chemotherapy-induced acral erythema: a clinical and histopathologic study of 44 cases. Actas Dermosifiliogr *99*: 281-290, 2008.
5 Webster-Gandy JD, How C and Harrold K: Palmar-plantar erythrodysesthesia (PPE): a literature review with commentary on experience in a cancer centre. Eur J Oncol Nurs *11*: 238-246, 2007.
6 Degen A, Alter M, Schenck F, Satzger I, Völker B, Kapp A and Gutzmer R: The hand-foot-syndrome associated with medical tumor therapy – classification and management. J Dtsch Dermatol Ges *8*: 652-661, 2010.
7 Martschick A, Sehouli J, Patzelt A, Richter H, Jacobi U, Oskay-Ozcelik G, Sterry W and Lademann J: The pathogenetic mechanism of anthracycline-induced palmar-plantar erythrodysesthesia. Anticancer Res *29*: 2307-2313, 2009.
8 Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M and Telser J: Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol *39*: 44-84, 2007.
9 Darvin M, Zastrow L, Sterry W and Lademann J: Effect of supplemented and topically applied antioxidant substances on human tissue. Skin Pharmacol Physiol *19*: 238-247, 2006.
10 Stahl W and Sies H: Antioxidant activity of carotenoids. Mol Aspects Med *24*: 345-351, 2003.
11 Wolfle U, Seelinger G, Bauer G, Meinke MC, Lademann J and Schempp CM: Reactive molecule species and antioxidative mechanisms in normal skin and skin aging. Skin Pharmacol Physiol *27*: 316-332, 2014.
12 Kluschke F, Martschick A, Darvin ME, Zastrow L, Chekerov R, Sehouli J and Lademann J: Application of an ointment with high radical protection factor as a prevention strategy against PPE. ASCO Annual Meeting, 2012.
13 Lademann J, Röwert-Huber HJ, Haas N, Kluschke F, Patzelt A, Zastrow L, Lange-Asschenfeldt B, Jung S, Sterry W and Sehouli J: Palmoplantar erythrodysesthesia-like skin symptoms in patients under various chemotherapeutics: preventive and therapeutic options. Skin Pharmacol Physiol *27*: 229-233, 2014.
14 Jacobi U, Waibler E, Schulze P, Sehouli J, Oskay-Ozcelik G, Schmook T, Sterry W and Lademann J: Release of doxorubicin in sweat: first step to induce the palmar-plantar erythrodysesthesia syndrome? Ann Oncol *16*: 1210-1211, 2005.
15 Darvin ME, Sandhagen C, Koecher W, Sterry W, Lademann J and Meinke MC: Comparison of two methods for noninvasive determination of carotenoids in human and animal skin: Raman spectroscopy versus reflection spectroscopy. J Biophotonics *5*: 550-558, 2012.
16 Darvin ME, Fluhr JW, Meinke MC, Zastrow L, Sterry W and Lademann J: Topical beta-carotene protects against infra-red-light-induced free radicals. Exp Dermatol *20*: 125-129, 2011.
17 Jung S, Darvin ME, Chung HS, Jung B, Lee SH, Lenz K, Chung WS, Yu RX, Patzelt A, Lee BN, Sterry W and Lademann J: Antioxidants in Asian-Korean and caucasian skin: the influence of nutrition and stress. Skin Pharmacol Physiol *27*: 293-302, 2014.
18 Lademann J, Köcher W, Yu R, Meinke MC, Na Lee B, Jung S, Sterry W and Darvin ME: Cutaneous carotenoids: the mirror of lifestyle? Skin Pharmacol Physiol *27*: 201, 2014.
19 Stahl W, Heinrich U, Jungmann H, von Laar J, Schietzel M, Sies H and Tronnier H: Increased dermal carotenoid levels assessed by noninvasive reflection spectrophotometry correlate with serum levels in women ingesting Betatene. J Nutr *128*: 903-907, 1998.
20 Meinke MC, Darvin ME, Vollert H and Lademann J: Bioavailability of natural carotenoids in human skin compared to blood. Eur J Pharm Biopharm *76*: 269-274, 2010.
21 Darvin ME, Sterry W, Lademann J and Patzelt A: Alcohol consumption decreases the protection efficiency of the antioxidant network and increases the risk of sunburn in human skin. Skin Pharmacol Physiol *26*: 45-51, 2013.
22 Darvin ME, Patzelt A, Knorr F, Blume-Peytavi U, Sterry W and Lademann J: One-year study on the variation of carotenoid antioxidant substances in living human skin: influence of dietary supplementation and stress factors. J Biomed Opt *13*: 044028, 2008.
23 Lademann J, Schanzer S, Meinke M, Sterry W and Darvin ME: Interaction between carotenoids and free radicals in human skin. Skin Pharmacol Physiol *24*: 238-244, 2011.
24 Miller KK, Gorcey L and McLellan BN: Chemotherapy-induced hand-foot syndrome and nail changes: A review of clinical presentation, etiology, pathogenesis, and management. J Am Acad Dermatol *71*: 787-794, 2014.
25 Common Terminology Criteria for Adverse Events v4.03. U.S. Department of Health and Human Services, National Institutes of Health, National Cancer Institute, Bethesda, MD, 2010.

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Complete list of publications

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Lademann J, Köcher W, Yu RX, Meinke MC, **Lee BN**, Jung S, Sterry W, Darvin ME. Cutaneous carotenoids: the mirror of lifestyle? Skin Pharmacol Physiol 2014;27:201.

Jung S, Darvin ME, Chung HS, Jung B, Lee SH, Lenz K, Chung WS, Yu RX, Patzelt A, **Lee BN**, Sterry W, Lademann J. Antioxidants in Asian-Korean and Caucasian Skin: The influence of Nutrition and Stress. Skin Pharmacol Physiol 2014;27:293-302.

Lee BN, Jung S, Darvin ME, Eucker J, Kühnhardt D, Sehouli J, Patzelt A, Fuss H, Yu RX, Lademann J. The influence of chemotherapy on the antioxidant status of the human skin. Anticancer Res 2016;36:4089-93.

Abstract

Lademann J, **Lee BN**, Engel S, Jung S, Sehouli J, Darvin ME, Fuss H. Investigation of cutaneous radical formation in patients receiving 5-fluorouracil, paclitaxel, docetaxel and capecitabine. ASCO Annual Meeting, Chicago, 2016.

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