Journal of Biomedical Optics

SPIEDigitalLibrary.org/jbo

Analyses of the correlation between dermal and blood carotenoids in female cattle by optical methods

Julia Klein
Maxim E. Darvin
Martina C. Meinke
Florian J. Schweigert
Kerstin E. Müller
Jürgen Lademann

Analyses of the correlation between dermal and blood carotenoids in female cattle by optical methods

Julia Klein,[a] Maxim E. Darvin,[b] Martina C. Meinke,[b] Florian J. Schweigert,[c] Kerstin E. Müller,[a] and Jürgen Lademann[b]
[a]Freie Universität Berlin, Faculty of Veterinary Medicine, Clinic for Ruminants and Swine, Königsweg 65, Berlin, Germany
[b]Charité—Universitätsmedizin Berlin, Center of Experimental and Applied Cutaneous Physiology (CCP), Department of Dermatology, Venerology, and Allergology, Charitéplatz 1, Berlin, Germany
[c]University of Potsdam, Institute of Nutritional Science, Department of Physiology and Pathophysiology, A. Scheunert-Allee 114-116, Bergholz-Rehbrücke, Germany

Abstract. Herd health programs for the maintenance of welfare and productivity in cattle need efficient tools for monitoring the health of individual animals. Recent reports demonstrate that the oxidative status is related to various stress conditions in dairy cows. Biomarkers, among other carotenoids, could serve as indicators of stress originating from the environment (e.g., heat stress or sun radiation) or from the animal itself (e.g., disease). To date, only invasive *in vitro* tests are available to assess the oxidative status in cattle. The present study compares the results of optical noninvasive *in vivo* measurements of dermal carotenoids in cattle udder skin using an LED-based miniaturized spectroscopic system (MSS) with those obtained by photometric analysis of beta carotene in whole blood samples using a portable device. Correlations between the concentrations of dermal and blood carotenoids were calculated under consideration of the nutritional status of the animals. Significant correlation ($R = 0.86$) was found for cattle with a moderate to obese body condition. Thus, the blood and skin concentrations of the marker substance beta carotene are comparable under stable stress conditions of the cattle. This demonstrates that the MSS is suitable for noninvasive assessment of dermal carotenoid concentrations in cattle. [DOI: 10.1117/1.JBO.18.6.061219]

Keywords: Raman spectroscopy; reflection spectroscopy; skin; antioxidants; free radicals.

Paper 12569SS received Aug. 31, 2012; revised manuscript received Nov. 8, 2012; accepted for publication Nov. 9, 2012; published online Dec. 12, 2012.

1 Introduction

Noninvasive spectroscopic technologies are gaining more and more importance for *in vivo* assessment of different compounds. Various studies in human and animal skin have proven the feasibility of noninvasive real-time measurements of carotenoids as possible biomarkers for the dermal antioxidant defense status.[1–4] Among all carotenoids, only beta carotene is found at high concentration in bovine tissue and milk.[5–7]

Carotenoids are powerful antioxidants that are able to neutralize free radicals before they can damage cells and cell compartments,[8–10] as well as possible marker substances for the whole antioxidative status of human skin.[11] All antioxidants form a protection chain in the human organism, acting as a safeguard against the destructive action of the free radicals,[12–14] if they are applied at an appropriate mixture and concentration.[15–18]

Innovative technologies for the *in vivo* determination of dermal carotenoid concentrations include reflection[3,19] and Raman spectroscopy.[20,21] Both technologies are suitable for the noninvasive monitoring of alterations in dermal carotenoid concentrations with time. Measurement procedures are easy and fast to perform and do not cause any pain. Using the latter technologies, a higher number of test persons could be examined to explore the effects of nutritional intake and subsequently follow the kinetics of carotenoid antioxidants, as well as their accumulation in the skin.[22–26]

A new technology using an LED-based miniaturized spectroscopic system (MSS) has been adapted for use in cattle, which allows the determination of dermal carotenoid concentrations based on reflection spectroscopy.[27]

The antioxidative status of cattle is influenced by different internal and external factors, such as birth, beginning of lactation,[28–30] and illness.[31] At present, it is unknown whether a reduction in the antioxidative status is one of the reasons for illness, or whether it occurs as a result of illness. Various disorders of cattle were associated with low plasma carotenoid concentrations.[32,33] The influence of external factors like stress caused by heat has also been discussed in the literature.[34]

Up to now, the determination of the antioxidant status, and especially of the beta carotene concentration, has relied on the examination of blood samples. These techniques are costly and labor-intensive; and for this reason, they do not allow sampling of a greater number of animals, or even repeated sampling of the same animals with time. An "ancient" method for estimation of the whole blood beta carotene status in cattle included visual inspection of serum samples based on a given color range.[35] The validity of the latter approach, however, is under consideration.[36] Today, high-pressure liquid chromatography (HPLC) is regarded as the gold standard for the determination of carotenoids in blood samples or tissue biopsy materials.[37,38] A recently developed cow-side test is available for the determination of beta carotene from whole blood samples in the field.[39]

Address all correspondence to: Maxim E. Darvin, Charité—Universitätsmedizin Berlin, Center of Experimental and Applied Cutaneous Physiology (CCP), Department of Dermatology, Venerology, and Allergology, Charitéplatz 1, Berlin, Germany. Tel: +49 30 450518208; Fax: +49 30 450518918; E-mail: maxim.darvin@charite.de

0091-3286/2012/$25.00 © 2012 SPIE

The aim of the present study was to compare the results of spectroscopic, noninvasive, *in vivo* measurements of the dermal carotenoid concentrations with the concentration of beta carotene, which was obtained invasively *in vitro* by a photometric method for whole blood.

2 Materials and Methods

2.1 *Animals*

In this study, 20 clinically healthy female cattle, aged between 2 and 11 years, were included. Of these animals, 4 were owned by the clinic for Ruminants and Swine (Faculty of Veterinary Medicine, Freie Universität Berlin, Germany), and 16 cows were kept on a commercial dairy farm. The breeds were as follows: 15 German Holstein, 4 Red Holstein, and 1 Jersey.

2.2 *In Vivo Measurements Using the Miniaturized Spectroscopic System*

For noninvasive determination of the dermal carotenoid concentrations, the skin of the bovine udder was chosen. Measurements were performed in triplicate using an LED-based MSS whose working principle is based on reflection spectroscopy.[27] The pattern of the reflection spectrum is determined by the presence of chromophores in the skin, which contain a high amount of carotenoids that absorb the emitted light at a distinct wavelength range. As the absorption spectrum of carotenoids lies in the blue-green range of the optical spectrum, the blue LED-emitting light in the range between 440 nm and 490 nm was used as a source of carotenoid excitation. The magnitude of the small dip in the reflected spectrum, measured in the range between 458 nm and 472 nm, is associated with the light absorption by dermal carotenoids. Thus, the light reflected from the skin contains information about the carotenoid concentrations. The carotenoid concentrations are expressed in arbitrary units that correlate with the carotenoid concentrations as determined during calibration of the system, using resonance Raman spectroscopy as the gold standard in earlier noninvasive experiments, including bovine skin samples obtained from the slaughterhouse.[27] A strong correlation ($R = 0.81$) was achieved. The reproducibility of these measurements on the same skin sites using the MSS exceeded 90%.

Measurements were performed on three distinct sites on the surface of the bovine udder of the left body site, each located about 10 cm from the teats on the dorsal side. Two sites were located right above the teats, and the third site was located halfway between the two sites mentioned earlier. The udder skin remained untreated and was carefully shaved without influencing the stratum corneum using a single-use razor shortly before the measurements were performed. Subsequently, the homogeneously pigmented skin sites of approx. 0.8 cm^2 were marked. Measurements were performed in triplicate on each site, and between the different measurements, the MSS was removed from the skin and was replaced for each subsequent measurement. This procedure was described in detail previously.[27,40] Figure 1 shows the measurement procedure.

2.3 *Examination of Blood Samples*

Following the measurements with the LED-based MSS, blood samples were obtained from the Vena/Arteria coccygea mediana into evacuated heparin-containing tubes (Vacutainer®, Becton Dickinson, Franklin Lakes, New Jersey). The samples were examined at the University of Potsdam Institute for Nutritional Science using the iEx™/iCheck™-Testkit (BioAnalyt GmbH, Teltow, Germany). The portable system, which allows the determination of the beta carotene concentrations from whole blood samples in the field, uses spectrophotometry (iCheck™) as the principal test method. It has been shown to produce reliable results when compared to those obtained by HPLC.[39]

Fig. 1 Measurement of dermal carotenoid concentrations on the bovine udder skin using a portable LED skin scanner system.

2.4 *Determination of Backfat Thickness*

As the lipophilic carotenoids are known to be accumulated in fat tissue, the backfat thickness of the animals was used as a measure for the body condition. Measurements were performed by ultrasound, as described by Schröder et al.[41] Based on the results of the latter measurements, the 20 cattle were assigned to either the group of "lean" cows or the group of "adipose" cows (Table 1), as proposed by Staufenbiel.[42]

Table 1 Assessment of body condition by description, body condition score (BCS), backfat thickness (BFT) and total body fat (TBF) content.[42]

Description	BCS[a]	BFT[b] (mm)	TBF[c] (kg)	Classification in the study
Emaciated	1.0	<5	<50	Lean
Very poor	1.5	5	50	Lean
Poor	2.0	10	76	Lean
Moderate	2.5	15	98	Moderate to obese
Good	3.0	20	122	Moderate to obese
Very good	3.5	25	146	Moderate to obese
Fat	4.0	30	170	Moderate to obese
Adipose	4.5	35	194	Moderate to obese
Obese	5.0	<35	<194	Moderate to obese

[a]Body condition score.
[b]Backfat thickness.
[c]Total body fat content.

2.5 *Statistical Analysis*

For statistical evaluation, SPSS 18.00 for Windows (IBM, Armonk, New York) was used. Differences were judged statistically significant at $P < 0.05$. As data were not normally distributed, the Spearman's Rank test was used. The Kruskal-Wallis test was applied when comparing more than two different groups.

3 Results

3.1 *Dermal Carotenoid Concentrations Versus Beta Carotene Concentrations in Plasma*

Dermal carotenoid concentrations, as expressed in arbitrary units, varied from 0.39 au to 1.53 au in 20 female dairy cattle. The carotenoid concentrations of the same skin areas showed highly significant differences between individual animals ($p < 0.001$). The median ($n = 20$) of the relative dermal carotenoid concentration was 0.80 ± 0.32 au, while beta carotene concentration in plasma ranged between 0.92 mg/L and 5.22 mg/L. These results are summarized in Table 2.

The correlation between both values dermal carotenoid concentrations and beta carotene levels in plasma of 20 female cattle was weak ($R = 0.23$). Figure 2 represents the obtained correlation.

As subcutaneous fat can influence the accumulation and storage of dermal carotenoid concentrations due to the lipophilic character of carotenoid molecules, the dermal fat content as determined by ultrasonography was included in the following considerations.

In 8 female cattle that were classified as poor, the correlation coefficient between the dermal carotenoid concentrations and beta carotene concentrations in plasma was calculated $R = -0.23$ (Fig. 3). However, in 12 female cattle that were classified from moderate to obese, the obtained correlation coefficient was $R = 0.86$, with $p < 0.05$ (Fig. 4).

The average dermal carotenoid concentration of the cows belonging to the Red Holstein breed was found to be

Table 2 Median and standard deviation of dermal carotenoid concentrations and plasma beta carotene concentrations measured on the udders of 20 cattle in triplicate.

Number of cows	Dermal carotenoid concentration median (au)	Dermal carotenoid concentration standard deviation (au)	Beta carotene in plasma (mg/L)
1	0.88	0.16	3.99
2	0.7	0.14	3.49
3	0.44	0.14	2.81
4	1.04	0.22	2.38
5	0.77	0.17	2.26
6	0.83	0.16	4.87
7	0.74	0.16	3.32
8	0.76	0.17	2.88
9	1.50	0.14	5.22
10	1.30	0.42	2.41
11	0.80	0.14	2.75
12	0.70	0.16	1.50
13	1.06	0.34	2.72
14	1.24	0.19	1.57
15	0.71	0.07	1.01
16	0.90	0.17	2.84
17	0.63	0.07	1.57
18	0.91	0.11	1.60
19	0.80	0.18	0.92
20	0.64	0.08	1.24

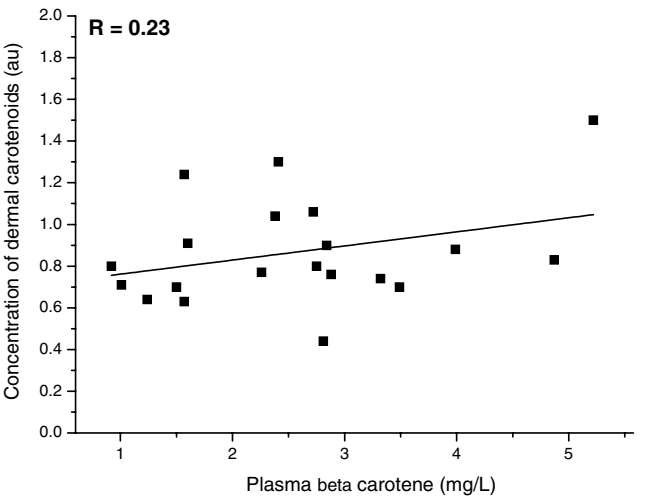

Fig. 2 Correlation between the dermal carotenoid concentration as expressed in arbitrary units and plasma beta carotene concentration for all investigated cattle ($n = 20$).

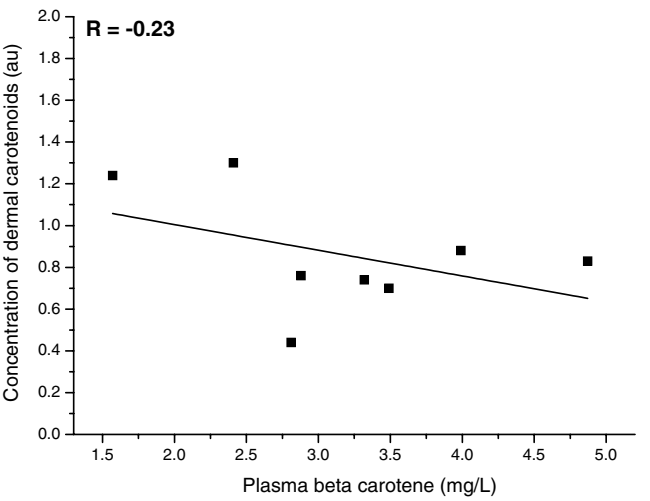

Fig. 3 Correlation between dermal carotenoid concentrations and beta carotene levels in plasma of cattle ($n = 8$) with a lean body condition.

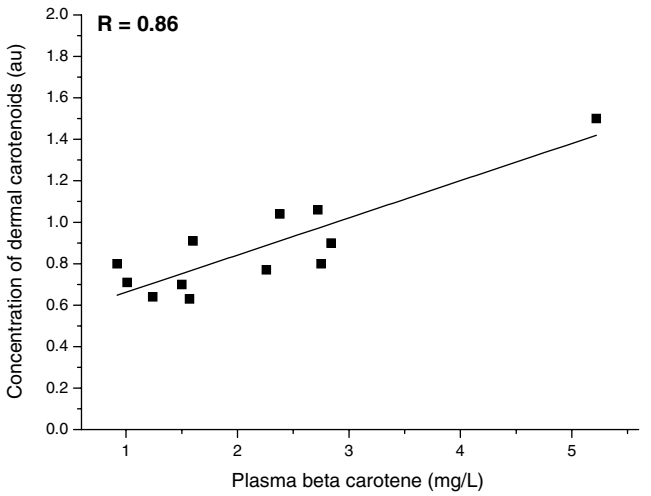

Fig. 4 Correlation between dermal carotenoid concentrations and beta carotene concentrations in plasma of cattle ($n = 12$) with a moderate-to-obese body condition.

significantly higher ($p < 0.05$) compared to the cows of the German Holstein breed. Nevertheless, due to the low number of investigated animals, the dependence of the dermal carotenoid concentration on the breed should be investigated as well.

4 Discussion

The measurements carried out with the MSS demonstrated that the individual dermal carotenoid concentrations of the 20 cattle investigated in the present study were significantly different. Similar results were reported by Klein et al. in cattle[40] and by Darvin et al. in humans.[43] The main reason for these differences could be a result of lifestyle and especially nutritional status.[44,45]

The absolute values of the beta carotene concentration in the blood of cattle show a strong variation. Herdt et al. found that a beta carotene concentration of blood in 3 mg/L in the serum is optimal.[46] Rosenberger reported a beta carotene concentration between 1.5 and 5 mg/L in the blood plasma of the cattle during the winter and spring periods. A subclinical deficit of beta carotene was determined between 0.6 and 1.5 mg/L.[47] In this study, the beta carotene values of the cattle were determined in the winter months. A higher beta carotene deficit was seen in 3 of the 20 cattle.

The average concentration of the 16 cattle at the commercial agricultural farm investigated in the present study was 2.78 mg/L, which was higher than the beta carotene concentration (1.41 mg/L) determined for the 4 cattle at the University Faculty of Veterinary Medicine. The feeding conditions of both groups of cattle, as well as the proficiency level of the milk production of all cattle investigated in the present study, were different. All 20 cattle were kept in tie-stalls without access to grazing land.

In bovine tissues, among all carotenoids, only beta carotene was found at higher concentrations.[5] Therefore, it was possible to determine the correlation between concentrations of the dermal carotenoids and beta carotene circulating in the blood.

The results of the present study show that beta carotene concentrations measured in the blood correlate ($R = 0.86$) with dermal carotenoid concentrations in a reproducible fashion in dairy cattle with a moderate to obese body condition ($n = 12$). A nutritional status below moderate is not desired, however, and may be an indicator of pathological events. In the group of "adipose" dairy cattle, however, a closer correlation was found between dermal carotenoid concentrations and blood beta carotene concentrations, which were demonstrated to be significant. More animals should be investigated in the future, especially animals containing high carotenoid concentration in their skin or blood, in order to make the obtained correlation more exact.

In humans, carotenoid concentrations in blood and skin are lower[4,48] but were shown to be influenced by the site where measurements were performed,[49] as the number of chromophores differ in the various regions of the body. The udder skin was chosen in the present study, as this part of the body in cattle can easily be approached and light-colored hair is sparse in this region compared to the rest of the body. Thus, hairs could be removed carefully without influencing the structure of epidermis. Results of measurements of dermal carotenoid concentrations in humans have been demonstrated to depend on skin thickness, fat deposition, and the vascularization in the various tissues, such as hemoglobin, have been shown to influence the carotenoid measurements by reflection spectrometry as used in the present study.[27] In humans, the accumulation of dermal carotenoids depends on the body mass index (BMI).[43] This finding is in accordance with reports by Noziere et al., which proposed that the percentage of fatty tissue in the cattle provides a significant storage location for beta carotene.[36] The latter pool is assumed to fulfill a buffer function, which guarantees the availability of antioxidants whenever the organism is exposed to oxidative stress. The thickness of the epidermis of cow udder skin does usually not exceed 200 μm.[50] Considering the penetration depth of blue light into the skin, which is approximately 150 to 200 μm, the influence of dermal chromophores such as melanin, hemoglobin, and bilirubin is negligible.[27] In the present study, however, it was shown that the lean and cachectic cows underlie constant changes in dermal carotenoid concentrations.

The accumulation of carotenoids in the skin depends on the nutritional carotenoid intake and only shows alterations after some delay with respect to the increase/decrease in blood levels. To this end, skin in contrast to blood serves as a reservoir for carotenoids.[48,51] The latter findings could explain the fact that even in adipose cattle, no close correlation was observed between dermal carotenoid concentrations and blood levels of beta carotene. This has also been demonstrated in humans.[48,52]

In addition, one to three days were needed until carotenoid antioxidants were present in the human skin following their oral uptake.[23,45] Decreases in the dermal carotenoid concentrations in humans, however, have been demonstrated as early as one day following stress exposition, such as UV and IR irradiation, endurance exercises, illness, fatigue, alcohol consumption, and smoking.[45,53,54]

When the body is exposed to stress conditions (e.g., metabolic stress), free radicals are formed, which are neutralized by various antioxidants, among these the carotenoids of the body. The nadir in the dermal carotenoid concentration was demonstrated using repeated measurements with time following stress exposition.

5 Conclusion

The present study demonstrates only a weak but still significant correlation between dermal carotenoid concentrations and beta carotene concentrations in the blood of cows exhibiting substantial subcutaneous accumulation of fat, which underlines the

buffer function for fat-soluble carotenoids. To this end, the LED-based MSS used in this study is suitable for monitoring the dermal carotenoid concentrations with time and for measurements in dairy cattle with a moderate or above body condition. In future, MSS could be used for daily milking parlor measurements to monitor the development of the carotenoid concentration.

Acknowledgments

We would like to thank Dr. Koecher, of Opsolution GmbH, Kassel, Germany, for making available the sensor systems, the technical supervision, and the intensely helpful discussions. We also thank the Foundation "Skin Physiology" of the Donor Association for German Science and Humanities for providing financial support.

References

1. J. Lademann et al., "Carotenoids in human skin," *Exp. Dermatol.* **20**(5), 377–382 (2011).
2. J. Lademann et al., "Interaction between carotenoids and free radicals in human skin," *Skin Pharmacol. Phys.* **24**(5), 238–244 (2011).
3. F. Niedorf, "Entwicklung eines Verfahrens zur quantitativen Auswertung nicht-invasiver reflexionsspektroskopischer Messungen von Beta-Carotin in der Haut," Ph.D. Thesis, Institut für Pharmakologie, Toxikologie und Pharmazie der Tierärztlichen Hochschule Hannover, Hannover (2001).
4. S. F. Haag et al., "Comparative study of carotenoids, catalase, and radical formation in human and animal skin," *Skin Pharmacol. Physiol.* **23**(6), 306–312 (2010).
5. K. A. Slifka et al., "A survey of serum and dietary carotenoids in captive wild animals," *J. Nut.* **129**(2), 380–390 (1999).
6. F. Calderon et al., "Variations in carotenoids, vitamins A and E, and color in cow's plasma and milk following a shift from hay diet to diets containing increasing levels of carotenoids and vitamin E," *J. Dairy Sci.* **90**(12), 5651–5664 (2007).
7. S. K. Jensen, A. K. B. Johannsen, and J. E. Hermansen, "Quantitative secretion and maximal secretion capacity of retinol, beta-carotene, and alpha-tocopherol into cows' milk," *J Dairy Res.* **66**(4), 511–522 (1999).
8. N. I. Krinsky, "Carotenoids as antioxidants," *Nutrition* **17**(10), 815–817 (2001).
9. P. Palozza and N. I. Krinsky, "Antioxidant effects of carotenoids *in vivo* and *in vitro*: an overview," *Meth. Enzymol.* **213**, 403–420 (1992).
10. W. Stahl and H. Sies, "Antioxidant activity of carotenoids," *Mol. Aspects Med.* **24**(6), 345–351 (2003).
11. S. F. Haag et al., "Determination of the antioxidative capacity of the skin *in vivo* using resonance Raman and electron paramagnetic resonance spectroscopy," *Exp. Dermatol.* **20**(6), 483–487 (2011).
12. M. Darvin et al., "Effect of supplemented and topically applied antioxidant substances on human tissue," *Skin Pharmacol. Physiol.* **19**(5), 238–247 (2006).
13. J. J. Thiele et al., "The antioxidant network of the stratum corneum," *Curr. Probl. Dermatol.* **29**, 26–42 (2001).
14. M. Wrona et al., "Cooperation of antioxidants in protection against photosensitized oxidation," *Free Radic. Biol. Med.* **35**(10), 1319–1329 (2003).
15. P. Palozza, "Prooxidant actions of carotenoids in biologic systems," *Nutr. Rev.* **56**(9), 257–265 (1998).
16. G. M. Lowe et al., "Lycopene and beta carotene protect against oxidative damage in HT29 cells at low concentrations but rapidly lose this capacity at higher doses," *Free Radic. Res.* **30**(2), 141–151 (1999).
17. M. E. Darvin et al., "The role of carotenoids in human skin," *Molecules* **16**(12), 10491–10506 (2011).
18. J. Lademann et al., "Uptake of antioxidants by natural nutrition and supplementation: pros and cons from the dermatological point of view," *Skin Pharmacol. Phys.* **24**(5), 269–273 (2011).
19. F. Niedorf, H. Jungmann, and M. Kietzmann, "Noninvasive reflection spectra provide quantitative information about the spatial distribution of skin chromophores," *Med. Phys.* **32**(5), 1297–1307 (2005).
20. T. R. Hata et al., "Non-invasive raman spectroscopic detection of carotenoids in human skin," *J. Invest. Dermatol.* **115**(3), 441–448 (2000).
21. M. E. Darvin et al., "Noninvasive detection of beta-carotene and lycopene in human skin using Raman spectroscopy," *Laser Phys.* **14**(2), 231–233 (2004).
22. U. Blume-Peytavi et al., "Cutaneous lycopene and beta-carotene levels measured by resonance Raman spectroscopy: high reliability and sensitivity to oral lactolycopene deprivation and supplementation," *Eur. J. Pharm. Biopharm.* **73**(1), 187–194 (2009).
23. M. E. Darvin et al., "Resonance Raman spectroscopy for the detection of carotenolds in foodstuffs. Influence of the nutrition on the antioxidative potential of the skin," *Laser Phys. Lett.* **4**(6), 452–456 (2007).
24. J. W. Fluhr et al., "Kinetics of carotenoid distribution in human skin *in vivo* after exogenous stress: disinfectant and wIRA-induced carotenoid depletion recovers from outside to inside," *J. Biomed. Opt.* **16**(3), 035002 (2011).
25. S. T. Mayne et al., "Noninvasive assessment of dermal carotenoids as a biomarker of fruit and vegetable intake," *Am. J. Clin. Nutr.* **92**(4), 794–800 (2010).
26. S. Rerksuppaphol and L. Rerksuppaphol, "Effect of fruit and vegetable intake on skin carotenoid detected by non-invasive Raman spectroscopy," *J. Med. Assoc. Thai.* **89**(8), 1206–1212 (2006).
27. M. E. Darvin et al., "Comparison of two methods for noninvasive determination of carotenoids in human and animal skin: Raman spectroscopy versus reflection spectroscopy," *J. Biophotonics* **5**(7), 550–558 (2012).
28. J. P. Goff, K. Kimura, and R. L. Horst, "Effect of mastectomy on milk fever, energy, and vitamins A, E, and beta-carotene status at parturition," *J. Dairy Sci.* **85**(6), 1427–1436 (2002).
29. R. Chawla and H. Kaur, "Plasma antioxidant vitamin status of periparturient cows supplemented with alpha-tocopherol and beta-carotene," *Anim. Feed Sci. Tech.* **114**(1–4), 279–285 (2004).
30. P. D. Katsoulos et al., "Long-term fluctuations and effect of age on serum concentrations of certain fat-soluble vitamins in dairy cows," *Vet. Clin. Path.* **34**(4), 362–367 (2005).
31. J. Klein et al., "Serial non-invasive measurements of dermal carotenoid concentrations in dairy cows following recovery from abomasal displacement," *PLoS One* **7**(10), e47706 (2012).
32. T. R. Batra et al., "Concentration of plasma and milk vitamin-e and plasma beta-carotene of mastitic and healthy cows," *Intl. J. Vit. Nut. Res.* **62**(3), 233–237 (1992).
33. C. Kawashima et al., "Relationship between plasma beta-carotene concentrations during the peripartum period and ovulation in the first follicular wave postpartum in dairy cows," *Anim. Reprod. Sci.* **111**(1), 105–111 (2009).
34. L. Locher et al., "Relevance, measurement and assessment of the antioxidative status in farm animals," *Berl. Munch. Tierarztl. Wochenschr.* **124**(9–10), 419–431 (2011).
35. F. J. Schweigert et al., "Rapid assessment of β-carotene status," *Intl. Dairy Top.* **6**(4), 15–17 (2007).
36. P. Noziere et al., "Variations in carotenoids, fat-soluble micronutrients, and color in cows' plasma and milk following changes in forage and feeding level," *J. Dairy Sci.* **89**(7), 2634–2648 (2006).
37. D. B. Rodriguez-Amaya, "Food carotenoids: analysis, composition, and alterations during storage and processing of foods," *Forum Nutr.* **56**, 35–37 (2003).
38. D. Talwar et al., "A routine method for the simultaneous measurement of retinol, alpha-tocopherol, and five carotenoids in human plasma by reverse phase HPLC," *Clin. Chim. Acta.* **270**(2), 85–100 (1998).
39. J. Raila et al., "Validation of a new point-of-care assay for determination of beta carotene concentration in bovine whole blood and plasma," *Vet. Clin. Pathol.* **41**(1), 119–122 (2012).
40. J. Klein et al., "Non-invasive reflection spectroscopic measurements of carotenoids in bovine udder skin," *J. Biomed. Opt.* **17**(10), 101514 (2012).
41. U. J. Schroder and R. Staufenbiel, "Methods to determine body fat reserves in the dairy cow with special regard to ultrasonographic measurement of backfat thickness," *J. Dairy Sci.* **89**(1), 1–14 (2006).
42. R. Staufenbiel, "Evaluation of body condition in dairy cows by ultrasonographic measurement of back fat thickness," *Prakt. Tierarzt.* **78**, 87–92 (1997).

43. M. E. Darvin et al., "Dermal carotenoid level and kinetics after topical and systemic administration of antioxidants: enrichment strategies in a controlled *in vivo* study," *J. Dermatol. Sci.* **64**(1), 53–58 (2011).
44. M. C. Meinke et al., "Influence on the carotenoid levels of skin arising from age, gender, body mass index in smoking/non-smoking individuals," *Free Rad. Antiox.* **1**(2), 15–20 (2011).
45. M. E. Darvin et al., "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**(4), 044028 (2008).
46. T. H. Herdt and H. D. Stowe, "Fat-soluble vitamin nutrition for dairy-cattle," *Vet. Clin. N. Am. Food A* **7**(2), 391–415 (1991).
47. G. Rosenberger, *Krankheiten des Rindes*, Blackwell Wissenschaft, Berlin (1994).
48. M. C. Meinke et al., "Bioavailability of natural carotenoids in human skin compared to blood," *Eur. J. Pharm. Biopharm.* **76**(2), 269–274 (2010).
49. M. E. Darvin et al., "Determination of beta carotene and lycopene concentrations in human skin using resonance Raman spectroscopy," *Laser Phys.* **15**(2), 295–299 (2005).
50. T. Ludewig et al., "Histological and histochemical investigations of the skin of the bovine mammary gland with special reference to changes during the perfusion in an *in-vivo* model.," *Deut. Tierarztl. Woch.* **103**(12), 501–505 (1996).
51. E. R. Greenberg et al., "A clinical trial of beta carotene to prevent basal-cell and squamous-cell cancers of the skin. The Skin Cancer Prevention Study Group," *N. Engl. J. Med.* **323**(12), 789–795 (1990).
52. W. Stahl et al., "Increased dermal carotenoid levels assessed by non-invasive reflection spectrophotometry correlate with serum levels in women ingesting Betatene," *J. Nutr.* **128**(5), 903–907 (1998).
53. M. E. Darvin et al., "Radical production by infrared A irradiation in human tissue," *Skin Pharmacol. Physiol.* **23**(1), 40–46 (2010).
54. Y. Kaya et al., "Accelerated atherosclerosis in haemodialysis patients; correlation of endothelial function with oxidative DNA damage," *Nephrol. Dial. Trans.* **27**(3), 1164–1169 (2012).