Whole Body UVA Irradiation Lowers Systemic Blood Pressure by Release of Nitric Oxide From Intracutaneous Photolabile Nitric Oxide Derivates

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Rationale: Human skin contains photolabile nitric oxide derivates like nitrite and *S*-nitroso thiols, which after UVA irradiation, decompose and lead to the formation of vasoactive NO.

Objective: Here, we investigated whether whole body UVA irradiation influences the blood pressure of healthy volunteers because of cutaneous nonenzymatic NO formation.

Methods and Results: As detected by chemoluminescence detection or by electron paramagnetic resonance spectroscopy in vitro with human skin specimens, UVA illumination (25 J/cm^2) significantly increased the intradermal levels of free NO. In addition, UVA enhanced dermal *S*-nitrosothiols 2.3-fold, and the subfraction of dermal *S*-nitrosoalbumin 2.9-fold. In vivo, in healthy volunteers creamed with a skin cream containing isotopically labeled ^{15}N-nitrite, whole body UVA irradiation (20 J/cm^2) induced significant levels of ^{15}N-labeled *S*-nitrosothiols in the blood plasma of light exposed subjects, as detected by cavity leak out spectroscopy. Furthermore, whole body UVA irradiation caused a rapid, significant decrease, lasting up to 60 minutes, in systolic and diastolic blood pressure of healthy volunteers by 11±2% at 30 minutes after UVA exposure. The decrease in blood pressure strongly correlated (R^2=0.74) with enhanced plasma concentration of nitrosated species, as detected by a chemiluminescence assay, with increased forearm blood flow (+26±7%), with increased flow mediated vasodilation of the brachial artery (+68±22%), and with decreased forearm vascular resistance (−28±7%).

Conclusions: UVA irradiation of human skin caused a significant drop in blood pressure even at moderate UVA doses. The effects were attributed to UVA induced release of NO from cutaneous photolabile NO derivates. (*Circ Res*. 2009;105:1031-1040.)

Apart from its effects on stroke, renal failure, and peripheral arterial disease, systemic arterial hypertension is a major risk factor for cardiovascular complications, including coronary artery disease, heart failure and sudden cardiac death.[1,2]

Interestingly, mean systolic and diastolic pressures and the prevalence of hypertension vary throughout the world. Many data suggest a linear rise in blood pressure at increasing distances from the equator. Similarly, blood pressure is higher in winter than summer.[3] Previously, it has been hypothesized that reduced epidermal vitamin D$_3$ photosynthesis associated with decreased UV light intensity at distances from the equator, alone or when coupled with decreased dietary calcium and vitamin D, may be associated with reduced vitamin D stores and increased parathyroid hormone secretion.[4] These changes may stimulate growth of vascular smooth muscle and enhance its contractility by affecting intracellular calcium, adrenergic responsiveness, and/or endothelial function. Thus, UV light intensity and efficiency of epidermal vitamin D$_3$ photosynthesis may contribute to geographic and racial variability in blood pressure and the prevalence of hypertension.[4]

However, there might exist another or additional supporting mechanism, respectively, by which ambient electromagnetic radiation may affect blood pressure. Furchgott et al noted as long ago as 1961 that exposure to sun light relaxed isolated arterial preparations,[5] although other types of smooth muscle tissue were much less sensitive.[6] The vascular photorelaxation was wavelength-dependent, increasing as wavelength was reduced from the visible into the UV range, and it

Non-standard Abbreviations and Acronyms	
CALOS	cavity leak out spectroscopy
CLD	chemoluminescence detection
cPTIO	1H-imidazol-1-yloxy-2-(4-carboxyphenyl)-4,5-dihydro-4,4,5,5-tetramethyl-3-oxide
EPR	electron paramagnetic resonance
FBF	forearm blood flow
FMD	flow-mediated vasodilatation
HR	heart rate
MAP	mean arterial blood pressure
MNIC	mononitrosyl-iron complex
RS-NO	S-nitroso thiols
RX-NO	nitroso compounds

was independent of the endothelium.[7] Furthermore, photorelaxation was markedly potentiated by solutions containing nitrite,[8–10] indicating that under certain circumstances nitrite may exhibit relaxing activities comparable to NO.

Nitrite is a constituent of sweat, assumed to be formed on the skin surface by commensural bacteria.[11] Furthermore, in human skin NOS-dependent production of nitric oxide (NO) potentially occurs in all dermal cell types.[12,13] Some of the NO molecules formed remain at or close to the point of their origin as nitroso compounds, eg, S-nitrosothiols (RS-NO) or mercuric chloride–nonsensitive nitroso compounds or as the oxidation products nitrite and nitrate.[14] UVA is known to penetrate deep enough into skin to reach the micro vessels.[15,16] Thus, in human skin photosensitive NO derivates like RS-NOs or nitrite may undergo photodecomposition when irradiated with UVA light,[17–19] resulting in the formation of bioactive NO.[14,20] Previously, we have demonstrated that UVA exposure of healthy skin specimens leads to an enzyme-independent high-output NO formation, reaching concentrations comparable or higher than found with maximal activity of the inducible NO synthase in cytokine-activated human keratinocyte cultures in vitro.[21] We now extend these previous results by investigating the effect of whole body UVA exposure on the systemic blood circulation in humans.

Methods

Details regarding materials and experimental procedures with respect to materials, volunteers, UV sources, cell cultures, human skin samples, UVA-induced decomposition of nitrite and S-nitroso albumin formation, detection of S-nitroso proteins by immunohistochemistry, Western blot analysis of S-nitrosothiol proteins in human dermis, collection of blood samples and determination of blood pressure, cGMP measurements, analysis of cutaneous vascular parameters, sample preparation for detection of ^{15}N-labeled nitroso compounds in human blood plasma by cavity leak out spectroscopy (CALOS), detection of NO, quantification of nitrite and nitroso compounds by chemoluminescence detection (CLD), electron paramagnetic resonance (EPR) spectroscopy, detection of ^{15}NO by CALOS, and statistical analysis are in the expanded Methods section in the Online Data Supplement, available at http://circres.ahajournals.org.

Results

UVA Irradiation of Human Skin Reduces Blood Pressure

Immediately after UVA irradiation, as well as up to 60 minutes after the light stimulus, the values of systolic as well as diastolic blood pressure were reduced in all subjects as compared to control values determined before the irradiation procedure. Figure 1 shows that mean arterial blood pressure (MAP) was significantly lowered after UVA illumination. The effect persisted for a considerable duration: relaxation toward previous resting state was observed on the timescale of about an hour ($-5.6\pm3.2\%$ immediately after UVA, $-11.9\pm1.8\%$ 15 minutes after UVA, and $-5.9\pm2.1\%$ 45 minutes after UVA); $P<0.005$ as compared to the controls).

UVA Irradiation of Human Skin Increases Plasma Nitroso Compounds and Nitrite Concentrations

The blood plasma of UVA-irradiated volunteers showed significantly enhanced nitroso compound (RX-NO) (Figure 2), as well as nitrite concentrations (Figure 3), in the time interval of 15 to 45 minutes after illumination (RX-NO: $74\pm16\%$ 15 minutes after UVA and $53\pm19\%$ 45 minutes after UVA; $P<0.005$ as compared to the controls; nitrite: $43\pm22\%$ 15 minutes after UVA, $59\pm32\%$ 45 minutes after UVA, and $40\pm26\%$ 75 minutes after UVA; $P<0.005$ as compared to the controls). As shown in Figure 2D, UVA-induced decreases in blood pressure highly correlated with plasma RX-NO ($R^2=0.74$) but did not correlate with plasma nitrite ($R^2=0.0071$) concentration (Figure 3D).

UVA Irradiation of Human Skin Alters Cardiovascular Parameters

Furthermore, UVA-induced decrease in blood pressure was paralleled by increased forearm blood flow (FBF), increased flow-mediated vasodilatation of the brachial artery (FMD$\Delta\%$), as well as decreased forearm vascular resistance. As shown in Figure 4, 15 minutes after UVA, a significant increase in FBF ($26.1\pm7.3\%$) and FMD$\Delta\%$ ($68\pm22\%$) and a significant decrease in forearm vascular resistance ($-28.1\pm7.5\%$) was detected. UVA challenge had no significant effects on heart rates of irradiated volunteers.

Plasma From UVA-Irradiated Volunteers Exerts NO-Dependent Biological Activity

cGMP responses of RFL-6 cells in the presence of superoxide dismutase (500 U/mL) and isobutyl methylxanthine (0.6 mmol/L) were used to determine the bioactivity of plasma obtained from nonirradiated as well as UVA-irradiated volunteers. As shown in Figure 4H, incubation of RFL-6 cells with plasma that was collected from UVA-exposed volunteers 30 minutes after the irradiation induced a significantly higher response in cGMP formation than plasma obtained from nonirradiated volunteers (7.07 ± 1.89 versus 2.65 ± 0.63 nmol/L cGMP per milligram of protein). These increases were significantly lower in the presence of the NO scavenger 1H-imidazol-1-yloxy-2-(4-carboxyphenyl)-4,5-dihydro-4,4,5,5-tetramethyl-3-oxide (cPTIO) (1.95 ± 1.23 nmol/L cGMP/mg protein).
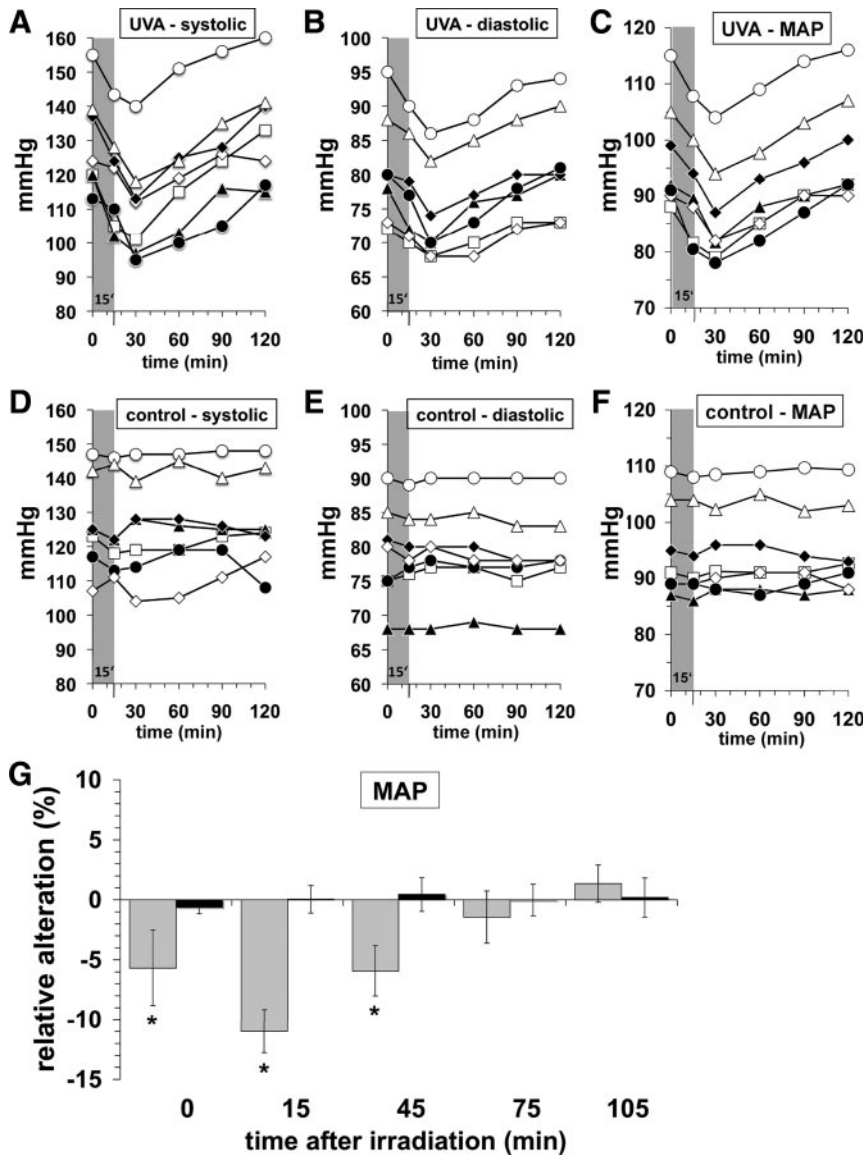
Figure 1. Effects of UVA irradiation of human skin on systolic and diastolic blood pressure. Healthy volunteers (○, no. 1; ●, no. 2; ◇, no. 3; ◆, no. 4; △, no. 5; ▲, no. 6; □, no. 7) were irradiated for 15 minutes (gray area in A through F) with UVA light (20 J/cm^2) or control-treated. Then, immediately after and 15, 45, 75, and 105 minutes after irradiation, systolic and diastolic blood pressure was detected. A, Systolic blood pressure in UVA-challenged volunteers. B, Diastolic blood pressure in UVA-challenged volunteers. C, MAP in UVA-irradiated volunteers. D, Systolic blood pressure in control-treated volunteers. E, Diastolic blood pressure in control-treated volunteers. F, MAP in control-treated volunteers. G, Relative alterations in MAP of irradiated (gray bars) and control volunteers (black bars) as compared to initial control values (0 minutes) indicated in C and F. Values are the means±SD of 7 individual experiments. *P<0.001.

Effects of Skin Temperature

During UVA irradiation, the ventral and lateral skin areas remained open to ambient air, and the skin temperatures of volunteers did not differ from controls (both 30.9±1.3°C). The dorsal skin areas were not ventilated by ambient air. Here, the skin temperature of irradiated volunteers (37.9±0.3°C) was slightly higher than that of controls (35.7±0.8°C) (Figure 5A). To exclude a possible artifact from skin temperature on blood pressure, we investigated the effect of a 15 minutes bath in 38°C instead of UVA irradiation. The warm bath did not affect blood pressure at any time points up to 105 minutes post bath. (Figure 5B).

Additionally, we measured capillary–venous oxygen saturation, blood filling, blood flow, and flow velocity in superficial (1 mm deep) and deeper (6 mm deep) microvessels of human skin before, immediately after and 30 minutes after exposure to UVA (20 J/cm^2) or 41°C warm water. As compared to nonirradiated skin, UVA exposure (20 J/cm^2) had no effects on the mentioned cutaneous vascular parameters (Figure 5C and 5D). As positive control, exposure of

human skin for 10 minutes to 41°C warm water significantly enhanced blood flow and blood velocity of superficial (1 mm deep) and deeper (6 mm deep) microvessels of human skin (Figure 5E and 5F).

Concentrations of Nitrite and *S*-Nitrosothiols in Skin Specimens and in Plasma of Volunteers

In parallel, immunohistological analysis of human skin specimens revealed consistently the ubiquitous presence of *S*-nitrosated proteins (Figure 6A), whereas UVA irradiation of skin specimens leads to a consistent strong increase in *S*-nitrosothiols (Figure 6B). In normal human dermis, *S*-nitroso thiols can be found at a concentration of 3.2±0.9 μmol/L. The amount of *S*-nitroso thiols significantly increases after UVA challenge by 2.3-fold to 7.5±1.2 μmol/L (Figure 6D). A similar UVA-induced 2.9-fold enhancement was found for *S*-nitrosoalbumin in skin specimens (Figure 6E). In the dermis of humans skin specimens incubated for 12 hours with 100 μmol/L nitrite, UVA irradiation leads to a 4.5-fold increase in *S*-nitrosoalbumin levels as compared to control specimens (Figure 6E).
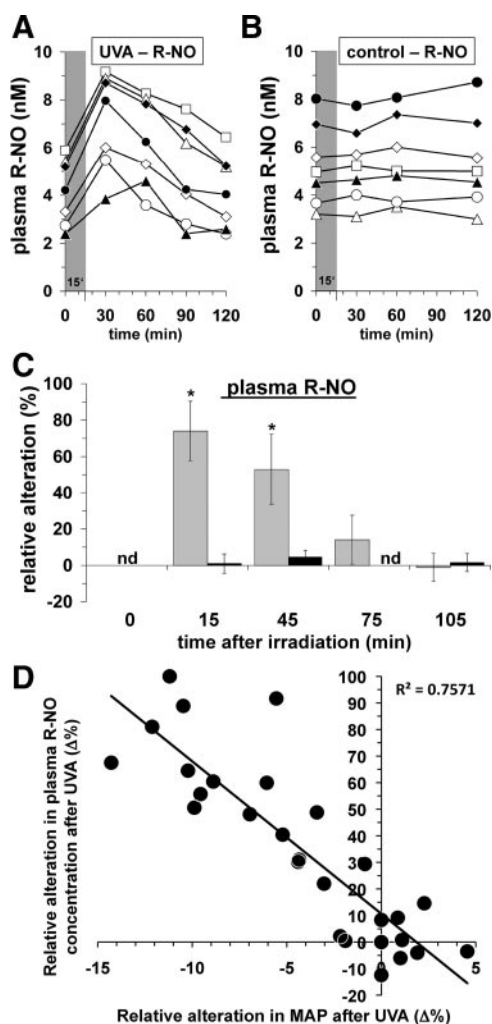
Figure 2. Effects of UVA irradiation of human skin on plasma concentrations of nitrosated compounds. Healthy volunteers ($\bigcirc$, no. 1; $\bullet$, no. 2; $\diamond$, no. 3; $\blacklozenge$, no. 4; $\triangle$, no. 5; $\blacktriangle$, no. 6; $\square$, no. 7) were irradiated for 15 minutes (gray area in A and B) with UVA light (20 J/cm^2) or control-treated. Then, 15, 45, 75, and 105 minutes after irradiation concentrations of nitrosated compounds (RX-NO) of plasma were detected by CLD. A, Plasma RX-NO concentrations of UVA-irradiated volunteers. B, Plasma RX-NO concentrations of control-treated volunteers. C, Relative alterations in plasma RX-NO concentrations of irradiated (gray bars) and control volunteers (black bars) as compared to initial control values (0 minutes) indicated in A and B. Values are the means$\pm$SD of 7 individual experiments. D, Correlation blot between MAP and plasma RX-NO concentration. With each volunteer, the calculated values of MAP after UVA irradiation as indicated in Figure 1G were correlated to the calculated values of relative alterations of plasma RX-NO as indicated in 2C. The correlation coefficient (R^2) is $R^2=0.7419$. *$P<0.001$.

To demonstrate UVA-dependent nonenzymatic NO formation from nitrite as well as UVA-induced nitrite-dependent *S*-nitroso-thiol formation in vitro, we irradiated nitrite-containing (10 μmol/L) and/or BSA-containing (10 mg/mL) solutions (PBS, pH 7.4) with UVA and detected nonenzymatic NO formation by CLD and *S*-nitroso-BSA formation by Western blot. As shown in Figure 6F, at the physiological pH 7.4, UVA radiation led to an apparent nitrite decomposition and a significant formation of NO. Furthermore, in the
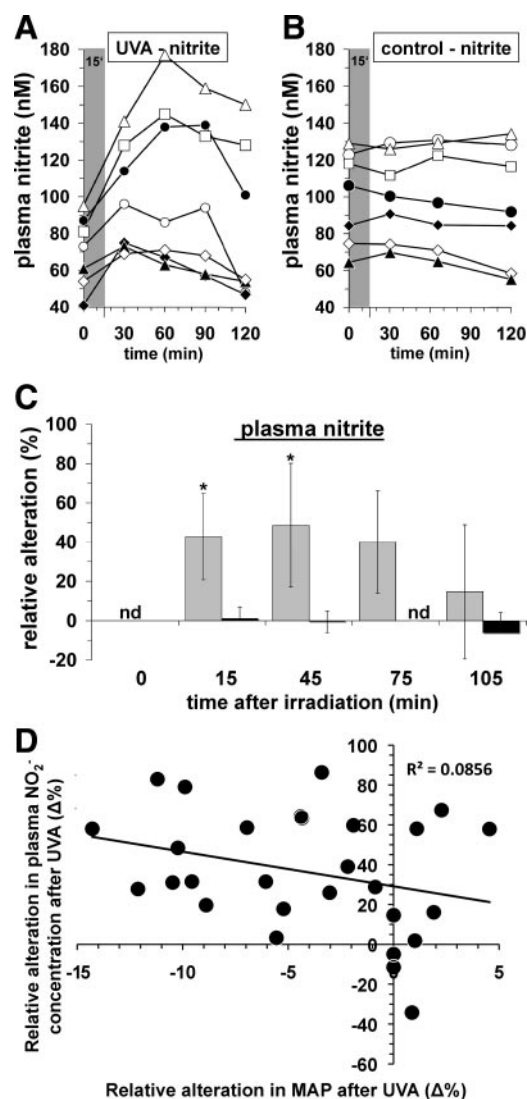

Figure 3. Effects of UVA irradiation of human skin on plasma nitrite concentrations. Healthy volunteers ($\bigcirc$, no. 1; $\bullet$, no. 2; $\diamond$, no. 3; $\blacklozenge$, no. 4; $\triangle$, no. 5; $\blacktriangle$, no. 6; $\square$, no. 7) were irradiated for 15 minutes (gray area in A and B) with UVA light (20 J/cm^2) or control-treated. Then, 15, 45, 75, and 105 minutes after irradiation nitrite concentrations of plasma were detected by CLD. A, Plasma nitrite concentrations of UVA-irradiated volunteers. B, Plasma nitrite concentrations of control-treated volunteers. C, Relative alterations in plasma nitrite concentrations of irradiated (gray bars) and control volunteers (black bars) as compared to initial control (c.) values indicated in A and B. Values are the means$\pm$SD of 7 individual experiments. D, Correlation blot between MAP and plasma nitrite concentration. With each volunteer, the calculated values of relative alterations of MAP after UVA irradiation, as indicated in Figure 1G, were correlated to the calculated values of relative alterations of plasma nitrite concentrations as indicated in 3C. The correlation coefficient (R^2) is $R^2=0.0071$. *$P<0.001$.

presence of BSA this UVA-dependent nonenzymatic NO production from nitrite led to an significant increase in *S*-nitroso-BSA formation, as detected by the *S*-nitroso-cysteine-specific antiserum (Figure 6G).

Release of Gaseous NO From Intact Skin and NO Spin Trapping in Human Skin Specimens

In a further experiment, an airtight chamber (16 cm^2) with a UVA transparent front window was placed on the forearm of
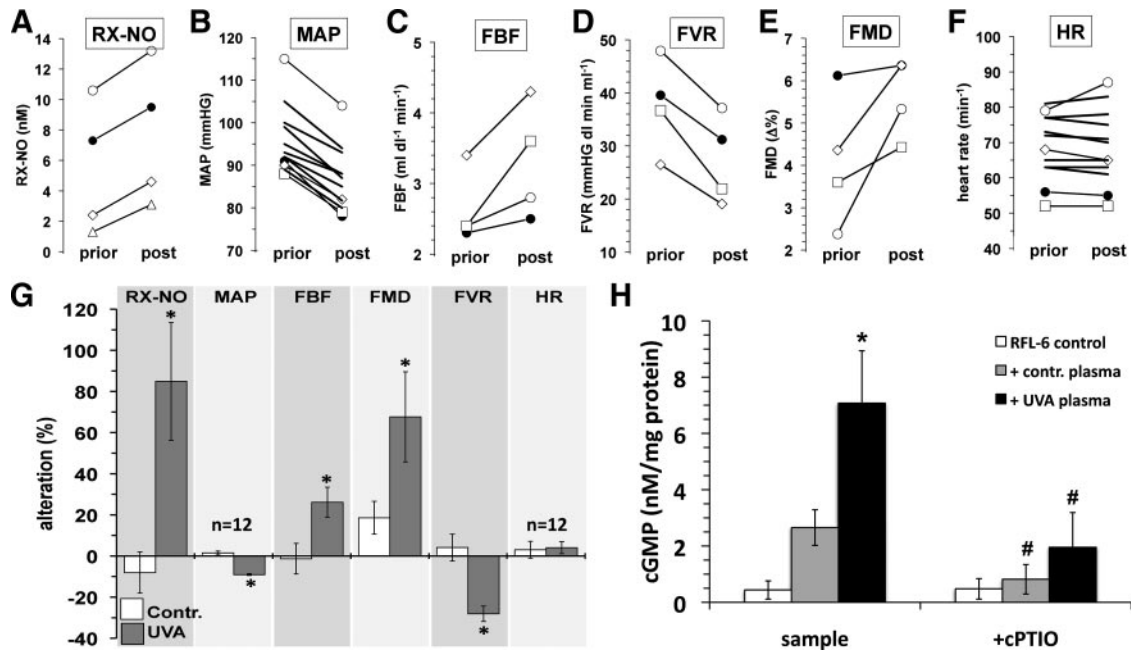
Figure 4. UVA irradiation of human skin alters cardiovascular parameters. Healthy volunteers (○, no. 1; ●, no. 2; ◇, no. 3; □, no. 7) were irradiated for 15 minutes with UVA light (20 J/cm²). Prior and 15 minutes after (post) irradiation plasma RX-NO concentration (A), MAP (B), forearm blood flow (FBF) (C), forearm vascular resistance (FVR) (D), the diameter of the brachial artery (FMDΔ%) (E), and heart rate (F) was detected in parallel. Values are the means±SD of 4 or 12 individual (in B and F) experiments, respectively. G, Relative alterations in plasma RX-NO concentration, MAP, forearm blood flow (FBF), forearm vascular resistance (FVR), the diameter of the brachial artery (FMDΔ%), and heart rate of UVA-irradiated, as well as control-treated, volunteers as compared to initial control values (0 minutes). Values are the means±SD of 4 or 12 individual experiments, respectively. H, cGMP production of RFL-6 cells (3×10⁵ cells) after 1 hour of incubation with plasma obtained from nonirradiated (gray bars), as well as UVA-irradiated (20 J/cm²), volunteers (black bars, blood samples were collected 30 minutes after UVA challenge). White bars represent the constitutive cGMP production of RFL-6 cells alone. Additionally, incubations were performed in the presence of the NO scavenger cPTIO (40 μmol/L). Values represent the means±SD of 3 individual experiments. *$P<0.001$ as compared to the controls; #$P<0.001$ as compared to the respective samples incubated in the absence of cPTIO.

volunteers. A gas flow of helium collected the gaseous NO emanating from the skin and was fed into the CLD analyzer (Figure 7A). In absence of UVA, a basal release of 29±25 fmol of NO per second per square centimeter was detected. Under UVA illumination with 20 J/cm², the release of gaseous NO was enhanced fourfold to 148±55 fmol of NO per second per square centimeter ($P<0.001$). After application of skin cream containing 10 μmol/L nitrite, the photo-induced yield of gaseous NO was again significantly enhanced to 334±112 fmol of NO per second per square centimeter (Figure 7A).

After illumination of Fe^{2+}-DETC–loaded human skin specimens for 30 minutes with UVA light (25 J/cm²), small sections of 200 to 250 mg were cut, immersed in strong HEPES buffer and snap frozen in liquid nitrogen. Before EPR analysis, the skin samples were reduced with dithionite (50 mmol/L for 15 minutes) to remove EPR signals from Cu^{2+}-DETC complexes.[22,23] The EPR spectra of figure 7B at test formation of mononitrosyl-iron complex (MNIC) adducts (¹⁴NO-Fe^{2+}-DETC, hyperfine triplet at g=2.035) in human skin. Spectra of mamma skin specimens (Figure 8A through 8C) routinely showed additional signal from nitrosy-lated ferrous hemoglobin (paramagnetic NO-Fe^{2+}-Hb)[24] and ceruloplasmin.[25]

From comparison with calibrated reference samples, we estimated formation of 63±7 pmol MNIC in 200 mg male abdomen skin after 30 minutes UVA illumination. In absence

of UVA, the MNIC yield remains below the EPR detection limit of ≈20 pmol. The MNIC yield could be enhanced to a massive 500 pmol by applying nitrite-loaded cream to the apical side of the skin specimens before UVA.

After splitting the skin samples horizontally with a razor blade, the apical outer layer had roughly threefold higher MNIC content than the endothermal inner layer. It shows that the outer layer is the main source of NO, as expected. Significantly, a large fraction of the total UVA induced NO has been trapped in the deeper skin layers, presumably because of diffusion of free NO through the skin tissue (The Fe-DETC traps and MNIC adducts themselves are immobilized in the lipid and protein compartments). After 30 minutes UVA, the MNIC concentration in the upper layers of male abdomen skin was ≈0.5±0.1 μmol/L. When the skin was pretreated with nitrite-spiked cream, the upper layers reached 6-fold higher MNIC concentration of ≈3.1±0.4 μmol/L.

These data suggest that NO is released from nitrite anions in the skin. Decomposition of nitrite was proven by application of cream with ¹⁵N-nitrite (I=1/2) before UVA. The isotopic doublet structure of Figure 8 proved that the ¹⁵NO ligand of MNIC derived from the ¹⁵N-nitrite of the cream. After subtraction of an experimental ¹⁵NO-Hb spectrum, we quantified the formation of 460 pmol ¹⁵NO-Fe^{2-}-DETC in 240 mg of mamma skin (Figure 8b).
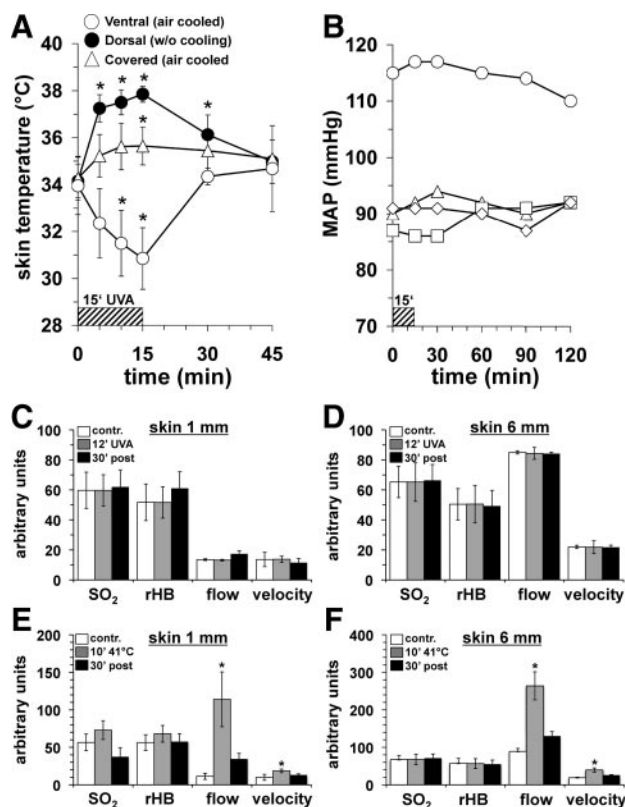
Figure 5. Effects of UVA irradiation on skin temperature, cutaneous blood flow, and influence of skin temperature on MAP. A, Skin temperature alterations were measured on UVA-exposed ventral and lateral air stream–ventilated skin areas and body sides (○), as well as UVA-irradiated dorsal skin areas that could not be cooled by the air stream (●). Additionally, skin temperature of control-treated subjects, which were covered during UVA exposure was measured (△). Values are the means±SD of 4 individual experiments. *$P<0.001$. The striped bar above the x axis indicates the time interval of light exposure. B, To exclude that the alterations in blood pressure after UVA challenge were the result of skin heating, blood pressure was determined during and after a 38°C bath for 15 minutes (○, ◇, □, △ represent the values of the respective volunteers). Striped bar indicates the time interval of warm water exposure. C and D, Effects of UVA radiation (20 J/cm²) on capillary–venous oxygen saturation (SO₂), blood filling (rHB), blood flow (flow), and flow velocity (velocity) in superficial skin regions (1 mm) (C) and deeper skin tissue (6 mm) (D) before UVA challenge (white bars), immediately after the light stimulus (gray bars), and 30 minutes after UVA irradiation (black bars). E and F, Effects of warm water bath (41°C) on capillary–venous oxygen saturation (SO₂), blood filling (rHB), blood flow (flow), and flow velocity (velocity) in superficial skin regions (1 mm) (E) and deeper skin tissue (6 mm) (F) before warm water exposure (white bars), immediately after (gray bars), and 30 minutes after the warm water exposure (black bars). Values are the means±SD of 4 individual experiments. *$P<0.001$.

UVA Irradiation of Human Skin Induces Transmigration of Nitrite-Derived NO From Skin Tissue Into Plasma

Additional experiments were performed to identify the source of the NO moiety in the metabolites circulating in the blood of irradiated volunteers. Following application of skin cream with ¹⁵N-nitrite (20 mL containing 100 μmol Na¹⁵NO₂, 5 mmol/L), whole body UVA irradiation (20 J/cm²) led to the formation of significant quantities of plasma ¹⁵N-nitrite (40±4 nmol/L in controls versus 62±4 nmol/L in irradiated

subjects, $P<0.001$) and *S*-nitrosothiols (RS-¹⁵NO) (0.4±0.4 nmol/L in controls versus 1.7±0.9 nmol/L in irradiated subjects, $P<0.001$) (Figure 8E and 8D). These quantities were determined by the isotope-sensitive CALOS method. The fraction of labeled to unlabeled nitrite or nitroso compounds remained undetermined in this experiment.

Discussion

The key finding of the present study is that UVA irradiation of healthy human skin significantly increases intracutaneous NO and *S*-nitrosothiol concentrations via decomposition of cutaneous photolabile NO derivates with the result of significantly enhanced concentration of plasma nitroso compounds and a pronounced decrease in blood pressure.

Our observations of systemic UVA response can be plausibly explained by a mechanism comprising 3 elementary steps. First, UVA liberates NO from photolabile intracutaneous NO metabolites. Second, a fraction of the highly mobile NO diffuses toward the outer surface, where it escapes into the ambient atmosphere. (This fraction is detectable with the airtight skin chamber.) Another NO fraction diffuses to deeper tissue layers, where it enters the capillary vessels and enhances local levels of RS-NO. These nitrosated species may be low-molecular-weight, such as glutathione-*S*-NO, or protein-bound high-molecular-weight, such as albumin-S-NO. Third, the fairly stable nitroso compounds are distributed via the blood circulation, where it may elicit a systemic response like a drop in blood pressure. We note that the vasodilating and hypotensive properties of *S*-nitrosothiols are well documented.[26] The observed release of free NO from UVA-irradiated skin lends strong support to this mechanism. Using isotopically labeled ¹⁵N-nitrite skin cream, CALOS spectroscopy demonstrated unequivocally that the photolysis of a photolabile NO derivate, here ¹⁵N-nitrite, in the epidermis by UVA contributes to the formation of nitrite and RS-NO species in the systemic blood circulation of volunteers. It provides proof of principle that NO moieties generated in the upper skin layers may migrate to the interior and translocate to NO moieties in the blood circulation for our proposed mechanism in vivo.

Human skin tissue is known to contain significant quantities of nitrite (4 to 6 μmol/L), RS-NO (≈2.6 μmol/L) and mercuric chloride-resistant, as well as UVA-resistant, nitroso species (1.3 μmol/L).[14] These concentrations exceed the human plasma concentrations by several orders of magnitude (nitrite ≈20-fold, RS-NO ≈300 fold). Every cell types in human skin is able to produce NO by at least one of three NO synthases. Therefore, enzymatically generated NO represents an important source of cutaneous photolabile NO derivates. Nevertheless, recently data presented by Mowbray et al gave evidence that dietary nitrite and nitrate represent a more important source for cutaneous NO derivates.[27] Because dietary nitrate increases circulating nitrite concentrations,[28] it appears possible and feasible that dietary nitrate may also represent an effective way to boost skin reservoirs of photolabile NO species.

Using EPR spectroscopy, we, for the first time, give direct evidence here for UVA-induced intracutaneous NO formation via photodecomposition of endogenous sources of pho-
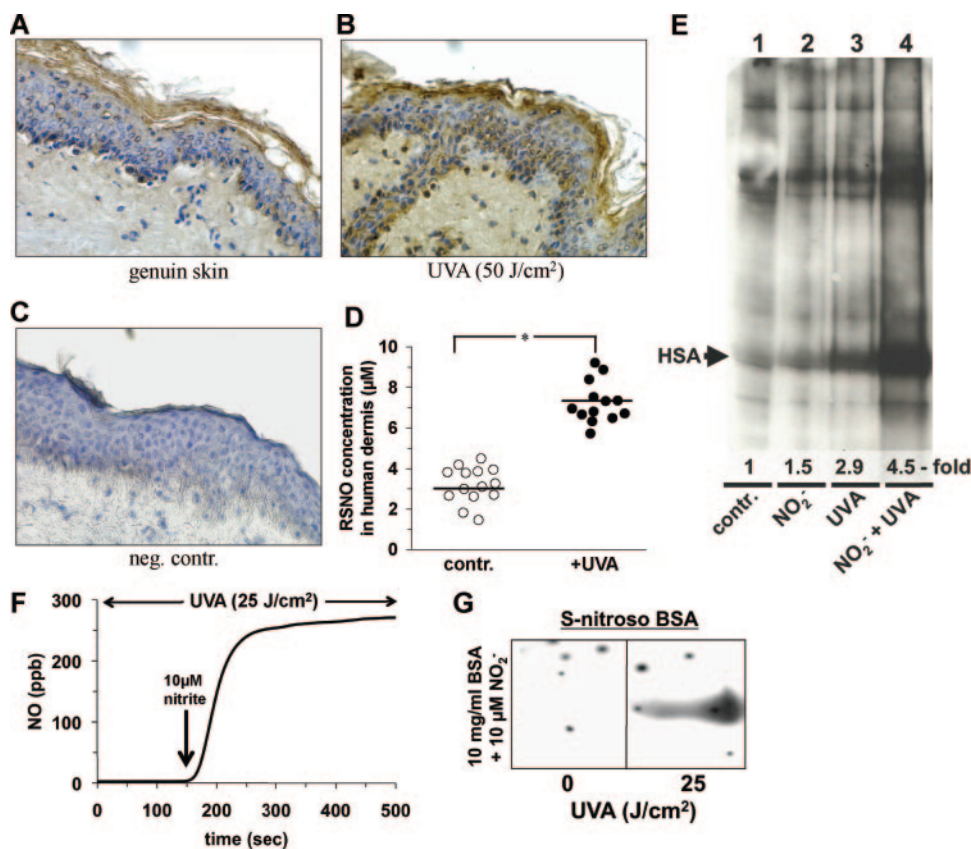
Figure 6. Analysis of S-nitrosothiols formation in human skin specimens, as well as in vitro and of UVA-induced photodecomposition of nitrite in aqueous solutions. In resting, as well as UVA-irradiated (25 J/cm^2), human skin specimens, obtained from mammoplastic surgery, S-nitrosation of proteins was detected by the S-nitrosocysteine-specific antiserum. A, Genuine human skin. B, UVA-irradiated skin specimens. C, For negative control, cryostat sections were denitrosated by a reducing solution (16 hours of incubation with 25 μmol/L CuCl$_2$ plus 1 mmol/L ascorbic acid in PBS, pH 7.4) before the antibody staining. A through C, Shown are representative pictures of 5 individual experiments. D, Detection of S-nitrosothiols in dermal tissue of genuine and UVA-irradiated human skin specimens detected by CLD in homogenates of genuine and UVA-irradiated human skin specimens. Values are the means±SD of 5 individual experiments. *P<0.001. E, Western blot analysis for S-nitroso protein formation in human dermis of genuine or UVA-irradiated (25 J/cm^2) human skin specimens maintained in the presence or absence of NaNO$_2$ (100 μmol/L). Shown is 1 representative graph of 3 individual experiments. F, In vitro nonenzymatic NO formation from UVA-irradiated (84 mW/cm^2) nitrite-containing solutions (10 μmol/L sodium nitrite in PBS, pH 7.4) detected by CLD. G, UVA-induced nitrite-dependent S-nitroso-thiol formation in vitro. UVA irradiation (25 J/cm^2) of PBS-containing (pH 7.4) nitrite (10 μmol/L sodium nitrite) and 10 mg/mL BSA resulted in an apparent S-nitroso-BSA formation, as detected by Western blot using a S-nitroso-cysteine–specific anti-serum.

tolabile NO derivates. The action of NO is largely determined by its rapid diffusion and its ability to penetrate cell membranes. The diffusion coefficient of NO at 37°C has been found to be 1.4-fold higher than that of oxygen or carbon monoxide and thus a diffusion distance of 500 μm was calculated for NO in tissue.[29] Thus, not surprisingly, with nitrite-enriched skin specimens, UVA-induced NO liberation could be found by EPR spectroscopy not only in apical skin regions but also in 2- to 3-mm deep regions of the dermis.

The penetration of photons into the skin strongly depends on the wavelength. It is known that UVA penetrates the epidermis and reaches even the deeper dermal regions down to 1 mm.[16] Approximately half of the UVA intensity can reach the depth of melanocytes and the dermal compartment,[30,31] and it has been estimated that the total solar energy deposited into the lower epidermis and upper dermis is 2 orders of magnitude higher for UVA than for UVB. In vitro studies have shown that UVA light at 340 to 360 nm induces the formation of NO by photolysis of nitrite anions, as well as S-nitrosated compounds, in aqueous solutions.[32–35] As shown by us previously,[14] UVA-induced photodecomposition of nitrite results in a modest but sustained release of NO. In contrast, irradiation of RS-NOs leads to a much elevated release of NO because of the far higher extinction coefficient of this species. Under high-UVA intensities, the release of

NO is short-lived because of rapid depletion of RS-NO (photobleaching). It should be noted that neither nitrite nor HgCl$_2$-resistant nitroso compounds, probably N-nitrosated species (RNNOs), contribute to UVA-provoked NO release from human skin.[14] Detailed analysis of the mechanism of light-induced nitrite decomposition revealed the formation of very reactive and potentially cytotoxic radical species like O$_2^{\cdot-}$, OH$^\cdot$, or NO$_2$.[17,32] The radical NO$_2^\cdot$ recombines rapidly ($k \approx 4.5 \times 10^8$ mol/L per second) with NO to N$_2$O$_3$. N$_2$O$_3$ and the catalytic action of transition metal ions represent very efficient nitrosating systems, in particular for thiols.[36,37] Via this reaction, NO$_2^\cdot$ decreases the yield of free NO from UVA-induced nitrite decomposition. In the presence of thiols such as glutathione, however, the NO-trapping capacity of NO$_2^{\cdot}$[38] will be counteracted via 3 reactions. First, N$_2$O$_3$ efficiently nitrosates thiols to RS-NO, which by itself is efficiently photolysed to NO and thiyl radicals ($^-$S$^\cdot$) under illumination by UVA. Secondly, NO$_2^\cdot$ will directly be reduced to nitrite by thiolates like GS$^-$. Thirdly, $^-$S$^\cdot$ reacts efficiently with GSNO to yield NO and a disulfide. In contrast, simple recombination of GS$^\cdot$ and NO$^\cdot$ has not been observed.[39] Therefore, reaction of thiols with both NO$_2^\cdot$ and with N$_2$O$_3$[38] will increase the formation of NO. The reaction of thiolate anions with NO$_2^\cdot$ is ≈10 times faster than the reaction with N$_2$O$_3$ (5×10^8 versus 6×10^7 mol/L per second) (reviewed elsewhere[38,40]).

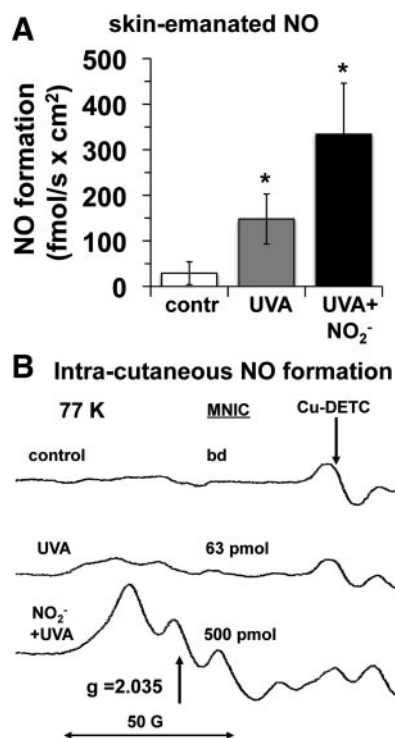## A skin-emanated NO

B Intra-cutaneous NO formation

Figure 7. UVA irradiation of human skin increases emanation of cutaneous NO, as well as intracutaneous NO formation. A, Using an airtight chamber (16 cm^2) with a UVA transparent front window, which was placed on the forearm of volunteers, we collected the gaseous NO emanating from the skin and was fed into the CLD analyzer. In absence of UVA, a basal release of 29 ± 25 fmol of NO per second per square centimeter was detected (white bar). Under UVA illumination with 20 J/cm^2, the release of gaseous NO was enhanced 4-fold to 148 ± 55 fmol of NO per second per square centimeter (gray bar). After application of skin cream containing 10 μmol/L nitrite, the photoinduced yield of gaseous NO was again significantly enhanced to 334 ± 112 fmol of NO per second per square centimeter (black bar). *$P<0.001$ as compared to the control (white bar). B, Fe^{2+}-DETC–loaded skin specimens from male abdomen were incubated for 30 minutes with 1 mmol/L N-iminoethyl-L-ornithine in the absence or presence of nitrite (100 μmol/L NaNO$_2$) and then were irradiated for 30 minutes with UVA light (25 J/cm^2). Intracutaneous formation of NO-Fe^{2+}-DETC complexes (MNIC) attributable to UVA-induced, nonenzymatic NO formation were detected by EPR spectroscopy. EPR spectra at 77 K of human skin specimens in HEPES buffer. The specimens are $\approx200\pm10$ mg each. In nonirradiated skin (control), MNIC signals are below the detection limit (bd) of ≈20 pmol. This spectrum shows the presence of ≈0.3 nmol of paramagnetic Cu^{2+}-DETC complexes. UVA irradiation of human skin tissue (UVA) induces the appearance of the EPR-typical triple signal for NO and a MNIC signal representing 63 ± 6 pmol of MNIC. In the presence of nitrite, UVA irradiation of human skin (NO$_2^-$+UVA) leads to a MNIC signal, corresponding to 500 ± 50 pmol of MNIC.
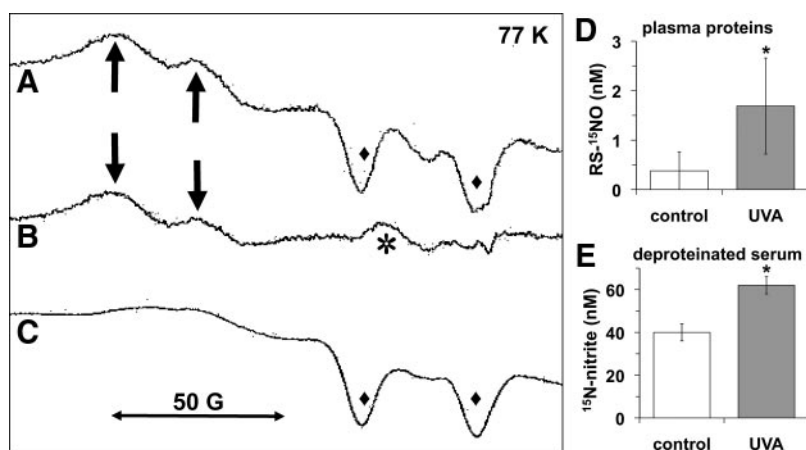
In parallel to UVA-induced intracutaneous NO formation, we observed a strong increase in cutaneous *S*-nitrosothiol formation in the epidermis, as well as in the deeper regions of the dermis. As shown by Western blot analysis, the dermal fraction of *S*-nitrosated compounds predominantly represent *S*-nitroso-albumin, which, because of absent circulation activity in the skin specimens, reflect the blood or serum filling, respectively, of cutaneous microvasculature. In vivo, of course, because of the excellent capillarization of the Stratum papillare, synthesized dermal *S*-nitroso-albumin will imme-

diately leave the skin compartment. Functioning as a transport form for NO, *S*-nitroso-albumin will favor its rapid systemic distribution as well as its vasoavailability. *S*-nitroso-albumin has been previously proposed to act as a reservoir of NO within the circulation, transporting and releasing NO into vascular beds to cause vasodilation.[41,42]

Photoproduction of NO has been observed previously at these wavelengths in vascular tissue of rats,[33] and the action spectra of this photoproduction implicated endogenous *S*-nitrosothiols and nitrite as the source of NO. The UVA dose of 20 J/cm^2, as used here, was applied by using a commercial tanning facility. This dose remains significantly below the minimal erythemal UVA dose of 66 ± 10 J/cm^2 reported for fair-skinned persons[43] and correlates with a sun exposure time of ≈45 minutes in a temperate climate zone.

UVA-induced effects on cardiovascular parameters, as well as the timescale of alterations, are in reasonable agreement with previous observations. Recently, Rassaf et al demonstrated that intravenous slow infusion of NO in healthy volunteers increased plasma levels of RS-NO and induced systemic hemodynamic effects at the level of both conduit and resistance vessels, as reflected by dilator responses in the brachial artery and forearm microvasculature, and elicits a simultaneous and significant drop in mean blood pressure. Interestingly, slow infusion of NO had no significant effects on heart rates of the treated volunteers.[44] These findings demonstrate that in humans, the pharmacological delivery of NO solutions results in the transport and delivery of NO as RS-NO along the vascular tree. Furthermore, in a pig model, Vilahur et al could show that low doses of *S*-nitroso glutathione (GS-NO), slowly administered, significantly reduced blood pressure.[45] In accordance with our observations, in both studies, heart rates were not significantly affected, neither by an NO nor low-dose RS-NO injection. In this context, it should be noted that the systemic response of the vascular system depends on whether the given dose is administrated by bolus injection or gradually with slow infusion. Thus, in the same study by Rassaf et al, an intravenous bolus injection of higher GS-NO amounts led to significantly enhanced heart rates.[44] Considering the time scale of UVA exposure, as well as of light-induced cardiovascular changes in our experimental setup, the underlying mechanism of our observations is less related to the high-dose GS-NO experiment of Rassaf et al but more to the mentioned NO and low dose RS-NO experiments.

As already mentioned, UVA radiation penetrates up to 1 mm into the skin. Therefore, hemodynamic changes shown here cannot be a direct result of cutaneous UVA exposure but rather are mediated by an UVA-induced factor. This assumption is strengthen by our observation that at the UVA doses used in our study, irradiation of skin did not show any significant local effects on cutaneous vasodilation or blood flow. Furthermore, we observed that an isolated irradiation of an arm, did not show any significant effects on blood pressure that was detected on this arm. On the other side, blood pressure detected on a nonirradiated arm of an otherwise UVA-irradiated volunteer shows the same results that were detected on the irradiated arm of the same volunteer (these

Figure 8. Effects of ^{15}N-nitrite cream on UVA-induced intracutaneous ^{15}NO formation and on RS-^{15}NO concentration in plasma of UVA-irradiated volunteers. Fe^{2+}-DETC–loaded human skin specimens, obtained from mammoplastic surgery, were treated apically for 30 minutes with 20 mL of a standard oil-in-water cream containing ^{15}N-nitrite (5 mmol/L $NaNO_2$) and then were irradiated for 30 minutes with UVA light (25 J/cm^2). Intracutaneous formation of ^{15}NO-Fe^{2+}-DETC complexes (MNIC) resulting from UVA-induced, nonenzymatic NO formation were detected by EPR spectroscopy. EPR spectrum of 240 mg of skin from female mamma was detected at 77 K. A, After reduction with dithionite, the sample shows a complex superposition of ^{15}N-MNIC (arrows) and ^{15}NO-Hb (♦). B, After numeric subtraction of an experimental spectrum of ^{15}NO-Hb (C), the difference spectrum (A minus C) shows ≈460 pmol of ^{15}N-MNIC (arrows) and some residual Cu^{2+}-DETC (*). C, The ^{15}NO-Hb spectrum used for the subtraction. D and E, Detection of ^{15}NO by CALOS. D, RS-^{15}NO concentrations in plasma of healthy volunteers, rubbed with a ^{15}N-nitrite–containing (5 mmol/L) cream before light exposure (20 J/cm^2 UVA). E, ^{15}N-nitrite concentrations in plasma of healthy volunteers, rubbed with a ^{15}N-nitrite–containing (5 mmol/L) cream before light exposure (20 J/cm^2 UVA). Values are the means±SD of 3 individual experiments. *$P<0.001$.

data are shown in the expanded Results section in the Online Data Supplement).

Furthermore, our control data strongly argue against an involvement of augmented ambient air temperature or skin temperature as an etiologic parameter for the effects on blood pressure observed after UVA challenge. In contrast to control-treated subjects, with UVA-irradiated volunteers, the permanent air stream exposure of ventral and lateral body parts, because of evaporation cooling, significantly decreases skin temperature. the surface of UVA-irradiated dorsal skin (not ventilated by cooling air), had a mean temperature of approx. 38±1°C. This is slightly higher than the skin temperature of fully covered subjects (35.7±0.8°C). Measuring capillary–venous oxygen saturation, blood filling, blood flow, and velocity of superficial (1 mm deep) and deeper (6 mm deep) microvessels of human skin clearly reveal that UVA exposure (20 J/cm^2) had no effects on the mentioned cutaneous vascular parameters, whereas, as positive control, exposure of human skin for 10 minutes to 41°C warm water significantly enhanced blood flow and blood velocity of superficial, as well as deeper, cutaneous microvessels. Moreover, mimicking skin temperature increases by a full-body bath in 38°C warm water for 15 minutes, none of the volunteers showed significant alterations in blood pressure. Thus, the influence of skin temperature-depended effect on blood pressure during UVA challenge can be neglected.

In conclusion, here, we give evidence that whole body UVA irradiation NO-dependently decreases blood pressure of healthy volunteers. These systemic effects are correlated with increased concentrations of nitroso compounds in the systemic circulation. We attribute the observed effects to photolysis of cutaneous nitrite and show that the physiological response may be enhanced by loading the skin with photolabile NO derivates before irradiation. Alternatively, endogenous photosensitive NO derivates may be modulated by control over dietary nitrate and nitrite intake.[46,47] These findings reveal the impact of light as an environmental parameter contributing to the phenomenon of "French paradox" and thus might have potential for the therapeutic applications in diseases with hypertension.

Disclosures

None.

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