The damaging effects of UVA radiation have been well-documented. UVA radiation is known to induce molecular, cellular, and clinical damage. Such harm may lead to photoaging, immune system depression, altered gene expression, or oncogene and tumor suppressor gene modulation, all of which are partly responsible for the development of skin cancer. In parallel to an increased understanding of the added damage caused by UVA radiation, progress has been made in sunscreen formulation. A variety of UVA filters are now available for formulators to combine with UVB filters to reach high-level photostable protection using a minimum concentration of active ingredients. The efficacy of products that contain these UV filter combinations usually is determined by noninvasive assessments, which cause either UVA-induced erythema or skin pigmentation. However, the biologic relevance of these end points for UVA radiation–induced skin damage is unknown.

In our study, we confirm that the assessment of UVA radiation–induced gene expression in skin specimens obtained from UVA-irradiated human skin by quantitative real-time polymerase chain reaction is a sensitive, reliable, and robust method to prove the efficacy of 2 daily moisturizers containing broad-spectrum sunscreen. Specifically, we demonstrate in vivo that topical application of a daily moisturizer with broad-spectrum sunscreen prevents UVA radiation–induced transcriptional expression of genes that are directly linked to skin aging (ie, matrix metalloproteinase 1 [MMP-1]) and also reflect the skin’s antioxidative stress defense response (ie, catalase [CAT], superoxide dismutase [SOD], glutathione peroxidase [GPx]). Furthermore, we demonstrate that the protection against UV-induced skin damage provided by products with different sun protection factor (SPF) but the same UVA protection factor (UVA-PF) is similar, which emphasizes the importance of high UVA protection to maintain unaltered essential biologic functions. These data indicate that the use of a daily moisturizer containing broad-spectrum sunscreen with a well-balanced SPF/UVA-PF ratio on a regular basis is beneficial for human skin.

the ambient diurnal UVA exposure ranges from 100 to 140 J cm\(^{-2}\).\(^3\) Comparing ambient diurnal UVB exposure in summer from Spain to Florida or Hawaii indicates 1.25- or 2.1-fold higher values.\(^4\) Ambient exposure provides upper limits on human exposure; accordingly, exposure of an unprotected face is approximately 20% of ambient exposure.\(^4\)

Short-term and long-term adverse effects to the skin, such as sunburn, pigmentation, immunosuppression, phototoaging, photodermatoses, and skin cancer, are induced by both acute and repeated sun exposure. For the last several decades, UVB radiation (290–320 nm) of the solar spectrum was considered to be harmful, though UVA radiation (320–400 nm) generally was believed to be safe. However, with the availability of high-intensity artificial UVA sources, it has been demonstrated that UVA radiation penetrates deeper into the skin, causing a wide variety of damaging biologic effects.\(^5\) UVA radiation mainly produces reactive oxygen species through interaction with endogenous and exogenous chromophores. These reactive oxygen species cause damage to DNA, cells, vessels, and tissues.\(^6\)-\(^10\) Similar to UVB, UVA also has been implicated in immune system depression and in the development of skin cancer including melanoma.\(^11\),\(^12\) Photoallergic and phototoxic reactions as well as photodermatoses are mainly UVA-induced.\(^13\)-\(^15\) Furthermore, biologic damage induced by cumulative suberythemal UVA exposure has been documented.\(^16\) Therefore, it is important to reduce UVA exposure to the skin by using a daily moisturizer containing a combination of UVA and UVB filters.

The efficacy of these products in blocking UVA radiation usually is demonstrated using noninvasive techniques such as immediate pigment darkening and persistent pigment darkening (PPD).\(^17\)-\(^19\) Nevertheless, surrogate biomarkers such as altered gene expression have been shown in vitro to accurately reflect clinical skin damage.\(^20\)-\(^23\) The expression pattern of the enzyme matrix metalloproteinase 1 (MMP-1), a cell marker for skin aging, is of particular interest. In addition, catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) represent biomarkers associated with oxidative stress; therefore, these enzymes are relevant for photoaging and photocarcinogenesis. Gene expression analyses in human skin biopsies (4 mm in diameter) obtained from UVA-irradiated human skin have been shown to be successful. This technique is a sensitive reliable method to test broad-spectrum protection at a biologic level in vivo.\(^24\) In this study we evaluate this method in determining the efficacy of daily moisturizers with broad-spectrum UV protection. In addition, we sought to assess protection against UV-induced skin damage, which may be provided by products with different sun protection factor (SPF) but the same UVA protection factor (UVA-PF).

**Methods**

**Study Design and Population**—This single-center, randomized, double-blind, placebo-controlled study of daily moisturizers containing broad-spectrum UV protection included 2 treatment groups. The study was conducted in accordance with the International Conference on Harmonisation Guideline for Good Clinical Practice. A validated computer program, which assigns treatments for participants, was used to perform randomization. The protocol was approved by the local ethics committee at Heinrich Heine University, Düsseldorf, Germany.

Following informed consent, 44 healthy participants with no personal history of skin cancer, photodermatoses, or recent (within the last 6 months) visits to a tanning salon were enrolled. In part 1 of the study, test areas of 4×4 cm were defined in 14 participants on the left and right buttock area. These skin sides were either sham exposed or exposed to increasing doses of UVA1 radiation from a Sellamed 2000 radiation system equipped with a Schott WG335/3-mm filter and 2 UG5/3-mm filters to obtain a spectrum between 340 and 400 nm. The UVA1 output was determined with a UVAMETER type II. In part 2 of the study, 4×4-cm buttock skin areas of the remaining 30 participants were left untreated and sham exposed, left untreated and UVA exposed (80 J/cm\(^2\)), or test products were applied and 20 minutes later the areas were exposed to UVA radiation. The products were topically applied according to standards of the European Cosmetic, Toiletry and Perfumery Association (ie, 2 mg/cm\(^2\)). Twenty-four or 72 hours after exposure, 4-mm punch biopsies were taken from each skin area and snap frozen in liquid nitrogen.

**Study Products**—The 2 daily moisturizers containing sunscreens that were evaluated during our study are commercially available in the United States. The first product had an SPF 15 and a UVA-PF 15 (determined by the PPD method), and the second product had an SPF 45 and a UVA-PF 15. The combination of UV filters in these products included avobenzone 2% or butyl methoxydibenzoylmethane, ecamsule 2% (Mexoryl) or terephthalylidene dicamphor sulfonic acid, and octocrylene (OC) 10% in the first moisturizer, and avobenzone 3% or butyl methoxydibenzoylmethane, homosalate 12%, octyl salicylate 5%, OC 2.35%, and oxybenzone 6% or benzophenone-3 in the second moisturizer.

**Assessment of Gene Expression by Quantitative Real-Time Polymerase Chain Reaction**—For assessment of gene expression, total RNA was extracted.
from frozen biopsies and gene expression was measured using quantitative real-time polymerase chain reaction as outlined in a prior study. In brief, for isolation of RNA from snap frozen skin biopsies, the samples were disrupted in lysis buffer from a peqGOLD Total RNA Kit using a Mixer Mill MM 300 three times for 3 minutes with 30 Hz. Fifty nanograms of total RNA were used for complementary DNA synthesis. Polymerase chain reactions were performed in an Opticon 1 using specific primer pairs (Table) and SYBR qPCR SuperMix With ROX. Polymerase chain reaction conditions were as follows: activation of hot-start *Thermus aquaticus* polymerase 94°C for 15 minutes followed by 45 to 50 cycles of denaturation at 95°C for 20 seconds, annealing at 55°C for 20 seconds, and extension at 72°C for 30 seconds. The \(2^{-\Delta\Delta CT}\) method was used. Expression was normalized to the housekeeping gene 18S ribosomal RNA, and unexposed areas were set equal to 1.

### Statistical Analysis
The Kruskal-Wallis 1-way analysis of variance by ranks (SigmaPlot Version 11) as a nonparametric test for the comparison of differences between measurements was used with \(\alpha = .05\) for statistical significance.

### Results

#### UVA Dose Determination for Gene Expression Modulation
UVA-induced gene expression is well-known to show interindividual variation. Therefore, we defined a UVA1 radiation dose \((n=4)\) and time \((n=10)\) that would yield a UVA-induced gene response in our participants that was large enough to serve as a reliable parameter to assess the efficacy of a daily moisturizer containing broad-spectrum sunscreen. We initially exposed buttock skin (a sun-protected anatomic site) to increasing doses of UVA1 radiation (340–400 nm; 20–80 J/cm²). This dose range was chosen due to its physiologic relevance, and depending on the given individual, it reflects a biologic dose range of 1 to 2 minimal erythema doses (MEDs) of UVA radiation. A UVA dose-dependent increase in transcriptional expression was detected in the tested genes (Figure 1). Maximal gene expression was achieved with a UVA1 dose of 80 J/cm² (Figure 1A) and was observed 24 hours after exposure (Figure 1B) with a mean (standard error of the mean) inducibility of 17-fold (5) for MMP-1, 3-fold (1) for CAT, 3-fold (1) for SOD, and 2-fold (0.5) for GPx when compared to sham-irradiated controls.

#### Evaluation of Photoprotection Provided by 2 Daily Moisturizers With Broad-Spectrum Sunscreen
We evaluated if topical application of the 2 daily moisturizers containing broad-spectrum sunscreens with different SPF (SPF 15 or SPF 45) but the same UVA-PF of 15 would affect UVA radiation–induced gene expression in vivo in human skin \((n=30)\). In comparison with untreated contralateral skin sites (eg, UVA), pretreated skin areas showed a significantly reduced UVA gene expression response independent of the applied products \((P < .05)\) (Figure 2).

### Primer Pairs for Quantitative Real-Time Polymerase Chain Reaction

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Pair 1</th>
<th>Primer Pair 2</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>18S ribosomal RNA</td>
<td>5'-GCCGCTAGAGGTGAAATTCTTG-3'</td>
<td>5'-CATTCTTGGAATTCTTGTCG-3'</td>
<td>McCallum and Maden (1985)</td>
</tr>
<tr>
<td>MMP-1</td>
<td>5'-GGGAGATCATCAGGGAGAACCTTG-3'</td>
<td>5'-GGGAGATCATCAGGGAGAACCTTG-3'</td>
<td>Goldberg et al (1986)</td>
</tr>
<tr>
<td>CAT</td>
<td>5'-CGGATGCGTTCATAGTCAGGAT-3'</td>
<td>5'-CGGATGCGTTCATAGTCAGGAT-3'</td>
<td>Quan et al (1986)</td>
</tr>
<tr>
<td>SOD</td>
<td>5'-GGTCAGATCGGAGTCGCCCAGGACT-3'</td>
<td>5'-GGTCAGATCGGAGTCGCCCAGGACT-3'</td>
<td>Sherman et al (1983)</td>
</tr>
<tr>
<td>GPx</td>
<td>5'-CCGACCGCAAGCTCATCA-3'</td>
<td>5'-CCGACCGCAAGCTCATCA-3'</td>
<td>Mullenbach et al (1987)</td>
</tr>
</tbody>
</table>

Abbreviations: MMP-1, matrix metalloproteinase 1; CAT, catalase; SOD, superoxide dismutase; GPx, glutathione peroxidase.
As expected, UVA radiation–induced gene expression showed intraindividual and gene-specific as well as gene-unrelated interindividual differences. However, even under these conditions, considerable inhibition of UVA radiation–induced gene transcription was observed for all genes assessed and similar results were obtained for the 2 daily moisturizers containing broad-spectrum sunscreens with different SPF (SPF 15 or SPF 45) but the same UVA-PF of 15. This observation suggests the importance of the UVA-PF compared to the SPF. The comparison of the 2 pretreated skin sites revealed that they had similar expression of MMP-1 (P = .3909), CAT (P = .0416), SOD (P = .0054), and GPx (P = .0183).

Our study was limited due to the lack of an evaluation of the contribution of UVB alone or solar-simulated radiation (UVB plus UVA) to gene expression to demonstrate if higher SPFs offer a notable increase in protection against induction of gene expression. It also would have helped to discriminate the relative contribution of the different organic filter mixtures toward gene expression caused by UVA, UVB, or a combination of both. However, obtaining 10 biopsies instead of 4 from each volunteer’s buttock is not ethical or feasible. In view of filter combinations, it already has been shown that addition of the UVB filter OC to the broadband avobenzone increases photostability of the latter and thereby increases UVA-PF in vivo.31 Synergistic effects have been described for combination of the broadband filters such as ecamsule (terephthalylidene dicamphor sulfonic acid) with either drometrizole trisiloxane or bis-ethylhexyloxyphenol methoxyphe- nyl triazine, which are approved in Europe and are under evaluation through a new regulatory process for obtaining marketing approval for over-the-counter products named Time and Extent Application from the US Food and Drug Administration.32,33

Comment
Repeated solar UV radiation exposure is a major environmental factor that contributes to clinical and histologic changes in routinely sun-exposed skin such as the face and hands. Acute suberythemal UVA
exposure during outdoor activities certainly is relevant in photoaging and skin cancer. In 2010 we demonstrated that erythema is an inadequate indicator of cutaneous damage for both acute and repeated exposures, as substantial molecular and cellular damage occurs at doses lower than 1 MED.16

In our prior study, we confirmed that UVA radiation (340–400 nm; 20–60 J/cm²) at both suberythemal and erythemal levels is a potent modulator of gene expression in human skin.24 Human skin samples, which reflect some of the major physiologic responses of human skin to UVA radiation, were characterized by intervariability for 1 gene between different individuals and for different genes in 1 individual. This observation was expected and most likely reflects differences in UVA radiation–inducible signaling pathways.24 We also confirm that the assessment of UVA radiation–induced transcriptional gene expression in skin specimens obtained from UVA-irradiated human skin by quantitative real-time polymerase chain reaction is a sensitive, reliable, and robust method to assess the efficacy of both broad-spectrum sunscreen and daily moisturizers containing broad-spectrum sunscreens. Specifically, we demonstrated in vivo in human skin that topical application of a daily moisturizer with broad-spectrum sunscreen prevents UVA radiation–induced transcriptional gene expression related to extracellular matrix degradation, which is directly linked to skin aging (ie, MMP-1), and genes that reflect the skin’s antioxidative stress defense response (ie, CAT, SOD, GPx).

By definition, SPF is a measure of protection against a single erythemal exposure. Sun protection factor does not account for long-term damaging effects of UVA. No consensus has been reached to measure the level of UVA protection. The PPD method, which evaluates the stable portion of pigmentation darkening of the skin following UVA irradiation, is possibly the most widely used method. The Boots star rating system is an in vitro spectrophotometric method used to describe the ratio of UVA to UVB protection offered by a product.34 Sunscreens and daily moisturizers containing broad-spectrum sunscreens are advocated to prevent skin cancer and photoaging. This theory has been demonstrated in animal studies, but the epidemiologic evidence in humans is less convincing.35 To our knowledge, there are no epidemiologic data on the role of sun protection in the prevention of photoaging in humans.

Conclusion
Our study aimed to assess the protection offered by 2 daily moisturizers with broad-spectrum sunscreens with different SPF but the same UVA-PF at the molecular level using transcriptional gene expression analysis. Analysis of the protection provided by the application of these products showed that the gene modulatory effects of UVA radiation could be partially or completely prevented. We envisage that the combination of this molecular assay with conventional noninvasive methods (SPF and UVA-PF measurements) will help improve the quality of efficacy claims of modern products containing filters and thereby may ultimately benefit the consumer.

Interestingly, the 2 daily moisturizers containing broad-spectrum sunscreens were similarly effective in reducing transcriptional gene expression levels independent of each moisturizer’s SPF value. Therefore, as with prior studies, we demonstrated at a molecular level that erythema is an inadequate indicator of cutaneous molecular damage because notable molecular damage occurs at doses lower than 1 MED (ie, 20 J/cm² of UVA) and similar protection was offered by SPF 15 and SPF 45 products. Therefore, the value of SPF is limited, as it does not account for the long-term damaging effects of UVA radiation.

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