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Tropospheric ozone and skin aging: Results from two German cohort studies

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ABSTRACT

During the last two decades, it has been well established that a short-term exposure to ozone (O_3) elicits an oxidative stress response in human and mouse skin, which leads to aberrant transcriptional expression of genes consistent with increased skin aging. Whether a long-term exposure to ambient O_3 is associated with any skin aging traits, has remained unclear. We addressed this question in two elderly German cohorts: the SALIA study (806 women aged 66–79 years), and the BASE-II study (1207 men and women aged 60–84 years). Five-year mean residential exposure to O_3 was modeled as the number of days with maximum daily 8-h mean O_3 concentrations $\geq 120\,\mu g/m^3$ per year in the wider neighborhood (5-digit postcode) of a participant's residence. Extrinsic (environmentally induced) skin aging traits – coarse wrinkles and pigment spots (lentigines) on the face – were assessed by means of SCINEXA™, a validated visual score previously shown to be well suited to measure extrinsic facial skin aging in cohort studies. We observed positive associations of O_3 exceedances with coarse wrinkles in the face, but not with pigment spots. These associations were present in each cohort as well as in the combined sample of both cohorts. They were independent of chronic ultraviolet radiation exposure as the most obvious confounder, and also of co-pollutants such as particulate matter and nitrogen dioxide. Thus, long-term exposure to elevated concentrations of tropospheric O_3 appears to contribute to skin aging.

1. Introduction

Recent studies have established a link between long-term residential exposure to traffic-related air pollution and extrinsic (environmentally induced) skin aging (Krutmann et al., 2014). Accordingly, long-term residential exposure to elevated levels of particulate matter (PM), soot, and nitrogen dioxide (NO_2) was found to be significantly associated with the number of pigment spots (lentigines) on the face in elderly Caucasian and Han Chinese women (Vierkötter et al., 2010; Hüls et al., 2016; Peng et al., 2017). Experimental in vitro evidence suggests that the interaction of skin cells with traffic particles can trigger expression of genes relevant for skin aging (wrinkle and pigment spot formation), including transcription factors relevant for inflammation and cytotoxicity, such as the aryl hydrocarbon receptor (AhR; Krutmann et al., 2014).

Traffic-related air pollutants are a heterogeneous mixture of particles and gases, which continuously interact with each other and produce secondary pollutants. One of these secondary pollutants with adverse effects on human health and ecosystem is tropospheric ozone (O_3). It is a growing public health concern, as ambient concentrations of tropospheric O_3 are increasing in many parts of the world. O_3 is formed in a chain of complex photochemical reactions, requiring the presence of nitrogen oxides, volatile organic compounds, other air pollutants, and solar radiation (European Environment Agency (EEA), 2016).

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With regard to human health effects, O_3 is one of the strongest known oxidants (Mustafa, 1990). Short-term exposure to O_3 is associated with reduced lung function, increased risks of cardiorespiratory disease and mortality, adverse central nervous system effects, and increased total mortality (Nyberg and Pershagen, 1996; United States Environmental Protection Agency (US EPA), 2013). Long-term exposure to O_3 was similarly linked to higher respiratory disease and mortality rates, elevated total mortality, adverse cardiovascular and reproductive/developmental effects (US EPA, 2013).

O_3 is probably the most powerful oxidant to which human skin is regularly exposed (Valacchi, 2010). A variety of adverse reactions in the upper and deeper skin layers, following short-term exposure to O_3, has been reported in animal and in vitro studies: oxidation of biomolecules, generation of radical species, depletion of antioxidant defense, cytotoxicity and cellular stress (Krutmann et al., 2017; Valacchi, 2010). It has therefore been hypothesized that long-term exposure to tropospheric O_3 could contribute to skin aging, similar to other air pollutants (Krutmann et al., 2017; Valacchi, 2010). However, it has remained unclear whether O_3 is associated with skin health effects in general or skin aging traits, in particular. In the present epidemiological study, we have addressed this question by studying two population-based cohorts located in densely urbanized areas of Germany: the SALIA cohort in the Ruhr Area and the BASE-II cohort in Berlin.

2. Material and methods

2.1. Cohort studies

The ***Study on the influence of Air pollution on Lung function, Inflammation and Aging*** (SALIA) was initiated in 1985–1994 and included 4874 women aged 55 years old from the highly industrialized Ruhr area, i.e. an environmentally heavily polluted urban area, and two northern counties, i.e. rural areas with very low pollution levels (Schikowski et al., 2005; Vossoughi et al., 2014). The first clinical follow-up examination was conducted in 2007–2010 of 834 women aged 67 to 80 years. The Medical Ethics Committee of the University of Bochum approved the follow-up examination (approval number 2732). This cohort has been successfully used in previous studies of the association between skin aging and air pollution (Vierkötter et al., 2010, 2015; Hüls et al., 2016).

The ***Berlin Aging Study II*** (BASE-II) is a study of aging with a multidisciplinary focus (Bertram et al., 2014; Gerstorf et al., 2016). In total, 2172 participants (~75% aged 60–84 years and ~25% aged 20–37 years) underwent clinical examinations in 2009–2014. Participants from the older subgroup were included in this analysis. All participants gave written informed consent. The Ethics Committee of the Charité-Universitätsmedizin Berlin approved the BASE-II study (approval number EA2/029/09).

Examinations in both cohorts were conducted in accordance to the recommendations for research on human subjects, adopted by the 18th World Medical Assembly, Declaration of Helsinki and later revisions (World Health Organization, 2001). All participants gave written informed consent.

2.2. Skin aging assessment

Skin aging is subdivided into extrinsic (environmentally induced) and intrinsic (chronological; Yaar, 2006). They are characterized by different phenotypes. The hallmarks of the extrinsic skin aging are coarse wrinkling and furrowing, pigment spots, skin thickening, solar elastosis, and other signs, observed at the exposed spots (e.g., face or back of the hand; Yaar, 2006). The intrinsic skin aging is a uniform process, not limited to specific areas of the skin, characterized by fine wrinkling and atrophy of skin and subcutaneous tissue (Yaar, 2006).

We used the Score of Intrinsic and Extrinsic skin Aging (SCINEXA™) tool to assess skin traits associated with extrinsic aging of the face: coarse wrinkles and pigment spots Vierkötter et al., 2009). SCINEXA™ is a validated visual score, which allows scoring of clinical signs of intrinsic and extrinsic skin aging and was shown to be well suited to assess extrinsic skin aging in epidemiological studies (Vierkötter et al., 2009, 2010, 2015; Li et al., 2015; Hüls et al., 2016; Peng et al., 2017). We scored the facial coarse wrinkles on the forehead, in the frown lines (between the eyebrows), in the outer eye corners (“crow's feet”), under the eyes, on the upper lip, and in the nasolabial fold, using the photoreference scales (Tschachler and Morizot, 2006). The score ranged from 0 (not present) to 5 (very severe). We counted the number of pigment spots ≥3 mm in diameter on the forehead and on the cheeks. The score was given as follows: 0 (no spots), 1 (1–10 spots), 2 (11–50 spots), and 3 (> 50 spots).

Trained investigators, using a standardized protocol (Vierkötter et al., 2009), performed skin aging assessment. In the SALIA cohort, scoring was done on-site as a part of the clinical follow-up investigation in 2007–2010, while in BASE-II we scored off-site, using photos of the study participants.

2.3. Environmental exposures at the residence

Background concentrations of air pollutants were obtained from the German Environmental Agency (Umweltbundesamt FG II 4.2), Berlin, derived by dispersion modelling with the chemical REM-CALGRID model (Stern, 2009), integration of monitoring data and smoothing by the method of optimal interpolation with a resolution of $7 \times 8\,km^2$. Exposure to high levels of O_3 was assessed as the count of days when the maximum daily 8-h mean concentration of O_3 was $\geq 120\,\mu g/m^3$ (“exceedance days”). According to the current legislation of the European Union (EU), 25 days of exceedance are allowed per year, averaged over three years (EEA, 2017). We added yearly concentrations of PM_{10} (particulate matter with aerodynamic diameter of $\leq 10\,\mu m$) and NO_2, obtained with the same modelling technique, as co-pollutants in multi-pollutant models.

We estimated residential exposure to the ultraviolet radiation (UV) as the UV-index in summer. The UV-index is based on the erythemal (skin reddening) action spectrum and serves as a proxy for acute exposure to high UV levels (Fioletov et al., 2010). We used the monthly means of the maximum daily UV-index from the National Aeronautics and Space Administration (2016), and the global radiation data from the German Meteorological Office (2009) to estimate the annual means of UV-index in summer at the 5-digit postcode level. We assessed the UV-index per postcode.

All environmental parameters were averaged over 5-year periods, corresponding to the investigation in the cohorts: 2009 to 2014 for BASE-II and 2006 to 2010 for SALIA. We assigned the 5-year mean concentrations to the geocoordinates of participants' addresses (SALIA) or to the 5-digit postcode where the participants resided (BASE-II) using ArcGIS Version 10 (Environmental Systems Research Institute, California, USA). We assigned the postcode level concentrations to the participants' residences, thus estimating the residential exposure in a wider neighborhood.

2.4. Statistical analyses

We analyzed the association of ambient O_3 with the markers of extrinsic skin aging using linear regression. Regression estimates were transformed to arithmetic mean ratios and presented as percent change in mean. We selected a fixed increment of 7 days/year of O_3 exceedance, based on the interquartile ranges in both cohorts. Adjusted models were defined a priori and included age, sex, body mass index, total years of education, smoking, alcohol consumption, and a dummy variable for the study cohort. We performed complete cases analysis with each skin aging parameter, as the number of missing values differed across the outcomes. In a sensitivity analysis, we additionally adjusted for exposure to second hand smoke, sensitive skin type

Table 1
Description of SCINEXA scores in SALIA, BASE-II, and in the pooled analysis dataset.

Parameter[1]	Individual cohorts		Pooled dataset (N = 2013)
	SALIA (*N* = 806)	BASE-II (*N* = 1207)	
Coarse wrinkles on the face			
Forehead	3.3 ± 1.2	2.9 ± 1.4	3.1 ± 1.3
Frown lines	3.4 ± 1.0	2.9 ± 1.3	3.1 ± 1.2
Crow's feet	2.9 ± 0.9	2.7 ± 1.1	2.8 ± 1.0
Under the eyes	3.6 ± 0.9	2.8 ± 1.0	3.1 ± 1.0
Nasolabial fold	3.7 ± 0.7	3.1 ± 0.9	3.3 ± 0.9
Upper lip	3.3 ± 0.9	2.0 ± 1.4	2.6 ± 1.4
Pigment spots on the face			
Forehead	1.1 ± 0.9	0.9 ± 0.7	1.0 ± 0.8
Cheeks	1.3 ± 0.7	0.8 ± 0.7	1.0 ± 0.7

[1] Mean ± standard deviation are presented for continuous parameters.

(Fitzpatrick, 1988), coal/biomass heating, self-reported information about use of sun beds and use of cosmetics with sun protection factor (available only in SALIA participants). We performed cohort-specific and pooled analyses, adjusting for cohort as a dummy variable. In two-pollutant models, we also adjusted for co-exposures to PM_{10}, NO_2, and UV-index. In addition, we tested for effect modification by sex and smoking status using product terms $O_3\times$ modifier. Analyses were performed with R version 3.4.0 (R Core Team, 2017).

3. Results

3.1. Description of the study cohorts and the distribution of the SCINEXA scores

We included 806 women from the SALIA cohort, and 1207 men and women from the BASE-II cohort. We observed higher SCINEXA scores in SALIA participants, compared to BASE-II participants (Table 1). The two cohorts differed in personal characteristics: BASE-II participants were on average younger, had lower body mass index, more participants studied 14 years or longer, there were more smokers, but fewer participants drank any alcohol compared to the SALIA cohort (Table 2). Most of study participants in both cohorts were retired, and < 4% had ever worked outdoors (see Supplementary Material "Employment and occupational exposure due to outdoor work activity in the study participants"). It is thus unlikely that study participants have had longer exposures overall as compared to the general population.

3.2. Residential levels of O_3 and other exposures

The average 5-year mean residential levels of O_3, NO_2 and PM_{10} as well as of ultraviolet radiation (UV) were higher in SALIA study participants, than in BASE-II participants, while the distribution range was wider in the latter cohort (Table 3). In the pooled dataset, the study participants experienced on average 17 days of O_3 exceedance per year, which was slightly below the current target value in the European Union (25 days of exceedances, averaged per 3 years, are permitted; EEA, 2017). Mean concentrations of PM_{10} and NO_2 were 23.0 $\mu g/m^3$ and 21.5 $\mu g/m^3$, correspondingly. The average UV-index in summer was 6.9 in the pooled dataset. The distribution of UV-index was very narrow in both study cohorts (Table 3).

Correlations of O_3 exceedances with other air pollutants and UV followed different patterns in the two cohorts: in the BASE-II study area, O_3 correlated positively with PM_{10} and NO_2, while in SALIA we observed inverse correlations (Table 3). This was probably due to the fact that, different from the urban population of BASE-II, about half of SALIA participants resided in a rural area, where other sources were the precursors for O_3, such as volatile organic compounds from green plants, which might have contributed to O_3 formation (Table 3). We found weak inverse correlation of UV-index with O_3 in SALIA, and no correlation in BASE-II, probably due to much coarser spatial variability of UV-index, compared to air pollutants (Table 3).

Table 2
Personal characteristics of study participants in SALIA, BASE-II, and in the pooled analysis dataset.

Individual characteristics[1]	Individual cohorts		Pooled dataset (N = 2013)
	SALIA (N = 806)	BASE-II (N = 1207)	
Age [years]	73.5 ± 3.1	68.3 ± 3.7	70.4 ± 4.3
Female	806 (100.0%)	662 (54.8%)	1468 (72.9%)
Body-mass index [kg/m^2]	27.3 ± 4.5	26.8 ± 4.2	27.0 ± 4.4
Years of education			
≥18	33 (4.1%)	341 (28.3%)	374 (18.6%)
14–17	113 (14.0%)	270 (22.4%)	383 (19.0%)
11–13	385 (47.8%)	486 (40.3%)	871 (43.3%)
< 11	271 (33.6%)	110 (9.1%)	381 (18.9%)
Smoking status			
Never	644 (79.9%)	583 (48.3%)	1227 (61.0%)
Former	140 (17.4%)	501 (41.5%)	641 (31.8%)
Current	22 (2.7%)	123 (10.2%)	145 (7.2%)
Passive smoking	486 (60.3%)	–[2]	–
Any alcohol use	603 (74.8%)	123 (10.2%)	726 (36.1%)
Sensitive skin[3]	455 (56.5%)	–	–
Sunbed use	145 (18.0%)	–	–
Use of cosmetic products with UV protection factor	491 (60.9%)	–	–
Coal/biomass heating	131 (16.3%)	–	–

[1] Mean ± standard deviation are presented for continuous parameters, N (%) are presented for categorical parameters.
[2] Not available.
[3] Skin type I or II after Fitzpatrick (1988).

Table 3
The distribution and correlations of the selected environmental parameters (5-year averaged values) in SALIA, BASE-II, and in the pooled analysis dataset.

Parameter	SALIA (N = 806)	BASE-II (N = 1207)	Pooled dataset (*N* = 2013)
Description[1]			
O_3 exceedance days [days/year]	17.6 ± 2.7 (11.0–21.0)	16.9 ± 1.4 (9.6–28.1)	17.2 ± 2.1 (9.6–28.1)
PM_{10} [$\mu g/m^3$]	23.5 ± 1.8 (21.3–29.4)	22.6 ± 1.4 (14.0–24.6)	23.0 ± 1.7 (14.0–29.4)
NO_2 [$\mu g/m^3$]	24.8 ± 5.5 (18.2–36.1)	19.3 ± 4.1 (7.2–25.2)	21.5 ± 5.4 (7.2–36.1)
UV-index in summer	7.3 ± 0.1 (7.0–7.5)	6.7 ± 0.1 (6.5–9.5)	6.9 ± 0.3 (6.5–9.5)
Correlations[2]			
O_3 exceedance days × PM_{10}	−0.58	0.53	−0.11
O_3 exceedance days × NO_2	−0.72	0.33	−0.19
PM_{10} × NO_2	0.83	0.81	0.82
O_3 exceedance days × UV-index in summer	−0.4	−0.05	0.08

[1] Mean ± standard deviation and range (minimum–maximum) are presented.
[2] Pearson's r.

3.3. Associations of O_3 exceedance days with markers of extrinsic skin aging

We found positive associations of O_3 exceedance with coarse wrinkles on the face. These associations were detectable in each cohort, as well as in the pooled sample. In the SALIA cohort, O_3 exceedance days were positively associated with severity of wrinkles on the forehead (7.69% increase (95%-confidence interval (CI) 0.86 to 14.5) per

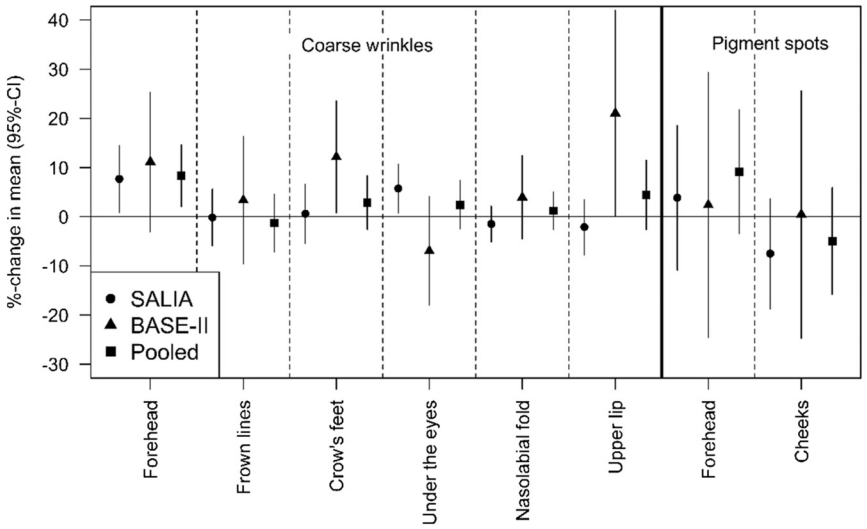

Fig. 1. Title: O_3 exceedance days [7 days/year] with scores for extrinsic skin aging in the study cohorts.
Legend: Associations of O_3 exceedance days with SCINEXA scores for extrinsic skin aging in SALIA (N = 806), BASE-II (N = 1207), and pooled analysis dataset (N = 2013), expressed as percent change in mean. Adjusted for: age, sex, body mass index, alcohol consumption, smoking, years of education, study sample (in the pooled analysis).

7 days of exceedance) and under the eyes (5.75% (95%-CI: 0.78 to 10.7; Fig. 1 and Supplementary Table S1). In the BASE-II cohort, we found positive associations with crow's feet (12.2% (95%-CI 0.75 to 23.6) and on the upper lip (21.1% (95%-CI: 0.11 to 42.0; Fig. 1 and Supplementary Table S1). We also found a positive association of O_3 exceedance days with wrinkles on the forehead in the pooled cohort (8.34% (95%-CI: 2.01 to 14.7; Fig. 1 and Supplementary Table S1).

The photochemical smog reactions to generate O_3 involve various air pollutants and require solar radiation, the major part of which is in the UV spectrum (wavelength < 400 nm; Mustafa, 1990; EEA, 2016). Since chronic exposure to UV radiation is known to cause coarse wrinkles in the face (Krutmann et al., 2017), we tested whether the observed associations of O_3 with coarse wrinkles were independent from co-exposure to UV radiation. As shown in the Supplementary Fig. 1c, the association between O_3 exceedance and more coarse wrinkles on the forehead was robust with the adjustment for UV radiation. Moreover, the association did not change after adjustment for the co-pollutants PM_{10} and NO_2 (Supplementary Fig. S1a, b). In further sensitivity analyses with the SALIA study (where this information was available), we found that the associations of O_3 with coarse wrinkles remained unchanged after adjustment for additional potential confounders, such as exposure to second hand smoke, skin type, self-reported use of sun beds and cosmetics with sun protection factor, and indoor air pollution exposure due to coal/biomass heating (Supplementary Table S2).

The observed association of O_3 with coarse wrinkles on the forehead was not modified by sex (Supplementary Table S3). However, we observed an effect modification by smoking: O_3 exceedance days were positively associated with wrinkles on the upper lip in current and former smokers, while no association was found in never smokers (interaction p = 0.049).

We did not detect an association with facial pigment spots (Fig. 1, Supplementary Table S1).

4. Discussion

In this first epidemiological study on long-term effects of tropospheric O_3 on skin aging, we found positive associations of long-term residential exposure to high O_3 levels with coarse wrinkles on the face in a sample of 2013 Caucasian men and women from two ongoing cohort studies in Germany. The associations were found in the following facial areas: on the forehead (pooled sample and the SALIA cohort), under the eyes (SALIA cohort), in the crow's feet area and on the upper lip (BASE-II cohort). Importantly, the health effects were demonstrated using a conservative exposure increment of 7 exceedance days per year, which was > 3 times smaller than the EU target value of 25 days. The associations with tropospheric O_3 were specific for coarse wrinkles, and were not detected with pigment spots, which in our previous studies were shown to be associated with chronic exposure to other air pollutants such as PM_{10} (Vierkötter et al., 2010) and NO_2 (Hüls et al., 2016).

The demonstrated skin aging effect, i.e. the association between O_3 and facial wrinkles is consistent with previous mechanistic studies. O_3 was shown to rapidly oxidize lipids and proteins in the skin, producing radical species, such as hydroxyl radical, and triggering oxidative stress (Krutmann et al., 2014; Valacchi, 2010). Over 2 decades ago, Thiele et al. (1997) reported that short-term O_3 exposure was effective in depleting antioxidants from the stratum corneum in murine skin. During the following years, the resulting stress response has been further characterized in human and murine skin (Krutmann et al., 2014; Valacchi, 2010). Altogether, these studies provide compelling evidence that the oxidative stress response elicited by O_3 is not restricted to the stratum corneum, but cascades down into deeper layers of the skin, where it can activate transcription factors such as NFkB and AhR (Krutmann et al., 2014; Valacchi, 2010).

In an experiment with hairless mice Valacchi et al. (2003) has found that O_3 activated the expression of matrix metalloproteinase 9 (MMP-9). Further investigation by the same research group has demonstrated an O_3-related increase in the expression of another enzyme of the same family - MMP-2 (Fortino et al., 2007). MMPs cleave various molecules in extracellular matrix, for example, collagen and elastin (McCawley and Matrisian, 2001). Also, they are involved in multiple skin pathologies, including skin aging, tumor development, and cutaneous lesions (Valacchi, 2010). Introduction of MMP-1 to unexposed skin culture triggered adverse changes in the extracellular matrix, similar to photoaging: collagen fragmentation and structure disturbances (Quan et al., 2009). Degradation of the extracellular matrix fibers (fragmentation, misshaping, loss of function, and deposition of elastotic material in the dermis) diminish the elasticity of the skin, promoting the wrinkle formation (Hildebrandt, 2010).

There are, to the best of our knowledge, no other studies on long-term effects of O_3 on the human skin. However, studies on short-term cutaneous effects of O_3 provide supportive evidence. Two controlled exposure studies with healthy volunteers, employing an environmentally realistic O_3 concentration of 0.8 ppm (corresponding to 1700 $\mu g/m^3$), reported acute responses in the skin: reduction in antioxidant content, lipid peroxidation, and a pro-inflammatory response in keratinocytes (He et al., 2006; Valacchi et al., 2017). In addition, two time-series studies have shown that acute increases in ambient O_3 concentrations were correlated with a number of emergency room visits

for adverse skin conditions, such as urticaria, eczema, and contact dermatitis in China (Xu et al., 2011) and Canada (Szyszkowicz et al., 2012).

The observed association of O_3 with coarse wrinkles on the face was robust to adjustment for solar UV radiation exposure in our study, so it is likely that the observed association was independent from the UV exposure. Pigment spots, the trait primarily linked to solar radiation, were not associated with O_3 exposure in our results, also suggesting that the observed effect of O_3 was independent from the UV radiation. However, we cannot completely rule out residual confounding, as UV exposure had much less spatial variability (reflected in a narrow distribution) than O_3. This is because UV is mainly dependent on latitude and altitude, while the concentrations of air pollutants depend on local sources.

The observed association was also independent of co-pollutants PM_{10} and NO_2, which in our previous studies have shown consistent associations with pigment spots (Vierkötter et al., 2010; Hüls et al., 2016). Considering the higher oxidative potential and shorter lifetime of the O_3 molecule, these discrepancies might indicate differences in the pathophysiologic pathways for various air pollutants, as previously suggested (Krutmann et al., 2014, 2017). However, having relied on modeled exposure data, we cannot completely rule out the misclassification by co-pollutants. So far, it is not feasible to conduct individual exposure measurements in a large epidemiologic study. At the same time, a study with a smaller sample, allowing exact exposure measurement, would not have enough statistical power to address the study question, as the investigated health effects are relatively small. In addition, since skin aging is a slow process, a panel study design with more exact exposure measurement would not allow the necessary length of the observations.

There are several strengths in our study. First, we were able to extend the study sample by pooling two cohorts together and obtaining a total sample of 2013 participants. Second, we assessed the extrinsic skin aging with the SCINEXA™ tool, validated to distinguish between extrinsic and intrinsic skin aging (Vierkötter et al., 2009). Third, we have employed multi-exposure models with UV and co-pollutants PM_{10} and NO_2 to show that the observed associations of O_3 with coarse wrinkles were independent from co-exposure to other air pollutants or UV.

However, our study also has some limitations. First, exposure assessment was based on modeled data, which although the most common way in epidemiological cohort studies, could have led to exposure misclassification with regard to personal exposure. However, considering that study subjects were mostly retired (mean age was 73.5 years in SALIA cohort and 68.3 years in BASE-II cohort), exposure assessment at the postcode level most likely reflected the individual exposure well, including not only air pollution concentrations at the residence, but also in the area of everyday mobility, such as the nearest shops, parks, etc.

Another limitation is related to the fact that the concentrations of O_3 change rapidly over time and space. O_3 is a secondary pollutant, which occurs from photochemical smog reactions, in the presence of solar radiation and precursors, such as NO_2. At nighttime and/or in the presence of scavengers, such as nitric oxide, O_3 can quickly decompose, to nitrogen dioxide and oxygen. The variability of short-term concentrations of O_3 poses a challenge to the analysis of long-term effects of ozone. The yearly average concentration, a measure commonly used with other pollutants, such as PM_{10} or NO_2 may underestimate the actual exposure to O_3 at its peak concentrations. To overcome this challenge, we have employed the exceedance days as a measure of high residential exposure to O_3. In addition, we have conducted multi-pollutant models, adjusting for NO_2, the most important precursor of O_3.

5. Conclusions

In this study with 2013 Caucasian men and women from two population-based cohort studies in Germany, we provide the first epidemiological evidence for an adverse role of ozone in coarse wrinkle formation, independently of other known environmental risk factors – UV, PM_{10} and NO_2. These findings were observed with the ozone levels not exceeding the current regulatory standards. In the light of ozone concentrations increasing alongside globally rising ambient temperature, the observed associations, if confirmed in further studies, provide important information about ambient ozone affecting skin health.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2018.12.047.

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