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Real-time trace gas sensing of ethylene, propanal and acetaldehyde from human skin *in vivo*

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Abstract

Trace gases emitted by human skin *in vivo* are monitored non-invasively and in real time using laser-based photoacoustic detection and proton-transfer reaction mass spectrometry. A small quartz cuvette is placed on the skin to create a headspace from which a carrier gas transports the skin emissions to the detection systems. The transparency of quartz to ultraviolet radiation (UVR) allows investigation of UVR-related trace gas emissions. As a demonstration of this measurement system, the effect of supplemental intake of systemic antioxidants on UVR-induced lipid peroxidation is investigated. The production by the skin of three biomarkers of UVR-induced lipid peroxidation (ethylene, acetaldehyde and propanal) is monitored. Although no significant effect of antioxidant intake was observed, the method presented here is a novel and promising technique for investigation of human skin *in vivo*.

Keywords: trace gas detection, dermatology, laser, mass spectrometry

1. Introduction

The detection of trace quantities of gaseous molecules has numerous applications in many fields of life science, such as biology (Hekkert *et al* 1998), environmental monitoring and medical diagnostics (Phillips 1992). An established method of trace gas detection is gas chromatography. However, this technique requires concentration of sample gases on a solid sorbent and is therefore not suited to online measurements. Furthermore, the choice of the proper sorbent material during the concentration step is critical. Compound specific differences in trapping efficiency of the sorbent material, and possible contamination due to 'bleeding'

may cause artifacts. To overcome this delicate and time consuming concentration step we propose to use alternative detection with laser-based photoacoustic detection (LPD) and proton transfer reaction mass spectrometry (PTR-MS). These techniques have such a high sensitivity that accumulation is no longer needed, and online measurements become possible.

LPD is able to monitor trace gas concentrations under atmospheric conditions with orders of magnitude better sensitivity than most gas chromatography instrumentation. In addition, they are able to monitor non-invasively and online under changing sample conditions (Harren *et al* 2000). The photoacoustic instrument used in this study has been developed previously at the Radboud University Nijmegen (Harren *et al* 2000). PTR-MS is a technique that is very well suited for measurements of aldehydes and other hydrocarbons in multi-component mixture in an online, non-invasive and highly sensitive fashion (Lindinger *et al* 1998, Steeghs *et al* 2004, Boamfa *et al* 2004, Steeghs *et al* 2006).

In this study, both LPD and PTR-MS are used for real-time monitoring of trace emissions from human skin *in vivo*. A novel headspace sampling system utilizing a quartz (SiO_2) skin cuvette, which is transparent to ultraviolet radiation (UVR), allows the investigation of physiological effects related to exposure to UVR.

Upon exposure to UVR, a variety of biologic effects can occur, both beneficial and harmful. It is commonly accepted that solar UVR causes almost all photoinduced damage of human skin. Part of the UVR-induced damage is due to the formation of free radicals in the skin (De Gruijl 1997, Kneepkens *et al* 1994). Pigmentation protects the skin by scattering UVR and by acting as a free radical scavenger. Despite this protection, exposure to UVR leads to an increase in lipid peroxidation (Harren *et al* 1999), causing polyunsaturated fatty acids to react with free radicals to form a variety of products, including pentane, ethane and ethylene (Sagai and Ichinose 1980). It was previously shown that trace gas detection of ethylene can be used to quantify UVR-induced lipid peroxidation *in vivo* (Harren *et al* 1999). Recently, two other biomarkers of UVR-induced lipid peroxidation, acetaldehyde and propanal, have been identified (Steeghs *et al* 2006).

Antioxidants act as scavengers of free radicals and may therefore protect the skin against UVR-induced lipid peroxidation by neutralizing reactive oxygen species (ROS) (Fang *et al* 2002). Therefore, we propose to monitor the effect of supplementation of the diet with antioxidants on UVR-induced lipid peroxidation. For this we employed LPD and PTR-MS to follow in real time the UVR-induced emission of ethylene, acetaldehyde and propanal from the skin of volunteers, some of which consumed the antioxidants, and others received a placebo.

2. Materials and methods

Our approach to sensitive measurement of trace gases from human skin consists of a LPD and a PTR-MS combined with a sampling system based around a quartz cuvette that is placed on the skin. The different elements of the total system are illustrated below.

2.1. Laser-based photoacoustic detection

The photoacoustic trace gas detector has been described in detail earlier (Harren *et al* 1990), and is schematically represented in figure 1. Briefly, the detector consists of a line-tunable CO_2-laser emitting radiation in the 9–11 μm region and a photoacoustic cell, in which the gas is detected. The requirement for gases to be detected is that they possess a high absorption strength and a characteristic absorption pattern in the wavelength range of the CO_2 laser. Inside the photoacoustic cell, traces of the sample molecule can absorb the laser radiation; the absorbed energy is released into heat, which creates an increase in pressure inside a closed

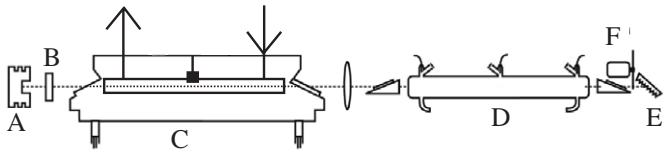

Figure 1. Schematic representation of the photoacoustic ethylene detector. The CO_2-laser (D) wavelength is determined by the angle of the grating (E). The chopper (F) modulates the laser at the resonance frequency of the photoacoustic detection cell (C). Shown here are the gas inlet and outlet, and the microphone, which records the ethylene signal, mounted in the middle of the wall of the resonator. A small fraction of the laser light is transmitted by a mirror (B) and quantified using a powermeter (A).

volume. By modulating the laser beam with a mechanical chopper, pressure waves (i.e., sound) are generated and detected with a sensitive miniature microphone.

Gas mixtures are sensitively measured by the laser-based photoacoustic detector due to the distinct fingerprint-like spectrum of different molecules in the CO_2-laser wavelength range. By comparing the photoacoustic signals on various laser lines (at which ethylene has different absorption strengths) the response of the compound under investigation can be distinguished from interfering background signals that do not show such an absorption spectrum. In the case of ethylene detection, the CO_2 laser is alternately tuned to the 10P14 (ν = 948.48 cm^{-1}) and 10P12 (ν = 951.19 cm^{-1}) laser lines, and at each line the photoacoustic signal is recorded. The different absorption coefficients of ethylene at these wavelengths add specificity to the calculation of the ethylene concentration. In addition, several measures were taken to prevent interfering gases from entering the detection cell in the first place. A sodalime-based scrubber and a KOH-based scrubber were used to remove CO_2 from the sample air. A $CaCl_2$-based scrubber was used to decrease the water content of the sample gas. Other potential interfering gases such as ethanol were removed using a liquid nitrogen cooled cryogenic trap at 125 K.

The amplitude of the acoustic waves is directly proportional to the concentration of sample molecule in the photoacoustic cell (Harren and Reuss 1997). The length of the resonator (10 cm) and the frequency of modulation (1600 Hz) of the laser are matched so that the generated sound resonates inside the detection cell. The diameter of the resonator is 6 mm. This resonance effect significantly increases sensitivity. The photoacoustic cell consists of a straight tube with a small microphone embedded in the middle, a gas inlet and a gas outlet. The small volume of the detection cell (30 ml) allows a fast response to changing gas concentrations because of the small sample volume of the photoacoustic cell. Due to their strong absorptions at CO_2 laser lines this system is capable of sensitive measurement of ethylene, ammonia, ozone and sulfurhexafluoride. As mentioned, for this study the photoacoustic detector was used to measure ethylene, in which case the sensor detection limit is 6 pptv (Harren and Reuss 1997) (part-per-trillion by volume).

2.2. Proton transfer reaction mass spectrometry

A custom-built PTR-MS system was used in this study (figure 2) and a detailed description of this system can be found elsewhere (Boamfa *et al* 2004, Lindinger *et al* 1998, Steeghs *et al* 2004, de Gouw *et al* 2003). Therefore, only a brief description is given here.

The instrument consists of an ion source, in which H_3O^+ ions are produced in a discharge in a mixture of water vapor and helium gas. Furthermore, the system houses a drift tube, a transition chamber and an ion detection section containing a quadrupole mass filter and a secondary electron multiplier. In the drift tube, the trace gases from the sample gas are ionized

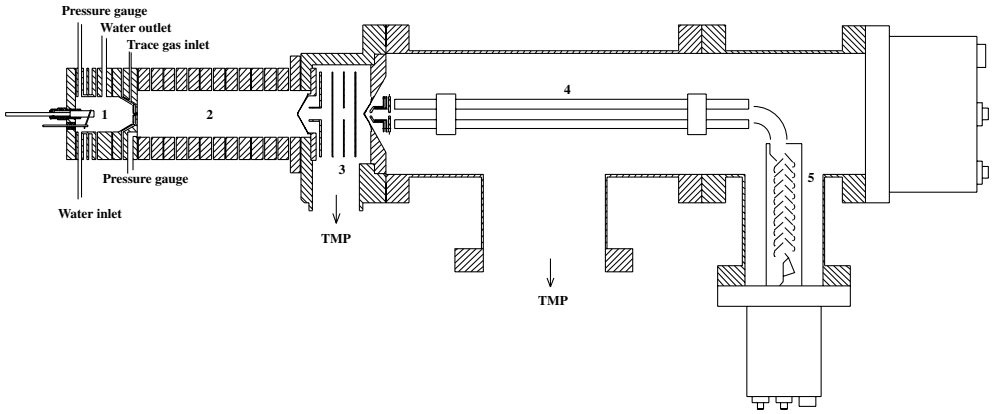

Figure 2. PTR-MS detection system. H_3O^+ primary ions are produced in a hollow cathode ion source (1), and are extracted to the drift tube (2). Here, they undergo proton-transfer reactions with the neutral compounds to be analyzed. After protonation, the ions are led through a transition chamber (3), to differentially pump the drift tube, toward the detection chamber (4). All pumps are turbo molecular pumps (TMPs). In the detection chamber a quadrupole mass filter mass-selects the ions before detection with a secondary electron multiplier (5).

by proton-transfer reactions with H_3O^+ ions, which will only take place when the proton affinity (PA) of the trace compound is higher than that of water (166.5 kcal $mol^{-1} = 7.16$ eV). The proton transfer rate is close or equal to the collision rate. Since the PA of water is higher than the PA of the main constituents in air (NO, O_2, CO, CO_2 and N_2), there is no interference from these compounds. Most typical organic compounds have a PA in the range between 7 and 9 eV, assuring a low excess energy of the reaction and a very low degree of fragmentation of the reaction products. Dissociation can occur (e.g. alcohols can split of a water molecule, which results in a fragment ion at molecular mass minus 17) and increases with carbon chain length. However, this results only in the formation of one or two fragments of significant intensity. Therefore, due to the soft ionization the matrix of signals is less complicated than with other mass spectrometry techniques.

The drift tube is a flow-through system kept at 2–2.5 mbar by an offline pressure controller. The present setup requires a minimal gas load of $\sim$0.5 l h^{-1} (all flow values are given at standard temperature and pressure (STP)) to get the optimal 2.25 mbar pressure in the drift tube and an E/N-value of 120 Townsend (E/N is the ratio between the electric field and the number density in the drift tube; 1 Townsend $= 10^{-17}$ V cm^2). This mass spectrometer is capable of online, real-time trace gas detection of many compounds, including aldehydes, alcohols, ketones, aromatics, acids and most other unsaturated or S, N, Cl, F and P-substituted hydrocarbons to the (sub) ppbv level. Here, the PTR-MS was applied to online measurement of acetaldehyde and propanal emissions from human skin *in vivo*.

2.3. Sampling system

A sampling method was developed that allows measurement of trace emissions from human skin in a real-time and non-invasive fashion, shown in figure 3. A quartz cuvette (5 cm diameter, 20 ml volume) is positioned on the skin. A reservoir of dry air is used as a carrier gas to transport the compounds produced by the skin to the trace gas detection systems. The flow through the cuvette is kept constant by a membrane pump and mass flow-controller (Brooks). This small flow, combined with a drop of water placed in between the edges of the cuvette

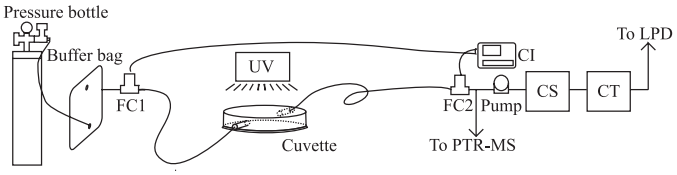

Figure 3. Schematic representation of the sampling system. Dry air from a pressure bottle is used to fill a buffer bag. A small membrane pump creates a flow of air through the two flow controllers (FC1 and FC2) and the quartz skin cuvette. FC1 is set to measuring mode. The system was considered closed if the flow through FC1 was at least 90% of the flow through FC2 (set to 0.7 l h^{-1}). A small portion of the flow (0.2 l h^{-1}) was led to the PTR-MS, which operates at low pressure, for measurement of volatile organic compounds. The control interface (CI) monitored the flow rates of both flow controllers, and recorded the total flow through the cuvette. The remaining portion of the flow (0.5 l h^{-1}) was led through chemical scrubbers (CS) to remove water ($CaCl_2$) and CO_2 (sodalime, KOH) from the sample air. After passage through a cold trap (CT) (125 K) to remove heavier hydrocarbons and water, the sample air entered the photoacoustic detection system.

and the skin, is enough to create a slight pressure drop which keeps the cuvette fixed firmly in place. From the skin cuvette the sample air is transported via Teflon PFA tubing to the trace gas detectors. The inlet and outlet tubing are shielded with aluminum foil to avoid UV-induced gas emissions from the tubing walls. During the whole experiment, a constant flow through the cuvette is maintained, which was split and led to both the LPD and the PTR-MS system. All gas handling parts (tubing, connections, needle valves, etc), except mass flow controllers, are made of Teflon PFA or Teflon PTFE (PolyFluor Plastics, Hoevestein, The Netherlands) to reduce adsorption effects on the tubing walls. Scrubbers and cooling trap, necessary for sensitive trace gas detection of ethylene were only used after the flow was split toward the LPD.

2.4. Determining the effect of systemic antioxidants on UVR-induced lipid peroxidation

We used the system to study the effect of systemic antioxidants on UVR-induced lipid peroxidation. The trace gas emission from the skin of volunteers was measured before and after consumption of antioxidants. A cocktail of antioxidants was supplied in the form of pills and a drink, to be taken orally after dinner during 8 days, as is discussed in detail later.

For the UVR measurements the quartz cuvette is placed on the buttock of a volunteer on a circular piece of bare skin (5 cm diameter) that will be irradiated with UVR. The buttock can be easily protected from unwanted exposure to UVR and provides a flat skin surface that allows a complete seal between the skin and the quartz cuvette. During the first 10 min the trace gas emission of the skin was measured without UVR exposure as a background value. Then only the skin surface covered by the quartz cuvette was exposed to UVR from a HPA 400S lamp (Philips) for 12 min at a UVB intensity of 2 mW cm^{-2} (UVA $>$ 20 mW cm^{-2}). During the measurement the headspace of the skin was continuously sampled by the trace gas detection systems. The carrier gas (dry air) was led through the skin cuvette at a flow of 0.7 l h^{-1}, of which 0.5 l h^{-1} was led to the LPD. The other 0.2 l h^{-1} was led to the PTR-MS, after it was diluted with 0.3 l h^{-1} pure nitrogen to reach the minimum flow rate required by the instrument.

During measurement the volunteers lay on their belly on a research bench, and the skin cuvette was placed on one buttock. The other buttock was irradiated in the second measurement to rule out any influence of adaptation of the skin, which may be caused by the first UVR exposure. The emission of the light source was mostly in the UVA region (see figure 4). Over this wavelength range the transmittance of quartz is nearly constant at approximately 90%.

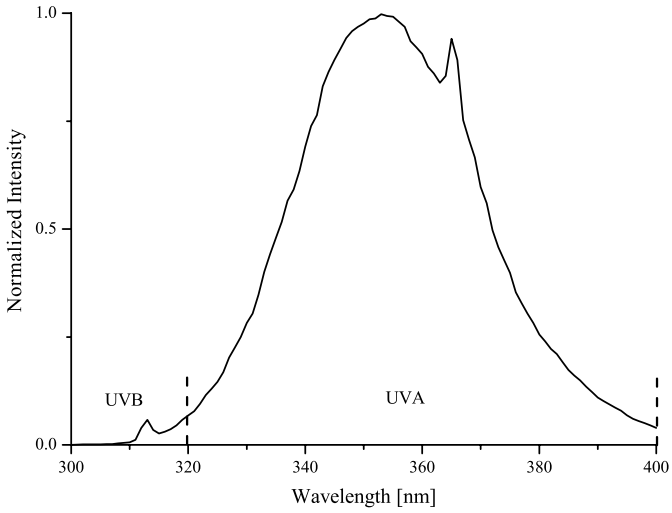

Figure 4. Emission spectrum of the UVR light source used for irradiation of the skin.

The population under study consisted of 17 healthy, non-smoking, white male volunteers, 20–35 years old and all students or employees of the Radboud University Nijmegen, with skin type 2 or 3, as shown in table 1. Volunteers were randomly assigned to groups receiving antioxidants (AOs) ($n = 11$) or a placebo ($n = 6$), which were identical in appearance. The study was performed in a double-blind fashion.

Volunteers were asked to supplement their diet with provided antioxidants during 8 days, to be taken orally after dinner. They were also asked not to smoke or drink alcohol for at least 24 h prior to measurements to minimize the influence of drinking and smoking on the emitted levels of the biomarkers. The duration of antioxidant uptake was based on previous results, where vitamin E supplementation led to an increase in blood levels and reached a steady state at 4 to 5 days after supplementation of 400–1200 IU/day (Dimitrov *et al* 1991). Furthermore, vitamins E and C during 8 days were effective in raising the minimal erythemal dose (MED)[3] of volunteers (Eberlein-Konig *et al* 1998). The AO supplements used here contained vitamin E (1000 international units (IU) dl-alpha-tocopherol, UMC St. Radboud), vitamin C (2000 mg ascorbic acid, UMC St. Radboud), selenium (200 μg selenomethione, Centrafarm) and N-acetylcysteine (NAC) (250 mg NAC, UMC St. Radboud). All AO supplements were in the form of pills, except for vitamin E for which a drink was used. Placebo supplements were identical to the antioxidant supplements except for the active compounds. Vitamin E is a fat-soluble vitamin with strong antioxidative properties (Topinka *et al* 1989), and is a generic term for a series of naturally occurring tocopherols and tocotrienols (alpha, beta, gamma, delta-homologues), from which alpha-tocopherol is the most bioactive form. The recommended daily allowance (RDA) is 15 mg of alpha-tocopherol. However, RDAs are based on preventing deficiency diseases, whereas a higher intake may have beneficial health effects. Vitamin C (ascorbic acid) is a water soluble vitamin with antioxidative activity, and it also recycles vitamin E (Fang *et al* 2002, Fuchs and Kern 1998). Selenium is a component of antioxidant glutathione peroxidase (Food and Nutrition Board 2000) and is often used in combination with vitamin E or vitamin C to reduce oxidative stress. N-acetylcysteine

[3] MED = the minimal effective UVR dose that causes a perceptible reddening of the previously unexposed human skin.

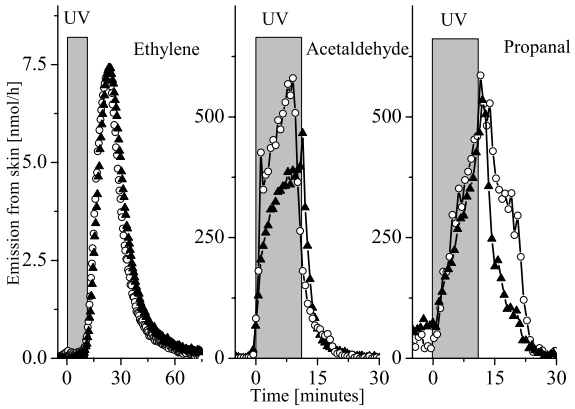

Figure 5. Example of trace gas measurements, before (black triangle) and after (open circle) intake of AOs. All concentration–time curves obtained during the measurements were standardized and a baseline was subtracted. Note the different time scales for LPD (ethylene) and PTR-MS (acetaldehyde and propanal). For ethylene measurements, the area under each curve was integrated from $t = 0$ to $t = 65$ min, representing the trace gas production during 65 min. For propanal and acetaldehyde measurements, the area under each curve was integrated from $t = 0$ to $t = 35$ min. Different integration times were used for the photoacoustic and PTR-MS measurements, because of the different gas delay and rise time for the two detection systems. To calculate the total production this outcome was corrected for total flow through the skin cuvette.

Table 1. Volunteers.

	AO group ($N = 11$)	Placebo group ($N = 6$)
Median age (range)	25 (20–29)	23 (20–24)
Median body mass index (BMI)[a] (range)	23 (21–26)	22 (20–26)
Skin type 2/3	5/6	4/2

[a] $\text{BMI} = \frac{\text{length}}{\text{weight}^2}$.

is an amino acid and a precursor of the strong antioxidant glutathione. Because uptake of glutathione after oral intake is poor, N-acetylcysteine is used to increase the glutathione level in the body (Moldeus *et al* 1986). The tolerable upper level of intake (UL) for adults is set at 1 g per day for vitamin E based on the adverse effect of increased tendency of hemorrhage (Food and Nutrition Board 2000), at 2 g per day for vitamin C (Food and Nutrition Board 2000), and at 400 μg per day for selenium, while for N-acetylcysteine no UL is set.

The measurement protocol was approved by the Medical Ethical Committee for Research on Human Subjects of the University of Nijmegen, The Netherlands and written consent was obtained from all participating subjects.

3. Results and discussion

3.1. Monitoring of trace emissions from human skin in vivo

The detection limits for both the PTR-MS detection system and the LPD were sufficient for online monitoring of acetaldehyde, propanal and ethylene. The time response of both systems was also suitable for the skin measurements, although improvements could be made

Table 2. Biomarker production in nmol (mean ± SD) before and after intake of antioxidants, and the mean difference.

	Antioxidants			Placebo			
Biomarker	Before	After	Mean diff.	Before	After	Mean diff.	Two sample *t*-test
Ethylene	0.054 ± 0.031	0.054 ± 0.036	0.0010 ± 0.020	0.036 ± 0.025	0.054 ± 0.036	0.020 ± 0.029	$P = 0.17$
Propanal	6.38 ± 2.92	7.10 ± 2.80	0.781 ± 2.92	7.99 ± 3.90	7.50 ± 3.60	3.70 ± 2.70	$P = 0.79$
Acetaldehyde	28.8 ± 22.5	33.5 ± 32.2	4.69 ± 14.0	42.6 ± 22.9	26.1 ± 9.91	−18.0 ± 20.0	$P = 0.021$

by reducing the volume of the gas sampling system. The flow through the cuvette and the total volume of the gas handling parts and detection cells (including buffer and scrubber volumes for LPD) determine the response time, and these settings were not optimized for both systems. The production rates observed in this study indicate the possibility of further increasing the flow and thus the time response. Figure 5 shows an example of concentration–time curves obtained with the trace gas detection systems.

3.2. The effect of antioxidants on UVR-induced lipid peroxidation

In total 15 sets of concentration–time curves (both before and after AO supplementation) were successfully obtained, of which 10 in the antioxidant group and 5 in the placebo group. Two sets of concentration–time curves were rejected due to leakage between the cuvette and the skin of the volunteer. The observed time delay, mainly in the LPD measurements, can be explained by the time response of the gas system. The biomarker production was calculated in nanomol for all concentration–time curves. Table 2 shows the average amounts of biomarkers that were induced by UVR. For ethylene the mean differences were equal for the antioxidant and the placebo group, $P = 0.17$ (t-test for equality of means) (95% CI: $-$ 1.0–0.2), which indicates that there was no significant effect of antioxidant intake on UVR-induced ethylene release. The only significant effect was observed for acetaldehyde, where the placebo group showed a significant increase in production in the second measurement. For the antioxidant group, one would expect a lower biomarker production in the second measurement due to a protective effect of the antioxidants, but such an effect was not observed. In contrast, in both the antioxidant and the placebo group the average biomarker emission was slightly higher after taking of antioxidants or placebo's, respectively. The variation in biomarker production is due to intra- and interindividual variability, although interindividual variation was limited somewhat by the high homogeneity in the study population. No information was available about diet, which can be of influence on the antioxidant status. We can assume the diet to remain constant during 8 days, but the baseline antioxidant status could have been higher in some subjects than in others. A 'saturation' effect could also be of influence: if the body is saturated with antioxidants, additional supplementation will not increase the bioavailability of antioxidants in the skin. No information on blood levels of antioxidants was available. Therefore, the ethylene production could not be related to blood levels of antioxidants. However, as mentioned previously, in earlier studies supplementation of antioxidants with comparable doses and duration did lead to an increased bioavailability (Dimitrov *et al* 1991, Eberlein-Konig *et al* 1998). In future studies information should be used on diet and antioxidant levels in skin and blood to asses the baseline antioxidant status of each volunteer.

4. Conclusion

The application of PLD and PTR-MS to detecting trace gases from human skin was demonstrated. Combined with the sampling system shown here, these detection techniques allow real-time and non-invasive measurement of many compounds emitted by human skin *in vivo*. As an application the effect of supplemental antioxidants on UVR-induced lipid peroxidation was studied. Although no effect of the antioxidant intake was observed, it was demonstrated that this system could be applied for similar experiments with volunteers. The real-time nature and high sensitivity of both detection systems allows investigation of dynamical processes on a short timescale and at low concentrations. For this reason the approach used here is applicable to numerous studies, for example: studying the wavelength-dependent effects of UVA and UVB light to create action spectra for various conditions,

assessing the efficiency of sun screen protection or to study the effects of UV treatment of skin diseases (e.g., psoriasis). It is therefore believed that laser-based photoacoustic detection and proton transfer reaction mass spectrometry can make an important contribution to the study of human skin *in vivo*.

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