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The effect modification by the Mediterranean Diet (MeDi) on skin aging in elderly women exposed to air pollution

Dissertation

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German summary

Die Effekt Modifizierung der mediterranen Diät (MeDi) auf die Hautalterung älterer Frauen die der Luftverschmutzung ausgesetzt sind

Autoren: V.Stender, C.Wigmann, H.Altug, J.Krutmann, T.Schikowski

Einleitung: Seit Jahrzehnten ist bekannt, dass UV-Strahlung und Rauchen extrinsische Hautalterung verursachen können. Aber auch Luftverschmutzung hat einen Einfluss auf die Hautalterung, insbesondere auf die Bildung von Falten und Pigmentflecken. Dies konnte zuerst in der SALIA Studie (Study on the influence of air pollution on lung function, inflammation and aging) gezeigt werden. Ernährung ist ein weiterer Faktor, der zur Hautgesundheit beiträgt. Der Aufnahme von Antioxidantien in Form von Obst und Gemüse sowie der Kalorien Restriktion wird nachgesagt, der Alterung entgegenzuwirken. Belege dafür sind allerdings kaum vorhanden.

Methoden: In der vorliegenden Arbeit wurde der Zusammenhang zwischen Hautalterung und mediterraner Ernährung bei 807 kaukasischen Frauen der SALIA Kohorte angeschaut. Dafür wurden Ergebnisse eines Fragebogens und eines erstellten MEDI Score basierend auf dem Score von Panagiotakos benutzt. Die Hautalterung wurde durch den SCINEXA™ Score beurteilt. Die Luftverschmutzung (NO_2, PM_{10}, $PM_{2.5}$, $PM_{2.5coarse}$, O_3) wurde anhand von Land-Nutzungs-Modellen bestimmt und anhand der Wohnadresse den einzelnen Probanden zugewiesen. Für die Statistik nutzten wir multiple logistische Regressionsmodelle, bezogen eine Interaktionsvariable (MEDI Score und Luftverschmutzungsparameter) ein und adjustierten für Kovariablen (Stadt oder Land, Sozialstatus, Alter, BMI, „Packyears“, Raucherstatus, Heizen mit fossilen Brennstoffen, Hauttyp, Lichtschutzfaktor, UV-Strahlung, Hormonersatztherapie, Sonnenbanknutzung).

Ergebnisse: Höhere Belastungen mit NO_2 korrelierten signifikant mit der Bildung von Pigmentflecken (Z-Scores). Höhere PM_{10} und $PM_{2.5}$ Werte waren signifikant assoziiert mit der Bildung von Falten auf der Oberlippe, ebenso mit der gleichmäßigen Pigmentierung auf der Unterarminnenseite. In den Modellen ohne Adjustierung für die UV Variablen korrelierten NO_2 und Ruß signifikant mit der Bildung von Nasolabialfalten. Der MEDI Score zeigte einzig einen schützenden Effekt für zigarettenpapierartige Fältelung auf dem Handrücken. Für viele andere Hautalterungsparameter zeigte der MEDI einen schädigenden Effekt. Der Antioxidantien Score zeigte nur für Pigmentflecken auf der Stirn einen schützenden Effekt. Die Interaktionsvariablen MEDI und PM_{10}, genauso wie MEDI und $PM_{2.5}$ zeigten eine schützende Tendenz auf die Faltenbildung (z-score).

Diskussion: Es gibt keine Zweifel daran, dass Luftverschmutzung negativ auf die Hautalterung wirkt. Antioxidantien und gute Ernährung sind weit verbreitete Verjüngungsmethoden. Die Studien dazu widersprechen sich allerdings, zeigen nur kleine Effekte und klinische Daten die die sichtbaren Effekte belegen fehlen bislang. Durch diese Studie versuchten wir Klarheit zu schaffen, allerdings bleiben Zweifel daran ob Ernährung überhaupt einen Einfluss auf die Hautalterung hat.

English summary

The effect modification by the Mediterranean diet (MeDi) on skin aging in elderly women exposed to air pollution

Authors: V.Stender, C.Wigmann, H.Altug, J.Krutmann, T.Schikowski

Introduction: For decades extrinsic skin aging is known as the result of UV radiation and smoking, but also air pollution has an impact on skin aging, presenting itself in wrinkle formation and pigment spots. This has been shown in the SALIA study (Study on the influence of air pollution on lung function, inflammation and aging) for the first time. Nutrition is a further factor contributing to skin health. Antioxidant uptake through fruits and vegetables as well as calorie restriction is rumored to delay aging effects. However, the evidence is very scarce.

Methods: We assessed the association of skin aging with an adherence to the Mediterranean diet (MEDI) in 807 Caucasian women from the SALIA cohort study using results of a questionnaire and transforming them to a MEDI score based on the score of Panagiotakos. Skin aging was evaluated by the SCINEXA score. Air pollution (NO_2, PM_{10}, $PM_{2.5}$, $PM_{2.5\ coarse}$, O_3) was evaluated by land use regression models and assigned to the home address of the study subjects. We applied multiple logistic regression models, included an interaction variable (MEDI score and air pollutant) and adjusted for covariates (urban or rural, social status, age, BMI, packyears, smoking status, heating with fossil fuels, skin type, sun protection factor, UV radiation, hormone replacement therapy, sunbed use).

Results: Higher NO_2 exposure was significantly associated with the formation of pigment spots in general (z-scores). Higher PM_{10} and $PM_{2.5}$ values were significantly associated with the formation of *wrinkles on the upper lip* as well as the *even pigmentation on the forearm inside*. In models without adjustment for UV covariates NO_2 and $PM_{2.5absorbance}$ correlated significantly with the formation of *nasolabialfolds*. The MEDI score showed a protective effect on *cigarette paper like skin on the back of the hands*. For many other skin aging parameters the MEDI score presented a harmful correlation. The antioxidant score showed a protective effect on *pigment spots on the forehead* only. The interaction terms MEDI and PM_{10} as well as MEDI and $PM_{2.5}$ showed a protective tendency on the wrinkle formation (z-score).

Discussion: There is no doubt that air pollution has a negative impact on skin aging. Antioxidants and healthy nutrition is broadly promoted as a rejuvenation method, but the studies contradict each other, or just show small effects and clinical data showing a visible effect are missing. In this study we tried to get clarity, but there is still remaining doubts if nutrition has an impact on skin aging at all.

List of Abbreviations

BMI	Body mass index
CW	Coarse wrinkles
ESCAPE	European Study of Cohorts for Air Pollution Effects
HRT	Hormone replacement therapy
IQR	Interquartile range (official unit for air particles)
IUF	Leibniz research institute for environmental medicine
HRT	Hormone replacement therapy
MEDI	Mediterranean diet score according to Pangiotakos
mMEDI	Modified Mediterranean diet score
NO_x	Nitrogen oxide
NO_2	Nitrogen dioxide
O_3	Ozone
$PM_{2.5}$	Particulate matter with an aerodynamic diameter smaller than 2.5 µg/m^3
$PM_{2.5absorbance}$	Measurement of blackness of PM2.5 filters, which is a proxy for elemental carbon
PM_{10}	Particulate matter with an aerodynamic diameter smaller than 10 µg/m^3
PS	Pigment spots
ROS	Reactive oxygen species
SALIA	Study on the influence of air pollution on lung function, inflammation and aging
SD	Standard deviation
SPF	sun protection factor
WHO	World health organization

Table of contents

1 Introduction

There are two forms of skin aging: extrinsic and intrinsic skin aging. Intrinsic skin aging is genetically determined and appears on all body parts at the same speed, whereas extrinsic skin aging is determined by the environment. Basically extrinsic aged skin presents itself in deep wrinkles, a leather like skin appearance with emphasis on the face and the hands. Intrinsic skin aging shows fine wrinkles, laxation of the skin and distributes over the whole body. The sun is the longest known factor influencing skin age, but also smoking, air pollution, nutrition, lack of sleep, stress, hormones, are known factors.

The topic of air pollution and its negative impacts on health emerged in the 1950s (1). Air pollution is known to impact the respiratory and cardiovascular system as well as the skin. The link between air pollution and skin aging was first discovered in 2010 with the SALIA study, an epidemiological study with elderly women which found that traffic related particulate matter (PM) promotes skin aging (2). Later further studies in Germany and in China indicated the relationship between air pollution and skin aging (3, 4). Even if the air pollution values have decreased in the past 50 years in the western world, it is a growing issue in developing countries and still remains a global challenge.

Nutrition is historically one of the earliest known factors influencing skin health. Following the rules of the Mediterranean diet (MEDI) pyramid is a popular lifestyle nowadays and consuming lots of fruits and vegetables is seen as a rejuvenation method in the general population. A few studies tried to prove the impact of certain diets or nutrients on skin age, but clear results are scarce. Nutrition may be relevant for the skin aging process, but the concrete extent that can be attributed to nutrition remains unclear.

In this thesis the influence of air pollution and nutrition on skin aging was analyzed. Air pollution is expected to act harmful on skin aging, for nutrition evidence is scarce. We expect the Mediterranean diet to delay skin aging and therefore influence the correlation between air pollution and skin aging.

1.1 The skin

1.1.1 Skin structure and function

The skin is our biggest organ and consists of three layers from outside to inside: the epidermis, the dermis and the subcutis. Figure 1 presents a scheme drawing of the structure of human skin.

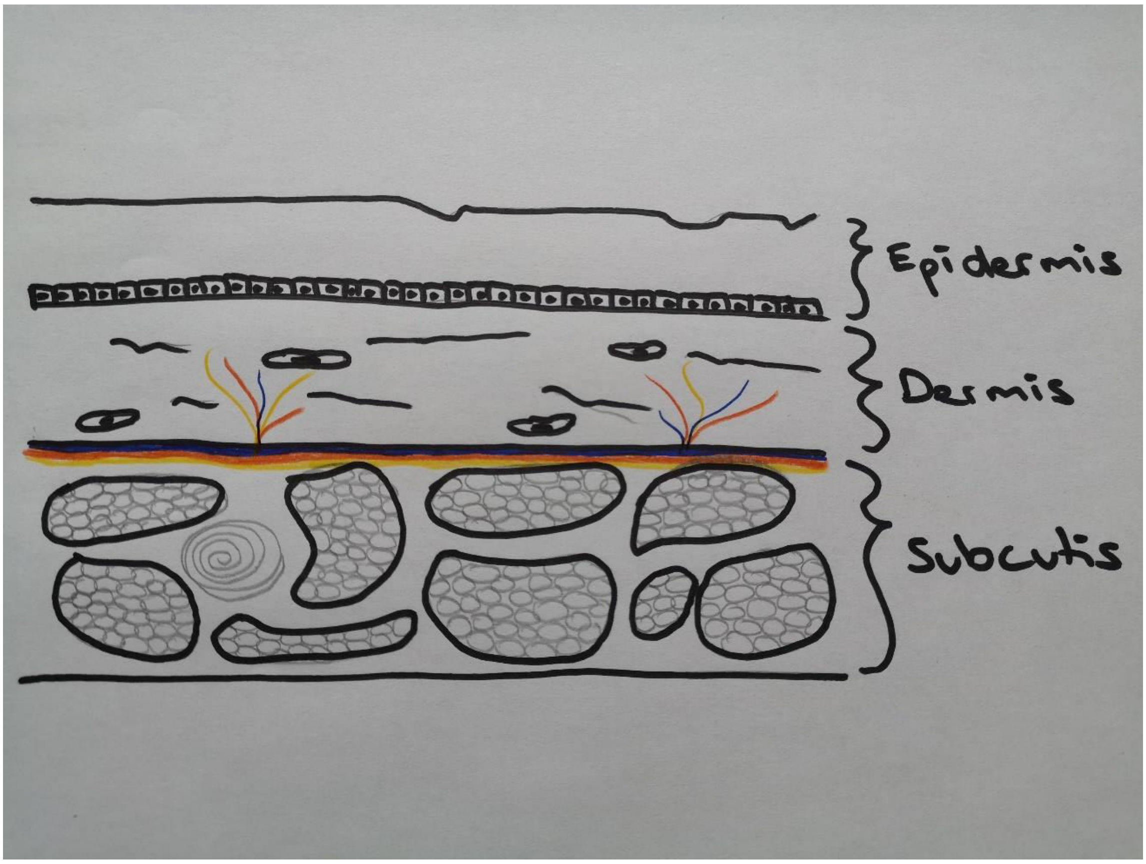


Figure 1: Scheme drawing of the human skin

The epidermis is the upper part which directly interacts with the environment and consists of 4, respectively 5 layers at the palms and bottom of the feet. Its main function is the barrier function between inner organs and environment. The majority, 90% of the cells are keratinocytes building a keratinized stratified squamous epithelium. The basic layer is the stratum basale, which directly interacts with the basal membrane. Here stem cells differentiate and keratinocytes start to wander through the upper layers - the stratum spinosum, stratum granulosum - until they finally reach the stratum corneum. Meanwhile they experience a process of proliferation until they become corneocytes in the end. At the palms and bottom of feet exists one additional layer, the stratum lucidum. Besides keratinocytes, the epidermis consists of Langerhans cells, Merckel cells and Melanocytes. Langerhans cells are dendritic cells that form part of the immune system, Merckel cells are tactually sensory organs and Melanocytes produce the skin pigment melanin and by that serve as a protection from sun.

The border between epidermis and the dermis is the dermoepidermal junction, a strong connection which fixes the epidermis on the dermis. The dermis itself consists of two layers: the papillary layer and reticular layer. It is meant to stabilize the epidermis and make the skin smooth and flexible. Therefore, it mainly consists of connective tissue but also contains some cells, the fibroblasts. Collagen fibers account for the biggest part of the dermis but also elastic fibers, proteoglycaans and glycosaminoglycans belong to the so called extracellular matrix. Most of the cells are fibroblasts producing the extracellular matrix, but also sensory- and immune cells can be found. Besides, blood vessels, nerve endings, hair roots and sweat glands are located here.

The subcutis is the deepest layer and consists of connective tissue, sensory bodies and a varying amount of adipose tissue, depending on the body mass index. Epifascial blood vessels and nerves lie here. It is the layer of movability within the skin and the adipose tissue serves as energy reservoir and thermal insulation (6, 7).

The skin is our biggest organ with great responsibility. First, it represents the border between inner organs and environment. Especially the horny layer protects of mechanical injury and its acidic PH of 5.5 serves as a chemical barrier against bacteria. Furthermore, the skin is important as temperature and humidity regulator with its blood vessels, nerves and sweat glands. It has the capability to protect from sun, by regulating the pigmentation via melanin. Immune cells like Langerhans cells represent the skin´s function for the immune system. Small sensory bodies and nerve endings make our skin a sensory organ and finally skin has an important social impact: It covers all our body and provides a first impression about one´s biological well-being, age, stress level and finally beauty.

1.1.2 Skin aging

Our skin is the border between environment and inner organs and has two different ways of aging. On the one hand it ages chronologically which is genetically determined and cannot be influenced easily (intrinsic). On the other hand, the skin as a barrier function has to bear a lot of environmental stressors that promotes skin aging (extrinsic). The most important environmental factor is the solar radiation, therefore extrinsic skin aging is often called photoaging. Table 1 shows a comparison of typical extrinsic and intrinsic skin aging features.

1.1.2.1 Intrinsic skin aging

Intrinsic skin aging is a chronological process that progresses slowly and steadily with increasing age. The changes are seen over the whole organ, but the best spots to differentiate extrinsic from intrinsic skin aging are body parts that are never exposed to the sun, like the underwear area or for people using the sunbed, just the tailbone. Extrinsic skin aging may otherwise hide the effects and often the visible outcome is difficult to differentiate. Intrinsic aged skin appears thin, atrophic, fine wrinkled and slightly silver-grey, with an even pigmentation (see Table 1). In the course of life it gets thinner in all three layers. This is due to a lack of growth factors stimulating the cells, which results in worse wound healing and reparation capacity in general. The subcutic adipose tissue as well as muscles decrease as well, which is why the skin seems to be lax and lifted skin folds need a longer time to come back into the original skin level. Intrinsic aged skin is more susceptible to minimal traumata. It looks dry, the healing of minimal wounds is slow and intracutaneous bleedings leave behind the visible hämosiderin, presenting itself as the clinical image of purpura senilis. Intrinsic aged skin also presents more benign tumors like verrucae seborrhoicae, decreasing numbers of sweat- and talc glands, as well as hair follicles which can result in pruritus and thin hair. The skin type according to Fitzpatrick does not have a strong influence here (8).

1.1.2.2 Extrinsic skin aging

The strongest influencer of extrinsic skin aging is the sun. Different skin types are differently able to cope with solar radiation because of the role of melanin. This is why two forms of extrinsic skin aging are differentiated: the atrophic- and milians zitrine form.

The atrophic form: This variation of skin aging is to be found mostly in lighter skin types. It is characterized by teleangiectasia in sun exposed areas, as for example on the cheeks, and finer wrinkles as in the milians zitrine form. But still the wrinkles are more severe than in intrinsic aged skin.

The milians zitrine form: This form occurs in all skin types and is the typical form of extrinsic skin aging. The main symbol is a hyperproliferation of the skin, as a protection of the chronical UV exposure. This results in a thickened skin with a leather like and yellowish appearance, coarse wrinkles, dryness and lack of elasticity (see Table 1). The cells as well as the dermal proteins hyperproliferate, the melanocytes vary in size and morphology which results in an uneven pigmentation. The neck, face, decolleté, forearm outsides, hands are areas where extrinsic skin aging is most severe. It usually starts with the formation of coarse wrinkles on the forehead, next to the outer corner of the eyes as crow´s feet, along the nasolabialfold and preauricular. The solar elastosis on the cheeks shows best visibility of extrinsic skin aging: The continuous inflammation of the dermal layer is responsible for its thickening with invasion of many immune cells, as well as the degradation and debris of collagen and elastic fibers. Responsible are matrixmetalloproteinases (MMPs) which are activated by solar radiation among others and degrade the dermal proteins resulting in accumulation and inflammation (8-10).

Table 1: Morphological characteristics of intrinsic and extrinsic aged skin (8)

Intrinsic (chronological)	**Extrinsic (environmentally induced)**
Fine wrinkles	Coarse wrinkles
Lax appearance	Solar elastosis
Even pigmentation	Uneven pigmentation

Thinning of skin layers	Thickening of skin layers
Few cell dysplasia	Much cell dysplasia
Few changes in size and organization of collagen and elastic fibers	Degeneration of elastic fibers and change of collagen fibers organization
Blood vessels: Purpura senilis	Blood vessels: Teleangiectasia, ecchymosis, inflammation
Reduction of hair follicles	Reduction of hair follicles
Reduction of sweat- and talc glands	Reduction of sweat- and talc glands
Normal amount of melanin	Increased amount of melanin, lentigines
Benign neoplasia	Benign neoplasia, malign neoplasia

1.1.3 Molecular mechanisms of skin aging

The mechanisms of skin aging are similar in extrinsic and intrinsic skin aging and mostly end in the same pathways. Figure 2 briefly shows the different existing pathways.

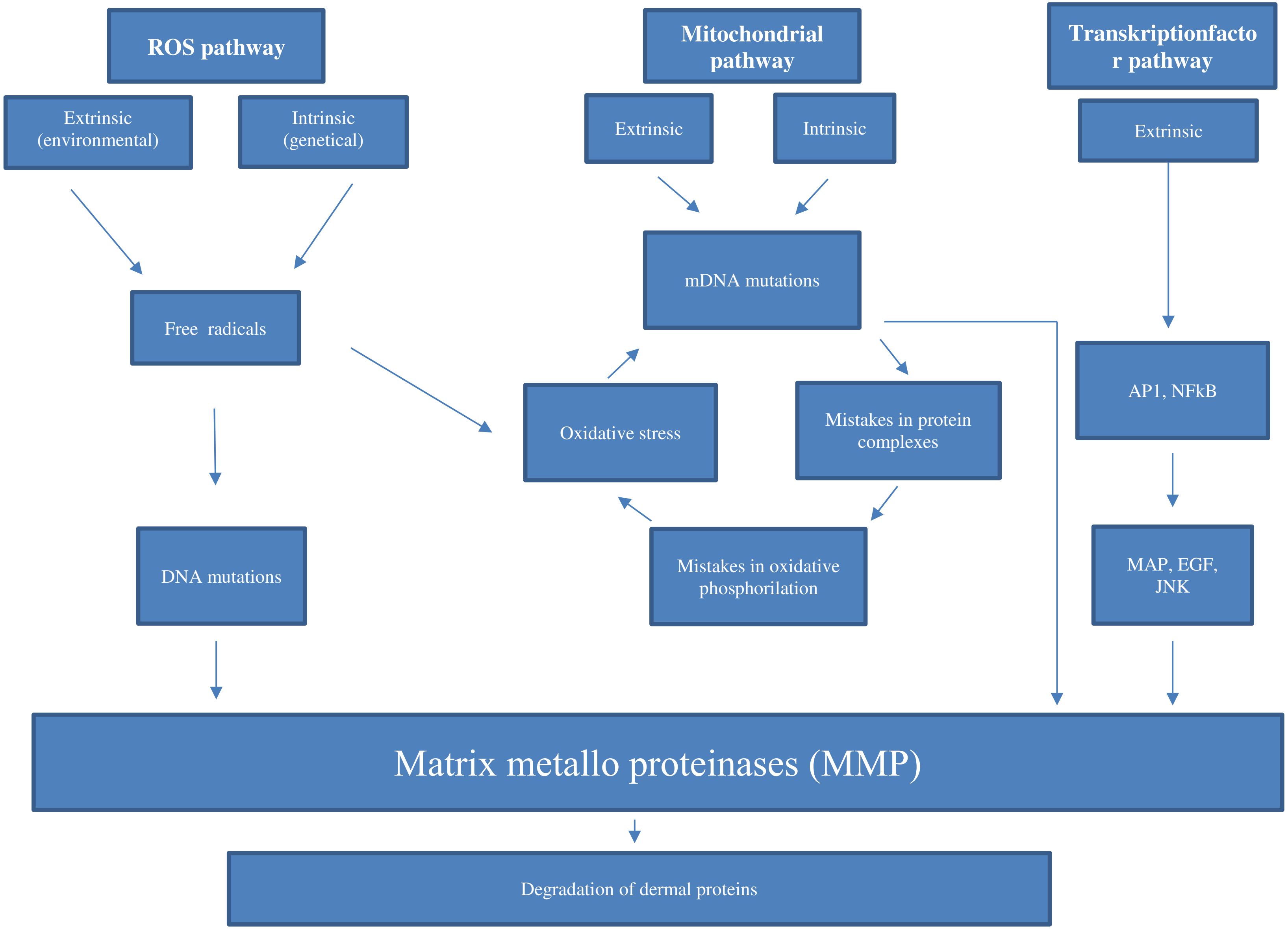
Already in 1965 Hayflick found that cell division is limited (11). After 52 times of division the capacity is reached and the cell undergoes an organized cell death, called apoptosis. The reason is the continuous shortening of telomeres in every division process. Telomeres are repetitive sequences at the end of chromosomes that do not contain genetic information but serve as a protection for the DNA. In every replication cycle the leading strand is replicated continuously from 3´ end to 5´ end. This is the only possible working direction for the DNA polymerase. The lagging strand needs to be replicated from 5´ to 3´ end, therefore small nucleoids called primers are used as a start point for the DNA polymerase which can only replicate discontinuously at the lagging strand. At the 3´ end a few bases get lost in each replication process, because the primer is not able to dock and the telomere shortens. When the tandem repetitions of the telomeres are gone, the cell goes into apoptosis. Stem cells and tumor cells are able to undergo this process with an enzyme called telomerase, which is able to renew the ends of chromosomes unlimitedly. The 3´ end can also be damaged by exogenous noxae like solar radiation, therefore the telomeres play and important role in intrinsic as well as extrinsic skin aging.

Another central mechanism of skin aging is reactive oxygen species (ROS). All living beings need oxygen to maintain their energy circle, but oxidative stress in form of reactive oxygen species can damage cells and chronical oxidative stress can promote the aging process. In 1956 Harman found the "free radical theory of aging" that says mistakes can happen in the metabolism while transforming oxygen into energy, leading to free radicals. It says, when decreasing the rate of ROS formation in a species, it increases its lifespan. Since it is known that oxidative stress increases with higher age, (12) this could be counteracted by reducing the caloric intake, eating less that easily oxidizes in the organism and decreases the temperature (13). Later also other studies showed that caloric restriction, resulting in lower energy metabolism, extends the lifespan in animals and humans (14). It is obvious that the oxidative stress is one factor promoting the aging process, but the body developed systems to cope with oxidative stress: radical catchers like Gluthathion, Catalase, cytochrom p450 and fenton reaction. This works as long as the system is balanced. With many free radicals at the same time and a continuous burden, the system gets satisfied and the imbalance leads to oxidative stress. In the course of the time the DNA gets damaged, proteins get oxidized and lose their function, which is irreversible. This is the common skin aging process that is genetically determined, but can also be induced by environmental noxae like solar radiation. UV light induces free radicals and promotes skin aging on that pathway, but it also directly damages the DNA. It often results in two DNA bases connecting with each other and forming dimers. Mostly the bases thymine and pyrimidine form the dimer. The DNA cannot be read anymore, which leads to apoptosis or mutation of the cell.

The compartment in the cell with the highest rate of metabolism is the mitochondria. It is the power plant of a cell, where the oxidative phosphorylation takes place. Basically the process uses oxygen to create energy. The respiratory chain is a complex of five proteins, located at the inner membrane of the mitochondria. Oxygen is used to establish a protone gradient over the complexes and the originating energy is used in the final step to transfer adensosindiphosphate (ADP) and phosphate to adenosintriphosphate (ATP), which is higher in energy. Every mistake in the production process leads to the creation of ROS and by that to oxidative stress. The mitochondrial DNA is a circular molecule that is mainly responsible for producing the protein complexes of the respiratory chain and is located directly next to it. More oxidative stress leads to more mutations in the mitochondrial DNA. This is the common procedure in the process of aging, but can also be directly induced by environmental noxae as sunlight. It results in a vicious circle: 1. Oxidative stress, 2. Mistakes in the mitochondrial DNA, 3. Mistakes in the protein complexes of the respiratory chain, which cannot work properly anymore, 4. Even more mistakes in the oxidative phosphorylation, which results in more oxidative stress (see Figure 2). Besides the "free radical theory of aging" another theory, the "mitochondrial theory of aging" was formed (15).

The mentioned processes lead to the induction of Matrix metalloproteinases (MMP), which are enzymes degrading the dermal proteins. They are important factors in the extrinsic skin aging and are activated via a direct and indirect way. Sunlight and other environmental noxae directly induce MMP. On the indirect pathway, noxae induce a signal cascade over the transcription factors AP-1 and NF.kB ending in the activation of MMP as well (Figure 2

Figure 2: Molecular mechanisms of skin aging



1.1.4 Factors influencing skin aging

1.1.4.1 Skin colour

The skin colour of an individual is caused by the pigment melanin. It is stored in the melanocytes, lying in the basal cell of the epidermis. The melanocytes produce melanin in cell organs called melanosomes and release it to the basal keratinocytes, which by that receive their pigmentation. The skin colour is an important influencing factor of skin aging. The darker the pigmentation the better the solar radiation gets absorbed and the better the skin is protected. Besides the dark eumelanin, there is the related pigment pheomelanin, which appears in blond- or red-haired individuals and does not protect as effectively. According to Fitzpatrick there are six different skin types from light skin (one) to dark skin (six).

Table 2: Skintypes according to Fitzpatrick (16)

Phototype	Skin description	Example
1	Pale white skin, very sensitive, always burns, never tans	Red hair with freckles
2	White skin, sensitive, burns easily, tans minimally	Blond haired, blue/green eyed Caucasians, northern Asians
3	Light brown skin, sometimes burns, slowly tans	Darker Caucasians, some Asians
4	Moderate brown skin, burns minimally, always tans	Mediterranean and Middle Eastern Caucasians, Southern Asians
5	Dark, brown skin, resistant, rarely burns, tans well	Some Hispanics and Africans
6	Dark brown to black skin, very resistant, never burns	Darker Africans, Indigenous Australians

1.1.4.2 Hormones

In the process of aging, hormone levels decline naturally. Estrogene is a sexual hormone that declines especially in women after the menopause and is an important factor influencing skin aging. Epidemiological studies showed that women, who received hormone replacement therapy have a perceived younger skin age (17) and others found also effects on the molecular level (18, 19). The molecular explanation is that estrogen receptors appear both in dermal fibroblasts and epidermal keratinocytes which was found in 1990 (20). Another study proved that a certain protein, p29, which is just found in estrogen sensitive cells, exists also within the skin (21). The natural decline of hormone levels is an intrinsic skin aging factor, the hormone replacement therapy on the other hand can be seen as an extrinsic influence.

1.1.4.3 Solar radiation (UV, infrared, visible light)

Solar radiation is the most important influencing factor of extrinsic skin aging. This was already found in 1969 by Albert Kligman (22). Solar radiation is composed of a spectrum of different wavelengths: UV radiation (290-400nm, 5%), visible light (400-700nm, 50%) and infrared light (700-4000nm, 45%) (Figure 3). The shorter the wavelength the stronger the radiation, but the longer the wavelength the deeper the radiation penetrates the skin. UV radiation makes 5% of the total spectrum and divides into UVA, UVB and UVC radiation. The shortest wavelength is UVC with 200-290nm, which is absorbed by the ozone layer and is not an issue for human skin. The UVB with its wavelength from 290-320nm is mostly absorbed by the ozone layer, but partly let through. It penetrates our skin superficially and induces changes just in the epidermis. UVA with 320-400nm wavelength is not absorbed at all by the ozone layer and completely hits our skin. It penetrates deeply, reaches the lowest layer of the epidermis and the dermis. Visible light and infrared light make up the biggest part of solar radiation and have long been considered to just minimally impact the skin. However studies could prove that both spectrums are able to induce MMP and ROS and by this induce matrix degeneration and skin aging (23, 24). Next to natural radiation, artificial radiation on sunbeds is an increasing problem. In the western world it is perceived as a prominent method to improve one´s appearance in the short run, but the long-term consequence is an aged, wrinkled appearance. For molecular mechanisms of solar radiation on skin see chapter “mechanisms of skin aging”.

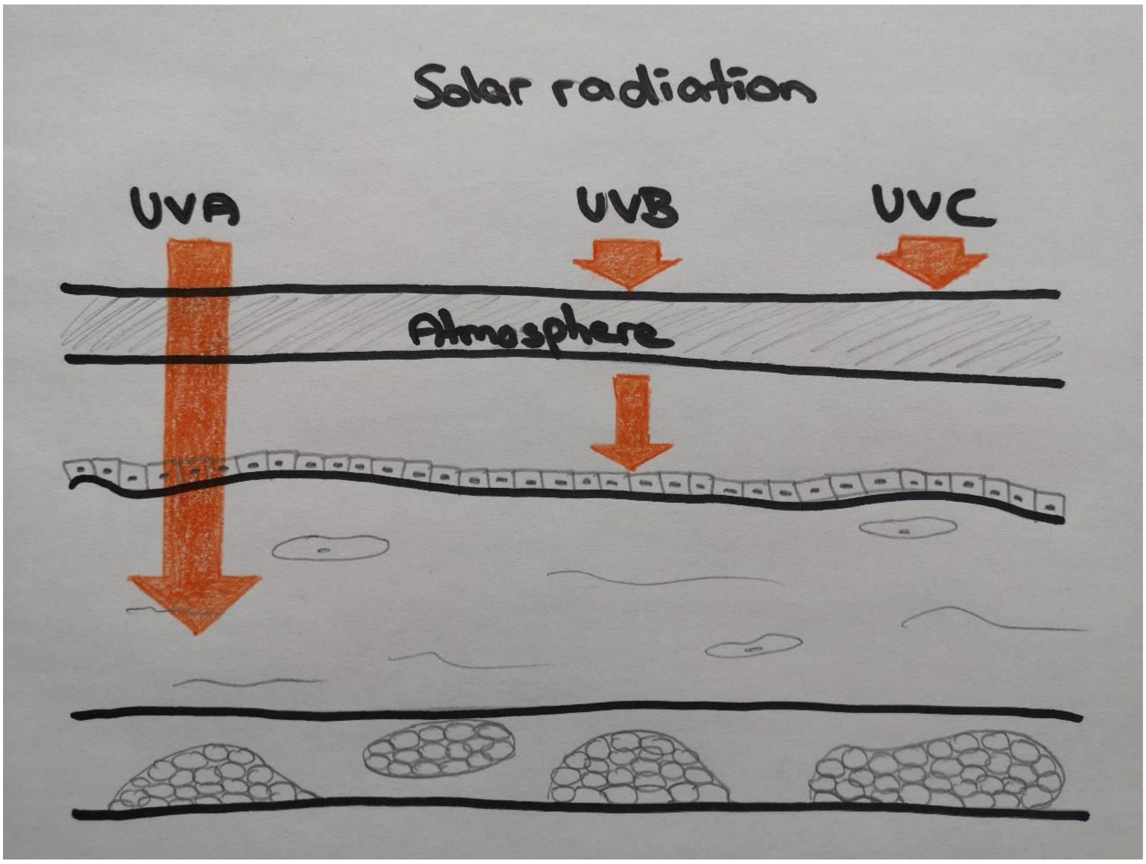


Figure 3: Scheme drawing of the penetration of solar radiation into the skin

1.1.4.4 Tobacco smoking

Studies could show that skin aging proceeds much faster in smokers than in nonsmokers, especially seen in wrinkles in the face and around the mouth (26-28). Also melanocytes are affected, since skin colour and pigmentation were observed to change (29, 30). This difference is visible in young smokers as well as in elderly. Cigarettes contain a variety of harmful chemical substances, mainly nicotine and carbon monoxide. The immediate effect on the body is a reduced microcirculation (31) but longtime consequences are similar to those induced by solar radiation, namely the induction of MMP (32).

1.1.4.5 Air pollution

The awareness for air pollution as a risk factor influencing skin aging emerged in the last years. Particles such as PM_{10} and $PM_{2.5}$ were shown to be able to penetrate the skin through hair follicles in clinical studies (8). There they act similar to other environmental noxae in triggering oxidative stress. Ozone as part of smog in high traffic areas during summer is known to decrease the content of antioxidants in the epidermis especially vitamin E. Besides, it induces MMP in deeper skin layers as well as an inflammation reaction (8).

1.1.4.6 Nutrition

Nutrition influences the skin, which is vividly shown by skin changes in the state of malnutrition (8). Supplementation in case of a lack of minerals, vitamins and essential fatty acids can again improve the skin condition. Until now it is unclear how nutritional substances operate on the skin, how they impact skin aging and especially if there is a positive effect in physiologic daily doses. Chapter 1.3 will focus on the impact of nutrition on skin aging in detail.

1.1.4.7 Stress, temperature, lack of sleep

Stress, temperature and lack of sleep are factors assumed to play a role in skin aging. Chronical stress can lead over different pathways to immune dysfunction, which is known to result in oxidative stress, a known influencer of skin aging (33). For lack of sleep an experimental study found that subjects look less healthy and less attractive (34). High temperatures are proven to impact the skin. In a study participants were exposed to solar radiation, but the radiation was filtered and just the heat could penetrate, it was seen, that also the heat alone induced MMP, and by that influenced the pathway of skin aging (35). Consequently, it is visible that miscellaneous impact the skin, but clear mechanisms are unknown until now.

1.2 Air pollution

The topic of air pollution and its negative impacts on health emerged in the 1950s (1). Air pollution is the release of substances in the air that can evidently harm humans and the environment. Among others these are smoke, soot, exhaust gases and odorants. Developing countries contribute to the biggest part to the worldwide air pollution nowadays (36). In the industrial countries, air pollution was at its highest in the 1950s but measures were taken to counteract this development. Our modern living standard asks for a lot of energy for production processes regarding groceries, clothing, furniture and more broadly for industries producing for public infrastructure. Also daily activities in the household like cooking consume a considerable amount of energy. Another factor is the increasing traffic, on the one hand emerging because products need to reach the customer, but on the other hand, because having a car has become a matter of course. Nowadays the traffic has the highest impact on air pollution in cities (37).

Air pollutants can be divided into primary and secondary pollutants. Primary pollutants derive from the direct emission of an air pollution source, whereas secondary pollutants derive from photochemical reactions between the primary pollutants. The main primary pollutants are particulate matter (PM_{10}, $PM_{2.5,}$ $PM_{0.1}$) and gases (CO_2, CO, SO_2, NO_2). Examples for secondary pollutants are ozone (O_3), peroxyacyl nitrates and nitric acid, which are the components of the typical “smog”.

Air pollutants of interest for this study include NO_2, PM_{10}, $PM_{2.5}$, $PM_{2.5coarse}$ and ozone (O_3). Nitrogen dioxide (NO_2) is one of the nitrogen oxides, which are produced by combustion processes. The main source for outdoor NO_2 is the traffic. NO is emitted by motor vehicles and reacts with oxygen in the atmosphere producing NO_2. Indoor NO_2 is produced mainly by unvented heating and cooking (38, 39). To particulate matter belong all solid or liquid particles suspended in the air. PM_{10}, $PM_{2.5}$ and $PM_{2.5absorbance}$ (measurement of blackness of PM2.5 filters, which is a proxy for elemental carbon (40)) belong to the coarse particles. They are produced by physical processes by the breakup of larger solid particles. These processes include resuspension of soil- and road dust, agricultural processes, mining operations and vehicular abrasion, as tyre- and brake wear. Mostly the particulates consist of sulfate and organic matter (38, 39). Ozone (O_3) is a secondary pollutant that protects us from solar radiation in high layers of the atmosphere, but can act harmful when being on ground level. Under the action of solar radiation NO_2 decomposes into NO and O. In a second step O reacts with O_2 to O_3 - ozone. Ozone levels are lower in urban areas since combustion processes produce NO, which again reacts with O_3 to NO_2 and O_2. By that ozone is captured. Hydrocarbons in the atmosphere are responsible for increasing ozone values on sunny afternoons. They are oxidized by OH in the atmosphere to peroxide radicals, which again react with NO to NO_2. NO cannot react with ozone anymore and its concentration increases (38, 39).

A lot of effort was done to minimize air pollution values until today, especially in the industrialized countries. The World Health Organization (WHO) introduced guidelines to offer help in reducing the health impacts of air pollution all over the world. Table 3 shows limits for all air pollutants of interest for this work. NO_2 values over 200µg/m^3 as a 1-hour-mean are toxic and therefore should be avoided completely. Ozone levels over 100µg/m^3 as a 8-hour-mean should be avoided, since a higher concentration was found to cause increased daily mortality (36).

Table 3: Limits for air pollution values set by the WHO

NO_2	Annual mean: 40µg/m^3 1-hour-mean: 200µg/m^3
PM_{10}	Annual mean: 20µg/m^3 Daily mean: 50µg/m^3
$PM_{2.5}$	Annual mean: 10µg/m^3 Daily mean: 25µg/m^3
Ozone	8 hour mean: 100µg/m^3

1.2.1 Effects of air pollution on skin aging

The pulmonary and cardiovascular system suffer most from air pollution exposure (41-43). Recently it has been shown that also the skin is affected by air pollution. In this context the

SALIA study (42) was the first study that found air pollution significantly correlated with extrinsic skin aging signs, especially with pigment spots in the face and less with wrinkle formation (2). This was found for soot in particular. Soot is the leftover particles in incomplete combustion of hydrocarbons. In Europe it comes mainly from traffic and chimneys in private households. Another study about indoor air pollution in China found that cooking with fossil fuels is associated with more severe wrinkles in the face and on the back of the hands in Chinese women (3). In addition, a study in a German and a Chinese cohort showed that NO_2 was significantly correlated with lentigines on the cheeks. The strongest association was found for women over 50 years. No correlation was found for lentigines on the back of the hands and forearms (4). Another study assessed the ozone exceedance and its impact on skin aging. Positive associations between ozone exceedance and coarse wrinkles in the face were found for one study cohort on the forehead and under the eyes, for the other study cohort in the crow´s feet area and on the upper lip (44).

In summary, there is good epidemiological evidence that small particles (PM_{10} and $PM_{2.5}$), soot ($PM_{2.5\ absorbance}$), NO_2 and ozone exceedance intensify skin aging.

1.3 Effects of nutrition on skin aging and the Mediterranean diet

Hundred years back in time food had a simple function: filling our stomach and keeping us satiated. Our whole body needs nutrition in order to grow, maintain its functions and refill our energy reservoirs. The idea that nutrition has more effects emerged later, when people did not suffer hunger anymore. At that point in time we could choose what to eat, depending on our preferences. The topic of additional functions of nutrients came up as well as the question about what is healthy and what is not. The Mediterranean nutrition pyramid demonstrates recommendations about lifestyle and healthy nutrition(45).

Nutrition was proven to have several health benefits. Healthy food is known to serve as prevention method against cardiovascular diseases, overweight, intestinal cancer, memory decline and many more (46-50). Interestingly, in the general population food is rumored to impact the skin and antioxidant-rich nutrition to be able to prevent skin aging. However, there are only few studies, which tried to prove the benefit of certain diets or forms of nutrition on skin aging. The results are vague, some show tendencies in favor of the theory, some against, but clear results are scarce.

One study examined whether nutrient intakes were correlated with skin wrinkling on sun exposed site and found less appearance of actinic keratosis with higher intake of vegetables, olive oil, legumes and fish. Consumption of meat, butter, dairy and sugar seemed to be harmful (51). Another study examined nutrient impact on the skin aging appearance in a cohort of middle-aged American women. Higher intakes of vitamin C were found to have a protective effect on wrinkles and senile dryness. High linoleic acid intake was associated with less senile dryness and skin atrophy. Higher fat and carbohydrate intakes were associated with more wrinkles and senile dryness (52). A study on the impact of fat, vegetables and antioxidant micronutrients on skin aging in Japanese women found an effect as well. High intake of fat was associated with more skin elasticity and wrinkle formation. The consumption of green and yellow vegetables was protective against the formation of wrinkles (53). A very recent Dutch study found that higher adherence to the Dutch guidelines of a healthy diet was associated significantly with less wrinkle formation in women, but not in men. The study separated the dietary patterns into four groups, and the red meat and snacks dominant group-, showed more facial wrinkles, whereas the fruit dominant group showed less wrinkles (54). Studies with controversial results were scarce.

Since the Mediterranean diet is one of the most famous diets in the world we expect a positive impact of this way of nutrition on skin aging. As explained before, the main principles of skin aging are the shortening of telomeres and the formation of reactive oxygen species (ROS). Studies found a positive correlation between the Mediterranean diet and the telomere length (55, 56). Other studies attribute a large anti-oxidative capacity to the Mediterranean diet (57-59). Thus, the Mediterranean diet should be beneficial on skin aging. To our knowledge there has never been a study on this topic before.

The Mediterranean diet was proven to have numerous health benefits. It is good against obesity and metabolic syndrome, cardiovascular diseases, diabetes, cognitive decline, cancer and many others (46-50, 60). This diet is a form of nutrition containing plant food as the basis, olive oil as main source of fat, with a moderate consumption of meat, fish, and dairy products (45). The famous diet pyramid puts physical activity, sleep and sociocultural activities at the very bottom, as basis for a healthy nutrition together with drinking water and pure tea (Figure 4). The first nutrition level contains plant food such as cereals, pasta, bread, rice, as well as vegetables in a large variety of colour and way of preparation (cooked/raw) and olive oil. These nutrients should be consumed with every main meal. The second level contains nuts, olives, seeds, herbs, spices, onion, garlic as a mean to increase the flavor in order to reduce salt. The third level contains low fat dairy. Nutrients from levels 2 and 3 should be consumed daily. Level 4 contains white meat, fish, eggs and legumes, which should be consumed weekly. Level 5 contains red meat, processed meat, as well as potatoes, because of their high glycemic index. These products should be consumed weekly. In the very top of the pyramid, stand sweets. The higher the level, the less often the nutrients should be consumed and the less healthy they are declared.

1.4 Aim of this study

The objective of this study was to investigate the protective role of the Mediterranean diet on skin aging in elderly women exposed to long-term traffic related air pollution. Two hypotheses and three research questions were formulated.

1.4.1 Hypothesis 1

Since the link between nutrition - and the Mediterranean diet in particular - and skin aging is not well established in the literature, this association is investigated first. We expect that the MEDI is going to have a protective effect on skin aging.

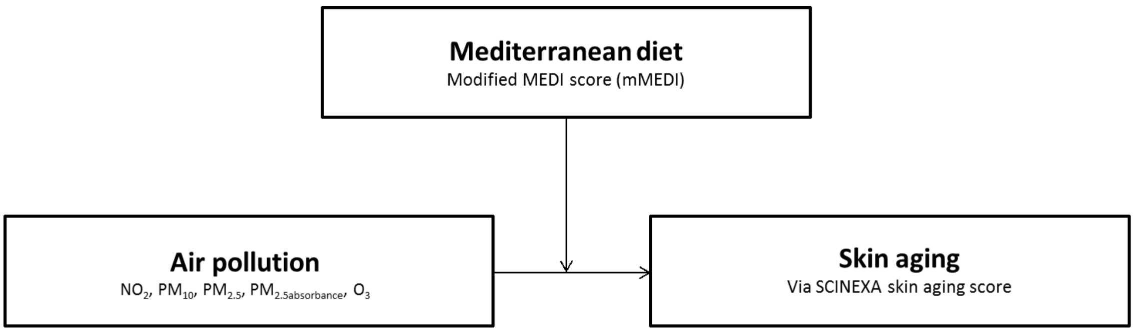
1.4.2 Hypothesis 2

Former research on this study population showed negative impacts of air pollution on skin aging, for pigment spots in the face and nasolabialfolds (2). We first want to reproduce the findings and second, we want to analyze if the MEDI has any modifying effect on air pollution induced skin aging. Based on the previous studies we expect the MEDI to attenuate the harmful effect of air pollution on skin aging.

1.4.3 Research questions

1. Is a higher adherence to the mMEDI associated with less pronounced extrinsic skin aging?
2. Does air pollution have a harmful impact on skin aging?
3. Does MEDI modify the association between air pollution (NO_2, PM_{10}, $PM_{2.5}$, $PM_{2.5absorbance}$, O_3) and extrinsic skin aging?

Figure 5: Visualization of research questions



2 Material and methods

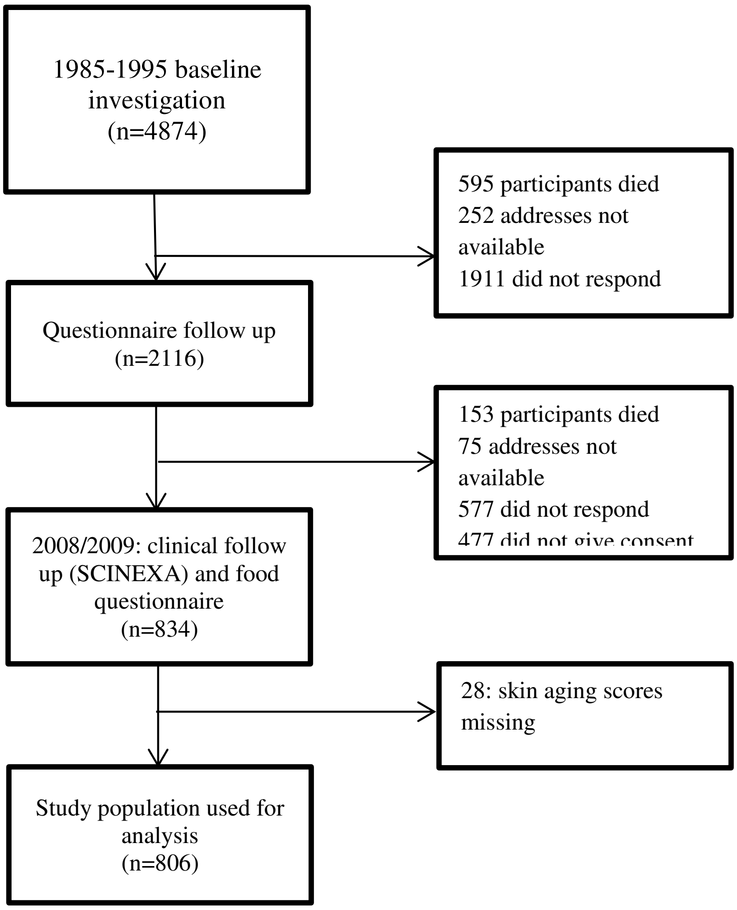
2.1 Study population

This work is a retrospective cohort analysis and is based on data of the SALIA study (Study on the influence of air pollution on lung function, inflammation and aging). The study was part of the Clean Air Plan introduced by the government of North-Rhine-Westphalia and was meant to compare the effects of air pollution in urban and rural areas on human health (42). The baseline investigations started between 1985 and 1994 and had several follow ups in 2006, 2008/2009, 2012/2013 and 2018/2019. Overall, 7 areas were chosen: 5 urban areas with high air pollution due to dense traffic and industry (Dortmund (since 1985), Duisburg (since 1990), Essen (since 1990), Gelsenkirchen (since 1985), and Herne (since 1986)) and 2 rural areas (Dülmen and Borken both since 1985) with few traffic and industry as reference areas (42).

All women at the age of 54 or 55 at time of recruitment were asked to participate. Men were excluded due to the bias of work in coal and steel industries. At the baseline investigations the women received a questionnaire about lung function and basic data like education, smoking habits, heating with fossil fuels etc. (42). Altogether 4874 women were included (see Figure 2). In 2006 there was a short questionnaire follow up with 2116 participants. Excluded were those who either did not respond, whose addresses were not available or who died. In 2008/2009 834 participants were left, others died, did not respond, did not give consent or their addresses were not available (41). A food questionnaire was introduced and further clinical examinations were conducted, including amongst others an extensive skin assessment (61). The skin aging assessment was performed by trained personnel via photo-reference scales (62). In 28 women a dermatological assessment was missing and therefore these women were excluded from further analyses. Finally, our analyses were conducted with 806 subjects.

The SALIA study has been approved by the Medicals Ethics Committee of the Ruhr Universität in Bochum, Germany in 2006 (registration No: 2732) and the Heinrich-Heine-Universität in Düsseldorf, Germany in 2010 (registration No: 3507). All participants have given written consent to participate in the study.

Figure 6: Flow chart of the SALIA population



2.2 Exposure to air pollution

Air pollution was evaluated in the context of the ESCAPE project (European Study of Cohorts for Air Pollution Effects) (40) for NO_2, PM_{10}, $PM_{2.5}$ and $PM_{2.5absorbance}$ in 2008/2009. Ozone values were measured by the German Environmental Agency (UBA) in 2005.

In the ESCAPE project concentrations of pollutants were measured at 40 sites in the study area for NO_2 and 20 sites for particulate matter (PM). Measurements took place in three periods of each two weeks: one in the cold season, one in the warm season and one in the intermediate season. Validated land use regression models (63) were used to assign the concentrations to each individual´s home address.

For this thesis the concentrations at the time of the baseline investigations in 1985-1994 were used. Since skin aging was assessed in 2008/2009 – at the same point as the air pollution measurements - and air pollution may have a long-term effect on skin aging, a certain time span was needed. Therefore, the exposure values were backextrapolated to the years of our baseline investigation. Annual means from background reference stations of the governmental monitoring system were used to receive annual mean concentrations for pollutants with available historic data. Within-city spatial variations decreased proportionally over time, so concentrations could be adjusted for the long-term trends using a pre-defined back-extrapolation algorithm, which is an established ESCAPE procedure (64, 65). The procedure is explained in detail in the ESCAPE manuals (www.escapeproject.eu). The ozone values were extracted from data gathered by the German environmental agency in 2005. They were derived by dispersion modeling with the chemical REM-CALGRID model (66), integration of monitoring data and optimal interpolation with a resolution of 2 x 2 km^2. Exposure to high ozone values was assessed as the number of days when the daily mean concentration exceeded 120µg/m^2 and are called exceedance days. According to the European union, 25 days of exceedance are allowed per year (36).

From the beginning of the SALIA study in 1985, a steep decline in air pollution could be observed, especially for particulate matter. The main reason for this was more awareness in the population and improvement of industrial combustion methods, but not because of improvement in traffic, whose impacts on air pollution remained stable (67).

2.3 Nutrition

2.3.1 Mediterranean diet score

At the time of the follow up examination in 2008/2009 the women filled out an extensive questionnaire about their eating habits of at least the last 12 months. The questionnaire involved 32 food items including beverages and alcohol. For each food item a frequency of consumption could be chosen: daily, several times a week, once a week, several times per month, once a month or never.

A modified Mediterranean dietary score was developed in order to evaluate the eating habits of the participants. This score was based on the validated Mediterranean diet score of Panagiotakos et al. (47). From now on the diet score by Panagiotakos is called MEDI and our modified score is called mMEDI.

The MEDI contained 9 food groups (Table 4): Non-refined cereals (whole grain bread, pasta, brown rice, etc.), fruit, vegetables, legumes, potatoes, fish, meat or meat products, poultry, dairy products (cheese, milk, yoghurt), additionally olive oil and alcohol (47). The suggested consumption frequencies are based on the Mediterranean diet pyramid (45). In order to score the frequency of consumption 0-5 points were given. The first 7 items are at the bottom half of the diet pyramid and should be consumed often. Therefore, 0 points were awarded if never consumed until 5 points if consumed daily. The last four food items have a position in the top half of the diet pyramid and should be consumed rarely. On that account, 5 points were awarded if consumed never and 0 points if very often. For alcohol intake and olive oil use in cooking the scoring was slightly different (see Table 2). A maximum adherence to the MEDI received the maximum score of 55.

For this work we modified the Mediterranean diet score, due to different regional and cultural characteristics of the SALIA population. All in all, both scores consisted of 11 food items. Most of the food items were similar. The SALIA questionnaire did not contain questions about whole grain products other than bread. Therefore, whole grain bread received an own category in the mMEDI as well as rice and pasta (see Table 3). The SALIA questionnaire differentiated between raw vegetables and cooked vegetables. Since both belong to the fundament of the Mediterranean diet both were included. The three items missing in the SALIA questionnaire were legumes, olive oil and alcoholic beverages. In Germany it is more common to drink beer instead of wine, but beer is not part of a typical Mediterranean diet, considering that fact alcoholic beverages were excluded. Legumes and olive oil were not part of the questionnaire and for that had to be excluded. All other items were included: *Whole grain bread, Pasta, Rice* (each as an own category) *Potatoes, Fruits, Vegetables (cooked), Salad or raw vegetables, Fish, Red meat, Poultry, Dairy products*. There were 6 answer categories in the SALIA questionnaire ranging from daily consumed to never consumed. For comparison of the MEDI and the mMEDI see Table 2 and Table 3. For point division to calculate the mMEDI score we summarized the answer categories to end up with 4 categories instead of 6. Never, once a month, and several times per month were put into one category. The others stayed the same. The new points reached from 0 to 3. Same as in the MEDI the beneficial items *Whole grain bread*, *Pasta, Rice, Potatoes, Fruits, Vegetables (cooked), Salad or raw vegetables* and *Fish* received 3 points when consumed daily, whereas the harmful items: *Red meat, Poultry and Dairy products* received 3 points if consumed once a month or less often. Finally, a maximum adherence to the mMEDI was awarded with 33 points in total.

Table 4: Mediterranean diet score of Panagiotakos (MEDI) (47)

How often do you consume	Frequency of consumption (servings/week)					
	Never	1-6	7-12	13-18	19-31	>32
Non refined cereals	0	1	2	3	4	5
	Never	1-4	5-8	9-12	13-18	>18
Potatoes	0	1	2	3	4	5
	Never	1-4	5-8	9-15	16-21	>22
Fruits	0	1	2	3	4	5
	Never	1-6	7-12	13-20	21-32	>33
Vegetables	0	1	2	3	4	5
	Never	<1	1-2	3-4	5-6	>6
Legumes	0	1	2	3	4	5
	Never	<1	1-2	3-4	5-6	>6
Fish	0	1	2	3	4	5
	Never	Rare	<1	1-3	3-5	Daily
Olive oil in cooking	0	1	2	3	4	5
	<=1	2-3	4-5	6-7	8-10	>10
Red meat	5	4	3	2	1	0
	<=3	4-5	5-6	7-8	9-10	>10
Poultry	5	4	3	2	1	0
	<=10	11-15	16-20	21-28	29-30	>30
Dairy products	5	4	3	2	1	0
	<300	300	400	500	600	>700 oder 0
Alcoholic beverages (ml/day)	5	4	3	2	1	0

11 food items = 0-55 points (0 = no adherence to MEDI; 55 = maximum adherence to MEDI)

Table 5: Modified MEDI score (mMEDI)

	Points in the mMEDI			
	Once a month or less often	Once a week	Several times per week	Daily
Whole grain bread	0	1	2	3
Pasta	0	1	2	3
Rice	0	1	2	3
Potatoes	0	1	2	3
Fruits	0	1	2	3
Vegetables (cooked)	0	1	2	3
Salad or raw vegetables	0	1	2	3
Fish	0	1	2	3
Red meat	3	2	1	0
Poultry	3	2	1	0
Dairy products	3	2	1	0

11 food items = 0-33 points (0 = no adherence to mMEDI; 33 = maximum adherence to mMEDI)

2.3.2 Antioxidant score

There is also evidence regarding oral antioxidants as a rejuvenation method (see 1.3) which is why we further added an antioxidant score. On basis of the mMEDI score we used the food items rich in antioxidants: *Fruits, Vegetables (cooked)* and *Salad or raw vegetables* and established a new score out of the three items. The point division stayed the same, since all three items are beneficial. Each item received 1 point (consumed every day) to 6 points (consumed never) and the questionnaire had a range from 3 to 18 points. The antioxidant score was low if the nutrition is rich in antioxidants and high if it is poor in antioxidants, in contrary to the mMEDI.

Table 6: Antioxidant score

	Frequency of consumption					
	Never	**Once a month**	**several times per month**	**once a week**	**several times per week**	**everyday**
Fruits	6	5	4	3	2	1
Vegetables (raw)	6	5	4	3	2	1
Vegetables (cooked)	6	5	4	3	2	1

3 food items = 3-18 points (3 = maximum antioxidant uptake, 18 = no antioxidant uptake)

2.4 Assessment of Skin aging

Skin aging was assessed by trained staff members of the IUF using validated photo-reference scales (62). The participants were asked not to use make up and cream on the day of the examination and to clean their face with sensitive cleaning wipes 15 min before the examination. The participant sat on a chair in a relaxed position, the eyes were closed, the face was relaxed and evenly illuminated. Pictures were taken for reproducibility. First, the extrinsic skin aging was evaluated containing pigment spots/solar lentigines (on forehead, cheeks, upside of the forearm and back of the hands), coarse wrinkles (on forehead, between the eyebrows, in crow´s feet area, under the eyes, on upper lip, on nasolabialfolds), solar elastosis and teleangiectasia. Second, intrinsic skin aging was evaluated containing: even pigmentation on the forearm insides, lax eyes, lax face (lower part) and cigarette paper like skin on the back of the hands (61).

Table 7: Parameters of the skin aging score (68)

Criteria	Location	Score
Extrinsic skin aging		
	Forehead	Number: 0 (0), 1-10 (1), 11-50 (2), >50 (3) According to Tschachler: 0-5 (+1)
	Cheeks	Number: 0 (0), 1-10 (1), 11-50 (2), >50 (3) According to Tschachler: 0-5 (+1)
	Forearm Topsides	Number: 0 (0), 1-10 (1), 11-50 (2), >50 (3)
Pigment spots/Lentigines solaris	Back of the hands	Number: 0 (0), 1-10 (1), 11-50 (2), >50 (3)
	Forehead	According to Tschachler: 0-5 (+1)
	Between eyebrows	According to Tschachler: 0-5 (+1)
	Crow´s feet region	According to Tschachler: 0-5 (+1)
	Under the eyes	According to Tschachler: 0-5 (+1)
	Upper lip	According to Tschachler: 0-4 (+1)
Coarse Wrinkles	Nasolabialfolds	According to Tschachler: 0-5 (+1)
Solar elastosis	Cheeks	Non (0), mild (1), moderate (2), severe (3)
Teleangiectasia	Cheeks	According to Tschachler: 0-5 (+1)
Intrinsic skin aging		
Even pigmentation	Forearm insides	Yes/No
	Eyelids	According to Tschachler: 0-5 (+1)
Lax appearance	Lower part of the face	According to Tschachler: 0-5 (+1)
Cigarette paper like skin	Back of the hands	Yes/No

2.4.1 Extrinsic skin aging

Pigment spots/solar lentigines were evaluated quantitatively on a scale from 0-3. No pigment spots were scored with 0, one to ten pigment spots with 1, eleven to fifty with 2 and over fifty pigment spots were evaluated with 3 (61). Besides, pigment spots on the forehead and cheeks were evaluated with the photo-reference scales by Tschachler (62) considering their size as follows: 0: no pigment spots or very few (<3mm), 1: one to several small pigment spots (=3mm), 2: one to several bigger pigment spots (4-8mm), 3: one very big pigment spot (>9mm, light pigmentation), 4: one very big pigment spot (>9mm, dark pigmentation), 5: several very big pigment spots (>9mm).

Coarse wrinkles were evaluated by validated photo-reference scales. The investigator had 5 validated photos (or 4 for wrinkles on upper lip) of women with different grades of skin wrinkling in the considered facial regions and should compare the study participant to the person on the photo and assign a value. A number of 0 meant no/very few skin aging and 5 meant severe skin aging (62). If the examiner evaluated the study participant´s skin between two photo-reference categories (for example 2 and 3) it was scored in between (2.5). If the skin aging was more severe than 5, it received one additional point.

Solar elastosis (the thickening of the skin) on the cheeks was evaluated with non visible solar elastosis (0), mild- (1), moderate- (2) and severe solar elastosis (3) in comparison to a reference photo with a severe form of solar elastosis.

Teleangiectasia (reddening of the face because of visible small blood vessels), was evaluated by the mentioned photo-reference scales with 0 (mild) to 5 (severe).

2.4.2 Intrinsic skin aging

The even pigmentation was considered on the inside of the forearm, because this side is rarely reached by the sun as the most important skin aging factor. It was evaluated with yes (even pigmentation) or no (uneven pigmentation).

The cigarette paper like skin on the back of the hands was assessed with yes or no, using a reference photo showing hands with thin, cigarette paper like skin.

The lax appearance was considered for the eyelids and the lower part of the face and was evaluated via the mentioned photo-reference scales (0-5).

Since the extrinsic and intrinsic skin aging outcome were not normally distributed, we established binary variables coding either for minor- or severe skin aging to be able to apply logistic regression. We calculated the means and all observations that were equal to the mean or higher were declared as severe skin aging and all observations below the mean were labeled few skin aging. The variables which already were binary (solar elastosis, even pigmentation, cigarette paper like skin) just stayed like this.

2.5 Covariates

In order to increase accuracy, we included all covariates that can also influence the skin aging and which were available in our dataset. We adjusted for age, body mass index, social status, home address, skin sensitivity, UV history, smoking history (including passive smoking and packyears), hormone replacement therapy and the heating system. Table 7 gives an overview of all parameters and their scales of measurement.

Age is the most important factor for intrinsic skin aging and was included as a continuous variable. A high body mass index (BMI) makes wrinkles in particular appear less severe because the subcutic adipose tissue serves as an additional layer plumping up the skin. Therefore, the BMI was included as a continuous variable as well. The social status is a categorical variable and was measured by years of school education in three categories: school education under ten years, school education of ten years and education longer than ten years. The residential address was important, considering whether the area was rural or urbanized, because of different air pollution exposures. The skin sensitivity is another categorical variable and refers to the skin type according to Fitzpatrick (16). Here the women were asked if they burn easily, become rather red and don't tan easily, tan easily and become rather brown and are only brown when exposed to the sun. The UV history considers three variables - first, it was considered if sun protection factor was used when being exposed to the sun and second, if ever used a sunbed, which are both binary variables. The third variable was the continuous value for the UV index at the home address. For the smoking history there were three variables included: First, if the person was either current smoker, ex-smoker or never smoker, as a categorical variable, second, the number of packyears, as a continuous variable and third, if the person was ever exposed to passive smoking either at home or at work, which was a binary variable. If ever received a hormone replacement therapy or not was another binary variable we adjusted for. Hormone replacement is known to delay skin aging. Finally, it was considered if a household had ever heated with fossil fuels, since this is source of indoor air pollution exposure. All these covariates were chosen on the basis of former studies (8, 10, 33, 69, 70).

2.6 Statistical analysis

All data analyses have been conducted using the statistical software R version 3.4.4.

2.6.1 Descriptive statistics

In order to describe the study population the arithmetic mean, the standard deviation and the range (min-max) have been calculated for numeric covariates. For categorical covariates total numbers and percentages have been calculated. For the air pollutants mean, standard deviation, range (min-max), as well as interquartile range (IQR) were calculated. The latter ones were referred to in this study, since it is common to report effect estimates in relation to a change of one IQR. The description of air pollutants was given separately for urban and rural areas. For the food items total numbers of answers per category as well as percentages were calculated. A modified MEDI Score (mMEDI) has been determined for each participant according to the score building rules explained before (see chapter 2.3.1). We calculated with the original continuous mMEDI in order to increase accuracy and did not establish a group with high adherence and one with low adherence.

2.6.2 Missing values

First, we excluded the participants without skin assessment from the calculations. Afterwards, there were still some values missing, presented in table 8. In the skin aging assessment there were 0-13 missing values observed for each outcome variable. These outcome values should not be replaced, so the calculations were done with varying numbers of participants, leaving out those without a value for the respective variable. In food items we had 1-11 missing values for each item and substituted them by the respective mean. The two missing values in education, were replaced by the most frequent category, which was 10 years of education. The two missing values in packyears, were substituted by the mean packyears of all smokers. For hormone replacement therapy we replaced the 3 missing values by “never”, since there were more women who never received a therapy. One missing ozone value was replaced by the mean ozone of the whole population.

Table 8: Missing values and their replacement

Variable	Missing values	Distribution	Replacement
Skin Aging	0 - 13		/
Nutrition	1 - 11		mean
Education	2	>10 years=266 =10 years=397 <10 years=143	10 years (middle category)
Packyears	2	160 smokers 646 nonsmokers	mean of all smokers
HRT	3	Ever: 323 Never: 483	never
Ozone	1		mean

2.6.3 Association between air pollution and skin aging and the modifying effect of the mMEDI score

Multiple logistic regression analyses have been performed to assess the impact of air pollution and the modifying effect of nutrition on skin aging. Since the outcomes in skin aging were not normally distributed we decided for logistic- instead of linear regression, which was used by former studies (2).

For each of the 18 skin aging outcomes we calculated 5 models, one for each air pollutant (NO_2, PM_{10}, $PM_{2.5}$, $PM_{2.5\ absorbance}$, O_3), each model including only one air pollutant. The result is significant if the p-value is <0.05. Results with a p-value $>=0.05$ were not taken into account for this thesis. The calculations were performed in a stepwise approach. We calculated models with all covariates, which might have an (possibly confounding) impact on skin aging and on which we had information through the SALIA questionnaire (see 2.5). Mediterranean nutrition was expected to have a positive modifying impact on skin aging (see chapter 1.3). Unfortunately, we found inverse effects and tried to find reasons for this development. Therefore, we calculated with different models: One model for the whole population with all covariates included and a second with an interaction variable between air pollutant and mMEDI. Here we standardized the mMEDI and the respective air pollutant before building an interaction term (value(mMEDI)–mean(mMEDI)):SD(mMEDI) and (value(air pollutant)-mean(air pollutant)):SD(air pollutant). We calculated a third model where the cohort was separated into urban and rural home address, fourth for smokers and non-smokers and a fifth model without UV covariates (UV index and sun protection factor). Since UV radiation has the highest impact on skin aging (33, 70-72) and may hide the effects of nutrition and air pollution, we excluded these covariates. Finally in a sixth model we established z-scores for pigment spots and coarse wrinkles, comprising all pigment spot variables in one summary variable and all coarse wrinkle variables in another one (see table 7). We did not use the binary variable here for the outcome skin aging, but the continuous values, which is why we applied linear regression models.

3 Results

3.1 Description and distribution of data

3.1.1 Study population

The study population consisted of 806 women with a mean age of 73.5 years at the time of the follow-up in 2008/2009 (see Table 9). The mean body mass index (BMI) was 27.3. Most of the women had an education of 10 years (49.26%). About half of the participants were living in the urban Ruhr area (54.34%) and the other half in rural areas (45.66%). Most of the women had never smoked (79.90%) and the current percentage of smokers was just 2.73%. Those who ever smoked had on average 20 packyears. Nevertheless, many women were exposed to passive smoking (60.3%) either at home or at work. Considering UV exposure the mean UV index was 3.7 with a small span (3.43-3.95). Most of the women did never use a sunbed (82.01%) and used sun protection factor (SPF) when exposed to the sun outdoors. Most of the women had a medium skin type according to Fitzpatrick (16) with category 2 or 3 (about 60%). Most of the women never received a hormone replacement therapy (HRT; 59.93%).

Table 9: Characteristics of the SALIA study population

806 subjects	Distribution	Mean	SD	Min - Max
Socialstatus	>10 years: 266 (33.0%) =10 years: 397 (49.3%) <10 years: 143 (17.7%)			
Urban/Rural	Urban: 438 (54.3%) Rural: 368 (45.7%)			
HRT	Ever: 323 (40.1%) Never: 483 (59.9%)			
SPF	Yes: 491 (60.9%) No: 315 (39.1%)			
Smoking	Non-smoker: 644 (79.9%) Ex-smoker: 140 (17.4%) Smoker: 22 (2.7%)			
Sunbed use	Ever: 145 (18.0%) Never: 661 (82.0%)			
Passive smoking	Ever: 486 (60.3%) Never: 320 (39.7%)			
Heating with fossil fuels	Ever: 212 (26.3%) Never: 594 (73.7%)			
Skin type	1=red: 148 (18.4%) 2=rather red: 307 (28.1%) 3=rather brown: 269 (33.4%) 4=brown: 82 (10.2%)	2.4	1.1	1 - 4
Packyears	160 persons different from 0	19.6 (of smokers)	21.3 (of smokers)	0 - 122.5
Age		73.5	4.3	55.7 - 79.8
BMI		27.3	9.6	16.9 - 45.7
UV index		3.7	0.2	3.4 - 4.0

3.1.2 Air pollution exposure

The mean exposure to air pollution was higher in the urban- than in the rural areas, consistently for all pollutants but ozone (see Table 10). The greatest difference was seen for NO_2 with a mean value of 45.52 µg/m^3 in urban areas and 28.42 µg/m^3 in rural areas. Considering its range NO_2 was occasionally twice as high in urban areas than in rural areas (84.14 vs. 41.50). Also, the mean concentrations of PM_{10} and $PM_{2.5}$ were much higher in the urban Ruhr area (53.2 µg/m^3 and 35.3 µg/m^3) in contrast to the rural areas (43.31 µg/m^3 and 28.78 µg/m^3). $PM_{2.5absorbance}$, had similar concentrations in urban (2.27 x 10^{-5}/m^3) and rural areas (2.04 10-5/m^3). Ozone was the only pollutant which had a higher impact in rural areas. Urban areas had on average 20 ozone exceedance days per year, whereas rural areas had 22 exceedance days.

Table 10: Exposure to air pollutants in the urban and rural areas of the SALIA population at baseline investigation (1985-1994) respectively in 2005 for ozone.

	Mean (IQR)	SD	Min-Max
Urban area			
NO_2 (µg/m³)	45.5 (14.4)	19.4	27.4 - 84.1
PM_{10} (µg/m³)	53.2 (5.1)	6.5	45.4 - 65.1
$PM_{2.5}$ (µg/m³)	35.3 (3.2)	3.6	30.4 - 41.3
$PM_{2.5absorbance}$ (10^{-5}/m)	2.3 (0.8)	1.5	2.2 - 6.4
O_3 (exceedance days/year)	20.0 (5.3)	6.8	6.7 - 26.7
Rural area			
NO_2 (µg/m³)	28.4 (6.5)	7.2	20.3 -41.5
PM_{10} (µg/m³)	43.3 (13.9)	8.5	32.2 - 54.9
$PM_{2.5}$ (µg/m³)	28.8 (9.1)	5.4	22.0 - 35.9
$PM_{2.5absorbance}$ (10^{-5}/m)	2.0 (0.7)	0.7	1.3 - 3.4
O_3 (exceedance days/year)	22.5 (5.0)	3.0	17.3 - 24.7

3.1.3 MEDI Score

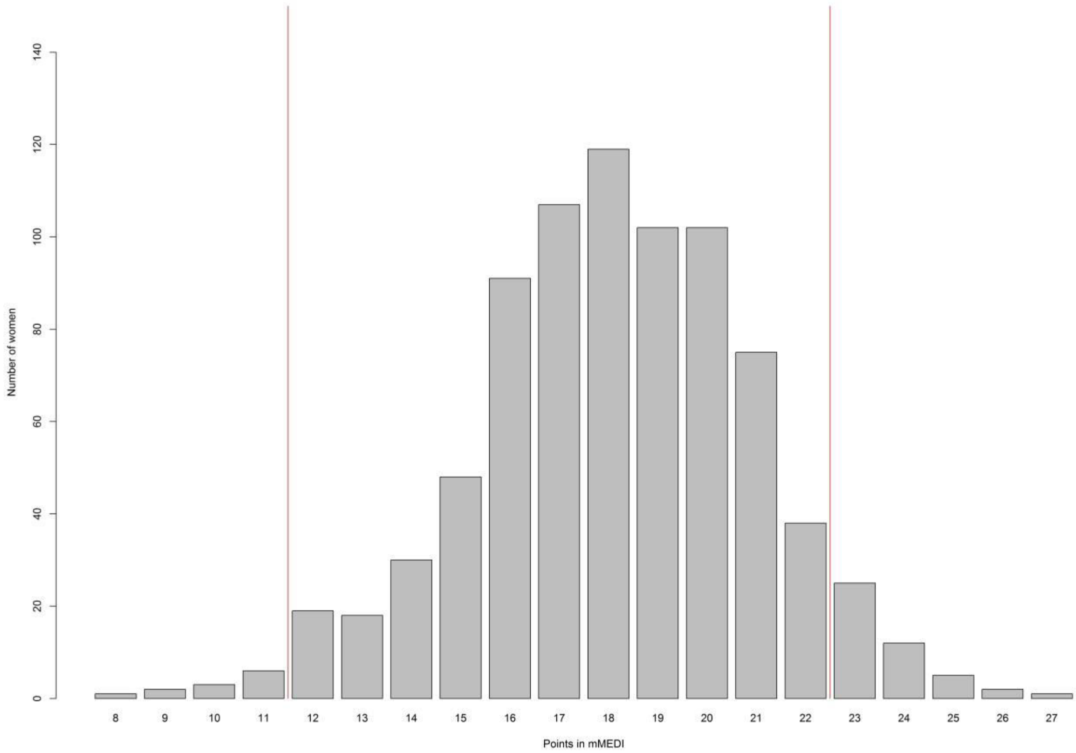
Table 11 presents the dietary habits of the SALIA population according to their answers in the food questionnaire. Potatoes were the basis of their nutrition and most of the women consumed them every day (51.6%), much more often than rice (only 2.2% consumed it every day) and pasta (only 2.7% consumed it every day). Also whole grain bread was consumed daily (72.5%). In most of the cases the meal was accompanied by vegetables. Cooked vegetables were mostly consumed several times per week (51.1%) as well as raw vegetables or salad (43.4%). Red meat was mostly consumed several times per week (40.8%) while poultry and fish were part of the meal once a week (poultry 34.6%, fish 48.4%). Interestingly, dairy products such as butter, milk, cheese and yoghurt were consumed everyday by 82.9% of the SALIA population. Finally, nearly all women ate fruits every day (86.4%).

Table 11: Reported frequency of consumption per food item

	Never	Once a month	several times per month	once a week	several times per week	everyday
Whole grain bread	30 (3.7%)	28 (3.5%)	14 (1.7%)	20 (2.5%)	130 (16.1%)	584 (72.5%)
Pasta	17 (2.1%)	99 (12.3%)	129 (16.0%)	323 (40.1%)	216 (26.8%)	22 (2.7%)
Rice	44 (5.5%)	166 (20.6%)	179 (22.2%)	269 (33.4%)	130 (16.1%)	18 (2.2%)
Potatoes	2 (0.2%)	9 (1.1%)	23 (2.9%)	29 (3.6%)	327 (40.6%)	416 (51.6%)
Fruits	11 (1.4%)	3 (0.4%)	9 (1.1%)	14 (1.7%)	73 (9.1%)	696 (86.4%)
Vegetables (cooked)	4 (0.5%)	4 (0.5%)	21 (2.6%)	44 (5.5%)	412 (51.1%)	321 (39.8%)
Salad or raw vegetables	10 (1.2%)	23 (2.9%)	38 (4.7%)	68 (8.4%)	350 (43.4%)	317 (39.3%)
Fish	43 (5.3%)	78 (9.7%)	98 (12.2%)	390 (48.4%)	184 (22.8%)	13 (1.6%)
Red meat	16 (2.0%)	34 (4.2%)	44 (5.5%)	120 (14.9%)	329 (40.8%)	263 (32.6%)
Poultry	42 (5.2%)	86 (10.7%)	125 (15.5%)	279 (34.6%)	242 (30.0%)	32 (4.9%)
Dairy products	16 (2.0%)	9 (1.1%)	10 (1.2%)	17 (2.1%)	86 (10.7%)	668 (82.9%)

For each woman the mMEDI score was built as explained in chapter 2.3.1. It had a mean of 18.11 and a range of 8-27 points (see Figure 3). Interestingly, no woman had a maximum adherence to the mMEDI with the maximum score of 33 points. For better visualization we divided the population into three groups: low adherence to the mMEDI (11 points or below), medium adherence (12 – 22 points) and high adherence (more than 22 points). A number of 12 women had a low adherence, 45 a high adherence and 749 had a middle adherence, which shows that nutrition is quite similar in the SALIA population.

Figure 7: Barplot of distribution of mMEDI in the SALIA population



3.1.4 Skin aging

Tables 12 to 14 present the distribution for pigment spots (PS), coarse wrinkles (CW) and other skin variables. The original scores were evaluated in a numeric way and via photo-reference scales as explained in section 2.4 and were transformed into new binary scores. Skin aging that scored equal to the mean score in the population or higher was declared as severe skin aging and skin aging below the mean as few skin aging. Most of the time the two groups were similar in size. For pigment spots on the forearms the group with severe skin aging (71.09%) was much bigger than the group with few (28.54%) (Table 10). All other distributions were at least 30% to 70%.

By calculating the mean from the original score and defining the mean or a value above as severe skin aging and everything below the mean as non-severe skin aging, we received the following distributions. For pigment spots on the forehead (numeric) just 31.0% had severe skin aging, whereas in the approach with photo-reference scale 43.1% of the women had severe skin aging (Table 12). Especially for pigment spots on the cheeks (photo-reference scale) many women showed severe skin aging (61.0%) as well as for pigment spots on the arms (71.1%).

Table 12: Distribution for pigment spots variables

Skin parameter	Originate score (%)	New score (severe skin aging)
PS Forehead (numeric)	0: 188 (23.3%) 1: 367 (45.5%) 2: 196 (24.3%) 3: 54 (6.7%)	yes: 250 (31.0%) no: 555 (68.9%)
PS Forehead (photo-reference scale)	0: 204 (25.3%) 1: 251 (31.1%) 2: 174 (21.6%) 3: 122 (15.1%) 4: 37 (4.6%) 5: 14 (1.7%)	yes: 347 (43.1%) no: 455 (56.5%)
PS Cheeks (numeric)	0: 83 (10.3%) 1: 434 (53.9%) 2: 234 (29.1%) 3: 54 (6.7%)	yes: 288 (35.7%) no: 517 (64.1%)
PS Cheeks (photo-reference scale)	0: 84 (10.4%) 1: 224 (27.8%) 2: 207 (25.7%) 3: 206 (25.6%) 4: 53 (6.6%) 5: 26 (3.2%)	yes: 492 (61.0%) no: 308 (38.2%)
PS Arms (numeric)	0: 41 (5.1%) 1: 189 (23.5%) 2: 327 (40.6%) 3: 246 (30.5%)	yes: 573 (71.1%) no: 230 (28.5%)
PS Hands (numeric)	0: 110 (13.7%) 1: 327 (40.6%) 2: 281 (34.7%) 3: 82 (10.2%)	yes: 363 (45.0%) no: 437 (54.2%)

PS: Pigment spots, numeric covariates (0: 0PS, 1: 1-10PS, 2: 11-50PS, 3: 50+ PS), covariates evaluated via photo-reference scale (0: no pigment spots or very few (<3mm), 1: one to several small pigment spots (=3mm), 2: one to several bigger pigment spots (4-8mm), 3: one very big pigment spot (>9mm, light pigmentation), 4: one very big pigment spot (>9mm, dark pigmentation), 5: several very big pigment spots (>9mm)

Table 13: Distribution for coarse wrinkle variables

Skin parameter	Originate score (%)	New score (severe skin aging)
CW Forehead	1: 36 (4.47%) 1.5: 24 (2.98%) 2: 126 (15.63%) 2.5: 63 (7.82%) 3: 156 (19.35%) 3.5: 82 (10.17%) 4: 123 (15.26%) 4.5: 59 (7.32%) 5: 137 (17.00%)	yes: 401 (49.75%) no: 405 (50.25%)
CW Eyebrows	0.5: 1 (0.12%) 1: 26 (3.23%) 1.5: 15 (1.86%) 2: 76 (9.43%) 2.5: 53 (6.58%) 3: 210 (26.05%) 3.5: 133 (16.50%) 4: 128 (15.88%) 4.5: 48 (5.96%) 5: 116 (14.39%)	yes: 425 (52.73%) no: 381 (47.24%)
CW Crow´s feet area	1: 28 (3.47%) 1.5: 38 (4.71%) 2: 153 (18.98%) 2.5: 82 (10.17%) 3: 246 (30.52%) 3.5: 120 (14.89%) 4: 93 (11.54%) 4.5: 19 (2.36%) 5: 27 (3.35%)	yes: 505 (62.66%) no: 301 (37.34%)
CW Under the eye	1: 2 (0.25%) 1.5: 9 (1.12%) 2: 62 (7.69%) 2.5: 64 (7.94%) 3: 195 (24.19%) 3.5: 94 (11.66%) 4: 195 (24.19%) 4.5: 93 (11.54%) 5: 92 (11.41%)	yes: 380 (47.15%) no: 426 (52.85%)
CW on upper lip	1: 18 (2.23%) 1.5: 11 (1.36%) 2: 103 (12.78%) 2.5: 47 (5.83%) 3: 234 (29.03%) 3.5: 116 (14.39%) 4: 182 (22.58%) 5: 95 (11.79%)	yes: 393 (48.76%) no: 413 (51.24%)
CW Nasolabialfold	0.5: 1 (0.12%) 1: 2 (0.25%) 1.5: 1 (0.12%) 2: 13 (1.61%) 2.5: 19 (2.36%) 3: 187 (23.20%) 3.5: 158 (19.60%) 4: 249 (30.89%) 4.5: 122 (15.14%) 5: 52 (6.45%)	yes: 423 (52.48%) no: 381 (47.27%)

CW: Coarse wrinkles, score values according to comparison with photo-references as explained in chapter 2.4.1.

For coarse wrinkle variables (Table 13) the distribution of severe skin aging and non-severe skin aging was very balanced. Taking coarse wrinkles on the eyebrows as an example, 52.73% of the women had severe skin aging and in 47.24% skin aging was non-severe. For coarse wrinkles in the crow´s feet area the skin aged part of the study population was a little bigger (62.66%).

Table 14: Distribution of other skin variables

Skin parameter	Originate score (%)	Current score (severe skin aging)
Solar Elastosis		yes: 321 (39.83%) no: 481 (59.68%)
Teleangiectasia	0: 133 (16.50%) 1: 190 (23.57%) 2: 177 (21.96%) 3: 135 (16.75%) 4: 90 (11.17%) 5: 78 (9.68%)	yes: 303 (37.59%) no: 500 (62.03%)
Even Pigmentation (Forearm inside)		yes: 590 (73.20%) no: 207 (25.68%)
Lax Eye	0: 3 (0.37%) 1: 4 (0.50%) 1.5: 1 (0.12%) 2: 36 (4.47%) 2.5: 15 (1.86%) 3: 134 (16.63%) 3.5: 95 (11.79%) 4: 275 (34.12%) 4.5: 128 (15.88%) 5: 112 (13.90%)	yes: 515 (63.90%) no: 288 (35.73%)
Lax Face (lower part)	1: 2 (0.25%) 1.5: 2 (0.25%) 2: 34 (4.22%) 2.5: 55 (6.82%) 3: 208 (25.81%) 3.5: 127 (15.76%) 4: 188 (23.33%) 4.5: 103 (12.78%) 5: 84 (10.42%)	yes: 375 (46.53%) no: 428 (53.10%)
Cigarette Paper like folds (Back of the hands)		yes: 522 (64.76%) no: 271 (33.62%)

Categories of originate score according to comparison with photo-references as explained in chapter 2.4.1

Table 12 presents all other skin aging variables. Solar elastosis, even pigmentation and cigarette paper like skin on the back of the hands were originally binary with just two manifestations and did not need to be transformed. Most of the women did not have solar elastosis on cheeks (59.68%), which is a severe extrinsic skin aging sign, but most had cigarette paper like skin on the back of the hands (64.76%), which is an intrinsic skin aging sign, occurring with age. Even pigmentation on the forearm inside, which is an intrinsic sign as well, was seen in 73.20% of the women. Considering the other variables, most women had very few teleangieactasia (23.57%), whereas for laxness of facial parts, the distribution of women with high and low skin aging was balanced.

3.2 Association between skin aging and air pollution as well as the modifying effect of nutrition

The following tables summarize the results of the different skin aging outcomes. The first column names the outcomes and the covariates of interest, mMedi and air pollutant. Columns two till six show the results (odds ratio and p-value) for the different models, including each another air pollutant (NO_2, PM_{10}. $PM_{2.5}$, $PM_{2.5coarse}$, O_3). The term “air pollutant” can be replaced by the respective air pollutant used in the model. The numbers written in bold print, mark results, which are statistical significant (p-value of 0.05). The figures present the odds ratios with 95% confidence intervals for our predictors (mMEDI, air pollutants). Again, five models stand next to each other, in order to compare the influences of the five air pollutants. Only figures indicating a statistical significant result are shown all other figures are presented in the appendix.

3.2.1 Models adjusted for UV covariates

Table 15 displays the results for calculations adjusted for the UV covariates: UV-index and sun protection factor. When referring to single skin aging variables, the outcomes will be written in cursive fonts in the wording given by the corresponding table from now on.

Considering the mMEDI, most of the results revealed that with a higher adherence to the mMEDI the probability for severe skin aging increased. Taking pigment spots as an example it can be claimed that with one additional point in the mMEDI the probability for *Pigment spots on cheeks (numeric)* increased about 8% (OR of 1.08). The effect was significant for all five air pollutant models with a p-value of 0.004. Similar tendencies could be seen for the outcome *Pigment spots on forehead (numeric)* and *Wrinkles on upper lip* in all five models.

The outcome *Cigarette paper like skin on the back of the hands* was the only one presenting an inverse relationship. With each additional point in the mMEDI the probability of having severe *Cigarette paper like skin on the back of the hands* decreased about 7% (OR:0.93).

Considering the impact of air pollution on skin aging it could be observed that for some of the outcomes the probability for skin aging increased with higher air pollution. Ozone had a harmful effect on the outcome *Wrinkles under the eyes*. The OR of 1.24 says that with one additional ozone exceedance day per year, the probability for *Wrinkles under the eyes* increased about 24%. PM_{10} and $PM_{2.5}$ acted harmful on the formation of *Wrinkles on upper lip*. One additional IQR of $PM_{2.5}$ raised the probability of severe *Wrinkles on upper lip* about 23.5%.

For many outcomes air pollution seemed to have a protective effect. This could be seen for *Telangiectasia on cheeks*, *Even pigmentation on forearm insides*, *Lax appearance (lower part of the face)* and *Cigarette paper like skin on the back of the hands*. All air pollutants with the exception of NO_2 seemed to have a protective effect on *Teleangiectasia on cheeks*. Taking PM_{10} as an example, the probability for *Teleangiectasia on cheeks* augmented about 22.7% with one additional IQR for PM_{10}. The air pollutants PM_{10} and $PM_{2.5}$ significantly raised the chance for *Even pigmentation on forearm insides* (OR: 1.23 for PM_{10}, 1.22 for $PM_{2.5}$). One additional IQR of PM_{10} enhanced the probability for severe skin aging in lax face around 15%, similar to $PM_{2.5}$. In the models for PM_{10}-, $PM_{2.5}$ and $PM_{2.5absorbance}$ those air pollutants had a protective effect on *Cigarette paper like skin on the back of the hands*. These effects were statistical significant with a p-value below 0.005% for PM_{10} and $PM_{2.5}$ and a p-value of 0.02 for $PM_{2.5coarse}$.

Table 15: Models adjusted for UV covariates

Outcomes		NO_2		PM_{10}		$PM_{2.5}$		$PM_{2.5abs}$		O_3	
		OR	p-value	OR	p-value	OR	p-value	OR	p-value	OR	p-value
Pigment spots											
Cheeks (numeric)	mMEDI	1,081	0,004	1,081	0,004	1,082	0,004	1,081	0,004	1,082	0,004
	Air pollutant	1,122	0,307	1,005	0,953	0,988	0,892	1,006	0,946	1,191	0,102
Cheeks (photoreference scale)	mMEDI	1,051	0,058	1,052	0,055	1,052	0,057	1,052	0,055	1,052	0,055
	Air pollutant	1,168	0,182	0,998	0,980	1,036	0,698	1,071	0,479	0,907	0,362
Forehead (numeric)	mMEDI	1,063	0,028	1,063	0,027	1,064	0,025	1,063	0,027	1,064	0,026
	Air pollutant	1,127	0,307	0,956	0,587	0,913	0,335	1,031	0,754	1,147	0,217
Forehead (photoreference scale)	mMEDI	1,041	0,119	1,042	0,110	1,043	0,107	1,042	0,113	1,042	0,112
	Air pollutant	1,109	0,350	0,959	0,590	0,941	0,499	0,996	0,970	1,139	0,204
Forearm topsides	mMEDI	1,013	0,642	1,014	0,632	1,013	0,647	1,014	0,618	1,015	0,607
	Air pollutant	1,186	0,170	1,073	0,400	1,096	0,344	1,082	0,444	1,136	0,241
Back of the hands	mMEDI	1,013	0,608	1,013	0,610	1,013	0,603	1,013	0,616	1,013	0,612
	Air pollutant	0,933	0,532	0,973	0,723	0,960	0,646	0,957	0,636	1,081	0,450
Wrinkles											
Forehead	mMEDI	1,021	0,408	1,023	0,380	1,024	0,361	1,021	0,408	1,021	0,407
	Air pollutant	1,014	0,902	0,865	0,058	0,843	0,053	0,970	0,740	1,026	0,802
Between eyebrows	mMEDI	1,034	0,198	1,033	0,201	1,033	0,204	1,034	0,197	1,034	0,195
	Air pollutant	1,004	0,975	1,032	0,685	1,038	0,671	1,005	0,959	1,075	0,477
Crow´s feet region	mMEDI	1,023	0,380	1,023	0,384	1,023	0,394	1,024	0,371	1,024	0,368
	Air pollutant	1,110	0,361	1,083	0,307	1,095	0,311	1,000	0,998	1,056	0,600
Under the eyes	mMEDI	1,039	0,137	1,039	0,133	1,039	0,130	1,038	0,140	1,039	0,138
	Air pollutant	0,953	0,662	0,932	0,357	0,930	0,410	0,974	0,775	1,238	0,037
Upper lip	mMEDI	1,066	0,014	1,065	0,016	1,063	0,018	1,066	0,013	1,066	0,014
	Air pollutant	1,061	0,592	1,191	0,024	1,235	0,018	1,136	0,169	1,054	0,608
Nasolabialfolds	mMEDI	0,999	0,969	0,999	0,978	1,000	0,985	1,000	0,993	1,000	0,989
	Air pollutant	1,144	0,227	1,035	0,651	1,005	0,956	1,136	0,175	1,047	0,649
Others											
Solar elastosis on cheeks	mMEDI	1,017	0,513	1,017	0,507	1,017	0,519	1,018	0,489	1,018	0,487
	Air pollutant	1,225	0,072	1,100	0,221	1,115	0,232	1,144	0,159	1,138	0,215
Teleangiectasia on cheeks	mMEDI	0,974	0,330	0,976	0,361	0,977	0,380	0,974	0,321	0,974	0,320
	Air pollutant	0,960	0,732	0,773	0,002	0,788	0,012	0,795	0,025	0,809	0,046
Even pigmentation on forearm insides	mMEDI	1,009	0,759	1,007	0,799	1,007	0,818	1,009	0,758	1,009	0,765
	Air pollutant	0,959	0,748	1,232	0,019	1,223	0,049	1,181	0,141	0,943	0,626
Lax appearance (eyelids)	mMEDI	1,046	0,095	1,047	0,088	1,046	0,092	1,047	0,090	1,047	0,090
	Air pollutant	1,144	0,255	0,965	0,659	1,014	0,885	1,026	0,790	0,954	0,662
Lax appearance (Lower part of the face)	mMEDI	0,980	0,427	0,981	0,446	0,982	0,467	0,979	0,410	0,979	0,412
	Air pollutant	0,845	0,129	0,854	0,042	0,832	0,042	0,890	0,211	1,077	0,465
Cigarette paper like skin on the back of the hands	mMEDI	0,932	0,017	0,932	0,018	0,934	0,020	0,931	0,016	0,932	0,017
	Air pollutant	0,876	0,297	0,678	0,000	0,682	0,000	0,790	0,023	0,930	0,526

Adjusted for: social status, urban/rural, hormone replacement therapy, sun protection factor, smoking status, sunbed use, passive smoking, heating with fossil fuels, packyears, age, BMI, skin type, UV index; NO_2: Nitrogen dioxide, PM_{10}/$PM_{2.5}$: Particulate matter suspended in the air with a diameter of 10µm/ 2.5µm, $PM_{2.5coarse}$: Proxy for elemental carbon, O_3: Ozone, OR: Odds ratio, numbers written in blue mark significant results to a p-value of 0.05

3.2.2 Models without UV covariates

Since the results in the full adjusted model were unexpected we calculated new models where the UV covariates UV index and sun protection factor were left out. Since sun exposure has the strongest impact on skin aging, it may hide the harmful effects of air pollution. In the following we will just refer to the results differing from the full model.

The effects of the mMEDI on the different outcomes remained similar: mMEDI acted harmful on pigment spots on cheeks- and pigment spots on the forehead variables as well as on the coarse wrinkles on the upper lip. Cigarette paper like skin was the only outcome, for which the Mediterranean Diet displays as effective.

In contrast to the full model, NO_2 seemed to have a significant harmful effect on *Pigment spots on cheeks (numeric)*: with each IQR more of NO2, the probability for a high number of *Pigment spots on cheeks* augmented about 23% (OR: 1.23, p-value: 0.05). This result is in accordance with earlier analyses (5).

The protective effects of PM_{10} and $PM_{2.5}$ on *Wrinkles on forehead* were highly significant (p value: 0.005). For the outcome *Wrinkles on upper lip*, there was just one significant result for $PM_{2.5}$. For *Wrinkles on nasolabialfolds* we saw a harmful effect of NO_2 and $PM_{2.5\ absorbance}$ in this model. This reproduced results from earlier analyses (5). For *Teleangiectasia on cheeks*, as well as *Lax appearance (lower part of the face)* effects vanished. NO_2 acted significantly protective on *Cigarette paper like skin on the back of the hands*, so that all air pollutants seemed to have a positive effect here.

In summary, air pollution promoted skin aging for the outcomes *Pigment spots on cheeks, Wrinkles on upper lip, Wrinkles on nasolabialfolds* and prevented skin aging for the outcomes *Wrinkles on forehead* and *Cigarette paper like skin on the back of the hands*.

Table 16: Models without UV covariates

Outcomes		NO_2		PM_{10}		$PM_{2.5}$		$PM_{2.5abs}$		O_3	
		OR	p-value	OR	p-value	OR	p-value	OR	p-value	OR	p-value
Pigment spots											
Cheeks (numeric)	mMEDI	1,083	0,003	1,083	0,003	1,083	0,003	1,084	0,003	1,085	0,003
	Air pollutant	1,232	0,049	1,105	0,162	1,097	0,272	1,137	0,117	1,141	0,211
Cheeks (photoreference scale)	mMEDI	1,052	0,056	1,052	0,052	1,052	0,055	1,052	0,053	1,053	0,051
	Air pollutant	1,179	0,130	1,024	0,735	1,058	0,496	1,086	0,319	0,902	0,328
Forehead (numeric)	mMEDI	1,063	0,027	1,064	0,025	1,065	0,023	1,064	0,025	1,065	0,024
	Air pollutant	1,156	0,187	0,996	0,961	0,959	0,630	1,069	0,430	1,126	0,282
Forehead (photoreference scale)	mMEDI	1,041	0,119	1,043	0,107	1,043	0,104	1,042	0,111	1,043	0,109
	Air pollutant	1,092	0,400	0,965	0,614	0,948	0,520	0,994	0,941	1,141	0,194
Forearm topsides	mMEDI	1,013	0,658	1,013	0,643	1,013	0,656	1,013	0,634	1,014	0,616
	Air pollutant	1,144	0,249	1,045	0,567	1,066	0,479	1,042	0,644	1,139	0,224
Back of the hands	mMEDI	1,016	0,539	1,015	0,554	1,015	0,552	1,015	0,545	1,016	0,537
	Air pollutant	0,990	0,921	1,034	0,628	1,023	0,775	1,030	0,708	1,080	0,447
Wrinkles											
Forehead	mMEDI	1,019	0,461	1,021	0,405	1,022	0,383	1,019	0,457	1,018	0,472
	Air pollutant	0,927	0,461	0,823	0,005	0,797	0,005	0,884	0,124	1,053	0,602
Between eyebrows	mMEDI	1,032	0,215	1,032	0,216	1,032	0,216	1,032	0,216	1,032	0,216
	Air pollutant	0,958	0,676	0,982	0,787	0,985	0,857	0,951	0,527	1,085	0,417
Crow´s feet region	mMEDI	1,021	0,433	1,021	0,427	1,021	0,427	1,021	0,421	1,021	0,431
	Air pollutant	0,974	0,805	0,967	0,633	0,971	0,722	0,879	0,109	1,105	0,328
Under the eyes	mMEDI	1,037	0,152	1,038	0,142	1,038	0,138	1,037	0,156	1,037	0,157
	Air pollutant	0,891	0,264	0,888	0,083	0,880	0,114	0,903	0,203	1,264	0,021
Upper lip	mMEDI	1,065	0,014	1,064	0,016	1,063	0,018	1,065	0,014	1,066	0,013
	Air pollutant	1,030	0,779	1,136	0,066	1,175	0,048	1,075	0,365	1,072	0,487
Nasolabialfolds	mMEDI	1,000	0,986	1,000	0,985	1,000	0,986	1,001	0,964	1,002	0,933
	Air pollutant	1,249	0,034	1,120	0,098	1,102	0,229	1,235	0,010	1,004	0,970
Others											
Solar elastosis on cheeks	mMEDI	1,016	0,551	1,016	0,535	1,016	0,540	1,016	0,530	1,017	0,524
	Air pollutant	1,116	0,297	1,022	0,756	1,031	0,709	1,023	0,777	1,168	0,133
Teleangiectasia on cheeks	mMEDI	0,972	0,287	0,975	0,345	0,976	0,360	0,973	0,298	0,971	0,261
	Air pollutant	0,881	0,249	0,753	0,000	0,756	0,001	0,762	0,002	0,834	0,081
Even pigmentation on forearm insides	mMEDI	1,010	0,736	1,007	0,801	1,007	0,818	1,009	0,752	1,010	0,735
	Air pollutant	1,027	0,821	1,242	0,007	1,246	0,019	1,213	0,050	0,922	0,495
Lax appearance (eyelids)	mMEDI	1,045	0,101	1,047	0,090	1,046	0,095	1,046	0,095	1,046	0,094
	Air pollutant	1,123	0,290	0,969	0,653	1,007	0,929	1,017	0,838	0,949	0,626
Lax appearance (Lower part of the face)	mMEDI	0,980	0,439	0,981	0,460	0,982	0,477	0,980	0,426	0,980	0,419
	Air pollutant	0,872	0,189	0,890	0,093	0,867	0,083	0,928	0,356	1,071	0,497
Cigarette paper like skin on the back of the hands	mMEDI	0,932	0,015	0,933	0,020	0,935	0,023	0,931	0,015	0,931	0,014
	Air pollutant	0,769	0,024	0,648	0,000	0,635	0,000	0,717	0,000	0,952	0,665

Adjusted for: social status, urban/rural, hormone replacement therapy, smoking status, sunbed use, passive smoking, heating with fossil fuels, packyears, age, BMI, skin type; NO_2: Nitrogen dioxide, PM_{10}/$PM_{2.5}$: Particulate matter suspended in the air with a diameter of 10µm/ 2.5µm, $PM_{2.5coarse}$: Proxy for elemental carbon, O_3: Ozone, OR: Odds ratio, numbers written in blue mark significant results to a p-value of 0.05

3.2.3 Models with an interaction variable (mMedi & Air pollutant)

The analyses including an interaction variable were performed without adjustment for UV covariates. When including an interaction term between mMEDI and the respective air pollutant the tendencies and significances for the covariates of interest stayed similar. Table 17 shows the results for the outcomes pigment spots and wrinkles and Table 18 the results for all other outcomes.

The mMEDI revealed a harmful effect in the models for *Pigment spots on cheeks (numeric)*, *Pigment spots on forehead (numeric)* and *Wrinkles on upper lip*. A protective impact could be seen for *Cigarette paper like skin on the back of the hands*.

Air pollutants acted protective in the models for *Wrinkles on forehead*, *Teleangiectasia on cheeks* and *Cigarette paper like skin on the back of the hands*. A harmful ramification of air pollutants could be observed for the *Pigment spots on cheeks (numeric)*, *Wrinkles on nasolabialfolds, Wrinkles on upper lip* and *Even pigmentation on forearm insides*.

The interaction term did not show any significant effects.

Table 17: Models with an interaction variable (Pigment spots and wrinkles)

Outcomes		NO_2		PM_{10}		$PM_{2.5}$		$PM_{2.5abs}$		O_3	
		OR	p-value	OR	p-value	OR	p-value	OR	p-value	OR	p-value
Pigment spots											
Cheeks (numeric)	mMEDI	1,258	0,003	1,266	0,002	1,264	0,003	1,259	0,003	1,266	0,002
	Air pollutant	1,183	0,050	1,148	0,157	1,111	0,261	1,134	0,118	1,100	0,233
	Interactionvariable: mMEDI & Air pollutant	0,975	0,748	0,882	0,137	0,893	0,179	0,970	0,712	1,025	0,736
Cheeks (photoreference scale)	mMEDI	1,154	0,057	1,158	0,052	1,156	0,055	1,154	0,057	1,168	0,041
	Air pollutant	1,142	0,133	1,029	0,762	1,063	0,508	1,080	0,343	0,922	0,302
	Interactionvariable: mMEDI & Air pollutant	0,998	0,984	0,938	0,419	0,967	0,672	0,936	0,425	1,091	0,239
Forehead (numeric)	mMEDI	1,195	0,025	1,195	0,024	1,196	0,023	1,193	0,025	1,196	0,025
	Air pollutant	1,123	0,193	0,995	0,962	0,955	0,631	1,068	0,433	1,094	0,282
	Interactionvariable: mMEDI & Air pollutant	0,945	0,492	0,962	0,640	0,993	0,932	0,954	0,580	0,993	0,927
Forehead (photoreference scale)	mMEDI	1,123	0,119	1,123	0,118	1,124	0,116	1,125	0,111	1,124	0,118
	Air pollutant	1,073	0,400	0,954	0,624	0,944	0,532	0,995	0,950	1,105	0,190
	Interactionvariable: mMEDI & Air pollutant	1,001	0,988	1,075	0,370	1,098	0,245	1,031	0,706	0,988	0,858
Forearm topsides	mMEDI	1,036	0,659	1,036	0,657	1,035	0,669	1,038	0,642	1,042	0,609
	Air pollutant	1,118	0,238	1,063	0,557	1,075	0,471	1,039	0,662	1,102	0,228
	Interactionvariable: mMEDI & Air pollutant	1,034	0,701	1,028	0,748	1,032	0,715	0,936	0,462	1,008	0,920
Back of the hands	mMEDI	1,045	0,546	1,043	0,562	1,043	0,565	1,045	0,548	1,042	0,577
	Air pollutant	0,995	0,949	1,047	0,620	1,027	0,762	1,028	0,721	1,062	0,427
	Interactionvariable: mMEDI & Air pollutant	1,052	0,509	1,026	0,741	1,044	0,581	0,972	0,724	0,967	0,632
Wrinkles											
Forehead	mMEDI	1,055	0,460	1,063	0,398	1,066	0,381	1,055	0,460	1,055	0,462
	Air pollutant	0,940	0,459	0,765	0,005	0,778	0,005	0,886	0,122	1,039	0,611
	Interactionvariable: mMEDI & Air pollutant	0,993	0,926	0,962	0,616	0,988	0,872	0,986	0,856	1,013	0,857
Between eyebrows	mMEDI	1,099	0,201	1,101	0,193	1,099	0,200	1,095	0,217	1,088	0,254
	Air pollutant	0,960	0,625	0,973	0,768	0,983	0,845	0,946	0,485	1,067	0,394
	Interactionvariable: mMEDI & Air pollutant	0,899	0,173	0,895	0,162	0,929	0,349	0,876	0,104	0,954	0,500
Crow´s feet region	mMEDI	1,061	0,431	1,063	0,418	1,063	0,416	1,061	0,433	1,069	0,372
	Air pollutant	0,973	0,748	0,951	0,596	0,964	0,688	0,876	0,095	1,073	0,359
	Interactionvariable: mMEDI & Air pollutant	0,908	0,219	0,898	0,168	0,907	0,209	0,888	0,146	1,066	0,373
Under the eyes	mMEDI	1,109	0,152	1,113	0,140	1,116	0,131	1,107	0,159	1,101	0,187
	Air pollutant	0,910	0,260	0,850	0,081	0,867	0,109	0,903	0,196	1,197	0,018
	Interactionvariable: mMEDI & Air pollutant	0,984	0,830	0,973	0,726	0,926	0,322	0,955	0,568	0,951	0,475
Upper lip	mMEDI	1,200	0,013	1,204	0,012	1,198	0,015	1,199	0,014	1,184	0,023
	Air pollutant	1,020	0,809	1,189	0,068	1,195	0,049	1,070	0,391	1,062	0,427
	Interactionvariable: mMEDI & Air pollutant	0,944	0,457	0,878	0,113	0,910	0,245	0,894	0,170	0,905	0,161
Nasolabialfolds	mMEDI	1,001	0,987	1,003	0,970	1,003	0,972	1,002	0,973	0,999	0,988
	Air pollutant	1,202	0,031	1,166	0,103	1,112	0,236	1,228	0,011	1,007	0,924
	Interactionvariable: mMEDI & Air pollutant	1,051	0,526	0,949	0,502	0,952	0,524	0,974	0,751	0,945	0,412

Adjusted for: social status, urban/rural, hormone replacement therapy, smoking status, sunbed use, passive smoking, heating with fossil fuels, packyears, age, BMI, skin type; NO2: Nitrogen dioxide, PM10/PM2.5: Particulate matter suspended in the air with a diameter of 10µm/ 2.5µm, PM2.5coarse: Proxy for elemental carbon, O3: Ozone, OR: Odds ratio, numbers written in blue mark significant results to a p-value of 0.05

Table 18: Models with an interaction variable (Others)

Outcomes		NO_2 OR	NO_2 p-value	PM_{10} OR	PM_{10} p-value	$PM_{2.5}$ OR	$PM_{2.5}$ p-value	$PM_{2.5abs}$ OR	$PM_{2.5abs}$ p-value	O_3 OR	O_3 p-value
Others											
Solar elastosis on cheeks	mMEDI	1,044	0,563	1,046	0,545	1,046	0,545	1,048	0,528	1,046	0,543
	Air pollutant	1,095	0,287	1,031	0,751	1,035	0,707	1,023	0,774	1,124	0,130
	Interactionvariable: mMEDI & Air pollutant	1,051	0,533	1,022	0,789	1,010	0,906	1,031	0,717	0,985	0,831
Teleangiectasia on cheeks	mMEDI	0,921	0,274	0,929	0,335	0,932	0,353	0,922	0,288	0,928	0,326
	Air pollutant	0,893	0,209	0,675	0,000	0,728	0,001	0,765	0,002	0,870	0,075
	Interactionvariable: mMEDI & Air pollutant	0,912	0,261	0,915	0,268	0,886	0,129	0,972	0,748	1,076	0,310
Even pigmentation on forearm insides	mMEDI	1,028	0,737	1,022	0,793	1,019	0,819	1,031	0,713	1,028	0,738
	Air pollutant	1,027	0,786	1,346	0,007	1,275	0,019	1,215	0,045	0,941	0,497
	Interactionvariable: mMEDI & Air pollutant	1,056	0,538	1,020	0,822	0,998	0,977	1,057	0,566	0,998	0,976
Lax appearance (eyelids)	mMEDI	1,133	0,105	1,139	0,090	1,137	0,095	1,135	0,100	1,136	0,098
	Air pollutant	1,095	0,311	0,955	0,633	1,005	0,959	1,014	0,868	0,962	0,629
	Interactionvariable: mMEDI & Air pollutant	0,953	0,556	0,950	0,516	0,933	0,379	0,939	0,444	0,991	0,903
Lax appearance (Lower part of the face)	mMEDI	0,945	0,435	0,950	0,488	0,952	0,499	0,943	0,423	0,933	0,347
	Air pollutant	0,896	0,196	0,849	0,085	0,852	0,077	0,927	0,340	1,058	0,452
	Interactionvariable: mMEDI & Air pollutant	1,021	0,789	0,920	0,295	0,938	0,416	0,956	0,577	0,929	0,293
Cigarette paper like skin on the back of the hands	mMEDI	0,818	0,015	0,818	0,018	0,825	0,022	0,814	0,014	0,815	0,014
	Air pollutant	0,809	0,024	0,550	0,000	0,604	0,000	0,719	0,000	0,969	0,711
	Interactionvariable: mMEDI & Air pollutant	0,998	0,977	0,935	0,409	0,957	0,589	0,915	0,311	0,966	0,667

Adjusted for: social status, urban/rural, hormone replacement therapy, smoking status, sunbed use, passive smoking, heating with fossil fuels, packyears, age, BMI, skin type; NO2: Nitrogen dioxide, PM10/PM2.5: Particulate matter suspended in the air with a diameter of 10µm/ 2.5µm, PM2.5coarse: Proxy for elemental carbon, O3: Ozone, OR: Odds ratio, numbers written in blue mark significant results to a p-value of 0.05

3.2.4 Models with z-scores

Table 19 and figures 8-11 show the results for the outcomes pigment spots and wrinkles.

It was statistical significant that the mMEDI enhanced the severance of facial pigment spots in all five models. The analyses of the pigment spots variable offered a statistical significant association between the mMedi and the severances of facial pigment spots (Figure 8). The higher the mMedi, the more likely was the formation of pigment spots in all models. Regarding the air pollutants, NO_2 alone had a harmful impact in a sense that it also promoted the formation of facial pigment spots (OR: 1.12, p-value: 0.02). When including an interaction term in the analysis, the tendencies and significances of mMEDIs´ and air pollutants´ effects on the z-score for facial pigment spots remained the same (Figure 9). Again, the interaction term did not show a significant effect.

In contrast to pigment spots, there existed no significant effect in the z-scores for coarse wrinkles (Figure 10).

Table 19: Z-scores

Outcomes		NO_2 OR	NO_2 p-value	PM_{10} OR	PM_{10} p-value	$PM_{2.5}$ OR	$PM_{2.5}$ p-value	$PM_{2.5abs}$ OR	$PM_{2.5abs}$ p-value	O_3 OR	O_3 p-value
Pigment spots											
Pigment spots	mMEDI	1,035	0,005	1,036	0,005	1,036	0,005	1,036	0,004	1,036	0,004
	Air pollutant	1,120	0,023	1,032	0,336	1,033	0,407	1,053	0,182	1,025	0,617
Pigment spots with interaction variable	mMEDI	1,104	0,005	1,106	0,004	1,105	0,005	1,105	0,004	1,109	0,004
	Air pollutant	1,096	0,024	1,043	0,348	1,036	0,414	1,051	0,191	1,017	0,643
	Interactionvariable: mMEDI & Air pollutant	1,000	0,992	0,973	0,468	0,986	0,699	0,981	0,617	1,017	0,610
Wrinkles											
Wrinkles	mMEDI	1,017	0,171	1,018	0,159	1,018	0,155	1,017	0,168	1,017	0,165
	Air pollutant	0,998	0,974	0,968	0,331	0,964	0,344	0,980	0,610	1,089	0,080
Wrinkles with interaction variable	mMEDI	1,050	0,167	1,053	0,142	1,053	0,141	1,049	0,176	1,048	0,189
	Air pollutant	0,997	0,932	0,954	0,303	0,958	0,320	0,978	0,550	1,067	0,075
	Interactionvariable: mMEDI & Air pollutant	0,968	0,391	0,934	0,069	0,941	0,103	0,932	0,067	0,984	0,623

Adjusted for: social status, urban/rural, hormone replacement therapy, smoking status, sunbed use, passive smoking, heating with fossil fuels, packyears, age, BMI, skin type; NO2: Nitrogen dioxide, PM10/PM2.5: Particulate matter suspended in the air with a diameter of 10µm/ 2.5µm, PM2.5coarse: Proxy for elemental carbon, O3: Ozone, OR: Odds ratio, numbers written in blue mark significant results to a p-value of 0.05

Figure 8: Z-score of facial pigment spots

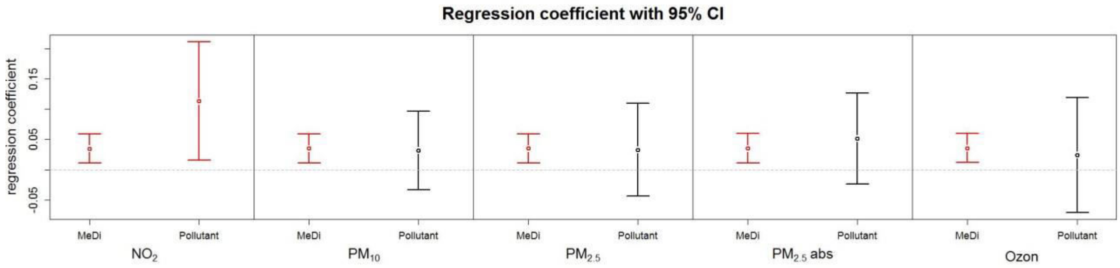


Figure 9: Z-score of facial pigment spots with interaction term

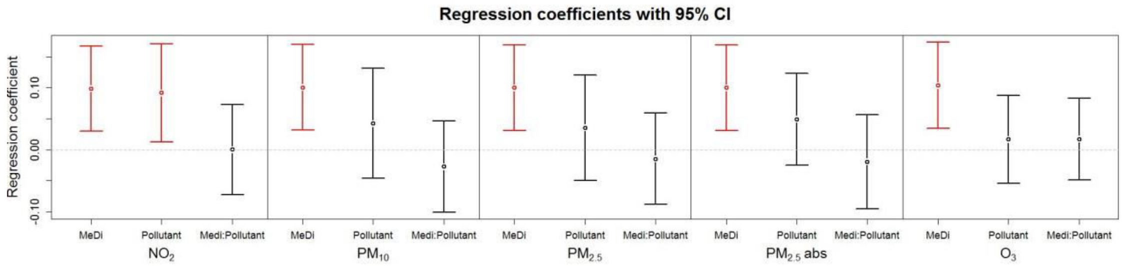


Figure 10: Z-score of coarse wrinkles

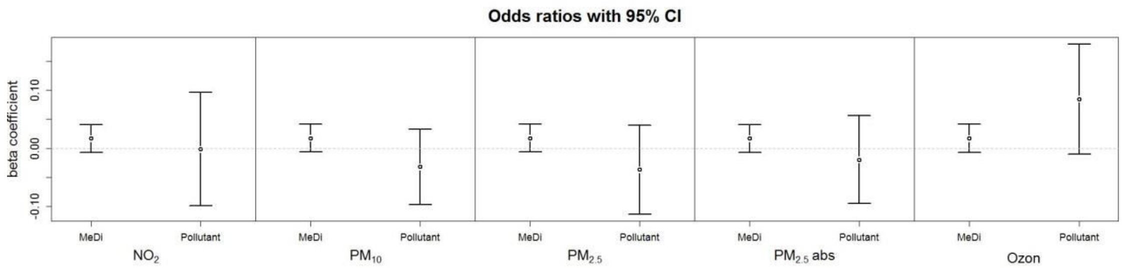
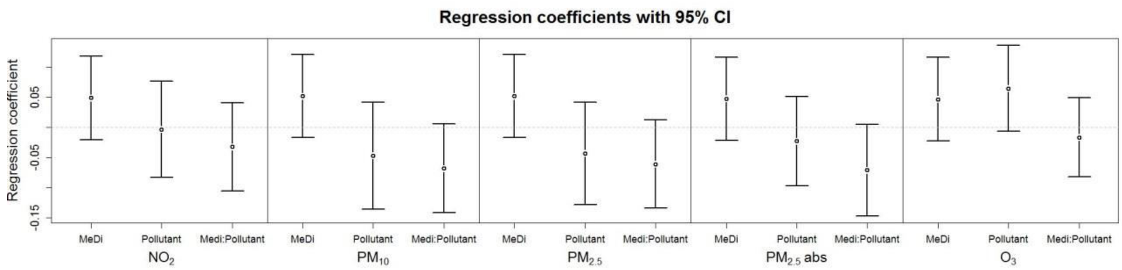


Figure 11: Z-score coarse wrinkles, interaction term



3.2.5 Sensitivity analyses

We also conducted some sensitivity analyses. (For detailed information see chapter 2.6.3). We stratified the population into a rural group and an urban group, a smoking and non-smoking group, changed the cut offs for severe skin aging and established an antioxidant score instead of the mMEDI. With these subgroups all analyses were repeated.

3.2.5.1 Urban and rural subgroups

It seems as if most of the significant effects were driven by the rural subgroup.

The marring effect of the mMEDI was only visible in the rural subgroup for the outcomes *Pigment spots on cheeks* and *Wrinkles on upper lip* (see table 20). The urban subgroup did not show these effects. The urban subgroup in contrast was the only group that exhibited the protective effect of mMEDI on *Cigarette paper like skin on the back of the hands*.

The separation into subgroups revealed that protective effects of air pollutants were driven mostly by the rural subgroup. This could be seen in the rural models for *Teleangiectasia on cheeks, Lax appearance (eyelids), Lax appearance (lower part of the face)* and *Cigarette paper like skin on the back of the hands*. In comparison to table 21, the urban subgroup did not show any tendencies regarding the air pollutants effects.

Table 20: Rural

Outcomes		NO2		PM10		PM2.5		PM2.5abs		O3	
		OR	p-value	OR	p-value	OR	p-value	OR	p-value	OR	p-value
Pigment spots											
Cheeks (numeric)	mMEDI	1,107	0,026	1,114	0,019	1,115	0,018	1,111	0,021	1,107	0,025
	Air pollutant	1,321	0,333	0,751	0,455	0,694	0,353	0,943	0,835	1,468	0,132
Cheeks (photoreference scale)	mMEDI	1,045	0,296	1,043	0,317	1,043	0,316	1,043	0,319	1,042	0,330
	Air pollutant	0,732	0,250	0,801	0,534	0,810	0,563	0,761	0,300	0,944	0,806
Forehead (numeric)	mMEDI	1,063	0,182	1,071	0,137	1,072	0,130	1,067	0,155	1,067	0,155
	Air pollutant	1,586	0,122	0,671	0,318	0,588	0,194	1,040	0,893	1,028	0,913
Forehead (photoreference scale)	mMEDI	1,019	0,660	1,020	0,644	1,019	0,649	1,021	0,626	1,019	0,652
	Air pollutant	1,305	0,329	1,333	0,422	1,346	0,416	1,237	0,419	1,310	0,256
Forearm topsides	mMEDI	0,980	0,657	0,977	0,609	0,977	0,614	0,978	0,627	0,975	0,579
	Air pollutant	0,854	0,594	1,155	0,713	1,097	0,816	0,985	0,958	1,325	0,258
Back of the hands	mMEDI	0,983	0,685	0,982	0,656	0,982	0,659	0,983	0,671	0,983	0,672
	Air pollutant	1,007	0,981	1,224	0,575	1,186	0,642	1,148	0,607	1,104	0,670
Wrinkles											
Forehead	mMEDI	1,060	0,159	1,061	0,157	1,061	0,154	1,058	0,172	1,058	0,173
	Air pollutant	0,790	0,378	0,706	0,323	0,684	0,292	0,888	0,648	0,920	0,716
Between eyebrows	mMEDI	1,081	0,071	1,078	0,080	1,078	0,082	1,080	0,074	1,079	0,077
	Air pollutant	0,918	0,756	1,208	0,602	1,252	0,542	1,021	0,938	1,144	0,567
Crow´s feet region	mMEDI	1,039	0,373	1,038	0,381	1,038	0,386	1,041	0,353	1,040	0,355
	Air pollutant	1,509	0,154	1,888	0,087	1,904	0,089	1,765	0,046	1,536	0,071
Under the eyes	mMEDI	1,038	0,367	1,039	0,359	1,040	0,346	1,037	0,378	1,034	0,421
	Air pollutant	0,865	0,591	0,760	0,438	0,690	0,306	0,887	0,648	1,378	0,163
Upper lip	mMEDI	1,101	0,028	1,098	0,034	1,097	0,035	1,103	0,025	1,103	0,025
	Air pollutant	1,390	0,240	2,074	0,047	2,053	0,054	1,328	0,296	1,251	0,349
Nasolabialfolds	mMEDI	1,018	0,670	1,015	0,725	1,014	0,731	1,015	0,709	1,015	0,710
	Air pollutant	0,909	0,720	1,284	0,474	1,304	0,457	1,254	0,380	1,154	0,530
Others											
Solar elastosis on cheeks	mMEDI	1,038	0,383	1,037	0,394	1,037	0,398	1,040	0,357	1,039	0,377
	Air pollutant	1,566	0,104	2,221	0,029	2,166	0,038	1,763	0,034	1,637	0,044
Teleangiectasia on cheeks	mMEDI	1,015	0,730	1,020	0,650	1,020	0,639	1,014	0,738	1,012	0,772
	Air pollutant	0,596	0,068	0,314	0,002	0,318	0,003	0,446	0,004	0,621	0,045
Even pigmentation on forearm insides	mMEDI	1,013	0,790	1,016	0,728	1,015	0,755	1,020	0,668	1,023	0,627
	Air pollutant	2,308	0,009	2,681	0,019	2,970	0,012	1,746	0,075	1,140	0,617
Lax appearance (eyelids)	mMEDI	1,045	0,307	1,047	0,284	1,049	0,273	1,043	0,332	1,041	0,349
	Air pollutant	0,604	0,074	0,460	0,037	0,427	0,025	0,633	0,097	0,821	0,414
Lax appearance (Lower part of the face)	mMEDI	0,975	0,546	0,977	0,574	0,978	0,591	0,973	0,511	0,970	0,467
	Air pollutant	0,609	0,071	0,448	0,026	0,423	0,020	0,586	0,045	0,975	0,914
Cigarette paper like skin on the back of the hands	mMEDI	0,959	0,349	0,958	0,341	0,958	0,346	0,954	0,292	0,952	0,266
	Air pollutant	0,457	0,009	0,246	0,001	0,255	0,001	0,484	0,015	1,098	0,708

Adjusted for: social status, urban/rural, hormone replacement therapy, smoking status, sunbed use, passive smoking, heating with fossil fuels, packyears, age, BMI, skin type; NO2: Nitrogen dioxide, PM10/PM2.5: Particulate matter suspended in the air with a diameter of 10µm/ 2.5µm, PM2.5coarse: Proxy for elemental carbon, O3: Ozone, OR: Odds ratio, numbers written in blue mark significant results to a p-value of 0.05

Table 21: Urban

Outcomes		NO_2		PM_{10}		$PM_{2.5}$		$PM_{2.5abs}$		O_3	
		OR	p-value	OR	p-value	OR	p-value	OR	p-value	OR	p-value
Pigment spots											
Cheeks (numeric)	mMEDI	1,062	0,081	1,061	0,089	1,060	0,091	1,060	0,093	1,062	0,083
	Air pollutant	1,128	0,422	1,003	0,983	0,958	0,772	0,974	0,800	1,153	0,344
Cheeks (photoreference scale)	mMEDI	1,052	0,149	1,050	0,166	1,052	0,155	1,052	0,152	1,051	0,160
	Air pollutant	1,082	0,615	0,950	0,709	1,045	0,776	1,035	0,750	0,952	0,749
Forehead (numeric)	mMEDI	1,057	0,121	1,058	0,118	1,056	0,127	1,057	0,122	1,058	0,120
	Air pollutant	1,117	0,476	1,122	0,397	1,087	0,588	1,058	0,600	1,299	0,103
Forehead (photoreference scale)	mMEDI	1,061	0,085	1,060	0,092	1,060	0,089	1,061	0,089	1,062	0,081
	Air pollutant	1,037	0,811	0,952	0,712	0,954	0,748	0,994	0,955	1,280	0,106
Forearm topsides	mMEDI	1,043	0,250	1,040	0,285	1,040	0,286	1,041	0,277	1,040	0,284
	Air pollutant	1,359	0,074	1,040	0,783	1,104	0,534	1,074	0,540	1,152	0,370
Back of the hands	mMEDI	1,033	0,334	1,034	0,326	1,034	0,325	1,033	0,332	1,034	0,323
	Air pollutant	0,984	0,916	1,024	0,855	1,053	0,723	0,997	0,974	1,124	0,430
Wrinkles											
Forehead	mMEDI	1,005	0,879	1,002	0,956	1,003	0,939	1,005	0,891	1,003	0,933
	Air pollutant	1,217	0,186	0,948	0,682	0,990	0,946	1,099	0,360	1,053	0,723
Between eyebrows	mMEDI	1,008	0,813	1,007	0,834	1,008	0,817	1,007	0,824	1,009	0,779
	Air pollutant	0,915	0,548	0,864	0,252	0,777	0,081	0,924	0,433	1,071	0,636
Crow´s feet region	mMEDI	0,999	0,977	0,999	0,972	0,999	0,980	0,996	0,919	0,998	0,962
	Air pollutant	1,029	0,851	1,011	0,934	1,069	0,656	0,912	0,367	0,959	0,781
Under the eyes	mMEDI	1,040	0,243	1,039	0,263	1,039	0,254	1,040	0,246	1,039	0,257
	Air pollutant	1,130	0,412	0,991	0,943	1,112	0,467	1,066	0,533	1,080	0,604
Upper lip	mMEDI	1,046	0,182	1,048	0,165	1,048	0,164	1,048	0,163	1,048	0,167
	Air pollutant	0,881	0,396	1,013	0,920	1,051	0,733	1,020	0,848	0,981	0,894
Nasolabialfolds	mMEDI	0,991	0,780	0,991	0,789	0,989	0,745	0,992	0,813	0,989	0,732
	Air pollutant	1,203	0,226	1,202	0,169	1,183	0,260	1,198	0,092	1,124	0,427
Others											
Solar elastosis on cheeks	mMEDI	1,001	0,985	1,000	0,992	1,000	0,996	1,001	0,982	0,999	0,982
	Air pollutant	1,178	0,283	1,120	0,391	1,290	0,088	1,117	0,300	1,117	0,466
Teleangiectasia on cheeks	mMEDI	0,942	0,095	0,937	0,072	0,941	0,088	0,938	0,074	0,941	0,088
	Air pollutant	1,020	0,898	0,790	0,102	0,904	0,522	0,842	0,133	0,855	0,313
Even pigmentation on forearm insides	mMEDI	1,001	0,982	1,004	0,922	1,002	0,954	1,005	0,899	1,002	0,964
	Air pollutant	0,838	0,294	1,081	0,613	0,922	0,631	1,112	0,388	0,849	0,364
Lax appearance (eyelids)	mMEDI	1,049	0,181	1,047	0,201	1,048	0,190	1,047	0,198	1,047	0,194
	Air pollutant	1,127	0,451	0,951	0,707	1,074	0,641	0,979	0,844	0,982	0,908
Lax appearance (Lower part of the face)	mMEDI	0,990	0,759	0,990	0,771	0,991	0,775	0,990	0,774	0,990	0,774
	Air pollutant	0,957	0,764	0,997	0,980	1,023	0,873	1,003	0,976	1,019	0,899
Cigarette paper like skin on the back of the hands	mMEDI	0,907	**0,017**	0,903	**0,014**	0,907	**0,017**	0,906	**0,015**	0,907	**0,017**
	Air pollutant	1,019	0,914	0,805	0,154	0,983	0,920	0,917	0,457	0,940	0,719

Adjusted for: social status, urban/rural, hormone replacement therapy, smoking status, sunbed use, passive smoking, heating with fossil fuels, packyears, age, BMI, skin type; NO2: Nitrogen dioxide, PM10/PM2.5: Particulate matter suspended in the air with a diameter of 10µm/ 2.5µm, PM2.5coarse: Proxy for elemental carbon, O3: Ozone, OR: Odds ratio, numbers written in blue mark significant results to a p-value of 0.05

3.2.5.2 Smoker and non-smoker subgroups

In general, more significant effects could be seen in the subgroup non-smokers (women, who had never smoked, table 22). For the smokers (women, who had ever smoked in their life or were current smokers, table 21) the results did not show any significant effects.

The marring tendency of the mMEDI could be seen in both subgroups and changed slightly depending on the outcome. The non-smokers were the group, being responsible for the protective effect of mMEDI in all earlier calculations.

In smokers, the only protective effect of air pollutants could be seen for *Wrinkles between eyebrows*, harmful tendencies were not to be seen at all. In non-smokers air pollutants had a protective effect for *Teleangiectasia on cheeks* and *Cigarette paper like skin on the back of the hands* and a harmful effect for W*rinkles on upper lip*.

Table 22: Smoker

Outcomes		NO_2		PM_{10}		$PM_{2.5}$		$PM_{2.5abs}$		O_3	
		OR	p-value	OR	p-value	OR	p-value	OR	p-value	OR	p-value
Pigment spots											
Cheeks (numeric)	mMEDI	1,156	0,033	1,162	0,026	1,163	0,025	1,161	0,026	1,158	0,031
	Air pollutant	1,190	0,489	0,889	0,522	0,872	0,546	0,888	0,597	1,015	0,947
Cheeks (photoreference scale)	mMEDI	1,130	0,071	1,136	0,058	1,131	0,068	1,134	0,062	1,151	0,043
	Air pollutant	1,258	0,373	0,985	0,936	1,131	0,587	1,063	0,786	0,815	0,381
Forehead (numeric)	mMEDI	1,208	0,013	1,211	0,012	1,215	0,010	1,210	0,012	1,210	0,013
	Air pollutant	1,029	0,917	0,867	0,476	0,807	0,378	0,899	0,679	0,973	0,911
Forehead (photoreference scale)	mMEDI	0,952	0,429	0,957	0,480	0,960	0,519	0,953	0,441	0,945	0,372
	Air pollutant	0,961	0,873	0,798	0,200	0,757	0,196	0,892	0,606	1,114	0,622
Forearm topsides	mMEDI	1,120	0,107	1,121	0,106	1,117	0,118	1,127	0,088	1,108	0,146
	Air pollutant	1,234	0,436	1,309	0,181	1,342	0,227	1,002	0,994	1,357	0,178
Back of the hands	mMEDI	1,096	0,149	1,087	0,186	1,089	0,177	1,086	0,188	1,075	0,258
	Air pollutant	0,654	0,115	0,939	0,718	0,883	0,566	0,946	0,799	1,230	0,353
Wrinkles											
Forehead	mMEDI	1,030	0,631	1,036	0,567	1,038	0,549	1,030	0,628	1,022	0,726
	Air pollutant	0,950	0,835	0,784	0,173	0,774	0,244	0,934	0,749	1,126	0,579
Between eyebrows	mMEDI	0,886	0,072	0,897	0,101	0,895	0,095	0,893	0,089	0,899	0,111
	Air pollutant	1,671	0,049	1,088	0,636	1,155	0,506	1,307	0,247	1,003	0,988
Crow´s feet region	mMEDI	0,957	0,531	0,959	0,553	0,961	0,571	0,957	0,532	0,959	0,556
	Air pollutant	1,229	0,448	0,933	0,716	0,880	0,583	1,088	0,721	0,953	0,842
Under the eyes	mMEDI	0,967	0,588	0,974	0,664	0,976	0,687	0,970	0,614	0,966	0,572
	Air pollutant	1,109	0,665	0,863	0,382	0,835	0,384	1,019	0,928	1,092	0,673
Upper lip	mMEDI	1,069	0,290	1,065	0,322	1,063	0,334	1,066	0,312	1,071	0,287
	Air pollutant	1,045	0,859	1,239	0,231	1,221	0,362	1,189	0,444	1,004	0,986
Nasolabialfolds	mMEDI	1,003	0,964	1,010	0,875	1,013	0,841	1,006	0,930	1,024	0,717
	Air pollutant	1,227	0,426	0,942	0,749	0,871	0,558	1,111	0,657	0,738	0,195
Others											
Solar elastosis on cheeks	mMEDI	0,946	0,388	0,951	0,428	0,950	0,418	0,949	0,408	0,940	0,337
	Air pollutant	1,302	0,298	1,093	0,621	1,125	0,598	1,233	0,346	1,297	0,251
Teleangiectasia on cheeks	mMEDI	0,958	0,538	0,965	0,603	0,961	0,564	0,967	0,629	0,959	0,550
	Air pollutant	1,172	0,560	0,921	0,665	1,031	0,898	0,831	0,440	1,078	0,751
Even pigmentation on forearm insides	mMEDI	0,967	0,619	0,965	0,605	0,960	0,545	0,970	0,655	0,994	0,934
	Air pollutant	1,367	0,278	1,401	0,076	1,556	0,054	1,275	0,334	0,705	0,171
Lax appearance (eyelids)	mMEDI	1,024	0,704	1,039	0,552	1,041	0,537	1,028	0,662	1,026	0,690
	Air pollutant	1,177	0,510	0,741	0,103	0,735	0,171	1,013	0,951	1,059	0,797
Lax appearance (Lower part of the face)	mMEDI	0,996	0,945	0,998	0,974	1,002	0,980	0,992	0,900	0,996	0,955
	Air pollutant	0,896	0,664	0,805	0,221	0,743	0,173	1,073	0,749	0,929	0,736
Cigarette paper like skin on the back of the hands	mMEDI	0,925	0,274	0,925	0,293	0,929	0,315	0,926	0,280	0,929	0,305
	Air pollutant	0,726	0,246	0,539	0,004	0,557	0,020	0,666	0,081	0,791	0,331

Table 23: Non-smoker

Outcomes		NO_2		PM_{10}		$PM_{2.5}$		$PM_{2.5abs}$		O_3	
		OR	p-value	OR	p-value	OR	p-value	OR	p-value	OR	p-value
Pigment spots											
Cheeks (numeric)	mMEDI	1,072	**0,020**	1,072	**0,021**	1,072	**0,021**	1,072	**0,020**	1,075	**0,016**
	Air pollutant	1,104	0,431	1,031	0,743	1,004	0,969	1,024	0,823	1,254	0,082
Cheeks (photoreference scale)	mMEDI	1,042	0,159	1,042	0,159	1,042	0,160	1,043	0,153	1,041	0,169
	Air pollutant	1,182	0,196	1,000	1,000	1,019	0,854	1,099	0,392	0,910	0,463
Forehead (numeric)	mMEDI	1,049	0,119	1,048	0,121	1,049	0,119	1,049	0,118	1,051	0,103
	Air pollutant	1,163	0,244	0,968	0,740	0,925	0,450	1,060	0,601	1,217	0,144
Forehead (photoreference scale)	mMEDI	1,068	**0,026**	1,068	**0,026**	1,068	**0,026**	1,068	**0,026**	1,069	**0,023**
	Air pollutant	1,165	0,219	0,999	0,992	0,984	0,874	1,025	0,819	1,145	0,278
Forearm topsides	mMEDI	1,001	0,968	1,001	0,966	1,001	0,972	1,002	0,953	1,002	0,953
	Air pollutant	1,195	0,203	1,025	0,804	1,046	0,672	1,117	0,355	1,040	0,766
Back of the hands	mMEDI	1,006	0,824	1,006	0,824	1,006	0,821	1,006	0,833	1,007	0,819
	Air pollutant	1,027	0,831	0,981	0,838	0,974	0,786	0,958	0,686	1,018	0,885
Wrinkles											
Forehead	mMEDI	1,025	0,385	1,025	0,380	1,026	0,370	1,025	0,389	1,025	0,377
	Air pollutant	1,044	0,727	0,886	0,184	0,867	0,140	0,972	0,792	1,041	0,745
Between eyebrows	mMEDI	1,058	0,053	1,058	0,052	1,057	0,053	1,057	0,054	1,059	0,048
	Air pollutant	0,898	0,387	1,028	0,761	1,028	0,781	0,952	0,643	1,103	0,431
Crow´s feet region	mMEDI	1,036	0,221	1,036	0,224	1,036	0,231	1,036	0,226	1,038	0,205
	Air pollutant	1,080	0,544	1,115	0,240	1,142	0,178	0,962	0,713	1,124	0,349
Under the eyes	mMEDI	1,045	0,122	1,045	0,120	1,045	0,119	1,045	0,125	1,048	0,100
	Air pollutant	0,910	0,447	0,940	0,495	0,945	0,560	0,944	0,594	1,288	**0,044**
Upper lip	mMEDI	1,067	**0,025**	1,066	**0,025**	1,066	**0,027**	1,068	**0,023**	1,067	**0,023**
	Air pollutant	1,072	0,574	1,187	0,062	1,231	**0,033**	1,132	0,236	1,061	0,630
Nasolabialfolds	mMEDI	1,000	0,990	1,000	0,995	1,000	0,997	1,001	0,966	1,002	0,938
	Air pollutant	1,127	0,334	1,064	0,494	1,034	0,732	1,150	0,191	1,164	0,215
Others											
Solar elastosis on cheeks	mMEDI	1,037	0,216	1,037	0,220	1,036	0,224	1,037	0,211	1,038	0,200
	Air pollutant	1,187	0,179	1,122	0,220	1,128	0,228	1,099	0,392	1,140	0,306
Teleangiectasia on cheeks	mMEDI	0,963	0,210	0,964	0,218	0,965	0,230	0,962	0,193	0,959	0,164
	Air pollutant	0,941	0,649	0,725	**0,001**	0,744	**0,004**	0,796	0,058	0,715	**0,009**
Even pigmentation on forearm insides	mMEDI	1,026	0,424	1,026	0,422	1,026	0,427	1,028	0,403	1,027	0,406
	Air pollutant	0,881	0,376	1,210	0,079	1,156	0,203	1,178	0,206	1,067	0,654
Lax appearance (eyelids)	mMEDI	1,048	0,123	1,047	0,124	1,047	0,129	1,048	0,122	1,047	0,133
	Air pollutant	1,150	0,299	1,024	0,809	1,084	0,433	1,036	0,754	0,899	0,422
Lax appearance (Lower part of the face)	mMEDI	0,971	0,308	0,972	0,320	0,972	0,329	0,970	0,291	0,973	0,331
	Air pollutant	0,804	0,081	0,851	0,083	0,849	0,097	0,813	0,056	1,105	0,418
Cigarette paper like skin on the back of the hands	mMEDI	0,927	**0,020**	0,924	**0,018**	0,926	**0,021**	0,925	**0,018**	0,926	**0,020**
	Air pollutant	0,914	0,529	0,682	**0,000**	0,698	**0,002**	0,803	0,065	0,921	0,556

3.2.5.3 Antioxidant score

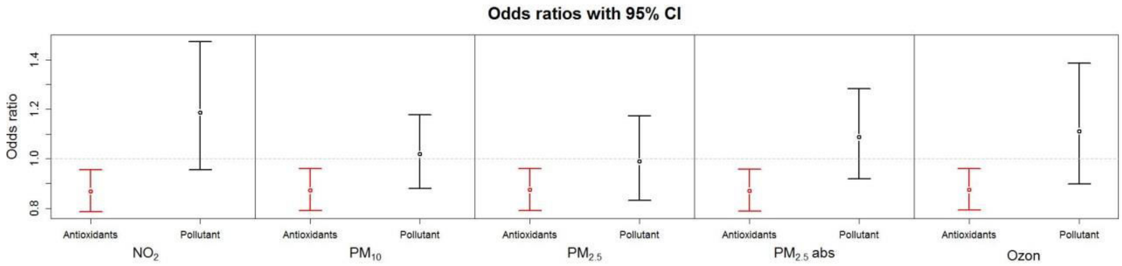
Moreover, we used an antioxidant score containing just fruits, raw vegetables and cooked vegetables. Here we found more protective effects of nutrition in the different models in comparison to the mMEDI. However, there were still many results that showed a harmful tendency.

Table 24 shows the effect of the antioxidant score on the different outcomes. A protective effect of antioxidants was seen for the outcome *Pigment spots on forehead (numeric)* in all air pollutant models, which was highly significant. This is the only model where nutrition revealed a significant protective effect on skin aging.

Table 24: Antioxidant score

Outcomes		NO2 OR	NO2 p-value	PM10 OR	PM10 p-value	PM2.5 OR	PM2.5 p-value	PM2.5abs OR	PM2.5abs p-value	O3 OR	O3 p-value
Pigment spots											
Cheeks (numeric)	Antioxidant score	0,970	0,491	0,970	0,493	0,971	0,498	0,971	0,511	0,977	0,591
	Air pollutant	1,244	0,039	1,116	0,122	1,115	0,195	1,143	0,103	1,133	0,236
Cheeks (photoreference scale)	Antioxidant score	0,996	0,934	0,999	0,981	0,997	0,951	0,998	0,961	0,999	0,990
	Air pollutant	1,187	0,115	1,029	0,680	1,067	0,434	1,090	0,302	0,901	0,320
Forehead (numeric)	Antioxidant score	0,869	0,005	0,873	0,006	0,874	0,006	0,871	0,005	0,874	0,007
	Air pollutant	1,187	0,121	1,019	0,802	0,989	0,895	1,087	0,326	1,112	0,337
Forehead (photoreference scale)	Antioxidant score	0,983	0,683	0,986	0,735	0,986	0,745	0,984	0,715	0,986	0,745
	Air pollutant	1,100	0,359	0,972	0,685	0,958	0,604	0,998	0,981	1,139	0,202
Forearm topsides	Antioxidant score	1,014	0,768	1,015	0,755	1,014	0,768	1,016	0,741	1,018	0,701
	Air pollutant	1,145	0,248	1,044	0,569	1,066	0,479	1,042	0,648	1,140	0,223
Back of the hands	Antioxidant score	0,956	0,292	0,954	0,273	0,954	0,277	0,955	0,281	0,957	0,300
	Air pollutant	0,998	0,982	1,041	0,555	1,034	0,683	1,036	0,660	1,076	0,467
Wrinkles											
Forehead	Antioxidant score	0,995	0,901	1,002	0,965	1,003	0,948	0,996	0,933	0,994	0,884
	Air pollutant	0,930	0,480	0,825	0,005	0,800	0,006	0,885	0,128	1,052	0,609
Between eyebrows	Antioxidant score	1,008	0,852	1,008	0,855	1,008	0,860	1,009	0,843	1,008	0,852
	Air pollutant	0,960	0,695	0,984	0,813	0,989	0,895	0,951	0,533	1,084	0,423
Crow´s feet region	Antioxidant score	1,017	0,705	1,018	0,688	1,018	0,693	1,020	0,654	1,017	0,697
	Air pollutant	0,975	0,808	0,967	0,634	0,972	0,730	0,879	0,107	1,105	0,328
Under the eyes	Antioxidant score	0,952	0,245	0,954	0,271	0,954	0,271	0,952	0,248	0,952	0,250
	Air pollutant	0,901	0,313	0,897	0,111	0,893	0,159	0,909	0,235	1,258	0,023
Upper lip	Antioxidant score	0,963	0,377	0,958	0,311	0,956	0,295	0,962	0,361	0,965	0,399
	Air pollutant	1,042	0,692	1,148	0,046	1,195	0,029	1,082	0,324	1,067	0,516
Nasolabialfolds	Antioxidant score	1,011	0,794	1,011	0,797	1,012	0,779	1,010	0,813	1,016	0,707
	Air pollutant	1,247	0,035	1,119	0,102	1,100	0,238	1,234	0,011	1,005	0,964
Others											
Solar elastosis on cheeks	Antioxidant score	1,035	0,426	1,036	0,406	1,036	0,411	1,037	0,402	1,039	0,367
	Air pollutant	1,113	0,307	1,020	0,780	1,029	0,732	1,021	0,797	1,171	0,126
Teleangiectasia on cheeks	Antioxidant score	1,025	0,573	1,037	0,414	1,036	0,428	1,031	0,493	1,020	0,652
	Air pollutant	0,875	0,225	0,748	0,000	0,748	0,001	0,758	0,002	0,837	0,086
Even pigmentation on forearm insides	Antioxidant score	1,063	0,222	1,053	0,300	1,054	0,297	1,058	0,258	1,063	0,225
	Air pollutant	1,021	0,865	1,235	0,009	1,237	0,024	1,206	0,057	0,925	0,512
Lax appearance (eyelids)	Antioxidant score	0,940	0,163	0,944	0,192	0,942	0,177	0,942	0,179	0,942	0,180
	Air pollutant	1,140	0,235	0,981	0,786	1,026	0,761	1,026	0,755	0,945	0,595
Lax appearance (Lower part of the face)	Antioxidant score	1,052	0,234	1,055	0,213	1,056	0,205	1,051	0,244	1,050	0,256
	Air pollutant	0,864	0,162	0,882	0,072	0,857	0,061	0,923	0,320	1,075	0,472
Cigarette paper like skin on the back of the hands	Antioxidant score	1,018	0,706	1,038	0,451	1,037	0,460	1,022	0,651	1,010	0,837
	Air pollutant	0,766	0,022	0,643	0,000	0,627	0,000	0,716	0,000	0,955	0,686

Figure 12: Antioxidant score, pigment spots on the forehead (numeric)



3.2.5.4 New Cut-Offs

We changed the cut-offs for the binary skin variables. For pigment spots variables the separation into few skin aging and severe skin aging was set at the middle category (see chapter 2). We evaluated 0-10 pigment spots as few skin aging and all numbers higher than 10 as severe skin aging. The effect of the mMEDI score did not change in these models, but the harmful effect of air pollution enhanced with clearer tendencies than before and highly significant results (p-value of 0.002 and an OR of 1.96 for *Pigment spots on cheeks (numeric)*).

Table 24 presents the new results for the pigment spot variables. The effects in the single models became stronger with the new cut-offs. For *Pigment spots on cheeks (numeric)*, significant harmful results occurred for all air pollutants except O_3, whereas before, only NO_2 showed a significant result. For *Pigment spots on forehead (numeric)* the results were not significant. For the outcomes *Pigment spots on cheeks (numeric)* and *Pigment spots on forehead (photo-reference scale)* the results presented clearer tendencies as well. With the old cut offs, no significant effects could be detected (see chapter 3.2.1).

Table 25: New cut-offs

Outcomes		NO_2		PM_{10}		$PM_{2.5}$		$PM_{2.5abs}$		O_3	
		OR	p-value	OR	p-value	OR	p-value	OR	p-value	OR	p-value
Pigment spots											
Cheeks (numeric)	mMEDI	1,081	**0,059**	1,085	**0,047**	1,084	**0,052**	1,085	**0,046**	1,087	**0,042**
	Air pollutant	1,960	**0,002**	1,295	**0,029**	1,379	**0,020**	1,565	**0,009**	0,922	0,629
Cheeks (photoreference scale)	mMEDI	1,069	0,106	1,072	0,088	1,071	0,095	1,072	0,087	1,075	0,077
	Air pollutant	1,925	**0,002**	1,289	**0,031**	1,370	**0,021**	1,571	**0,008**	0,960	0,805
Forehead (numeric)	mMEDI	1,093	**0,003**	1,094	**0,003**	1,093	**0,003**	1,094	**0,003**	1,094	**0,003**
	Air pollutant	1,160	0,234	1,037	0,655	1,043	0,656	1,047	0,626	1,142	0,249
Forehead (photoreference scale)	mMEDI	1,078	**0,011**	1,080	**0,009**	1,079	**0,009**	1,080	**0,009**	1,080	**0,009**
	Air pollutant	1,227	**0,098**	1,048	0,548	1,046	0,628	1,095	0,332	1,072	0,543
Forearm topsides	mMEDI	1,135	**0,029**	1,140	**0,024**	1,138	**0,027**	1,139	**0,025**	1,148	**0,018**
	Air pollutant	1,569	0,106	1,150	0,385	1,235	0,265	1,316	0,217	0,755	0,253
Back of the hands	mMEDI	1,007	0,860	1,010	0,796	1,011	0,774	1,008	0,827	1,008	0,831
	Air pollutant	1,157	0,352	0,891	0,246	0,856	0,192	0,973	0,815	1,001	0,996

3.2.6 Research question 1

We did not find the expected association between the mMEDI and skin aging. We presumed the mMEDI to positively influence skin aging but found rather the contrary for most skin aging signs (see above). Only for the outcome *Cigarette paper like skin on the back of the hands* a protective effect of mMEDI in all 5 models could be seen (see Table 30, Figure 21). All other skin aging outcomes showed either a harmful effect of mMEDI or an effect fluctuating slightly around an odds ratio of 1.

Since the first hypothesis could not be verified we made a sensitivity analysis for mMEDI and established the antioxidant score containing just fruits and vegetables (see chapter 2.3.2). We noticed a significant protective effect of the antioxidant score on *Pigment spots on the forehead (numeric)* (see Table 94, Figure 85). But for the other outcomes the antioxidant score revealed different tendencies. In some models it showed a protective tendency (*Wrinkles under eyes* and *Wrinkles on upper lip*, *Pigment spots on back of the hands*, *Pigment spots on cheeks (photo-reference scale)*, *Pigment spots on forehead (photo-reference scale)* and *lax appearance (eyelids)*. In other models it showed a harmful tendency (*Pigment spots on forearms*, *Wrinkles on eyebrows*, *Wrinkles in crow´s feet area, Wrinkles on nasolabialfolds, lax appearance (lower part of the face), Even pigmentation on forearm insides, Solar elastosis on cheeks, Teleangiectasia and Cigarette paper like skin on the back of the hands*).

Neither the mMEDI score nor the antioxidant score showed a clear protective effect of nutrition on skin aging. Rather an adverse effect of the mMEDI on skin aging could be detected.

3.2.7 Research question 2

We were able to reproduce the findings that traffic related air pollution has a negative impact on skin aging, especially for particles. $PM_{2.5}$ and PM_{10} exposed a significant harmful effect on *Wrinkles on upper lip* (Table 23, Figure 14) as well as on the *Even pigmentation on forearm insides* (Table 27, Figure 18). All findings were for the full model, adjusted for all covariates. Since the earlier studies (2) did not include UV covariates, we repeated our calculations without the covariates UV index and sun protection factor. NO_2 and $PM_{2.5\ absorbance}$ were significantly harmful for the outcome *Wrinkles on nasolabialfolds* (Table 34, Figure 25) as found by previous analyses as well. Nevertheless, contradictory results were pointed out, showing a protective effect of air pollution on skin aging in *Teleangiectasia*, *lax appearance (lower part of the face)* and *Cigarette paper like skin on the back of the hands*.

Our hypothesis, that air pollution has negative impacts on skin aging could be confirmed partly.

3.2.8 Research question 3

In order to see the modifying effect of mMEDI on air pollution induced skin aging, we included an interaction variable between the considered air pollutant and mMEDI (see chapter 2.6.3). We detected that all significant effects (of air pollutants as well as mMEDI) vanished.

All z-score models pointed at a positive correlation between mMEDI and skin aging (the higher the mMEDI the more skin aging), but interestingly the interaction term showed a protective effect on coarse wrinkles, when both mMEDI and air pollution values were high, which was borderline-significant for the models PM_{10} and $PM_{2.5\ absorbance}$ (Table 59, Figure 50).

4 Discussion

This study aimed at investigating the protective role of the Mediterranean diet on skin aging in elderly women exposed to long-term air pollution. For the first research question it was hypothesized that the mMEDI would have a protective effect on skin aging. The second research question should show the harmful impact of air pollution on skin aging and the third research question should assess an attenuating impact of mMEDI on air pollution induced skin aging. The results of this study show that air pollution and especially particles have a negative impact on some skin aging outcomes, but seem to have a protective effect on others. The mMEDI did not show a protective effect in all but one analysis. It rather had adverse effects. Using an interaction term, the mMEDI was not able to attenuate the effects of air pollution on skin aging in a significant way. There are attenuating tendencies, but no significances.

4.1 Adherence to the Mediterranean diet and skin aging

Since the Mediterranean diet is one of the most popular diets in the world we expected a positive impact for this way of nutrition on skin aging. The main principles of skin aging are the shortening of telomeres and the formation of reactive oxygen species (ROS). Studies found a positive correlation between the Mediterranean diet and the telomere length (55, 56). Other studies attribute a large anti-oxidative capacity to the Mediterranean diet (57-59). Thus, the Mediterranean diet could be expected to act beneficial on skin aging. Unfortunately, this study could not prove this hypothesis. We did not see a protective link between the adherence to the Mediterranean diet and skin aging. To our knowledge this was the first study on the Mediterranean diet on skin aging.

Considering healthy ways of nutrition in general, only few studies examined the impact of healthy diets on skin aging until now. One study examined whether nutrient intakes were correlated with skin wrinkling on sun exposed site and found less appearance of actinic keratosis with higher intake of vegetables, olive oil, legumes and fish. Consumption of meat, butter, dairy and sugar seemed to be harmful (51). In our study we did not emphasize single healthy nutrients more than others. Our mMEDI score according to Panagiotakos differentiated only between healthy nutrients and unhealthy ones. Taking rice as an example it received the same number of points in the score as raw vegetables. But rice as well as potatoes and wheat contain much more carbohydrates than raw vegetables and another study could show that high consumption of carbohydrates has adverse impacts on skin age (52). Additionally, fresh fruits and vegetables contain a higher amount of vitamins. Especially Vitamin C was proven to have a protective effect on wrinkles and senile dryness (52) and by that acts protective on skin aging. Also cereal products contain vitamins and minerals, but those do not play a huge role in skin aging. Another point is that fresh fruit and vegetables need to be consumed immediately, they cannot be stored as rice and potatoes to be eaten months later. Besides, fresh nutrients contain the most vitamins and minerals and this amount declines already after a few days of storing. Unfortunately, olive oil and legumes were not taken into account for this study, since they were only part of our questionnaire in later follow ups. Especially the moderate intake of olive oil seems to have a huge impact on skin aging proved by many studies (50, 57, 73-76) and also the Mediterranean nutrition pyramid puts emphasis on olive oil as a main source of fat. The lack of olive oil might have negatively impacted our results, but on the other hand, one teaspoon of olive oil per meal per person is the healthy amount according to the Mediterranean diet pyramid. One teaspoon is very few and very difficult to dose in daily life. When consumed in higher amounts, which is probably the case in daily life, then the positive effects vanish, since high intake of fat was proven to act controversial on skin aging (52-54). Furthermore, pure virgin olive oil is very expensive and many of the olive oils in cheap discounters are mixed oils with only a small amount of olive oil and large amounts of other vegetable oils like sunflower or raps. Those on the other hand were not proven to be beneficial for skin health. It would have been difficult for this study to include olive oil, because the quality of different oils differs and the mMEDI score was based only on self-report. According to Panagiotakos, sugar and sweets were not part of the MEDI, therefore they were not evaluated in this study. Other studies on different diets on skin aging took sugar into account and found a negative impact on skin aging (Zitat). Also dairy products need to be differentiated more clearly, since it is a difference in carbohydrates, fat and sugar, based on which products are consumed. Greek yoghurts with 10% fat or fruit yoghurts containing a lot of sugar, are probably more controversial than simple basic yoghurts with 1.5% fat.

We assumed that the MEDI score of Panagiotakos might not be beneficial when considering the impact of the Mediterranean diet on skin aging. On that account, we repeated the calculations taking into account just the fresh single nutrients of raw vegetables/salad, cooked vegetables and fruits and established an antioxidant score. This score was able to show that eating more fruits and vegetables results in less pigment spots on the forehead. All other outcomes remained similar. Antioxidants were long rumored to be protective for skin aging, since they attenuate the formation of ROS, which is a central mechanism in intrinsic as well as extrinsic skin aging. But while there are several studies that prove antioxidants´ ability to protect from sunburn when consumed or topically applied (8, 77-80), clinical data that show a visible effect is missing (81). Some studies even suggest that oral supplementation of antioxidants may act harmful on health. One study found a higher probability for lung cancer and death due to cardiovascular diseases (82) and another showed higher incidence of melanoma with high doses of antioxidants (83).

Therefore, a good balance has to be found, which is probably best done by diet and not by supplementation.

On the one hand this indicates that our assumption that there need to be a gradation of beneficial nutrients in a diet score is correct. Rice and salad do not act equally positive on skin aging, otherwise we would not have seen this protective effect of antioxidants on wrinkles on the forehead. This is why the modified MEDI score according to Panagiotakos might partly explain the results, but other influencing factors need to be considered as well. Unfortunately, all other results remained controversial, which are findings that go along with the literature. Here it says as explained above, that antioxidants protect against sunburn and since the sun is the factor most responsible for extrinsic skin aging, antioxidants might only act as an indirect factor preventing skin aging.

Considering the applied nutrient scores, we conclude that the effects of the antioxidant score on skin aging are bigger than the influence of the mMEDI score on skin aging, but might just have an indirect effect and might not be as large as expected. Nevertheless, other components than the applied scores that might influence the result of this study need to be taken into account.

In other studies the healthy diet was evaluated by a large number of components. The SALIA study was set mainly to investigate the effect of air pollution on lung function. Therefore, the questionnaire was not as extensive as others with respect to diet. Especially olive oil and legumes were missing, as well as sweets and sugar. Another point is that the amount of food intake was not taken into account. Taking the food category salad/raw vegetables as an example, it could make a difference if the participants eat a big salad as a main meal, or eat salad in just small amounts as a side dish. Most of the women reported to eat salad or raw vegetables every day, but since the cohort consists of elderly German women it is likely that the way of nutrition is typically German with a division of three components: First meat or fish, second carbohydrates as potatoes, rice or pasta, and third vegetables or salad as side dish. Also the amount of meals per day and snacks in between might be an important information, since another study proved the hours between nutritional intake as relevant for skin age (54). Furthermore, the information on diet was based only on self-report. Self-report is always subjective and cannot be verified. Probands could have misunderstood the question, lied out of shame for bad nutritional habits or have dementia, which was not taken into account when judging the answers or they simply forgot what they ate.

The subjectivity might be one of the biggest problems in this study, as well as the applied Mediterranean score according to Panagiotakos. Another reason for the unexpected results might be that antioxidants, which were expected to be beneficial for skin aging, do simply not have the expected direct effect on skin aging. The rumors on antioxidants´ effects on skin aging may lie in their role as a mediator. In high doses they might delay sunburn and by that prevent skin aging as a longterm effect, but it seems as if there is no direct and short-term effect of antioxidants on skin aging.

4.2 Effects of air pollution on skin aging

Recently it has been shown that air pollution has negative impacts on the skin. In this context the SALIA study (42) was the first study that found air pollution significantly correlated with extrinsic skin aging signs, especially with pigment spots in the face and less with wrinkle formation (2). These results could be reproduced by this study. When applying z-scores it became evident that NO_2 increased the probability for a higher number of facial pigment spots as well as a bigger size. The wrinkle formation did not correlate significantly with air pollution. Another study in a German and a Chinese cohort focused on the impact of air pollution on single body sites and showed that NO_2 was significantly correlated with lentigines on the cheeks. The strongest association was found for women over 50 years. No correlation was found for lentigines on the back of the hands and forearms (4). Also this study considered 18 different skin aging outcomes in different body regions. The probability for a higher number of lentigines, which are the same as pigment spots, increased with higher NO2 levels. But in this study NO_2 and $PM_{2.5abs}$ also increased the probability for coarse wrinkles in nasolabialfolds, and $PM_{2.5}$ increased the probability for coarse wrinkles on the upper lip. PM_{10} and $PM_{2.5}$ finally increased the probability for an even pigmentation on the forearm inside, which was not found by other studies before. Another study assessed the ozone exceedance and its impact on skin aging. Positive associations between ozone exceedance and coarse wrinkles in the face were found, for one study cohort on the forehead and under the eyes, for the other study cohort in the crow´s feet area and on the upper lip (44). This study could partly reproduce these findings. A higher number of ozone exceedance days increases the probability for coarse wrinkles under the eyes. Other significant effects of O_3 on skin aging were not visible.

Most of the study results on the topic of the impact of air pollution on skin aging could be reproduced by this study. However, we found adverse effects giving the impression that air pollution acts beneficial on skin aging in some cases: All air pollutants other than NO_2 seem to be protective for teleangiectasia on the cheeks, PM_{10} and $PM_{2.5}$ seem to be protective for the lax appearance and all other than O_3 seem to be protective for cigarette paper like skin on the back of the hands. These results were unexpected, since studies usually focus on the positive results. We did not find a single study presenting adverse effects. There could be several explanations that we considered in our calculations and will discuss in the following: The solar radiation, the distinct places of residence (urban/rural) and the smoking status of the participants. Starting with the solar radiation, one reason for these results could be that UV exposure, as the most important factor in extrinsic skin aging, hides the effects of air pollution. UV radiation is known to act harmful on the skin since 1969, which was shown by Albert Kligman (22) and afterwards by several other studies (2, 3, 84). This association is strong, much stronger than the association between air pollution and skin aging. And it is very difficult to differentiate between what actually made the skin changes, since the study participants where all exposed to the sun during their whole life. The sun is an influencing factor that cannot be switched off in a human study. In order to get an impression of how other factors alone, here air pollution, impact the skin aging, another prospective study needs to be done. Two study groups need to be built, one study group is kept inside since birth for a long period of time. Solar radiation needs to be prevented completely. This group needs to be exposed to natural air pollution under laboratoric conditions. The control group needs to live a normal live, outside exposed to all possible factors that influence skin aging. Since this is completely unethical, such a study will never be possible in humans, but it may be possible in animal studies.

We repeated the calculations without the solar covariates (SPF and solar index) but the adverse effects stayed similar. Therefore, we considered the distinct places of residence (urban/rural) in the SALIA cohort a reason for the adverse effects of air pollution on skin aging. Thus, we separated the cohort into an urban and a rural group, repeated the calculations and found that all effects – the harmful and the protective – of and air pollutants on skin aging, only appeared in the rural group, but not in the urban one. An explanation could be that air pollution levels are very low in the rural areas (see chapter 3.1.2) while people in rural areas might have more outdoor activities than those in the cities and by that are exposed to the sun more frequently. Gardening, cycling and enjoying the nature are typical rural activities, while spending time indoors in restaurants, cafes, or shopping malls are typical when living close to the city center. Of course there are always exceptions, but in general it can be expected that people that love nature and calmness, settle rather in rural areas and people, who are more attracted to entertainment, prefer city centers and by that spend their time more outdoors or more indoors. This strengthens the thesis that solar radiation hides the effects of air pollution. On the other hand, people living next to a big street in the city center, have a high air pollution burden 24 hours per day. It could be assumed that these high air pollution values impact the skin aging. Unfortunately, this study could not find any significant impacts in the urban group, so that it is questionable if the effect of air pollution on skin aging is as big as expected.

Smoking is another well-known factor influencing skin aging (26-28) and it is possible that there might be an interaction with air pollution. Therefore, we repeated the calculations with a smoking group, containing all women, who ever smoked in their life and a non-smoking group with never smokers. Smoking is the second best known factor impacting skin aging. This is why the smoking status might as well be a factor hiding the effects of air pollution or being responsible for the unexpected protective tendencies of air pollution on skin aging in some analyses. We did not find a huge difference in the outcomes when separating into a smoking and non-smoking group. In this study it seems as if the smoking status is not influencing the impact of air pollution on skin aging.

4.3 The attenuating impact of mMEDI on skin aging in women exposed to air pollution

There have never been studies on the attenuating effect of neither the Mediterranean diet, nor other healthy diets, on air pollution induced skin aging before. Unfortunately, we did not find any attenuating effects of mMEDI on air pollution induced skin aging in elderly German women.

4.4 Future implications for industry, individual consumers and research

Our results are not as expected. The Mediterranean diet could not show protective effects on skin aging in this study, but showed rather adverse effects. Only a diet rich in antioxidants, attained from fruits and vegetables showed an attenuating probability for pigment spots on the forehead. This shows that eating vegetables and fruits every day is the essence of every healthy diet. The choice of the so called “unhealthy nutrients” like consuming meat or dairy products every day, seems to be secondary in terms of skin aging. Nevertheless, the Mediterranean diet has several well investigated protective effects on cardiovascular health and overweight. Therefore, the food industry should start to advertise for a healthy diet, and not only for processed food which are the most profitable for them. It should not necessarily be advertised in terms of skin aging, but in terms of general health. Cardiovascular diseases and overweight are responsible for a wide range of secondary diseases, which can be easily prevented by each individual´s choice of diet.

Further research needs to be done to get a clearer impression on the topic of healthy diets on skin aging. Therefore, it is not necessary to stick to the Mediterranean diet, but to go through many nutritional studies and choose the diet components and factors, which were proven to be beneficial or controversial on health. These are for example nutrients like olive oil and legumes as beneficial items and the consumption of fat and sugar as controversial items. Then, a gradation of item-benefits is needed. Obviously raw vegetables prevent skin aging more than pasta does, therefore a score is needed, which distinguishes not only between good and bad, but establishes more categories. In this study the consumption of dairy products was evaluated as a bad nutrient. But a gradation could distinguish between low fat products and normal fat or high fat products, since it is difficult to believe that low fat products have a negative impact on skin aging. Additionally, the meals per day and snack habits in between need to be considered, which were proven to have an impact on skin aging as well (54). Finally, also the caloric intake and the amount of nutrients, measured for example in grams per day needs to be minded. As explained before this is important in order to recognize if for example salad is eaten as a side dish or main dish every day. Another point is important in this context, namely the subjectivity of the diet questionnaire. The women in the SALIA study could have bias the results out of shame for bad eating habits or simply forgot what they ate. There was no possibility to control their statements. Therefore, objective methods considering diet are needed, which are more effortful but will provide clearer and more comparable results.

Also the effect of air pollution on skin aging needs to be examined more clearly. As explained before, there are two factors impacting the skin age even more than air pollution: Solar radiation and cigarette smoking. The results of this study give the impression that at least the high impact of the sun is hiding the effects of air pollution on skin aging. Even when applying sensitivity analyses, this study could not completely explain the adverse effects of air pollution. Since the sun is an irremovable factor, studies excluding the solar radiation completely won´t be possible in humans. In animal studies another prospective study could be done. Two study groups of mice need to be built, one study group is kept inside since birth without ever being exposed to solar radiation and cigarette smoking. This group needs to be exposed to natural air pollution as it appears outside, but under laboratoric conditions. The control group needs to be kept outside in a stable, exposed to the sun and all other environmental factors that influence skin aging. If the inside-group of mice presents extrinsic skin aging signs after a certain period of time, it can be said that these are definitely the effect of air pollution. But also this study idea has limitations, since mice skin is similar to human skin, but not comparable in all points. Another problem is the fur, which may prevent the solar radiation and also other environmental influencers to penetrate the skin in the same amount as in humans.

Nevertheless, this study allowed us to get a critical mindset about facts that seem to be unquestionable true. Only complex and especially numerous studies are able to verify a thesis and since the advantages of healthy nutrition on skin aging are not well documented, but are a common assumption, this study showed that this connection is not obvious.

4.5 Conclusion

In 2010 the SALIA study could first prove negative effects of air pollution on skin aging. This study could partly agree with these findings. Therefore, the limits for air pollution values set by the World Health Organization should decrease even further in the future, not only to benefit the skin, but also improve our cardiovascular and health.

It has long been rumored that nutrition might impact skin aging. Since study results from epidemiological studies are scarce, we tried to prove this theory. Unfortunately, our study could not prove the beneficial effects of the Mediterranean diet on skin aging. Building an antioxidant score, we could show that a diet rich in antioxidants is able to attenuate the formation of Pigment spots on the forehead. In our study we found that air pollution has a negative impact on skin aging. Applying z-scores, NO_2 increases the probability for the number and the size of facial pigment spots. PM_{10} and $PM_{2.5}$ act harmful on the formation of coarse wrinkles on the upper lip as well as on the even pigmentation on the forearm insides. PM_{10} and $PM_{2.5absorbance}$ act harmful on the formation of nasolabialfolds and O_3 increases the probability for coarse wrinkles under the eyes. Taking into consideration the effect of mMEDI on air pollution induced skin aging, we did not find an attenuating effect. Finally, the Mediterranean diet might have positive impacts on other diseases such as cardiovascular diseases, diabetes or cancer, but effects on skin aging were not visible in our study population. Further research on this topic is needed, with a more extensive questionnaire, gradation of items, concrete amounts of intake and perhaps with an objective, verifiable evaluation method for the assessment of dietary habit.

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Apendix

SOP Hautalterung, SALIA study 2012

I Lentigines

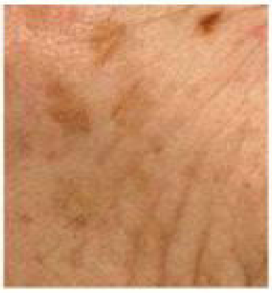
0: no lentigines

1: one or more small lentigines (at least Ø=3)

2: one or more larger lentigines (Ø 4-8mm)

3: one very large lentigo, rather pale (Ø > 9 mm)

4: one very large lentigo, dark (Ø > 9 mm)



5: large (Ø 4-8mm) and very large (Ø > 9mm) lentigines

II

Wrinkles on forehead

0

1

2

3

4

5

III Inter sourciliar wrinkles

0

1

2

3

4

5

rides sous les yeux

0

1

2

3

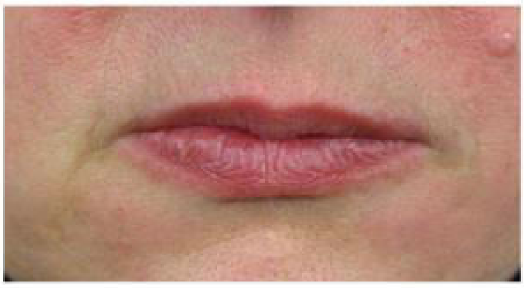
4

5

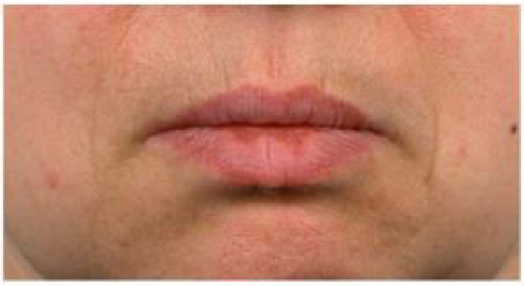
IV Ride de la patte d'oie

Wrinkles on upper lips

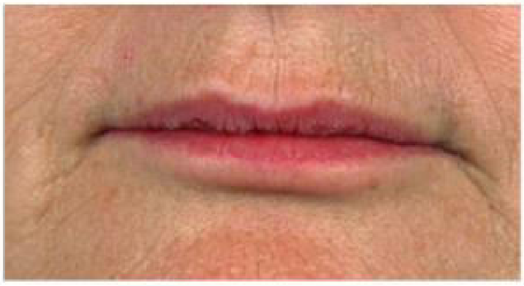
0



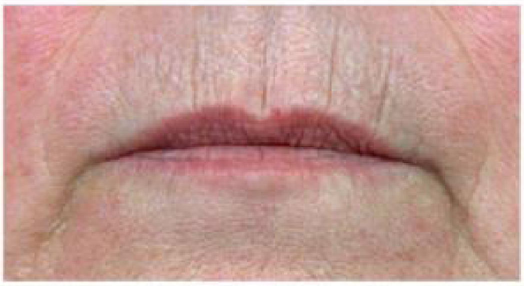
1



2

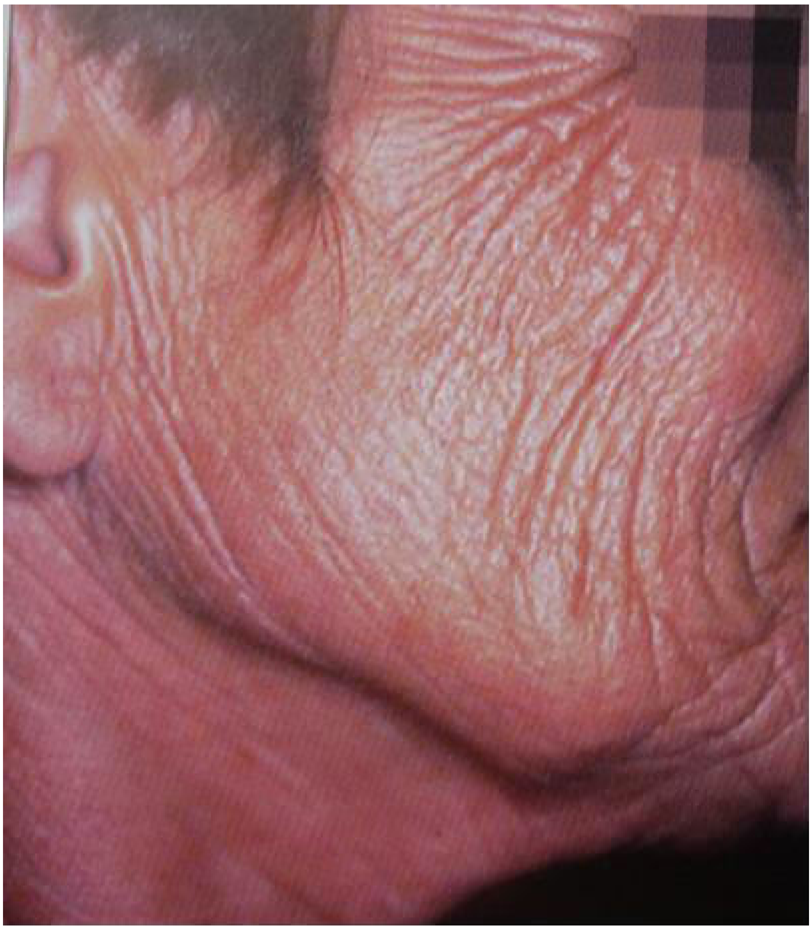


3



4


Nasolabial-
falten
0
1
2
3
4
5



Solar elastosis

Cigarette paper like skin

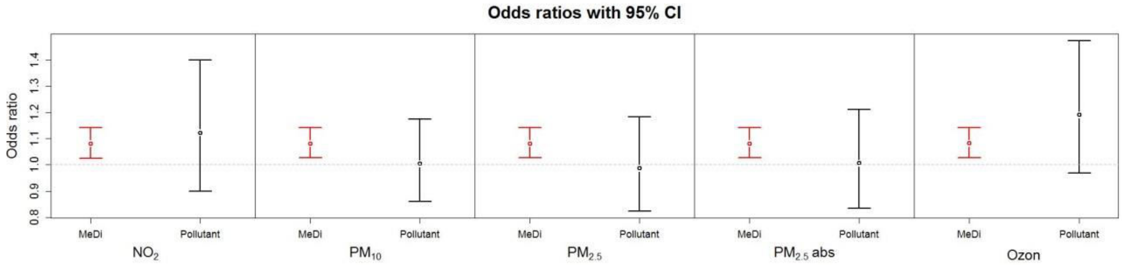
affaissement de la paupière supérieure


affaissement
de l'ovale
du visage
0
1
2
3
4
5

Figures

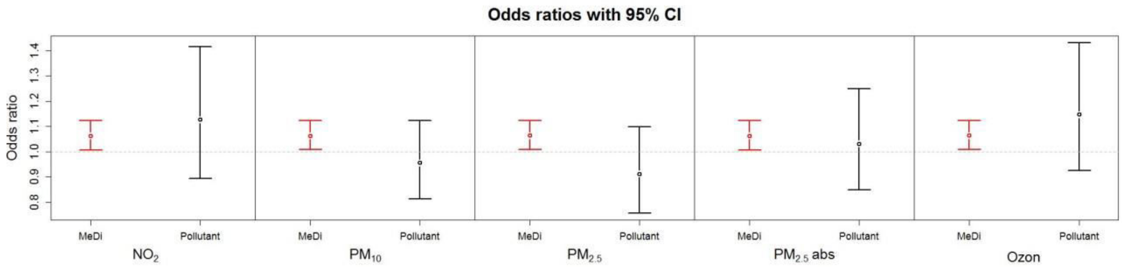
Models adjusted for UV covariates

Pigment spots on the cheeks (numeric)

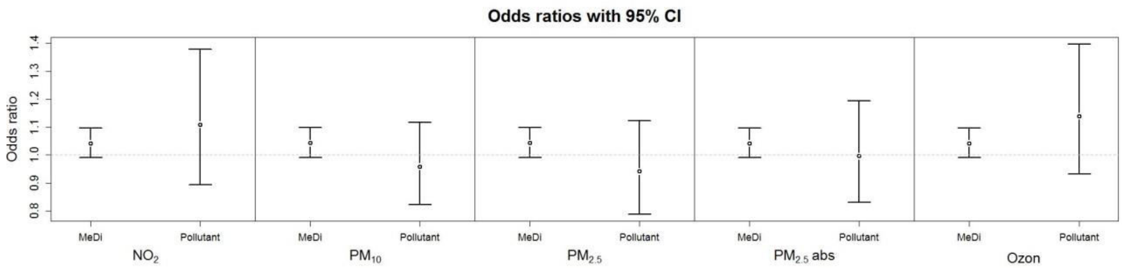


Adjusted for: social status, urban/rural, hormone replacement therapy, sun protection factor, smoking status, sunbed use, passive smoking, heating with fossil fuels, packyears, age, BMI, skin type, UV index; NO_2: Nitrogen dioxide, PM_{10}/$PM_{2.5}$: Particulate matter suspended in the air with a diameter of 10µm/ 2.5µm, $PM_{2.5coarse}$: Proxy for elemental carbon, O_3: Ozone, red colour: Significant results

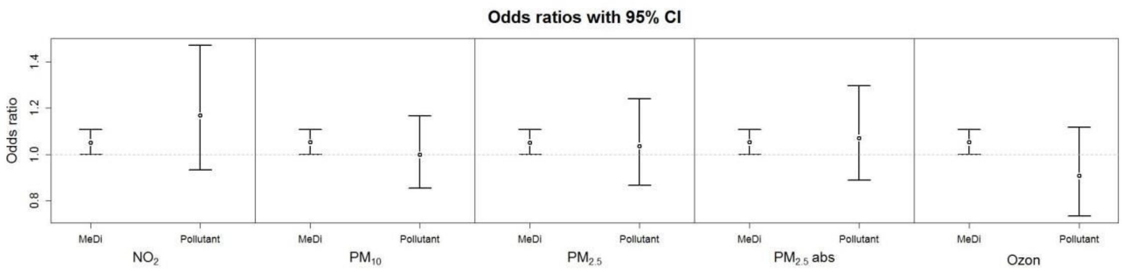
Pigment spots on forehead (numeric)



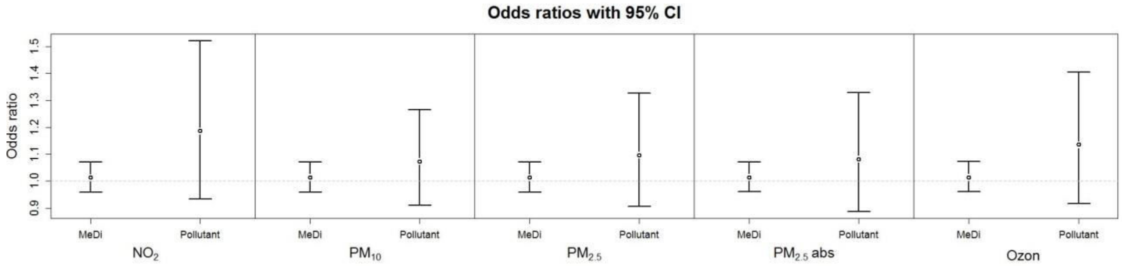
Pigment spots on forehead (photo-reference scale)



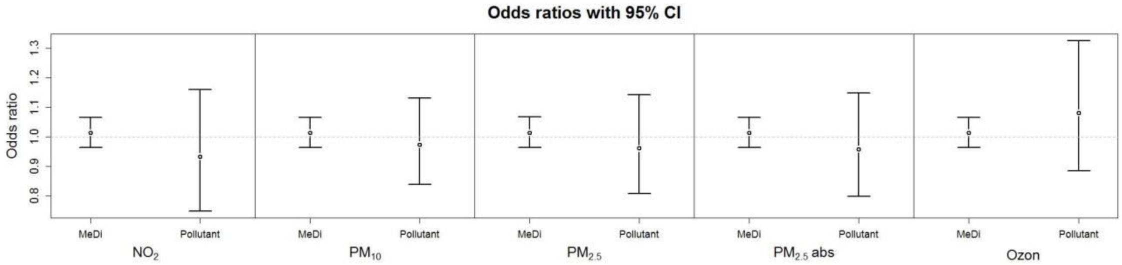
Pigment spots on cheeks (photo-reference scale)



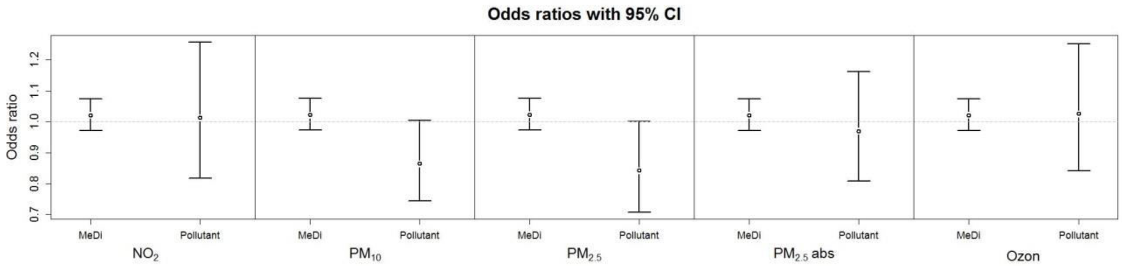
Pigment spots on forearms



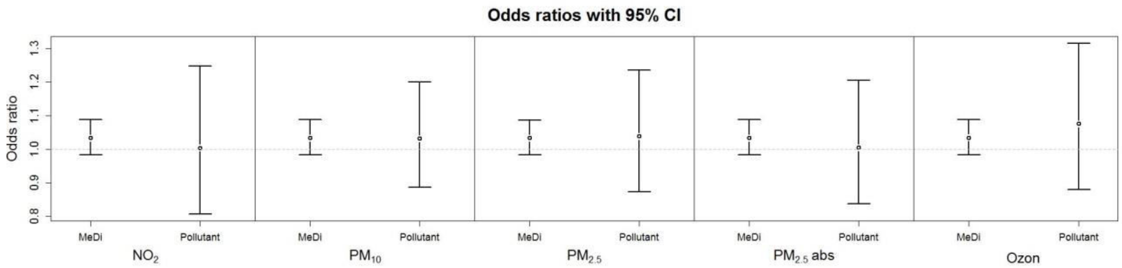
Pigment spots on the back of the hands



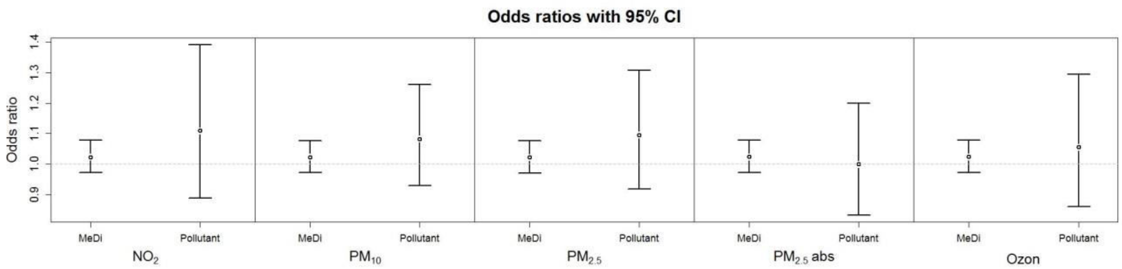
Coarse wrinkles on forehead



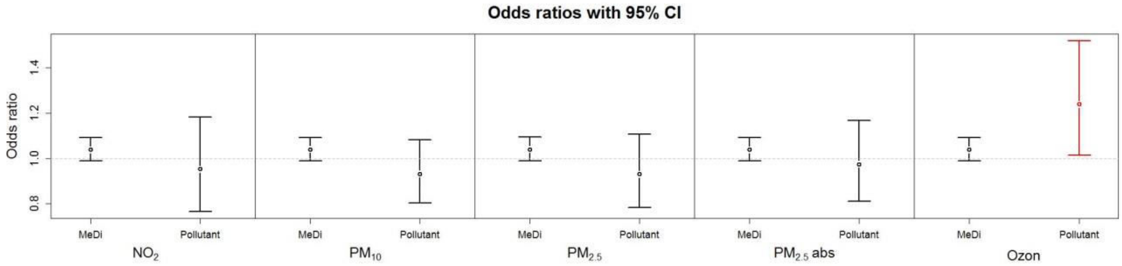
Coarse wrinkles on eyebrows



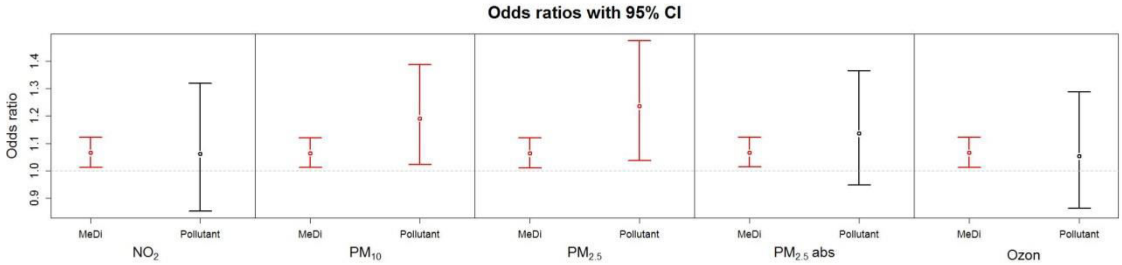
Coarse wrinkles in crow´s feet area



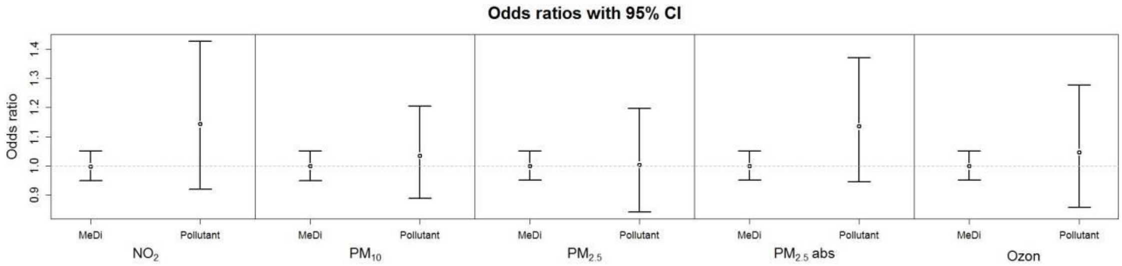
Coarse wrinkles under the eyes



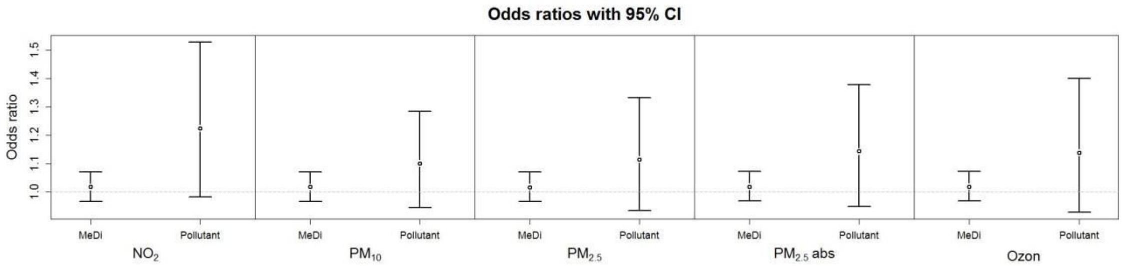
Coarse wrinkles on upper lip



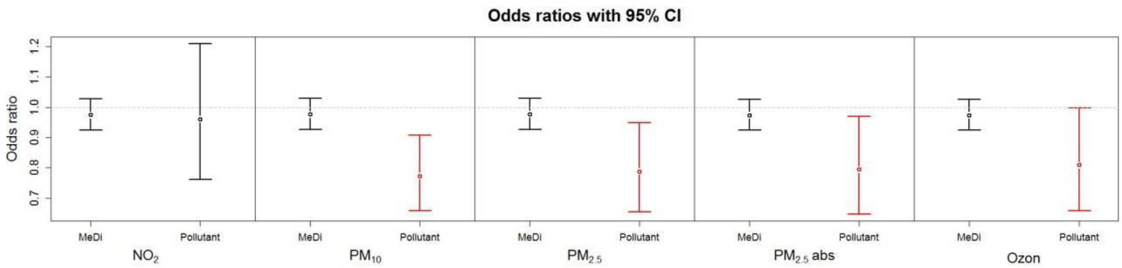
Coarse wrinkles on nasolabialfolds



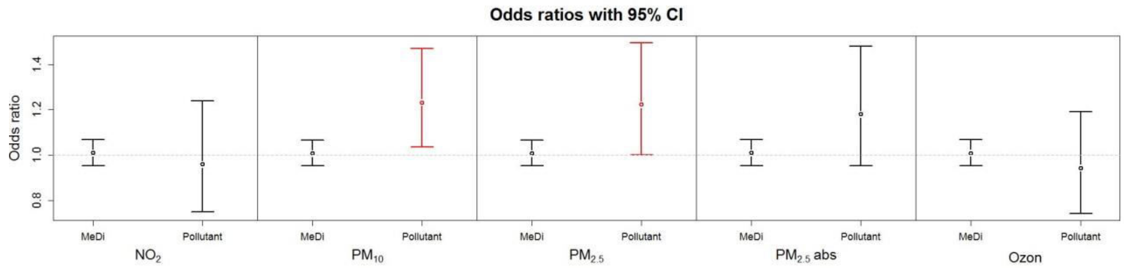
Solar elastosis on cheeks



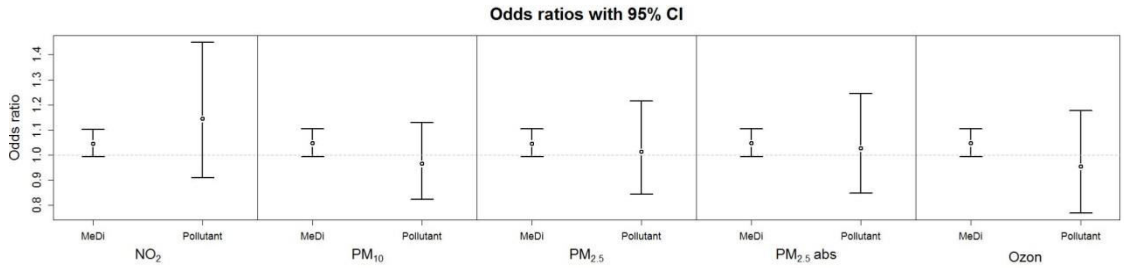
Teleangiectasia



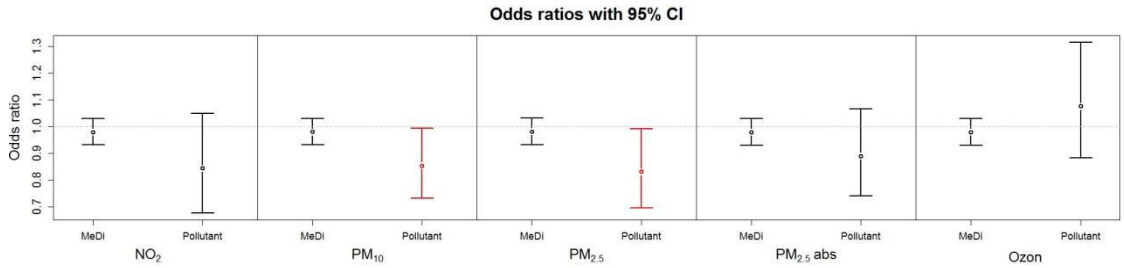
Even pigmentation (forearm inside)



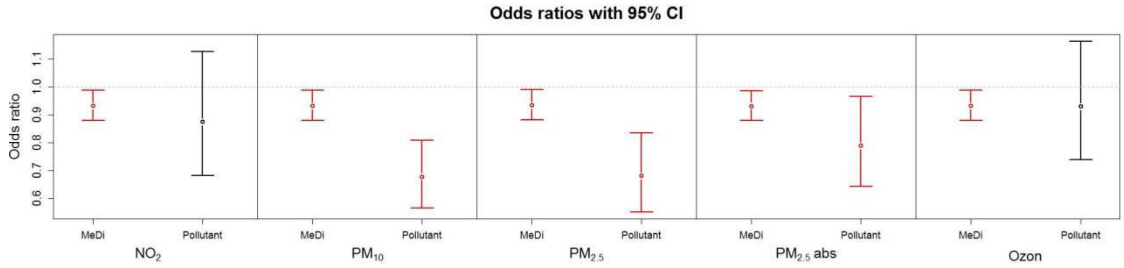
Lax eyes



Lax face (lower part)

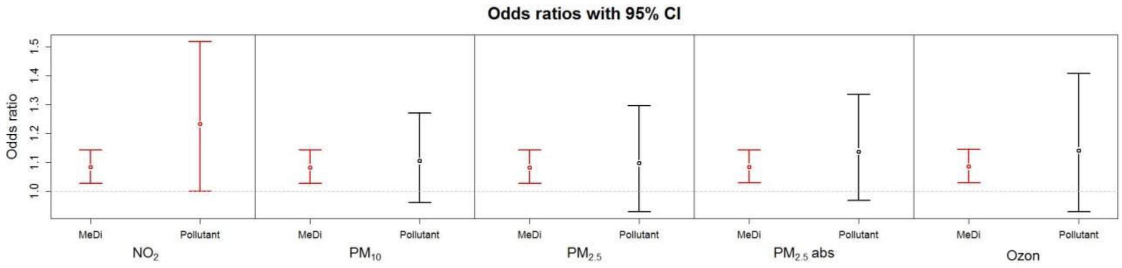


Cigarette paper like skin on the back of the hands

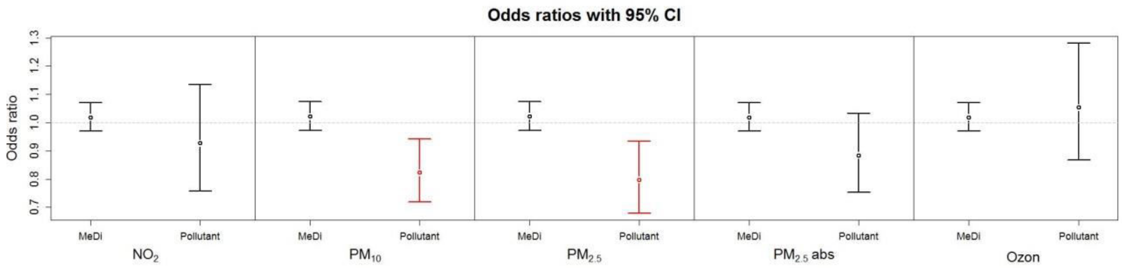


Figures without UV covariates

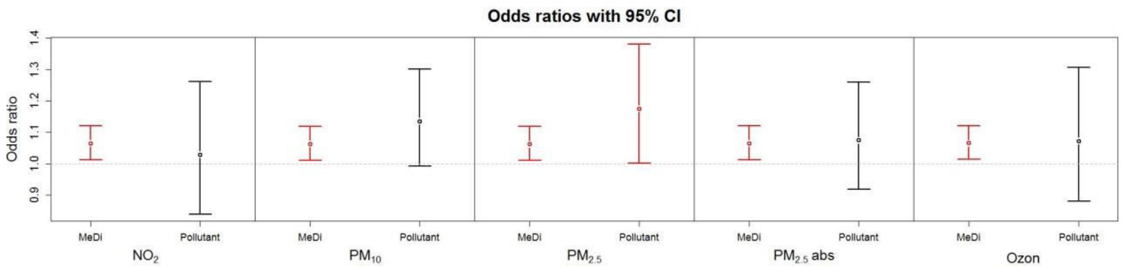
Pigment spots on the cheeks



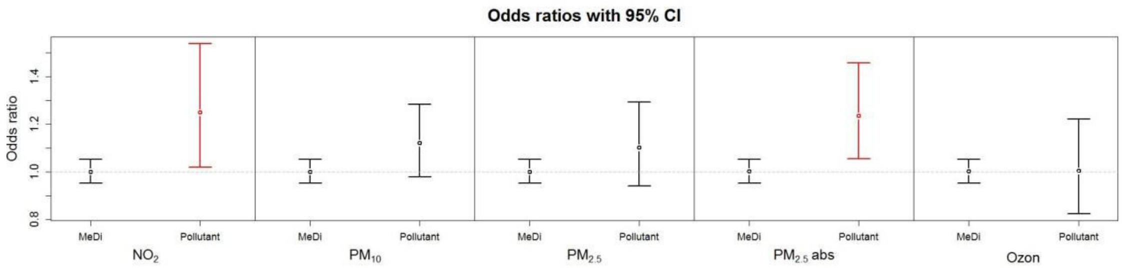
Coarse wrinkles on forehead



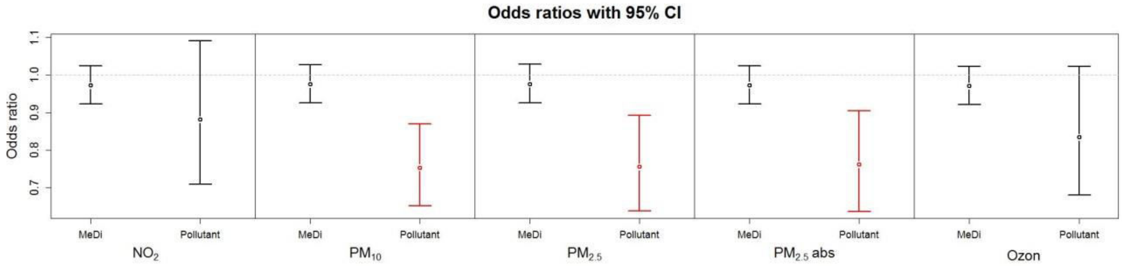
Coarse wrinkles on upper lip



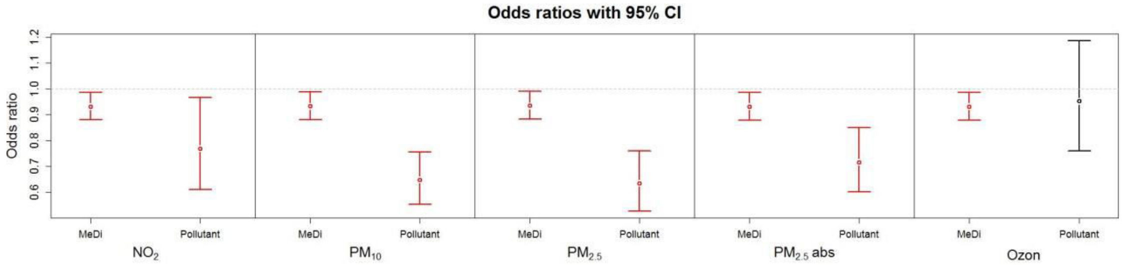
Coarse wrinkles on nasolabialfolds



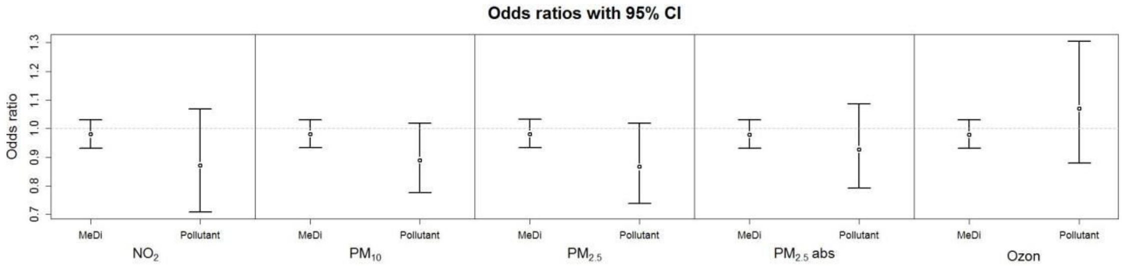
Teleangiectasia on cheeks



Cigarette paper like skin on the back of the hands

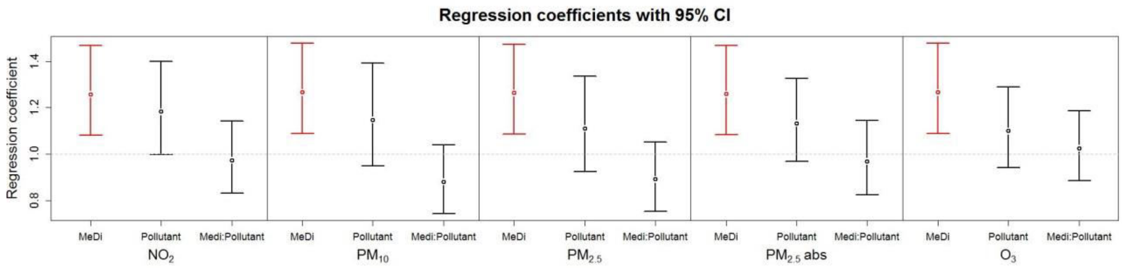


Lax face

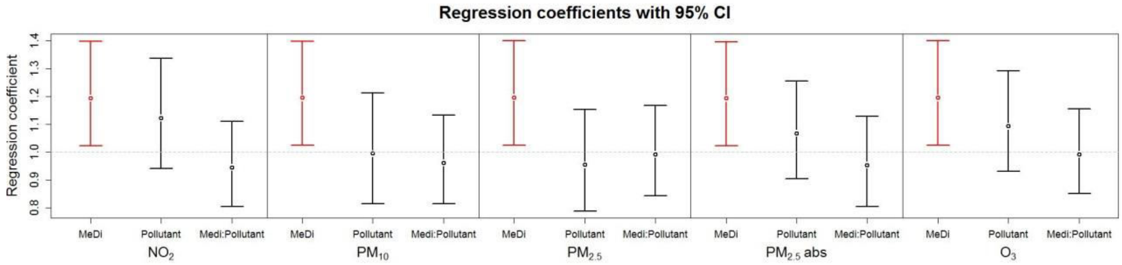


Figures with interaction variable

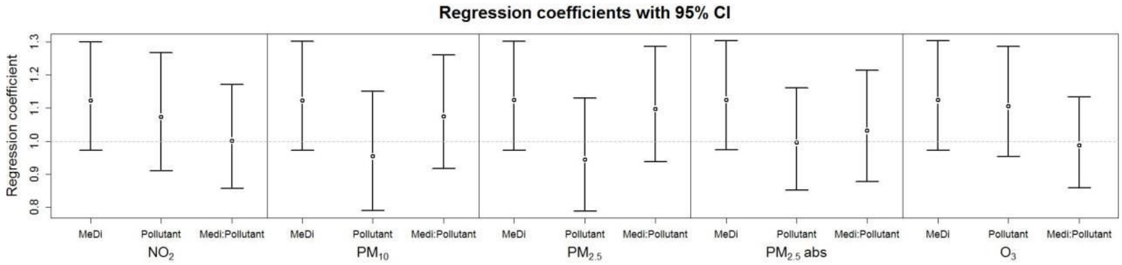
Pigment spots on the cheeks (numeric)



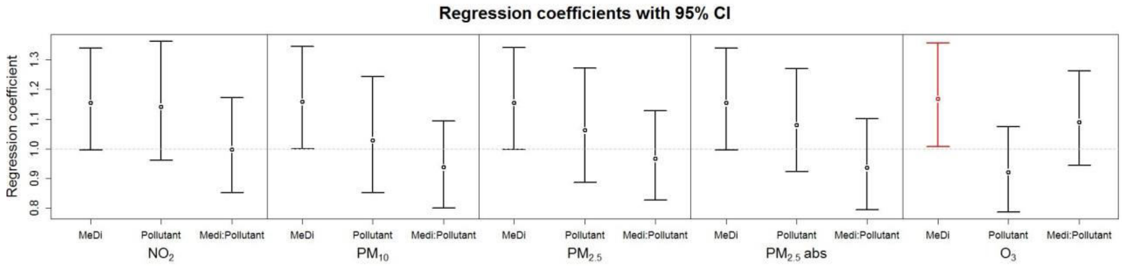
Pigment spots on the forehead (numeric)



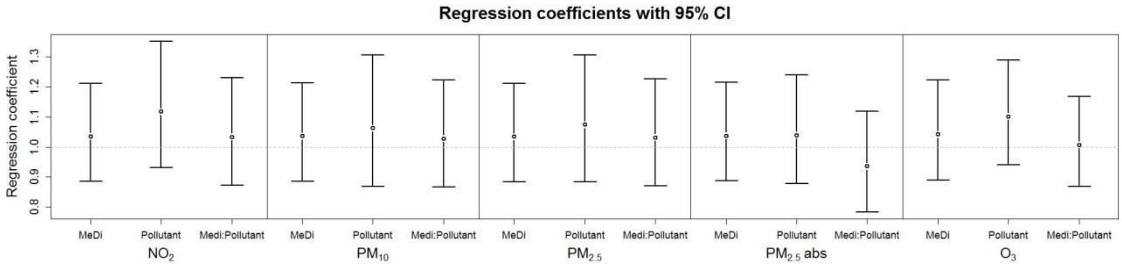
Pigment spots on the forehead (photo-reference scale)



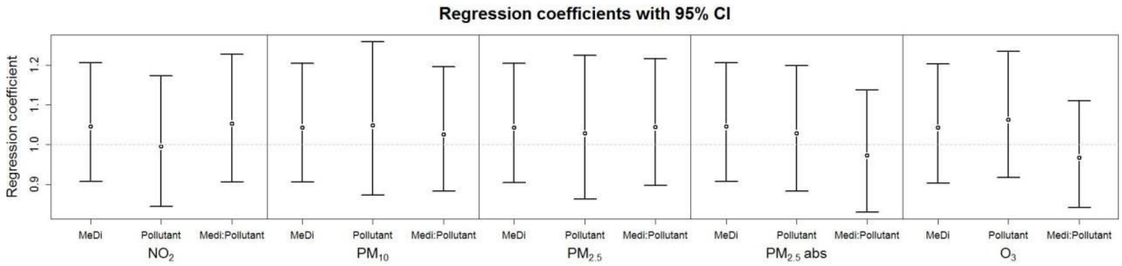
Pigment spots on the cheeks (photo-reference scale)



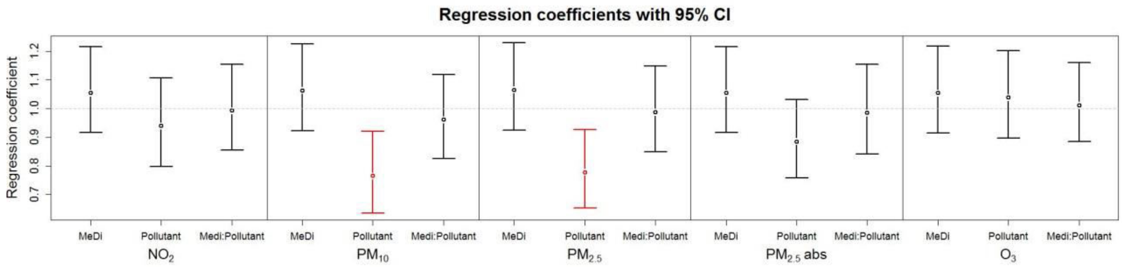
Pigment spots on the forearm



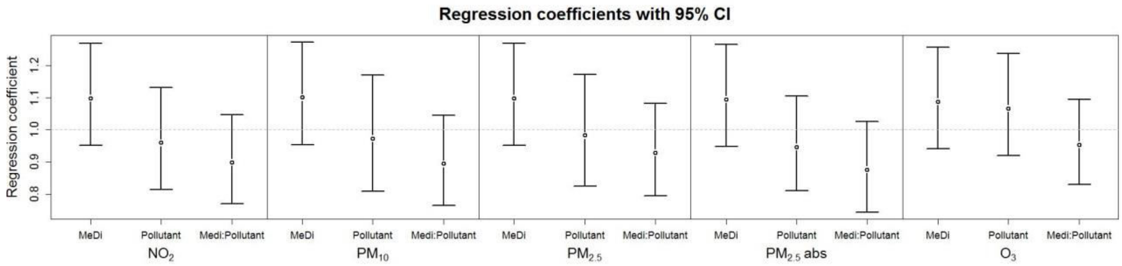
Pigment spots on the back of the hand



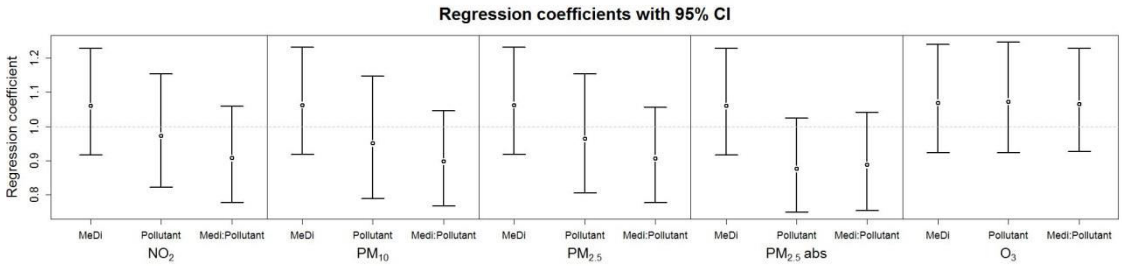
Coarse wrinkles on the forehead



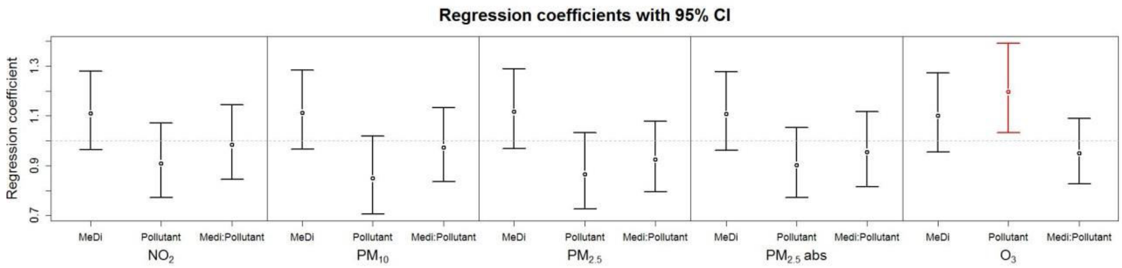
Coarse wrinkles on the eyebrows



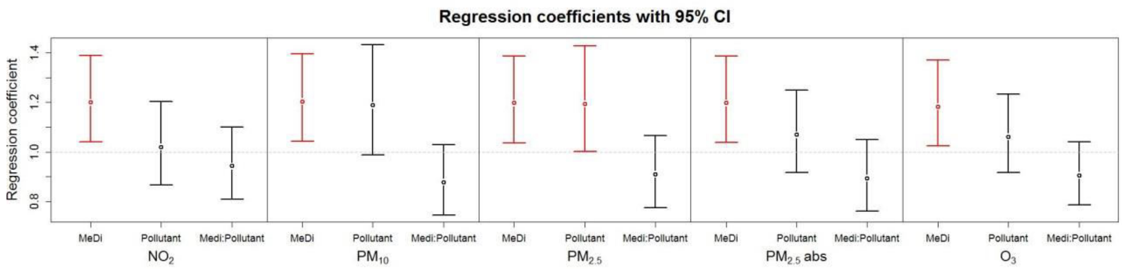
Coarse wrinkles in the crow´s feet area



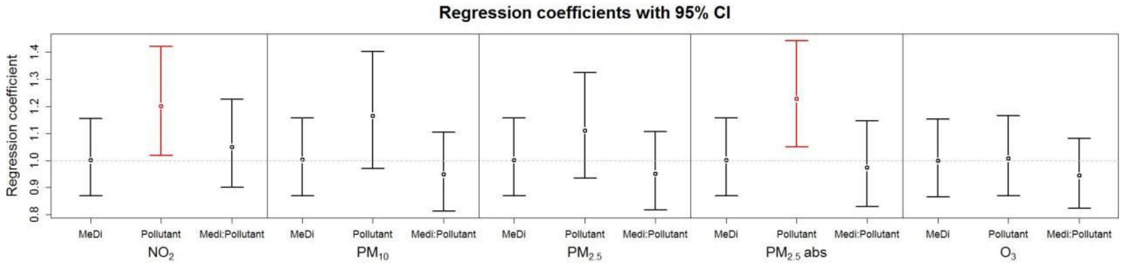
Coarse wrinkles under the eyes



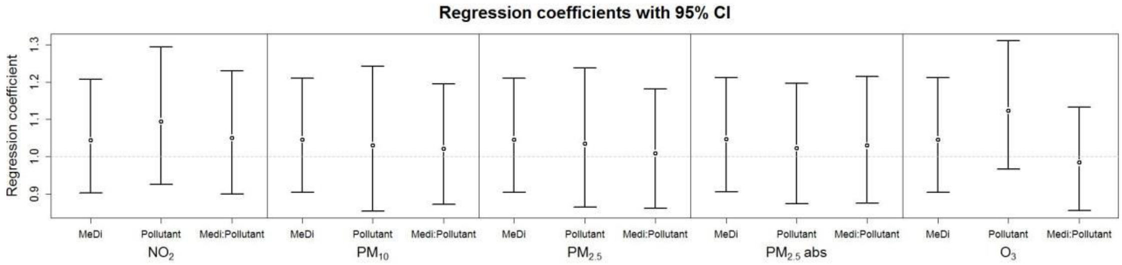
Coarse wrinkles on upper lip



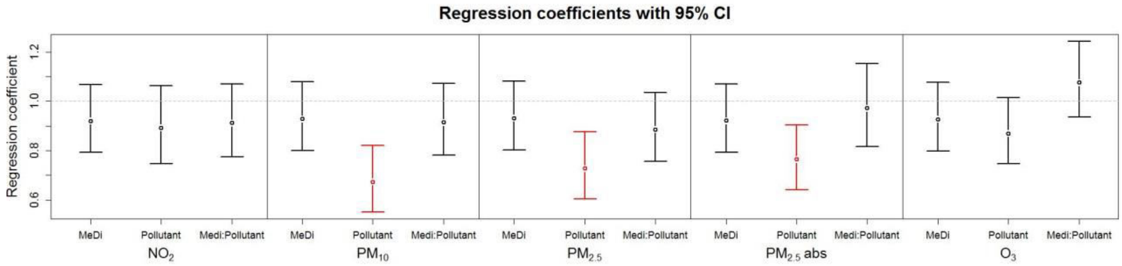
Coarse wrinkles on nasolabialfolds



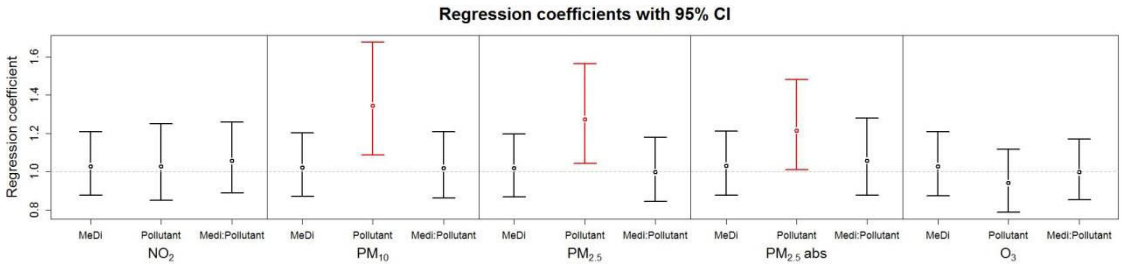
Solar elastosis on the cheeks



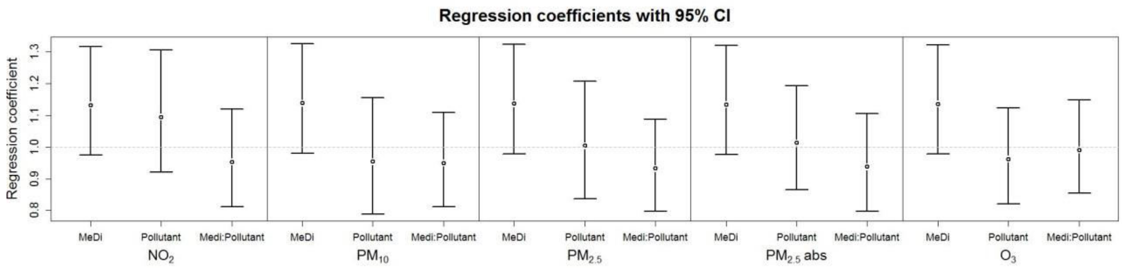
Teleangiectasia



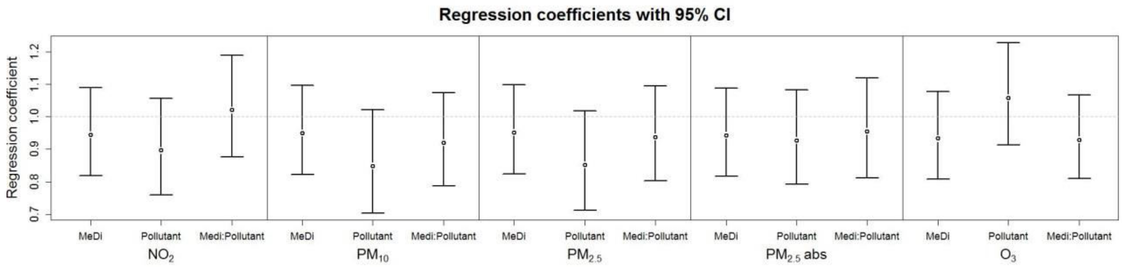
Even pigmentation on forearm inside



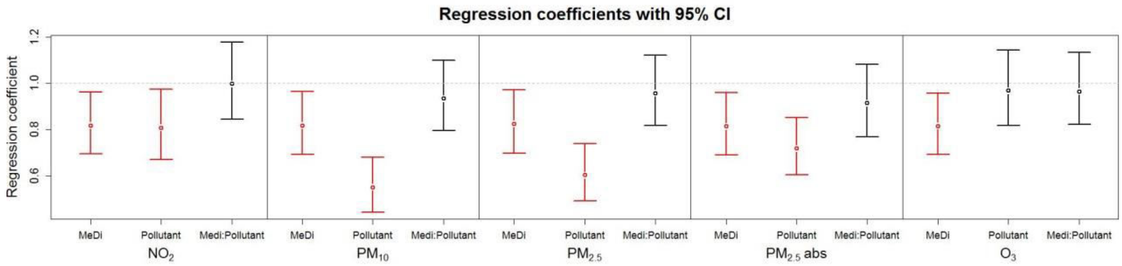
Lax eyes



Lax face

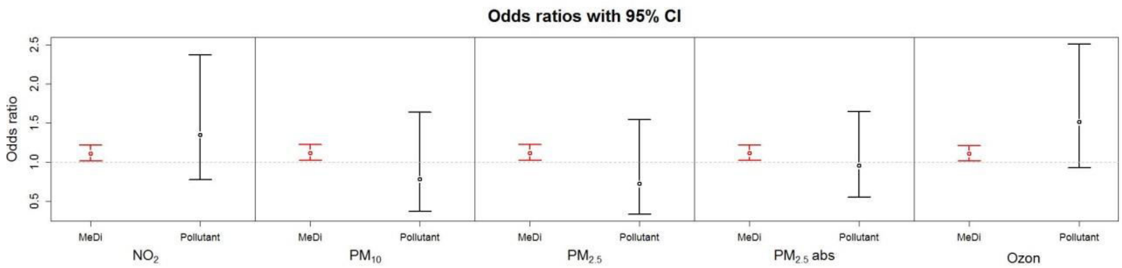


Cigarette paper like skin on the back of the hands

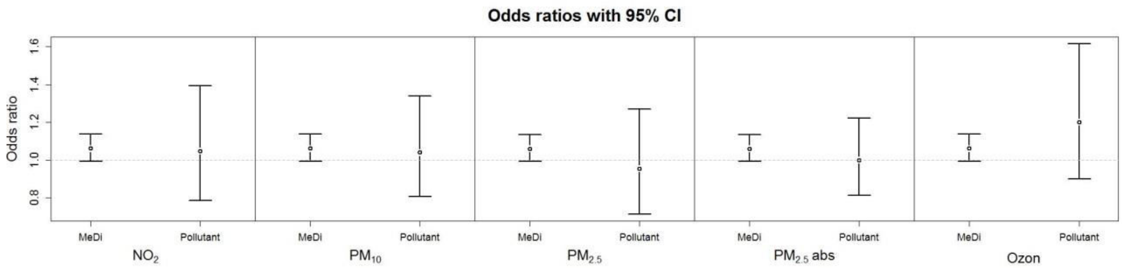


Figures rural/urban

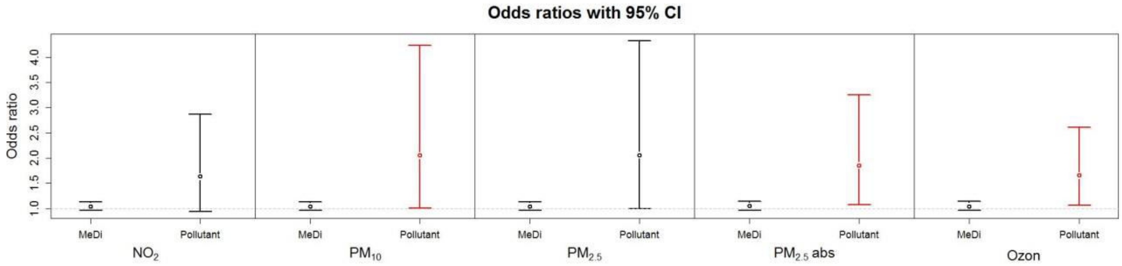
Rural subgroup, pigment spots on the cheeks (numeric)



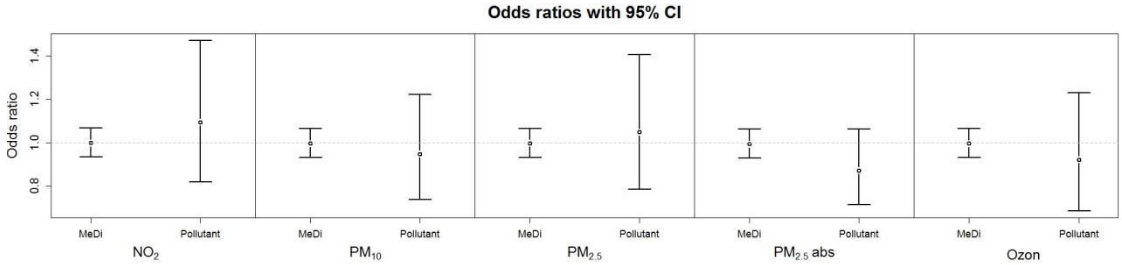
Urban subgroup, pigment spots on the cheeks (numeric)



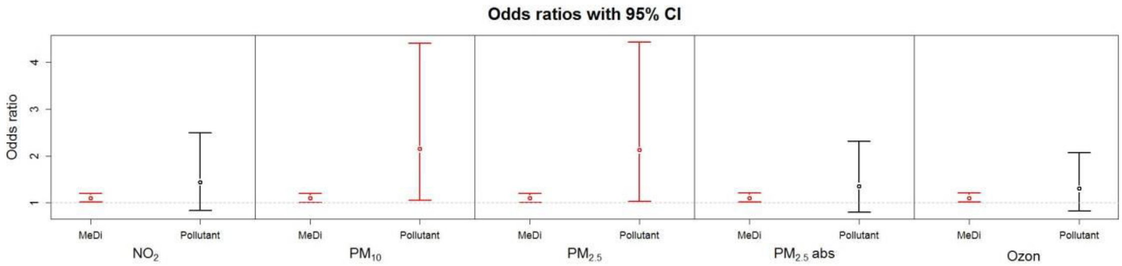
Rural subgroup, coarse wrinkles in crow´s feet area



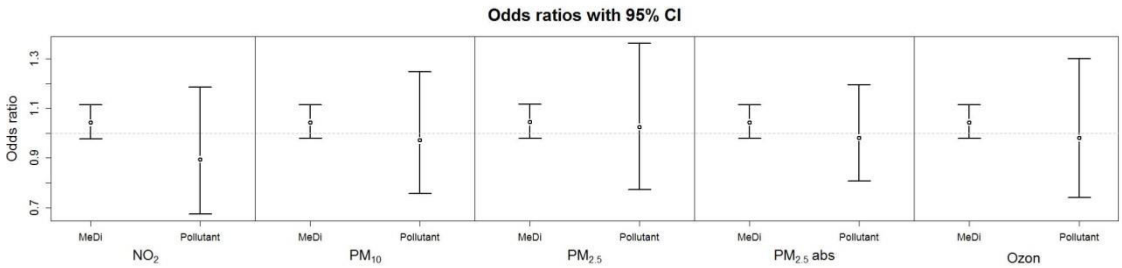
Urban subgroup, coarse wrinkles in crow´s feet area



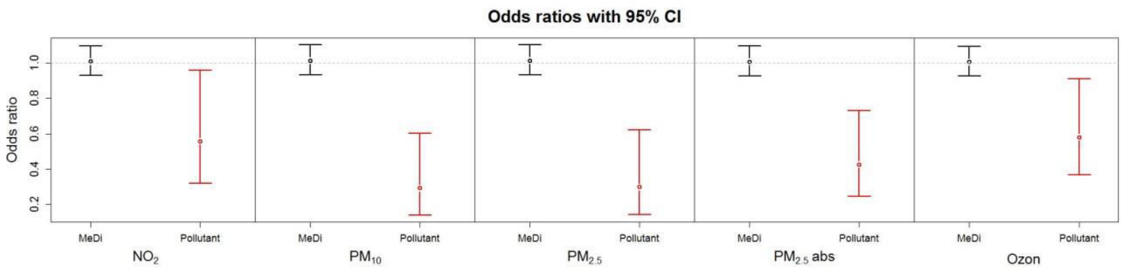
Rural subgroup, coarse wrinkles on upper lip



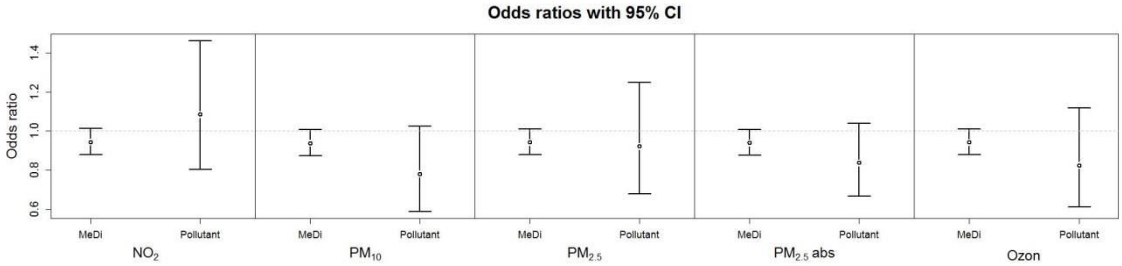
Urban subgroup, coarse wrinkles on upper lip



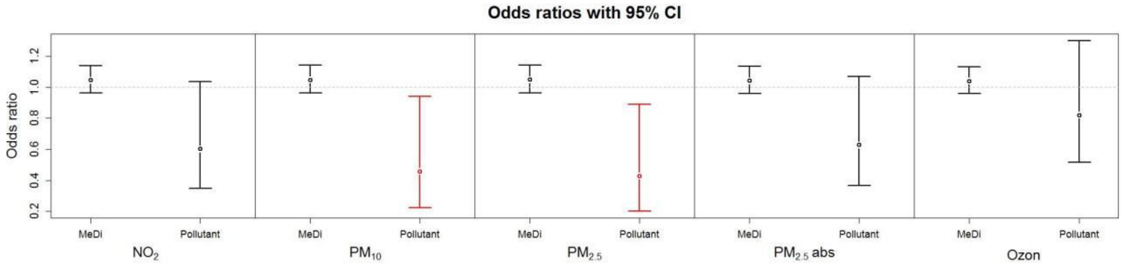
Rural subgroup, teleangiectasia



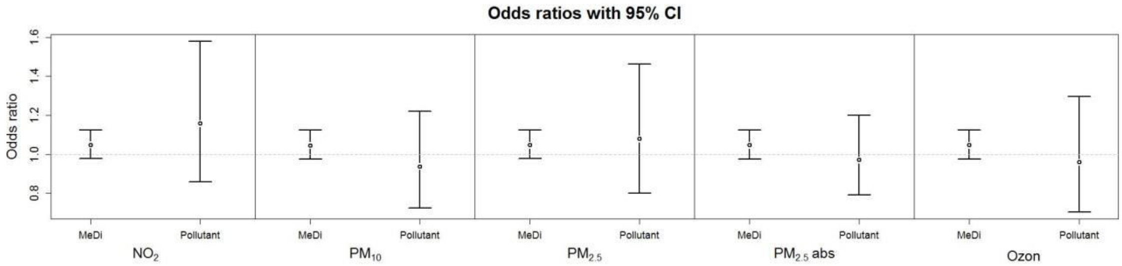
Urban subgroup, teleangiectasia



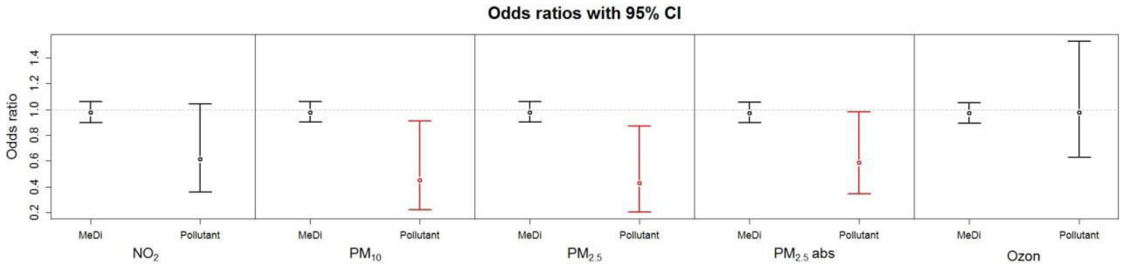
Rural subgroup, lax eyes



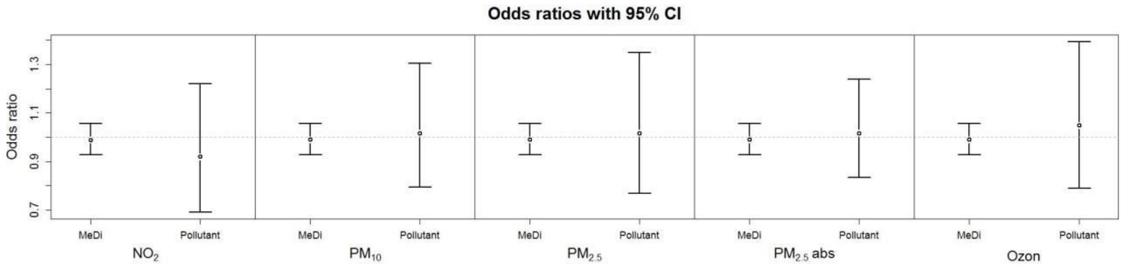
Urban subgroup, lax eyes



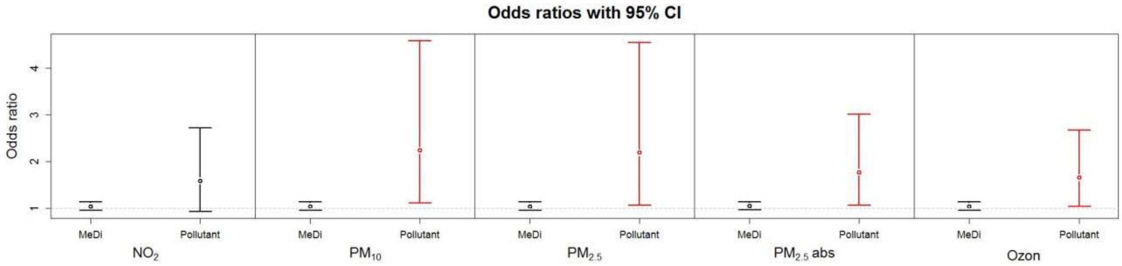
Rural subgroup, lax face



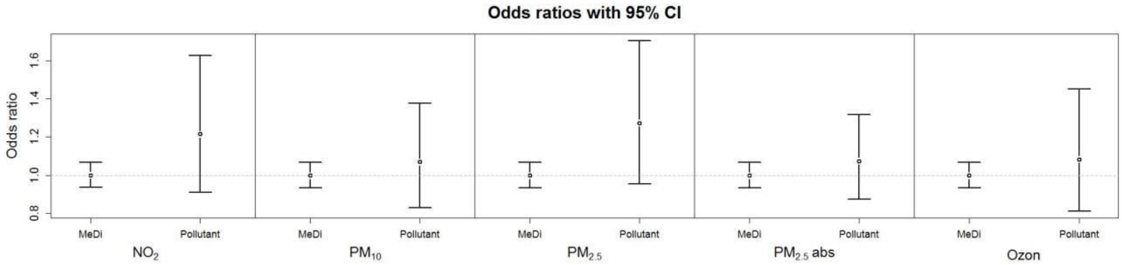
Urban subgroup, lax face



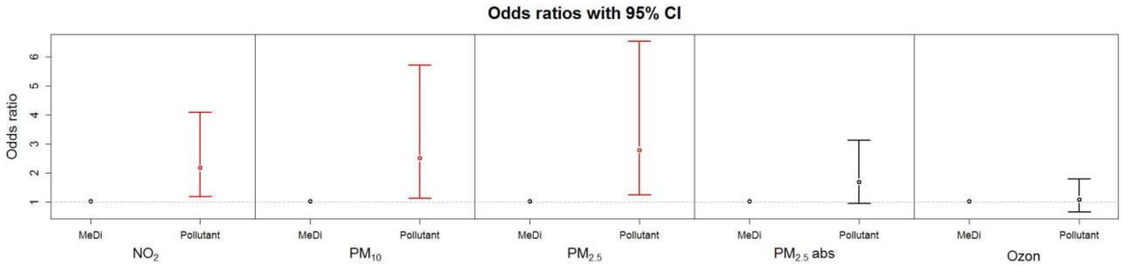
Rural subgroup, solar elastosis



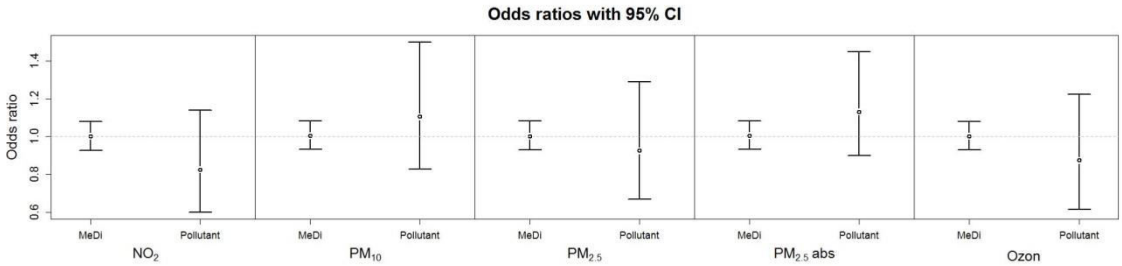
Urban subgroup, solar elastosis



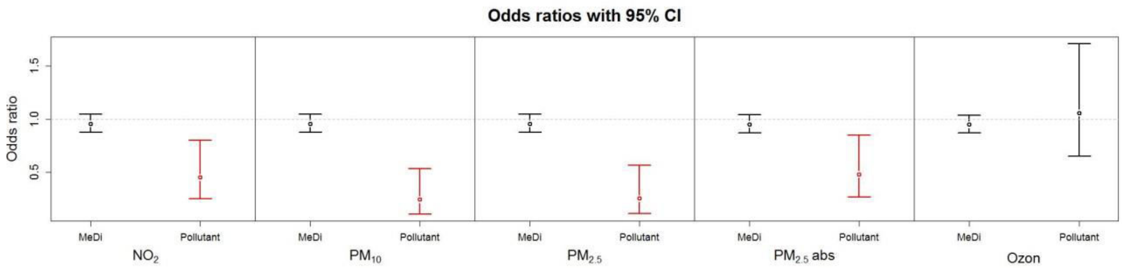
Rural subgroup, even pigmentation on the forearm inside



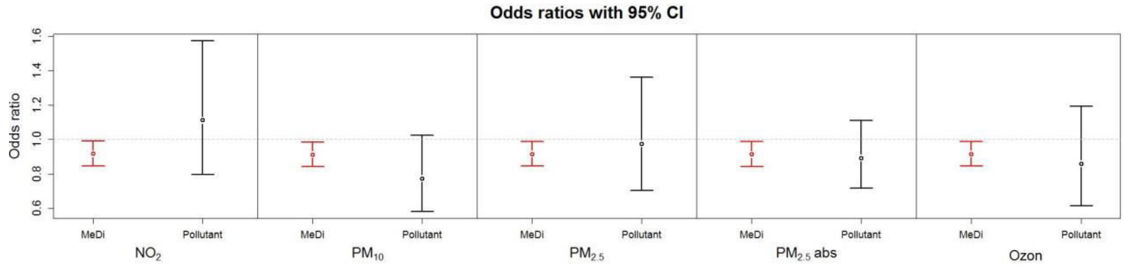
Urban subgroup, even pigmentation on the forearm inside



Rural population, cigarette paper like skin on the back of the hands

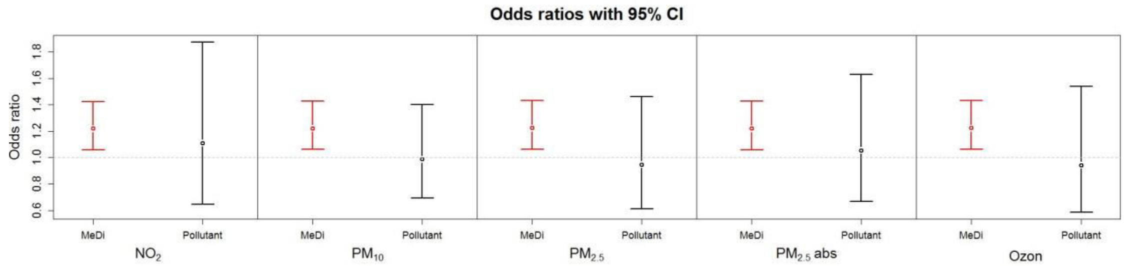


Urban population, cigarette paper like skin on the back of the hands

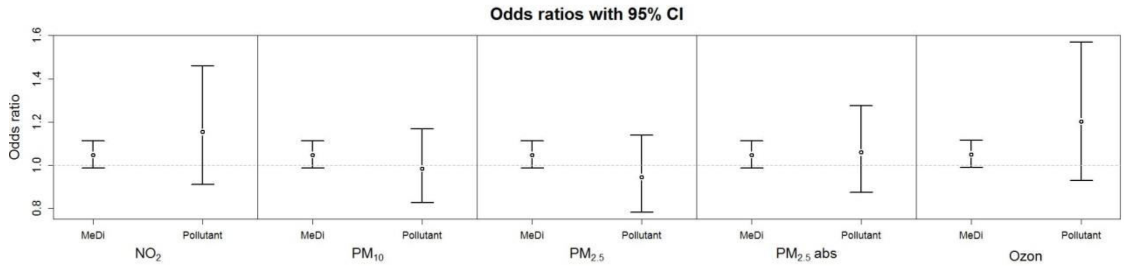


Figures Smoker/non-smoker

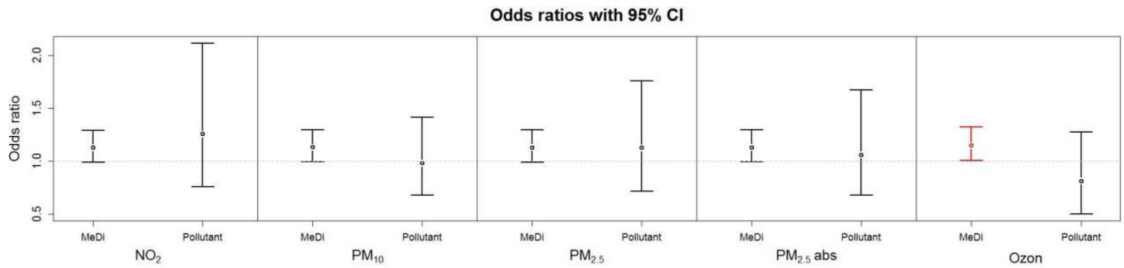
Ever-smoker, pigment spots on the forehead



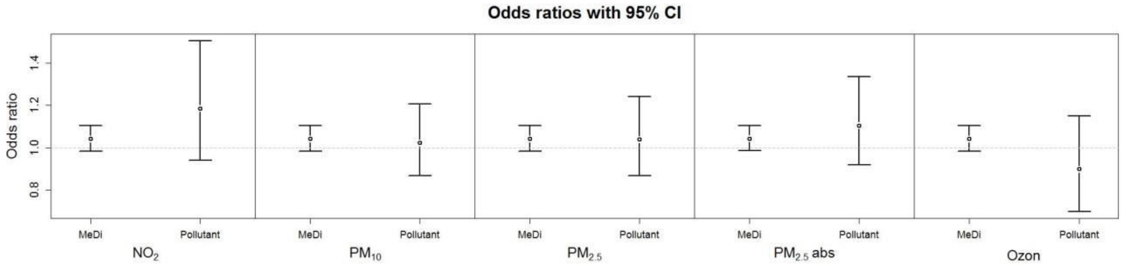
Non-smoker, pigment spots on the forehead



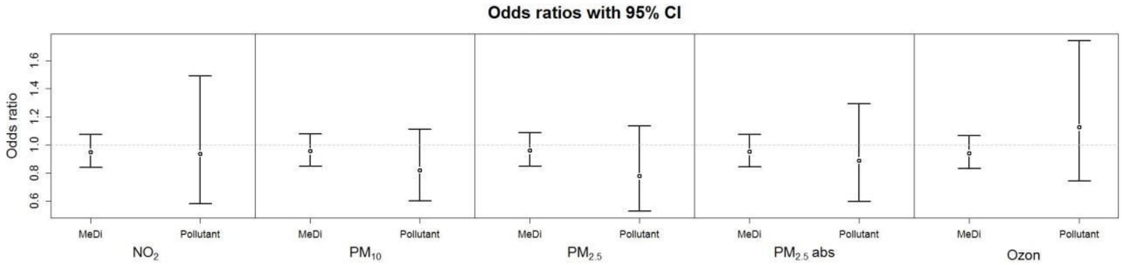
Smokers, pigment spots on the cheeks (photo-reference scale)



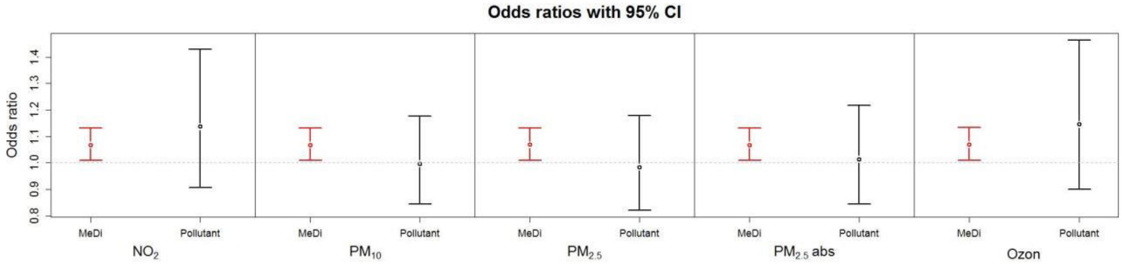
Non-smokers, pigment spots on the cheeks (photo-reference scale)



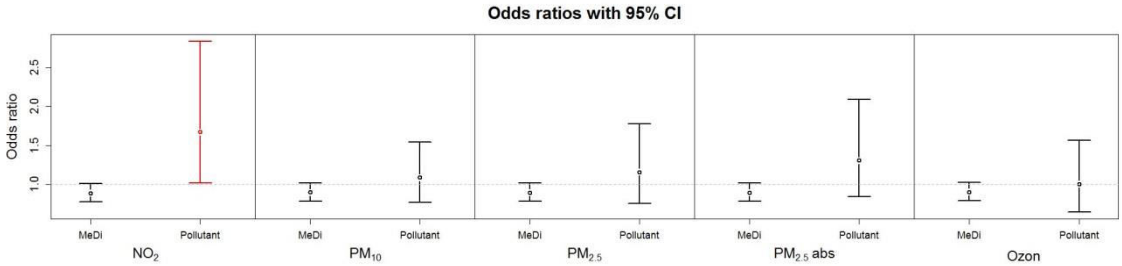
Smokers, pigment spots on the forehead (photo-reference scale)



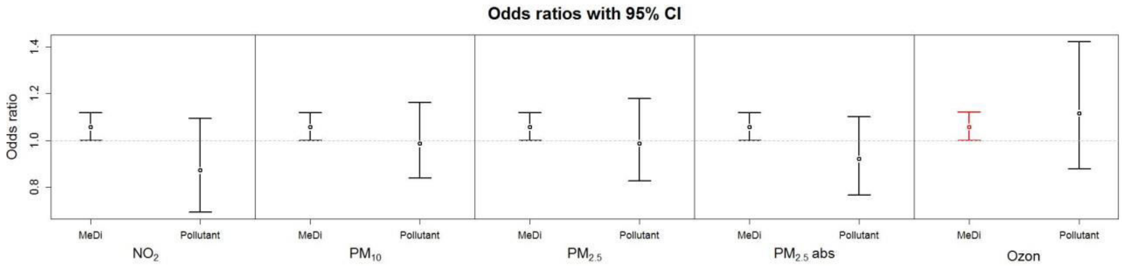
Non-smokers, pigment spots on the forehead (photo-reference scale)



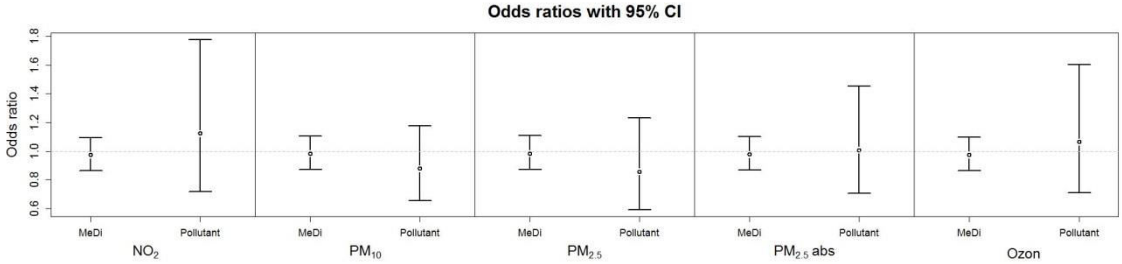
Smokers, coarse wrinkles on the eyebrows



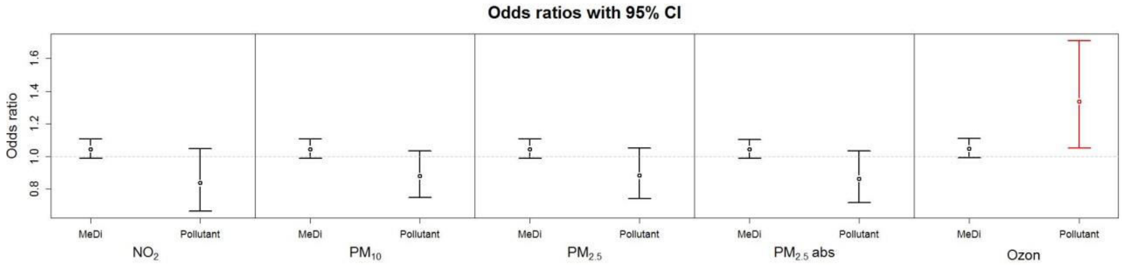
Non-smokers, coarse wrinkles on the eyebrows



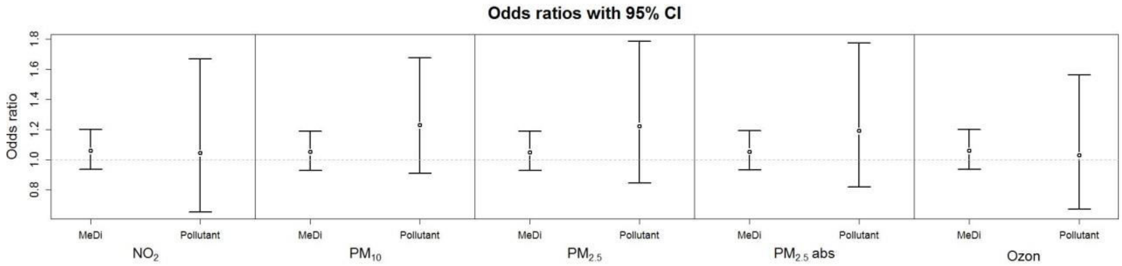
Smokers, coarse wrinkles under the eyes



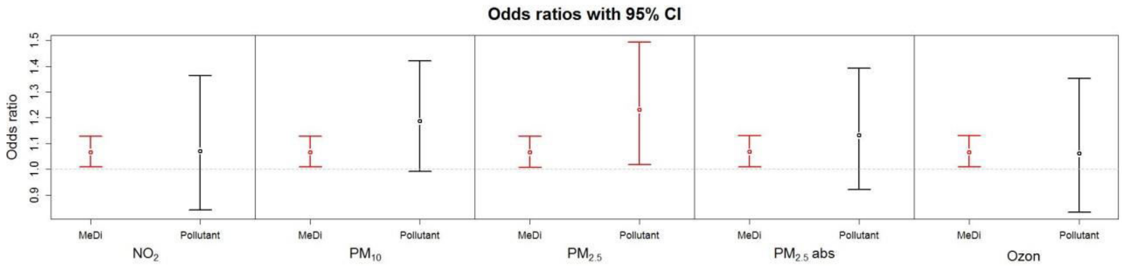
Non-smokers, coarse wrinkles under the eyes



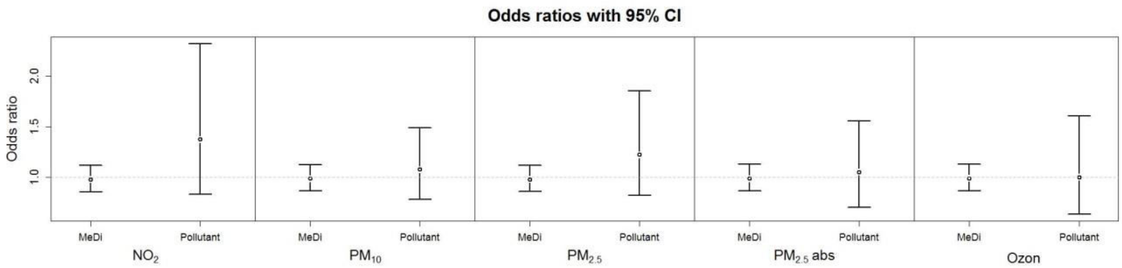
Smokers, coarse wrinkles on upper lip



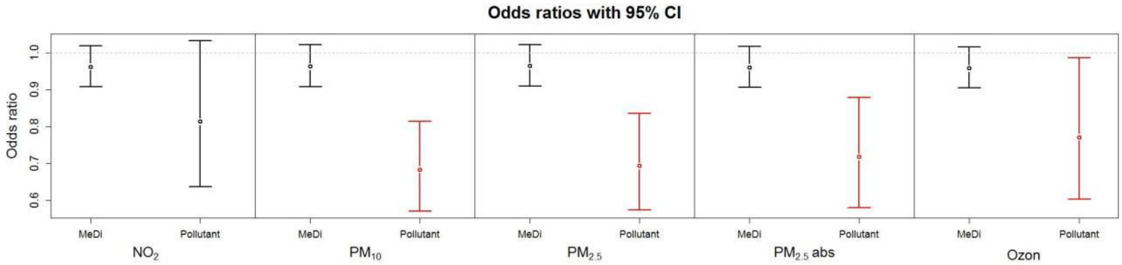
Non-smokers, coarse wrinkles on upper lip



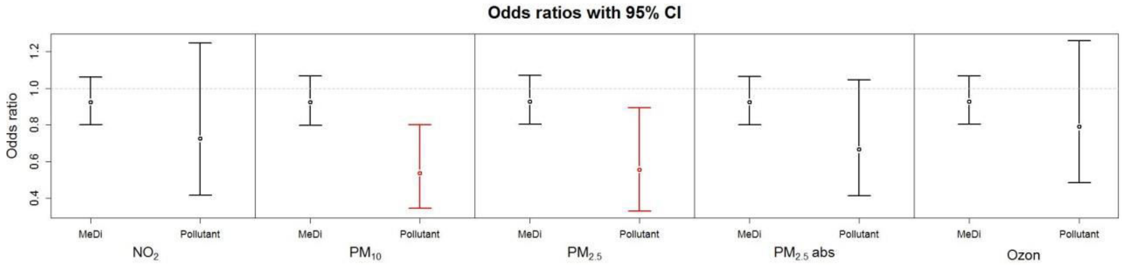
Smokers, teleangiectasia



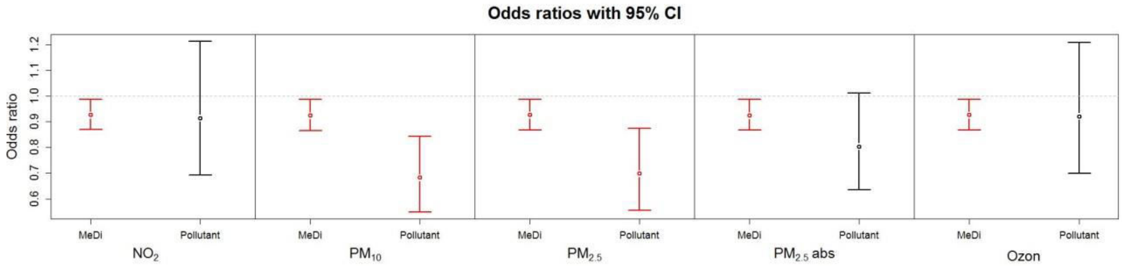
Non-smokers, teleangiectasia



Smokers, cigarette paper like skin on the back of the hands



Non-smokers, cigarette paper like skin on the back of the hands



Food-Questionnaire

CORE MODUL J: FOOD FREQUENCY

J 1	Wie häufig nehmen Sie die folgenden Nahrungsmittel zu sich?

	Fast täglich	Mehrmals in der Woche	Etwa einmal in der Woche	Mehrmals im Monat	Einmal im Monat oder seltener	Nie
Fleisch, Wurst (ohne Geflügel)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Geflügel	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Fisch	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Roher Fisch	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Kartoffeln	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Teigwaren (z. B. Nudeln)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Reis	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Sojabohnen	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Salat od. Gemüse (roh zubereitet)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Gemüse (gekocht)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Frisches Obst	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Schokolade, Pralinen	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Kuchen, Gebäck, Kekse	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Sonstige Süßwaren (Bonbons, u.ä.)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Salzige Knabbereien wie z. B. Chips, Erdnüsse	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Weißbrot, Mischbrot, Toastbrot	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Vollkornbrot, Schwarzbrot, Knäckebrot	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Haferflocken, Müsli, Cornflakes	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Milch, Jogurt, Quark, Käse	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Eier	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Margarine (als Brotaufstrich)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Margarine Sorte: ______________________	☐ 1	☐ 2	☐ 3			

1 - Margarine “normal”
2 - Margariene “fettreduziert”
3 - Andere

	Fast täglich	Mehrmals in der Woche	Etwa einmal in der Woche	Mehrmals im Monat	Einmal im Monat oder seltener	Nie
Butter (als Brotaufstrich)	☐1	☐2	☐3	☐4	☐5	☐6
Diätlimonade, sonst. Diätgetränke	☐1	☐2	☐3	☐4	☐5	☐6
Obstsäfte, Gemüsesäfte	☐1	☐2	☐3	☐4	☐5	☐6
Sonstige Erfrischungsgetränke (Limonade etc.)	☐1	☐2	☐3	☐4	☐5	☐6
Mineralwasser	☐1	☐2	☐3	☐4	☐5	☐6
Alkoholische Getränke (Bier, Wein)	☐1	☐2	☐3	☐4	☐5	☐6
Starke alkoholische Getränke (Schnaps)	☐1	☐2	☐3	☐4	☐5	☐6

J 2	Wie viele Tassen Kaffee und wie viele Tassen schwarzen oder grünen Tee trinken Sie gewöhnlich am Tag? **INT:** Angaben für 'normale' Tassen umrechnen! Wenn Proband keinen Kaffee oder Tee trinkt, 0 eingeben. Wenn nur 1 bis 2 Tassen pro Woche, dann auch 0 eingeben.		Anzahl Tassen Kaffee/Tag: |___|___| Anzahl Tassen schwarzer Tee/Tag: |___|___| Anzahl Tassen grüner Tee/Tag: |___|___|	
J 2.1	Wie viele Jahre ernähren Sie sich in der angegebenen Weise?		|___|___| Jahre	
J 3	Wenn Sie so zurückdenken (ab Ende Schulzeit), wie häufig haben Sie alkoholhaltige Getränke durchschnittlich getrunken?	☐1 ☐2 ☐3 ☐4	Nie Gelegentlich Regelmäßig / (fast) täglich Trinkverhalten geändert	**K 1** **K 1** **J 3.1** **J 3.2**
J 3.1	Wie viel Bier, Wein/Sekt, Spirituosen haben Sie dann pro Tag getrunken? (Mehrfachnennung möglich)		Bier (in 0,3l Gläsern): |___|___| Wein/Sekt (in 0,2l Gläsern): |___|___| Spirituosen (in 2cl Gläsern): |___|___|	
J 3.2	In welchem Alter haben Sie Ihr Trinkverhalten geändert?		im Alter von: |___|___|	

J 3.2.1	In welcher Weise haben Sie Ihr Trinkverhalten geändert?	☐ 1 ☐ 2	ich habe früher regelmäßig / (fast) täglich getrunken, trinke jetzt aber nur noch gelegentlich oder gar nicht mehr ich habe früher gar nicht oder nur gelegentlich getrunken, trinke jetzt aber regelmäßig / (fast) täglich	
J 3.2.2	Warum haben Sie Ihr Trinkverhalten geändert?	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5	geschmackliche Veränderung ärztliches Anraten persönliche Einstellung eigenes gesundheitliches Bedenken Anderes: ______________________	
J 3.2.3	Als Sie regelmäßig / (fast) täglich getrunken haben oder wenn Sie jetzt regelmäßig / (fast) täglich trinken, wie viel Bier/Wein/Sekt/Spirituosen trinken Sie oder haben Sie pro Tag getrunken? (Mehrfachnennungen möglich)		Bier (in 0,3l Gläsern): |___|___| Wein/Sekt (in 0,2l Gläsern): |___|___| Spirituosen (in 2cl Gläsern): |___|___|	

Acknowledgement

An dieser Stelle möchte ich all jenen danken, die mich bei der Anfertigung dieser Arbeit tatkräftig unterstützt haben:

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