# Airborne Particle Exposure and Extrinsic Skin Aging

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For decades, extrinsic skin aging has been known to result from chronic exposure to solar radiation and, more recently, to tobacco smoke. In this study, we have assessed the influence of air pollution on skin aging in 400 Caucasian women aged 70–80 years. Skin aging was clinically assessed by means of SCINEXA (score of intrinsic and extrinsic skin aging), a validated skin aging score. Traffic-related exposure at the place of residence was determined by traffic particle emissions and by estimation of soot in fine dust. Exposure to background particle concentration was determined by measurements of ambient particles at fixed monitoring sites. The impact of air pollution on skin aging was analyzed by linear and logistic regression and adjusted for potential confounding variables. Air pollution exposure was significantly correlated to extrinsic skin aging signs, in particular to pigment spots and less pronounced to wrinkles. An increase in soot (per $0.5 \times 10^{-5}$ per m) and particles from traffic (per 475 kg per year and square km) was associated with 20% more pigment spots on forehead and cheeks. Background particle pollution, which was measured in low residential areas of the cities without busy traffic and therefore is not directly attributable to traffic but rather to other sources of particles, was also positively correlated to pigment spots on face. These results indicate that particle pollution might influence skin aging as well.

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## INTRODUCTION

According to the United Nations Department of Economic and Social Affairs Population Division, the proportion of the world's human inhabitants who can be classified as aged is increasing dramatically (Department of Economic and Social Affairs (ESA), 2002). Aging is accompanied by progressive deterioration of structure and function of all tissues, including visible signs of both intrinsic and extrinsic skin aging. In this regard, skin aging is of particular importance, because it has medical, psychological and social consequences. Among all organs skin is the most visible, and skin aging directly impacts individual self-esteem (Gupta and Gilchrest, 2005). This is illustrated best by the fact that the current market for cosmetic and medical products devoted to the prevention and treatment of skin aging has 15 billion US$ worth of sales worldwide (Yarosh, 2008).

Aging results from the combined action of intrinsic and extrinsic factors. From a preventive point of view, the latter are of particular interest because they can be modified more easily. In the case of skin, extrinsic skin aging can be clearly distinguished from intrinsic skin aging at a clinical, histological, and molecular level. Clinical symptoms of extrinsic skin aging include coarse wrinkles, irregular pigment spots, and elastosis (Yaar *et al.*, 2003). For decades, it has been thought that extrinsic skin aging results predominantly from exposure of skin to solar radiation, and the terms extrinsic and photoaging have been used synonymously. There is, however, growing evidence that other environmental factors may contribute to skin aging as well. In particular, exposure of skin to tobacco smoke was found to be an independent pathogenic factor (Schröder *et al.*, 2006).

Ambient particulate matter (PM) represents another environmental threat to which millions of humans worldwide are exposed. Adverse effects of PM on human health are currently a serious concern and have been shown to include a higher risk for cancer, pulmonary, and cardiovascular diseases (Beelen *et al.*, 2008; Castano-Vinyals *et al.*, 2008). The health effects of ambient PM exposure on human skin in general, and on skin aging in particular, have not yet been studied.

A major mechanism by which ambient PM exerts its detrimental effects is through the generation of oxidative stress (Donaldson *et al.*, 2005), an important contributor to extrinsic skin aging (Schröder *et al.*, 2006). Particles in the nanosize range, especially those from traffic sources, are considered among the most harmful components of ambient PM. These nanoparticles cause oxidative stress in part because their physical properties, i.e. small size but large surface per unit mass, make them highly reactive toward biological surfaces and structures (Donaldson *et al.*, 2005). It has been postulated that these particles can serve as carriers for organic chemicals and metals that are capable of

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*Abbreviations: PAH, polycyclic aromatic hydrocarbon; PM, particulate matter; SCINEXA, score of intrinsic and extrinsic skin aging*

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localizing in mitochondria and generating reactive oxygen species (Li *et al.*, 2003). In particular, polycyclic aromatic hydrocarbons (PAHs) are adsorbed on the surface of suspended PM in air of urban areas (Menichini, 1992). PAHs can activate xenobiotic metabolism, which converts PAHs to quinones. Quinones are redox-cycling chemicals, which produce reactive oxygen species, and are therefore thought to be key compounds in PM toxicity (Penning *et al.*, 1999).

In view of the recent evidence that human skin (1) is exposed to increased levels of ambient PM, which can penetrate skin either through hair follicles or transepidermally (Lademann *et al.*, 2004), and (ii) possesses the necessary armamentarium to respond to PM-bound PAHs (Fritsche *et al.*, 2007; Jux *et al.*, 2009), we hypothesized that long-term exposure to air pollution might lead to extrinsic skin aging through oxidative stress generated by the particles themselves or by associated PAHs. We tested the first part of this hypothesis in an epidemiological study using a cohort of Caucasian women aged 70–80 years (SALIA study, study on the influence of air pollution on lung function, inflammation, and aging).

## RESULTS

### Study subjects and study areas

We investigated skin aging signs in 400 women in the SALIA study cohort in 2008/2009. In Table 1 a description of all relevant data of these study subjects is given separately for the participants investigated in the Ruhr area and in the rural area (Borken). And in Table 2 general information of the study area cities as well as of other European cities is given. Table 1 shows that the participants were almost equally distributed between rural and urban areas. The women from the Ruhr area were slightly older (arithmetic mean (AM) = 74.3 years) than the women from Borken (AM = 74.0 years). We also asked about known influencing factors of skin aging. In this regard, about 17% had <10 years of school education, indicating a worse social status. The mean body mass index (BMI) was 27.6 in both study regions. Hormone replacement therapy (HRT) was taken by 44.1% of the women from the Ruhr area and by 29.1% of the women from Borken. There were more women who ever smoked in the Ruhr area (23.2%) than in the rural area (13.2%). More than 50% of the women had skin type I or II according to Fitzpatrick. Concerning sun exposure behavior, women from the Ruhr area more often reported to have had sunburns before the age of 21 years (52.1%) than women from Borken (32.3%). Also women from the Ruhr area more often used sunbeds (16.1%) than women from Borken (10.6%). Exposure to PM was determined by four objective measurements as described in detail in the Materials and Methods section. Briefly, we determined the distance of participants' residence to the next busy road. Here, nearly 20% of the women lived 100 m or less away from a busy road. The mean level of traffic-related particle

**Table 1. Description of SALIA study subject characteristics**

| | | Ruhr area | Rural area (Borken) |
|---|---|---|---|
| Sample size | *N* | 211 | 189 |
| *Data from questionnaire* | | | |
| Age range | AM (Min, Max) | 74.3 (70.9–79.2) | 74.0 (68.6–78.8) |
| <10 Years of school education | %Yes (*n*) | 16.1 (34) | 18.0 (34) |
| BMI | AM (95% CI) | 27.6 (27.0–28.3) | 27.6 (26.9–28.2) |
| Ever used HRT | %Yes (*n*) | 44.1 (93) | 29.1 (55) |
| Ever smoked | %Yes (*n*) | 23.2 (49) | 13.2 (25) |
| Light skin type (Fitzpatrick skin type I or II) | %Yes (*n*) | 55.5 (117) | 54.0 (102) |
| At least one sunburn before the age of 21 | %Yes (*n*) | 52.1 (110) | 32.3 (61) |
| At least one sunbed use | %Yes (*n*) | 16.1 (34) | 10.6 (20) |
| *Data from GIS-based models (2000/2003)* | | | |
| Distance ⩽100 m from a busy road | | | |
| (>10,000 cars per day) | %Yes (*n*) | 21.8 (46) | 15.3 (29) |
| *Emission inventory 2000* | | | |
| Particles from traffic ($kg\,a^{-1}\,km^{-2}$) | AM (IQR) | 899.9 (736.8) | 225.7 (215.7) |
| Soot ($10^{-5}m^{-1}$) | AM (IQR) | 2.2 (0.3) | 1.7 (0.1) |
| *Measurement at single stations from 2003–2007* | | | |
| $PM_{10}$ background concentration in ambient air ($\mu g\,m^{-3}$) | AM (IQR) | 27.9 (3.4) | 25.2 (0) |

Abbreviations: AM (95% CI), arithmetic mean (95% confidence interval); BMI, body mass index; HRT, hormone replacement therapy; IQR, interquartile range; Max, maximum; Min, minimum; %Yes (*n*), percentage and number of study subjects with respective characteristic.

**Table 2. Data about population density, latitude, temperature, and particle pollution by traffic of all cities of the SALIA study area and further cities for comparison**

| City | Country | Population density per km$^2$ at the end of year 2008 | Latitude | Mean of daily lowest temperature in the year 2009 | Mean of daily highest temperature in the year 2009 | Soot ($10^{-5}$ m$^{-1}$) in the year 2000 |
|---|---|---|---|---|---|---|
| *Cities of SALIA study area* | | | | | | |
| Borken | Germany | 260.9 | 51.9 | 7.1 | 15.9 | 1.66 |
| Duisburg | Germany | 2122.2 | 51.4 | 9.3 | 17.2 | 2.12 |
| Essen | Germany | 2756.6 | 51.5 | 7.2 | 14.2 | 2.43 |
| Gelsenkirchen | Germany | 2499.2 | 51.6 | — | — | 2.20 |
| Dortmund | Germany | 2084.2 | 51.5 | 7.5 | 14.3 | 2.00 |
| *Other European cities for comparison*[1] | | | | | | |
| Munich | Germany | 4275.0 | 48.0 | 5.7 | 14.9 | 1.84 |
| — | Netherlands | 397.2 | 50–54 | 5.6 | 13.5 | 1.64 |
| Stockholm | Sweden | 4252.0 | 59.4 | 2.5 | 9.9 | 1.28 |

[1]Adapted from Brauer *et al.* (2003).

emission was 899.9 kg a$^{-1}$ km$^{-2}$ in the Ruhr area and 225.7 kg a$^{-1}$ km$^{-2}$ in Borken. The mean absorbance as a measure of soot concentration in fine particles was around $2.0 \times 10^{-5}$ per m and the 5-year mean of the background PM with an aerodynamic diameter of 10 μm ($PM_{10}$) concentration was approximately 26.5 μg m$^{-3}$. In comparison to other European cities, the cities of the Ruhr area are very highly polluted (Table 2).

**Clinical signs of skin aging**

Skin aging was assessed by means of the SCINEXA (score of intrinsic and extrinsic skin aging). This score includes skin aging signs that are characteristic for extrinsic and intrinsic skin aging, and we previously showed that this score is suitable to determine and differentiate between extrinsic and intrinsic skin aging (Figure 2; Vierkötter *et al.*, 2009). The occurrence of clinical signs of skin aging in the SALIA study cohort is shown in Table 3. The distribution of the score values of pigment spots and seborrheic keratosis was log-normally distributed and therefore the geometric mean was given. The geometric mean of the score values of pigment spots was about 3.0 on forehead up to more than 20.0 on forearm. The mean score value of seborrheic keratosis on the upper part of the body was 3.2. The distribution of the wrinkle grades, the grades of telangiectasia as well as the laxity grades were normally distributed, and in these cases the AM was given. The AM for all wrinkles on different locations on the face was around 3.0. The mean grade of telangiectasia was nearly 2.0 and the mean laxity grade of the face was nearly 4.0. Solar elastosis on the cheek was present in approximately 37% of women.

**Association between exposure to airborne particles and occurrence of skin aging signs**

A significant association was found between traffic-related airborne particles and signs of extrinsic skin aging, i.e., pigment spots on face and nasolabial fold. All adjusted mean ratios and odds ratios (OR) are presented in Table 4.

**Table 3. Description of skin aging signs evaluated with SCINEXA**

| | | |
|---|---|---|
| Extrinsic skin aging signs | | |
| *Pigment spots* | | |
| On forehead | GM (95% CI) | 3.3 (2.7–4.1) |
| On cheeks | GM (95% CI) | 8.1 (7.2–9.0) |
| On upper side of the forearm | GM (95% CI) | 22.7 (20.1–25.6) |
| On back of the hand | GM (95% CI) | 9.4 (8.3–10.8) |
| *Coarse wrinkles* | | |
| On forehead | AM (95% CI) | 3.2 (3.1–3.3) |
| In the crow's feet area | AM (95% CI) | 2.8 (2.8–2.9) |
| Under the eyes | AM (95% CI) | 3.6 (3.5–3.6) |
| On upper lip | AM (95% CI) | 3.4 (3.3–3.5) |
| Nasolabial fold | AM (95% CI) | 3.7 (3.7–3.8) |
| Solar elastosis | %Yes (*n*) | 36.8 (147) |
| Telangiectasia | AM (95% CI) | 1.9 (1.8–2.1) |
| Intrinsic skin aging signs | | |
| Laxity | AM (95% CI) | 3.6 (3.5–3.7) |
| Seborrheic keratosis[1] | GM (95% CI) | 3.2 (2.8–3.7) |

Abbreviations: AM (95% CI), arithmetic mean (95% confidence interval); GM (95% CI), geometric mean (95% confidence interval); %Yes (*n*), percentage and number of study subjects with respective skin aging symptom.
[1]Seborrheic keratosis was evaluated in 368 study subjects.

There were 22% more spots on forehead and 20% more spots on cheeks per increase of one interquartile range (IQR) of the $PM_{2.5}$ absorbance (= soot). For particles from traffic,

**Table 4. Association between different skin aging signs and exposure to airborne particles**

| | Distance ⩽100 m from a busy road | | | Soot (per increase of $0.5\times10^{-5}\,m^{-1}$) | | | Traffic-associated particles (per 475 $kg\,a^{-1}\,km^{-2}$) | | | $PM_{10}$ (per 5 $\mu g\,m^{-3}$) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Skin aging sign** | **MR/OR** | **95% CI** | ***P*** | **MR/OR** | **95% CI** | ***P*** | **MR/OR** | **95% CI** | ***P*** | **MR/OR** | **95% CI** | ***P*** |
| *Pigment spots* | | | | | | | | | | | | |
| On forehead[1] | 1.35 | 0.98–1.86 | 0.068 | **1.22** | **1.03–1.45** | **0.022** | **1.16** | **1.06–1.27** | **0.002** | 1.07 | 0.99–1.15 | 0.078 |
| On cheeks[1] | 1.15 | 0.86–1.54 | 0.362 | **1.20** | **1.03–1.40** | **0.019** | **1.17** | **1.08–1.27** | **0.000** | **1.08** | **1.01–1.15** | **0.027** |
| On forearm[1] | 0.95 | 0.70–1.30 | 0.756 | 1.08 | 0.92–1.27 | 0.334 | 1.05 | 0.97–1.15 | 0.243 | 1.02 | 0.95–1.09 | 0.634 |
| On back of hands[1] | 1.13 | 0.80–1.58 | 0.484 | 1.12 | 0.94–1.34 | 0.200 | 1.09 | 0.99–1.20 | 0.072 | 1.02 | 0.95–1.10 | 0.529 |
| *Wrinkles* | | | | | | | | | | | | |
| On forehead[2] | 0.97 | 0.88–1.06 | 0.504 | 0.96 | 0.91–1.01 | 0.078 | 0.99 | 0.96–1.02 | 0.390 | 0.99 | 0.97–1.01 | 0.153 |
| Crow's feet[2] | 0.99 | 0.92–1.06 | 0.731 | 0.98 | 0.94–1.01 | 0.208 | 0.98 | 0.96–1.00 | 0.114 | 0.99 | 0.97–1.00 | 0.077 |
| Under the eyes[2] | 1.00 | 0.94–1.07 | 0.830 | 0.99 | 0.96–1.03 | 0.662 | 0.99 | 0.97–1.01 | 0.287 | 0.97 | 0.97–1.00 | 0.054 |
| On upper lip[2] | 1.01 | 0.94–1.08 | 0.736 | 1.03 | 0.99–1.06 | 0.168 | 1.01 | 0.99–1.03 | 0.333 | 1.01 | 0.99–1.02 | 0.320 |
| Nasolabial fold[2] | 1.04 | 1.00–1.08 | 0.056 | **1.04** | **1.01–1.06** | **0.001** | **1.03** | **1.01–1.04** | **0.000** | **1.01** | **1.01–1.02** | **0.020** |
| *Further skin aging symptoms* | | | | | | | | | | | | |
| Solar elastosis[1] | 0.81 | 0.47–1.40 | 0.451 | 1.15 | 0.87–1.53 | 0.327 | 1.02 | 0.87–1.18 | 0.849 | 1.32 | 0.73–2.40 | 0.363 |
| Telangiectasia[1] | 0.83 | 0.64–1.01 | 0.067 | 0.91 | 0.81–1.01 | 0.069 | 0.95 | 0.90–1.01 | 0.082 | 0.94 | 0.73–1.15 | 0.572 |
| Laxity[1] | 1.03 | 0.98–1.09 | 0.270 | 1.00 | 0.97–1.03 | 0.913 | 1.00 | 0.99–1.02 | 0.744 | 1.00 | 0.94–1.06 | 0.955 |
| Seborrheic keratosis[1] | 1.17 | 0.68–1.65 | 0.504 | 1.13 | 0.87–1.39 | 0.325 | 1.01 | 0.87–1.15 | 0.898 | 1.18 | 0.63–1.73 | 0.523 |

Abbreviations: BMI, body mass index; 95% CI, 95% confidence interval; HRT, hormone replacement therapy; MR, mean ratio; OR, odds ratio.
[1]Adjusted for age, skin type, sunburns, sunbed use, and smoking,
[2]Adjusted for age, BMI, HRT, skin type, sunburns, sunbed use, and smoking.
Significant associations with a *P*-value $<0.05$ are marked in bold.

we found 16% more spots on forehead and 17% more spots on cheeks per increase of one IQR. There was also a slight increase of spots on cheeks per one IQR of background $PM_{10}$ concentrations of 8%. Furthermore, soot, particles from traffic, and to a lesser extent the $PM_{10}$ background concentrations were associated with a slightly more pronounced nasolabial fold. Distance of 100 m or less from a busy road was also associated with 35% more pigment spots on forehead and 15% more pigment spots on cheeks. However, it was not significant, because the number of study subjects living close to a busy road was too small.

The statistical models used accounted for other factors known to influence skin aging. In the following the main results for the influence of these factors are presented as arithmetic or geometric mean ratio (AMR/GMR) and its 95% confidence interval (95% CI). It was found that HRT was associated with fewer wrinkles under the eyes (AMR: 0.93; 95% CI: 0.88–0.98) and less pronounced nasolabial folds (AMR: 0.97; 95% CI: 0.93–1.00). Women with a skin type I or II had significantly less spots on forehead (GMR: 0.72; 95% CI: 0.56–0.92) and on cheeks (GMR: 0.69; 95% CI: 0.55–0.86); fewer wrinkles under the eyes (AMR: 0.95; 95%CI: 0.89–1.00) and on upper lips (AMR: 0.95; 95% CI: 0.89–1.00); and less solar elastosis (OR: 0.62; 95% CI: 0.41–0.95), but more pronounced telangiectasia (AMR: 1.12; 95% CI: 0.98–1.27) in comparison to skin type III or IV. Sunburns in childhood and sunbed use were associated with more spots, e.g., 40–50% more spots on forehead. Smoking (current or prior) was associated with more wrinkles on upper lips (AMR: 1.13; 95% CI: 1.07–1.20), increased solar elastosis (OR: 2.11; 95% CI: 1.24–3.58), more pronounced telangiectasia (AMR: 1.26; 95% CI: 1.07–1.45), and laxity of the face (AMR: 1.06; 95% CI: 1.00–1.11). Social status had no further influence on skin aging when all influencing factors were taken into account in one model.

## DISCUSSION

This study provides epidemiological evidence that traffic-related PM represents an important environmental factor that contributes to extrinsic skin aging in humans. This conclusion is based on the present observation that not only (i) an increase in soot, but also (ii) an increase in particles from traffic, and (iii) higher $PM_{10}$ background concentrations were associated with more pigment spots on the face and more pronounced nasolabial folds. The distance of residence to the closest busy road was also associated with more pigment spots, but this effect did not reach significance.

For determination of traffic-related PM, we applied the most up to date state-of-the-art method according to Brauer *et al.* (2003). Here, the exact geographic coordinates of each study participants' address were determined by geographic information system, and the respective PM concentrations were assigned for this address using measurements and land use regression. Furthermore, as the SALIA study participants were (i) mainly housewives, (ii) almost all remained at the same address for the last 30 years, and (iii) the pattern of pollution of the different investigated cities remained the same over the last decades, exposure of residence reflects long-term exposure. Even in the hypothetical case that we have studied only a random subgroup of all women willing to participate, it is very unlikely that we have introduced bias because (i) participation did not depend on air pollution (Ranft *et al*., 2009) and (ii) participation could not depend on skin aging signs as this aim of the study was not known to participants beforehand.

The association between PM and skin aging symptoms was strongest for pigment spot formation. The pathogenesis of pigment spots is not very well understood. It has been suggested that solar radiation is an important pathogenetic factor and skin aging-associated pigment spots are therefore also called lentigines solaris. In support of this hypothesis is the finding that pigment spots are mainly present in chronically sun-exposed areas of the skin (Garbe *et al*., 1994), and that after chronic exposure to UVB radiation a delayed induction of pigmented spots has been observed in the skin of mice (Kadono *et al*., 2001). Here, we confirm that UV exposure is significantly associated with a more pronounced occurrence of pigment spots. UV exposure, however, does not explain why pigment spots are the leading skin aging symptom in Asians (Tschachler and Morizot, 2006), who, in contrast to Caucasians, avoid sun exposure and thus should have less rather than more pigment spots. In this regard, it is of interest that a number of recent mechanistic studies indicate that skin pigmentation may occur in the absence of UV radiation. For example, treatment of melanocytes with selected oligonucleotides was able to induce tyrosinase activity and subsequent melanin synthesis in the absence of UV exposure (Eller and Gilchrest, 2000). Of particular interest to this study, aryl hydrocarbon receptor ligands, such as dioxin or PAHs, have recently been shown to induce melanocyte proliferation and thereby skin tanning in mice (Krutmann *et al*., 2008). PAHs are frequently bound to the surface of combustion-derived PM and this mechanism may therefore provide a scientific rationale for the association between pigment spots and exposure to traffic-related PM that we have observed. Accordingly, the strongest effect was seen for soot, which carries a high concentration of surface-bound PAHs.

The ability of particles to penetrate into skin is a matter of debate. Different skin penetration studies have used a variety of nanoparticle types as well as different experimental models (Tinkle *et al*., 2003; Toll *et al*., 2004; Baroli *et al*., 2007; Nohynek *et al*., 2007; Rouse *et al*., 2007). It is therefore not surprising that conflicting results have been obtained. To the best of our knowledge, however, no skin penetration studies have been carried out with ambient PM, and more specifically with the fraction of combustion-derived nanoparticles in the PM mixture. In general, there is no doubt that particles can penetrate into the hair follicles depending on their size (Lademann *et al*., 2004). Through this pathway ambient particles may be able to reach viable cells in deeper skin layers such as melanocytes and thus serve as Trojan horses by releasing surface-bound PAHs and/or directly affecting the function of skin cells. Further mechanistic studies are required to determine the relative contribution of such particles themselves versus particle-bound substances to extrinsic skin aging.

In this study, in addition to traffic-related PM, we studied a number of other factors thought to influence skin aging. All these influencing factors were analyzed together with the influence of air pollution in a multivariate statistical model. Here, the use of HRT was associated with fewer wrinkles, as previously described (Dunn *et al*., 1997). We also observed that a light skin type was associated with less pigment spots, less coarse wrinkles and elastosis, but more pronounced telangiectasia. This is again consistent with previous reports that these particular skin types show different characteristics of extrinsic skin aging than darker skin types (Lober and Fenske, 1990). In this study, reported sunburns in childhood and sunbed use were associated with more pigment spots, but not with more wrinkles, although both symptoms are known to result from chronic UV exposure. It is therefore possible that a more detailed UV exposure history is required to detect the effect of UV exposure on wrinkles. However, it is unlikely that the effects of air pollution on skin aging are confounded by sun exposure, as all investigated cities lay next to each other and the general climate and UV radiation flux is essentially identical in these cities. Moreover, the effect estimates for air pollution were not changed after including assessments characterizing sun exposure in the models. A smoking history was associated with more wrinkles, more elastosis, and more pronounced telangiectasia, and these observations are in agreement with the existing literature (Kennedy *et al*., 2003; Schröder *et al*., 2006). Taken together these results indicate that the design and protocol chosen for this study are suitable to test influencing factors on skin aging with perhaps minor limitations for the UV exposure effects.

To our knowledge, this is the first study to describe an association between airborne particles and extrinsic skin aging. Further studies should be conducted to confirm these results not only in Caucasians, but also for other ethnic groups such as Asian populations, where extrinsic skin aging is predominantly characterized by the development of pigment spots.

## MATERIALS AND METHODS

### Study design and study participants

The SALIA study was initiated as a cross-sectional study between 1985 and 1994 as part of the Environmental Health Survey, which was an element of the Clean Air Plan introduced by the Government of North-Rhine Westphalia in Germany. A detailed description of the SALIA study has been previously provided by Schikowski *et al*. (2005). The study areas were chosen from the Ruhr district in Germany and two rural counties north of the Ruhr district. They represent a range of exposures to airborne PM from traffic and steel and coal industries. In Table 2 the investigated cities are presented with data about population, geographical position, climate, and air

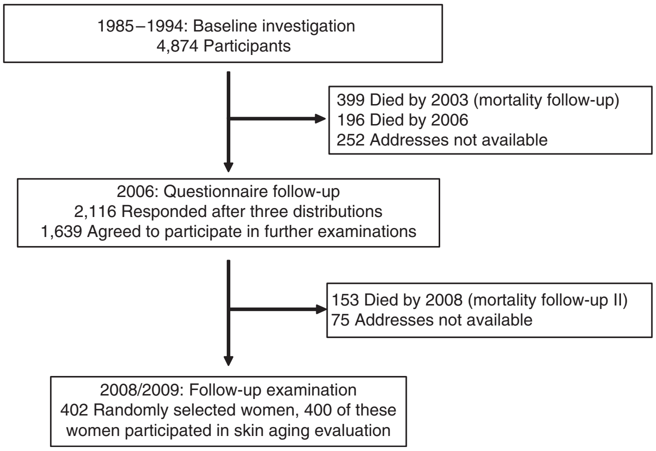


**Figure 1. Flowchart showing the SALIA cohort from baseline until follow-up in 2008/2009.**

| Skin aging signs: | Localization: | Scoring: |
|---|---|---|
| *Extrinsic signs* | | |
| Pigment spots[1] | On forehead | 0 (0), 1–10 (5), 11–50 (30), >50 (75) |
| | On cheeks | 0 (0), 1–10 (5), 11–50 (30), >50 (75) |
| | On upper side of the forearm | 0 (0), 1–10 (5), 11–50 (30), >50 (75) |
| | On back of the hand | 0 (0), 1–10 (5), 11–50 (30), >50 (75) |
| Coarse wrinkles[2] | On forehead | Grade 0 to 5 |
| | Wrinkles in crow's feet area | Grade 0 to 5 |
| | Under the eyes | Grade 0 to 5 |
| | On upper lip | Grade 0 to 5 |
| | Nasolabial fold | Grade 0 to 5 |
| Solar elastosis | On cheeks | Yes/no |
| Telangiectasia | On cheeks | Grade 0 to 5 |
| *Intrinsic signs* | | |
| Laxity[2] | Ovality of the face | Grade 0 to 5 |
| Seborrheic keratosis[1] | On upper part of the body | 0 (0), 1–10 (5), 11–50 (30), >50 (75) |

[1]Scoring of spots and seborrheic keratosis with counts in paratheses, [2]Grading with photoreference scales: 0 = sign not present, up to 5 = sign very severely present.

**Figure 2. Applied skin aging score on the basis of SCINEXA (score of intrinsic and extrinsic skin aging; Vierkötter *et al.*, 2009).**

pollution conditions. For comparison reasons we provide these data for other European city regions that were not investigated in the SALIA study. In comparison with these European cities, the cities of the Ruhr area are the areas most highly polluted with soot. In the baseline investigation, all women aged 54–55 living in predefined areas were asked to participate. The study was restricted to women because the aim was to investigate the effects of exposure to airborne PM, which would have been difficult in men of this region, who had predominantly worked as miners with high PM exposure at their work places. In Figure 1, a flow chart presents the SALIA study cohort from baseline to follow-up in 2008/2009. In 2008 and 2009, we conducted a follow-up of the SALIA study, in which we investigated 402 women randomly selected out of all surviving women, which gives their agreement for further investigations. The women were now 70–80 years old. Of these, 400 women participated in the skin examination. The Medical Ethics Committee of the Ruhr University Bochum, Germany approved the follow-up study. The Declaration of Helsinki Principles was followed and all study participants were informed in detail by written form and have given written consent.

### Assessment of skin aging symptoms and its influencing factors

Skin aging symptoms, which are characteristic for intrinsic and extrinsic skin aging, were evaluated on the basis of a validated skin aging score, called SCINEXA (Vierkötter *et al.*, 2009), with slight modifications to the original version. In Figure 2 the modified SCINEXA for the SALIA study is shown. Extrinsic skin aging was represented by pigment spots (lentigines), coarse wrinkles, elastosis, and telangiectasia, whereas laxity and seborrheic keratosis indicated intrinsic skin aging.

Pigment spots and seborrheic keratosis were given a value of 0 if there were no spots or seborrheic keratosis, 5 indicated 1–10 spots or seborrheic keratosis, 30 represented 11–50 spots or seborrheic keratosis, and 75 indicated more than 50 spots or seborrheic keratosis. Coarse wrinkles, telangiectasia, and laxity were scored from 0 (not present) to 5 (very severely present) according to photoreference scales (Tschachler and Morizot, 2006). Solar elastosis was evaluated as Yes (present) or No (not present). The study subjects were asked not to use any skin care products or cosmetics on the day of examination.

Other variables, which might influence skin aging, were determined by standardized interviews. These contained questions about UV exposure (e.g., sunburns in childhood and sunbed use), skin type according to Fitzpatrick (1988), social status (years of school education), BMI, smoking history, and intake of HRT.

### Assessment of exposure to airborne PM

Different approaches for the assessment of airborne PM were used. First, based on the subject's residential address and on available traffic counts for the year 2000, distance of the residential address to the next busy road with more than 10,000 cars per day was determined using geographic information system. If a subject lived 100 m or less to a busy road, they were considered to have high exposure to traffic-related particles. Second, we assessed the exposure to motor vehicle exhaust by using emission inventories from the year 2000 provided by the State Environment Agency of North Rhine Westphalia (LANUV). These inventories are given in a 1 km grid and estimate particle emission per square kilometer. Third, blackness of fine particle filters was used to estimate soot concentration from traffic-related sources and was then assigned to each individual's address by land-use regression models (Hochadel *et al.*, 2006). This exposure assessment was identical to those in the "Traffic-Related Air Pollution and Childhood Asthma" study (Brauer *et al.*, 2003; Cyrys *et al.*, 2003). Here, $PM_{2.5}$ absorbance was determined as a marker for soot according to ISO 9835. Fourth, measurements of total suspended particles or PM with an aerodynamic diameter of 10 µm ($PM_{10}$) were provided by monitoring stations distributed over the Ruhr district in an 8 km grid, which have been maintained by the State Environment Agency for more than 25 years. Total suspended particle measurements were converted into $PM_{10}$ estimates using a factor of 0.71 (Gehring *et al.*, 2006). These measurements mainly reflect broadscale background variations in air quality. The individual exposure to this background air pollution was estimated by the $PM_{10}$ concentrations of the monitoring station next to the participant's residential address averaged over the years 2003–2007.

Therefore, the total ambient PM was classified as (i) motor vehicle exhaust, which was assessed indirectly by the subject's distance from a busy road and appropriate emission inventories; (ii) soot, as estimated by blackness of $PM_{2.5}$ filters and; (iii) total suspended particles ($PM_{10}$), a value provided by long-term monitoring stations.

### Statistical analysis

A descriptive analysis summarizing evaluated skin aging indicators, known influencing factors of skin aging, and air pollution data was performed.

To analyze the effect of airborne particles on skin aging symptoms adjusted for further factors influencing skin aging, we used linear and logistic regression models. The adjusted regression coefficients were transformed to GMR for log-normally distributed symptoms with 95% CI, for normally distributed symptoms to adjusted AMR with 95% CI (Schikowski *et al.*, 2005; Vierkötter *et al.*, 2009), and for categorical variables to adjusted OR with 95% CI. The formulas for GMR (equation 1), AMR (equation 2), and OR (equation 3) are the following:

$$\mathrm{GMR}_i = \exp^{(\beta_i)} \quad (1)$$

$$\mathrm{AMR}_i = \frac{\beta_i}{M_{\mathrm{total}}} + 1 \quad (2)$$

$$\mathrm{OR}_i = \exp^{(\beta_i)} \quad (3)$$

where $\beta_i$ represents regression coefficient and $M_{\mathrm{total}}$ the total mean.

The GMR and AMR are relative values for continuous variables and are comparable in their meaning to the OR. They are more easily interpreted than a simple regression coefficient. They describe the relative change in skin aging signs when exposure is increased by one unit. As exposure units, we used the IQR observed in the population. An IQR means the difference of the 75th quartile and the 25th quartile of the distribution of the particle pollution variables (soot: $0.5 \times 10^{-5}\,\mathrm{m}^{-1}$; traffic emissions: $475\,\mathrm{kg\,a}^{-1}\,\mathrm{km}^{-2}$ and $PM_{10}$: $5\,\mu\mathrm{g\,m}^{-3}$). Like the OR, a GMR or AMR of 1 means that there is no association, a GMR or AMR $<1$ means a negative association, and a GMR or AMR $>1$ means a positive association.

In all models, data were adjusted for age (by year), Fitzpatrick skin type (light vs. dark skin type), number of sunburns before the age of 21, sunbed use (yes or no), and smoking history. BMI ($\mathrm{kg\,m}^{-2}$) and HRT were additionally included in the model for coarse wrinkles. The mutually adjusted association was defined as significant if the *P*-value was $<0.05$. The statistical computing was carried out using SAS 9.2 (SAS/STAT Software; SAS Institute, Cary, NC, 2002–2003).

## CONFLICT OF INTEREST

The authors state no conflict of interest.

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