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Controlled full-body human exposure to ozone increases malondialdehyde on skin

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ABSTRACT

Malondialdehyde (MDA), a product of lipid peroxidation, is a biomarker of oxidative stress. We investigated the relationship between ozone exposure and MDA in skin wipes in a series of three controlled full-body chamber studies. Twenty-nine volunteers were exposed under different levels of temperature, relative humidity, ozone concentration, clothing coverage, and ventilation rate. One study also examined age-related differences among teenagers, young adults, and seniors. Exposures lasted between 3 and 6 h. Skin wipes were collected from the volunteers' forearms before and after exposure and analyzed for MDA and, in some cases, squalene. To characterize baseline variability, skin wipes were collected from 3 unexposed reference persons twice a day for 5 days. Two skin wipes were collected daily from two additional persons; one from each person was subsequently exposed to chamber air, the other was unexposed. This was to distinguish ozone chemistry on collected skin oil from processes occurring directly on skin. Ozone exposure significantly increased MDA on skin. A higher post/pre-exposure MDA ratio was observed with a longer exposure duration. MDA concentrations were an order of magnitude higher on skin-oil-loaded wipes exposed to ozone than on wipes collected from volunteers exposed to ozone. This suggests that ozone reactions with skin lipids can generate MDA even in the absence of an inflammatory process. Inter- and intra-person variability in skin MDA can be attributed to the combined influence of environmental and personal factors, which drive the production and transformation/uptake of MDA on skin. The lack of association between squalene depletion and MDA production suggests an intricate balance between parallel, possibly interconnected, ozone-initiated formation and loss mechanisms responsible for the measured concentrations of the two compounds.

1. Introduction

Ozone is one of the most reactive chemicals to which the skin is exposed in the environment. Ozone reacts with skin surface lipids, depleting protective antioxidants and resulting in lipid hydroperoxides

in the stratum corneum in a dose-dependent manner (Weber et al., 2001; He et al., 2006; Petracca et al., 2021). Such oxidative stress effects may lead to perturbations of the skin's barrier function and could trigger inflammatory responses (Thiele et al., 1997). Although ozone does not penetrate deeper in the skin, Pryor et al. (1995) suggested that lipid

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ozonation products are diffusible, stable and may act as signal transduction molecules through activation of specific lipases, which trigger the release of endogenous mediators of inflammation in deeper tissues not directly exposed to ozone. Adverse effects of ozone in the deeper functional layers of the skin have been demonstrated in murine models (Valacchi et al., 2002, 2004). Prolonged oxidative stress can lead to premature skin aging and various skin diseases (Bickers and Athar, 2006; Kammeyer and Luiten, 2015). Epidemiological studies have also underlined the contribution of ozone exposure to skin disease (Xu et al., 2011; Kousha and Valacchi, 2015).

Malondialdehyde (MDA) is generated from reactions of reactive oxygen species (ROS) and cell membrane lipids (lipid peroxidation) (Draper and Hadley, 1990; Janero, 1990). Polyunsaturated fatty acids oxidation is initiated by free radicals, forming complex intermediates such as bicyclic endoperoxides, which subsequently decompose to MDA and other products (Yin et al., 2011). Ozone can also initiate similar oxidative pathways and ROS formation can be substantial even in common indoor environments. Moreover, ROS can continue to form even in the absence of ozone, through mechanisms such as free radical initiated autoxidation of skin lipids (Morrison et al., 2023; Wang et al., 2025). Human sebum contains unsaturated triglycerides and fatty acids and 10–20% squalene by weight. While sebum, and thus squalene, is continuously excreted on the skin surface, squalene is also rapidly oxidized, for example by ozone (Wisthaler and Weschler, 2010; Zhou et al., 2016). Unsaturated fatty acids and their esters are also oxidized during ozone exposure, as evidenced by the formation of decanal and other terminal ozonation products (Wang et al., 2022). ROS initiates the oxidation of squalene to the metabolite squalene monohydroperoxide (SQOOH) (Pham et al., 2015), which along with MDA, is considered a biomarker of oxidative stress.

Real-world studies have found positive associations of air pollution with MDA in nasal fluid, exhaled breath condensate, or urine, suggesting the induction of lipid peroxidation. Concentrations of MDA in exhaled breath condensate and urine changed with the levels of PM_{2.5}, elemental carbon, SO₂, CO, NO₂, but not ozone, during the Beijing Olympics (Gong et al., 2013). On the other hand, Romieu et al. (2008) reported increased MDA in exhaled breath condensate with increasing PM_{2.5} as well as ozone. In another study, average personal PM_{2.5} and ozone exposure was associated with increased nasal, but not urinary, MDA (He et al., 2020). In the same study, however, an increase in personal ozone exposure was associated with decreased salivary MDA (He et al., 2022).

Only a few studies have examined the effect of ozone on human lipid peroxidation in the skin and even fewer have done so *in vivo*. He et al. (2006) reported an increase in lipid hydroperoxides in the stratum corneum, indicative of moderate state of oxidative stress, after 2h exposure to ozone. Curpen et al. (2020) developed a method using MDA and squalene measurements on skin to evaluate the effect of both gaseous and particulate air pollutants on the skin and the anti-pollution claims of cosmetics products. The authors found a significant effect of 2h ozone exposure on skin squalene (decreasing) and SQOOH and MDA (increasing), while the effects of particulate matter were smaller, reflecting the lower oxidative power of particles. Increasing concentrations of the pollutants were associated with higher oxidative stress, indicating a dose-dependent relationship between ozone and the biomarkers on the skin. While they observed an inverse relationship between MDA and squalene upon ozone exposure, it is unclear if this is due to a biological oxidative stress reaction or abiotic ozonation and autoxidation chemistry. Valacchi et al. (2017) found a significant increase of lipid peroxidation byproducts 4HNE protein adducts and 8-iso-prostaglandin-F(2a) in human skin compared with control tissues using biopsy analysis after ozone exposure. None of these studies used full-scale controlled human exposure to ozone levels common in indoor and outdoor environments.

Ozone reactions on the skin occur in skin surface lipids and the underlying stratum corneum. Changes to the lipid base of the stratum corneum is a determinant of the barrier function. A lack of free fatty

acids, cholesterol or ceramides can result in an increased transepidermal water loss (TEWL), a marker of skin barrier function (Mao-Qiang et al., 1996). Reactions of ozone with skin lipids may thus result in a disturbed skin barrier. However, the effect of ozone exposure on TEWL has not been previously studied. Exposure to NO₂ and particulate matter has been shown to increase TEWL (Shamsipour et al., 2020; Oh et al., 2021), but controlled short-term exposure studies are lacking.

In this paper, we examine the concentration of MDA in skin wipes before and after controlled exposure of volunteers to ozone by combining results from three different chamber studies. In one study, the formation of MDA in skin wipes (off skin) was studied to test the possibility of abiotic formation of MDA. Two of the studies also measured squalene on skin to examine the relationship between squalene and MDA on skin.

2. Methods

The three chamber studies described here applied similar but not identical methods of exposure, sample collection, and analyses. Therefore, we describe each separately and highlight differences. A summary of the methodological differences can be found in Table S1. The experimental conditions can be found in Table S2.

2.1. DTU study

Two groups of four healthy volunteers (age 20–34 years) were recruited. They were instructed not to drink alcohol or eat spicy food, garlic, lozenges, chewing gum, or mint drops one day prior to and during the days of the experiments. They were asked to avoid exposure to tobacco smoke and keep a somewhat consistent diet and mode of morning transport to the laboratory throughout the entire experimental session. They were instructed not to use any cosmetics, skin care products or other care products on the forearms for at least 24 h before the first experiment and until the end of all the experiments. They were provided with shampoo and soap to be used during the experimental period, and they were asked not to use any of it within 12 h before each experiment. They were also asked not to shower or wash their forearms in the morning before experiment. The volunteers wore a t-shirt inside the chamber in order for the forearms to be uncovered throughout the experiments.

The volunteers were exposed in a 22.5 m³ stainless steel climate chamber to 5 experimental conditions: 1) reference: 35 ppb ozone, 30% relative humidity (RH); 2) elevated RH: 35 ppb ozone, 60% RH; 3) elevated ozone: 70 ppb ozone, 30% RH; 4) no ozone: 0 ppb ozone, 30% RH; and 5) extended exposure: 35 ppb ozone, 30% RH, 6 h exposure. Exposure duration was 3 h, except for condition 5. Experiments with conditions 1–4 were performed with one group in the morning and the other group in the afternoon. Experiments with condition 5 (6 h duration excluding a 15 min lunch break) were performed with the two groups on two separate days. The chamber was ventilated with an air change rate (ACR) of 3.2 h⁻¹. A mixing fan was used to mix the air in the chamber. The occupancy exceeded the cooling capacity of the system and caused increases in the indoor temperature above the set point, especially in the afternoons. The temperature in the chamber was ~25 °C during morning sessions, ~28.5 °C during afternoon sessions. The actual average RH was between 25 and 37%, except for condition 2 (60%). To generate ozone, pure oxygen was delivered through a Jelight 600 UV ozone generator (Jelight Company Inc). The default target ozone concentration was ~100 ppb in the unoccupied chamber, with steady state ozone concentration in the occupied chamber around 35 ppb. One condition was without ozone in the chamber and one with elevated ozone level (~70 ppb in the occupied chamber, ~190 ppb in the unoccupied chamber). The ozone concentration was slightly below the target concentration under condition 2 (elevated RH) (17–26 ppb).

The volunteers arrived in the lab 45 min before entering the chamber. After about 20 min of acclimatization in a room with temperature

23.5 °C (SD 0.3 °C) and RH 35% (SD 5.8%), the transepidermal water loss (TEWL), indicative of the skin's barrier function, was measured on the right dorsal and volar forearm (corresponding to the skin wipe sampling site) of two of the four volunteers using a Tewameter TM HEX (Courage + Khazaka Electronic GmbH, Germany) as described in Rogiers (2001). The probe is a small hollow cylinder with 30 sensors. It was placed on the skin until the TEWL graphs became stable (up to 2 min).

Before entering the chamber, skin wipe samples were collected from the right forearm of each volunteer. Nonwoven medical compress (5 × 5 cm, 30 g) was used as the skin wipe (Mesoft®, Mölnlycke Health Care AB, Sweden). Skin wipe samples were collected (20 strokes) from 25 cm² of the dorsal forearms using the compress soaked with isopropanol; the investigator wore disposable nitrile gloves. The sampling area was marked by holding a plastic sheet with a 25 cm² hole (5 × 5 cm) onto the forearm. The sheet was cleaned with disposable wipes (Kimtech Science™ Kimwipes™; Kimberly-Clark Worldwide, Inc) wetted with isopropanol after each use. Clean pads treated in the same way as those used for the skin wiping but not used for sampling were used as field blanks. In 25% of sampling, a duplicate sample was taken from a skin area adjacent to the primary sampling location. Duplicate samples were strongly correlated ($r = 0.66$, $p = 0.002$). Afterwards, the wipes were placed in Eppendorf vials and stored in a freezer (−20 °C).

After exposure, the volunteers exited the chamber and rested for 30 min in order to acclimatize and extend the time between exposure and skin sampling. Curpen et al. (2020) observed the largest change in the concentration of MDA on skin when sampling was carried out between 1 and 3 h after a 2 h ozone exposure. Skin wipe samples after exposure were taken from the left forearm and TEWL measurements were done on the same arm as before exposure.

Two skin wipe samples were also collected from two experimental staff members on each experimental day. One of these samples from each person was exposed to chamber air by placing it on clean aluminum foil inside the chamber with the wiping surface facing upwards during the afternoon group exposure. The other sample was unexposed, placed in a vial and frozen immediately after collection. Additionally, two skin wipes were collected daily from three other colleagues (reference persons) over five consecutive days (one in the morning, one in the afternoon) to observe the everyday variability of MDA on skin. There were no restrictions on the behavior of the reference persons.

2.2. ICHEAR study

The Indoor Chemical Human Emissions and Reactivity (ICHEAR) study examined the impact of humans on indoor air chemistry of exhaled and dermally emitted human bioeffluents under a set of environmental conditions. The study design and methodology have been described elsewhere (Bekö et al., 2020; Langer et al., 2024). Briefly, in a 22.5 m³ stainless steel climate chamber ventilated with an ACR of 3.2 h^{−1}, groups of four volunteers were exposed to different temperatures (set points 25 and 31 °C), RH (set points 25% and 65%), clothing level (short or long), and ozone conditions. The groups consisted of either young adults (age 19–30), seniors (age 68–73) or teenagers (age 13–15). Two mixing fans operated in the chamber to achieve mixing of the air in the chamber. The volunteers were sitting in the chamber either with ozone absent from the chamber air (3h exposure; 3 half-day conditions as described in Langer et al. (2024)) or they were in the chamber with ozone absent in the morning (3h exposure) followed by a short lunch break and a period with ozone present (2.5h exposure; 14 full-day conditions). Pure oxygen was delivered through a Jelight 600 UV ozone generator (Jelight Company Inc). The target ozone concentration was ~100 ppb in the unoccupied chamber. Steady state ozone concentration in the occupied chamber was between 35 and 40 ppb.

Sterile 10 × 10 cm gauze pads were precleaned with dichloromethane for 24 h using Soxhlet extraction. Prior to sampling, they were wrapped in aluminum foil and stored in plastic zip lock bags. Skin wipe

samples were collected from 100 cm² of forearms using the gauze pads soaked with isopropanol (7 strokes). Samples were collected from the right forearm of each volunteer in the morning prior to chamber exposure and from the left forearm after exposure. Clean pads treated in the same way as those used for the skin wiping but not used for the sampling were used as field blanks. After the sampling, they were put in glass vials and stored in a freezer (−20 °C).

2.3. EPFL study

In a series of experiments within a 62 m³ climate-controlled chamber at the École Polytechnique Fédérale de Lausanne (EPFL), 6 young adults (age 21–29) were exposed to various environmental conditions in a fashion similar to ICHEAR. For details, see Yang et al. (2024). Briefly, four conditions were tested in 8 experiments, with replicate runs of each condition. The conditions included three ACRs (0.5, 1.5, and 3 h^{−1}) with mixing fans operating in the chamber to ensure full air mixing, and an additional condition with ACR 3 h^{−1} with the mixing fans off. The air temperature was 24 °C and the relative humidity was 50 %. The experiments with the two higher ACR lasted 3h in the morning without ozone and 3h after a lunch break in the afternoon with ozone being generated in the chamber. The experiments with ACR 0.5 h^{−1} lasted 5 h with ozone present in the chamber air during the whole time. Ozone was generated in the supply air duct using a Jelight 600 UV generator (Jelight Co. Inc., USA). The steady-state ozone level in the occupied chamber was between 25 and 35 ppb (Yang et al., 2024).

Sterile 5 × 5 cm cotton pads were precleaned with ethanol using solid-liquid extraction three times at Duke University. Then they were stored in vials with ethanol until skin sampling. Skin wipe samples were collected by wiping a 5 × 5 cm area of the lower forearm for 1 min. The wipes were then placed in an Eppendorf tube. 1 mL methanol was added and the tubes were stored in a freezer until shipping to Duke University for analyses. Skin wipe samples were collected from one arm before the volunteers entered the ozone-laden chamber (afternoon sessions with higher ACRs; long session from the morning with lowest ACR) and from the other arm after the volunteers left the chamber.

2.4. Chemical analyses of the wipes

In the DTU study, skin wipes were analyzed for MDA, squalene, and cholesterol. Internal standards were prepared by dissolving 5 mg of d₆-squalene and 5 mg of d₇-cholesterol with 2.5 mL methanol (2 mg/mL). The two solutions were mixed, resulting in a 1 mg/mL solution for each target compound. Each wipe was placed into a 15 mL tube. We used previously established methods to extract lipids from skin wipes (Lin et al., 2024) and analyze MDA (Cui et al., 2018). 10 µL internal standard mixture was added. Then 2 mL of methanol was added, and the tubes were vortexed for 15 min and centrifuged. 150 µL supernatant was transferred to a 1.5 mL amber vial for MDA analysis. We mixed the extract with 750 µL phosphoric acid (440 mM) and 250 µL thiobarbituric acid (TBA) solution (42 mM). The mixture was incubated at 80 °C for 1 h during which MDA-TBA adducts were formed. The solution was then cooled to room temperature and injected into a Waters 2695 HPLC system with a multi λ fluorescence detector (Waters 2475). We used a Hypersil Gold column (C8, 5 µm, 4.6 × 150 mm, Waters) for chromatographic separation. The excitation and emission wavelengths were 532 nm and 553 nm, respectively. For each batch of 40 samples, a laboratory blank and a positive control sample were prepared. Results of blank and positive control samples are shown in Table S3.

8 mL methanol was then added to the tubes with the wipes. They were vortexed for 15 min, centrifuged, and then stored at −20 °C until analyses for squalene and cholesterol at the University of North Carolina. The remaining sample extracts were blown down to dryness and reconstituted with 1 mL hexane. N-Methyl-N-(trimethylsilyl)tri-fluoroacetamide (MSTFA; derivatizing agent for cholesterol) and squalene (internal standard) were added and the samples were incubated at

60 °C for 1 h. Samples were analyzed by gas-chromatography, mass spectrometry in selected ion mode. Detailed methods, chromatography information and calibrations can be found in the Supplementary Material (Table S4).

In the EPFL study, the vials with the wipes soaked in 1 mL methanol were analyzed for MDA as described above. In ICHEAR, the wipes from three volunteers per condition were cut into two halves and extracted and analyzed separately for either squalene and cholesterol, or pyroglutamic acid as described in Langer et al. (2024). The subsamples for the analysis of squalene and cholesterol were extracted with pentane/hexane mixture and cholesterol was derivatized using MSTFA. The subsamples for the analysis of pyroglutamic acid were extracted by ultrasonication in 15 mL of methanol for 30 min, twice. The combined extracts were dried under a gentle stream of nitrogen at room temperature to approximately 2 mL, divided into two 1 mL portions (by weighing). One portion was used for the analysis of pyroglutamic acid while the second portion was shipped to Duke University and analyzed for MDA as described above. MDA concentrations were normalized by sampled skin area. Squalene concentrations were normalized by skin area and cholesterol concentrations, a compound much less reactive with ozone (Langer et al., 2024).

It is important to note that sample analyses in the three studies were performed over a 4-year period. When examining the batch effects, we found that the MDA response factor in the 2021 standard curve was 3-fold higher than the 2024 standard curve. The difference is due to the degradation of 2024 standard curve which was confirmed using a newly purchased standard. Therefore, data from all subsequent analyses were corrected for batch effect to the 2021 values using the ratio of the slopes of the standard calibration curves, assuming a stable relationship between MDA concentrations and its fluorescence. See text and Figs. S1 and S2 in the Supplementary Material for details.

2.5. Data analyses

Post-exposure to pre-exposure ratios for MDA, squalene and TEWL were calculated. Two-sample *t*-test (with equal or unequal variances after variance ratio test) and two-sample Wilcoxon rank-sum (Mann-Whitney) test were used to compare post-/pre-exposure ratios between different experimental conditions. Pre- and post-exposure means were compared using paired *t*-test and Wilcoxon matched-pairs signed-ranks test. Pre-vs. post-exposure correlations and correlations among measured variables (MDA vs. squalene, MDA vs. TEWL) were analyzed using univariate linear regression providing a coefficient of determination (R^2). Additionally, four linear regression models were used to test the relationship between changes in MDA and changes in squalene on skin during exposure. Two modeled post-exposure MDA concentrations with pre-exposure MDA and either post/pre squalene ratio or Δ squalene as independent variables. The other two modeled Δ MDA with either Δ squalene or post/pre squalene ratio as independent variables. In these analyses, Δ MDA and Δ squalene were post-minus-pre-exposure values normalized by pre-exposure concentrations. For the analyses of the relationship between MDA and squalene on skin, the data from ICHEAR and the DTU study were merged. $P < 0.05$ was considered statistically significant. Statistical analyses were performed in Stata software (StataCorp LLC, College Station, Texas, USA).

In the analyses of TEWL in the DTU study, the mean of the last 10 readings (10 s) was used for each measurement. Three measurements were made at each location, and their results were averaged to obtain a TEWL value per location on the skin and measurement session.

Participants in all three studies provided informed consent before participation. The EPFL study was approved by the Human Research Ethics Committee (HREC) at EPFL. The ICHEAR and the DTU studies did not require specific ethical approval, as the Technical University of Denmark has blanket approval to conduct noninvasive experiments that take place under normally occurring indoor conditions (KA 04741, Regional Committee for Research Ethics – Copenhagen).

3. Results

Since the three chamber studies did not use identical protocols, the main results are presented separately for each study.

3.1. DTU study

The concentration of MDA in skin wipes was significantly higher after exposure to ozone than before (Table 1). Average post-exposure MDA levels were not significantly different from pre-exposure levels when there was no ozone present in the chamber air (Table S5). Mean post/pre-exposure MDA ratio with ozone present was significantly higher than that with ozone absent (Fig. 1). Compared to the reference condition (30% RH, 35 ppb O_3 , 3 h exposure), the ratio was smaller with no ozone ($p < 0.05$) and higher when ozone concentration doubled ($p > 0.05$) or when exposure duration was 6 h ($p < 0.01$) (Fig. 1, Table S5). The ratio was significantly lower for the elevated RH condition, although it should be noted that ozone concentration in the chamber was about 50–70% of the other conditions with ozone. A slight increase in MDA on skin also occurred without ozone present during exposure.

An order of magnitude more MDA was measured on wipes with skin oil collected from two staff members and subsequently exposed to ozone in the chamber compared to wipes collected after volunteers were exposed to the chamber air. Significantly more MDA was also measured on wipes exposed to chamber air without ozone than on non-exposed wipes (Table 2), further supporting the possibility that MDA generation on skin may also occur without ozone exposure. Additionally, the average ratio of MDA in staff skin wipes exposed to chamber air to their counterparts not exposed to chamber air (collected from same arm at the same time) was 1.7 with ozone absent (1.7 for both staff members) and 11.5 with ozone present in the chamber air (15.1 and 7.0) ($p < 0.05$ for exposed/unexposed pairs). It was 9.8 with 35 ppb ozone and 14.9 with 70 ppb ozone. These results, however, rely on a small number of samples. We did not perform control experiments with blank dry or isopropanol-soaked pads exposed to ozone.

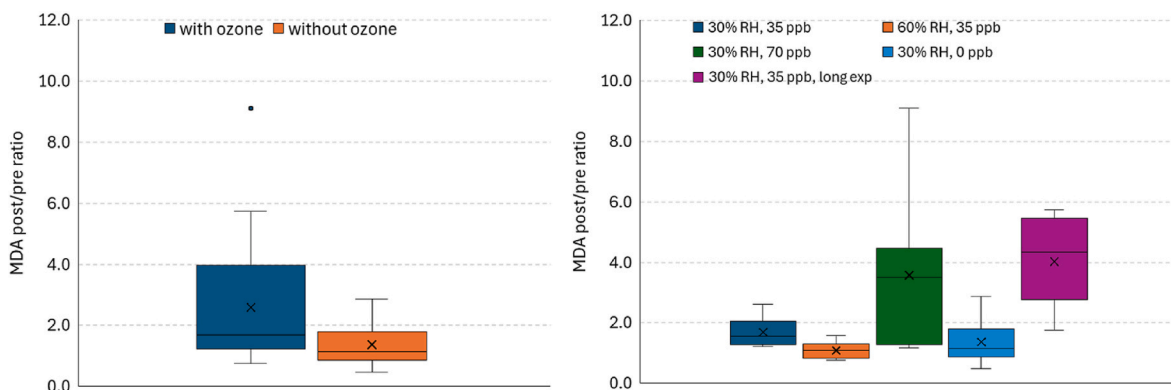
We observed considerable inter-and intrapersonal variability in MDA concentrations on skin wipes. MDA concentrations in pre-exposure wipes across the 8 subjects and 5 experimental days ranged between 0.01 and 0.47 ng/cm² (mean 0.14 ± 0.08 ng/cm²; Table S6). MDA concentrations in wipes collected from 3 reference persons in the morning and afternoon over 5 consecutive days ranged between 0.12 and 0.89 ng/cm². Mean (0.35 ng/cm²) and median (0.3 ng/cm²) morning and afternoon values were nearly identical, and the median of the afternoon/morning ratio was 1.1. However, this ratio ranged from 0.3 to 3.4. It is noteworthy that blank samples had detectable levels of MDA. The amount of MDA in them was significantly lower than in pre-exposure skin wipes and skin wipes from reference persons ($p < 0.001$) (Table S7). However, some skin wipe samples had low levels, comparable to some blank samples.

Pre-vs. post-exposure squalene concentrations in the skin wipes normalized by cholesterol are shown in Fig. 2. The slope of the relationship with ozone was slightly smaller than with ozone absent. However, average post/pre-exposure ratios were similar and below unity for all experimental conditions (average 0.78 with ozone, 0.82 without ozone). The ratio was lowest for Condition 5 with extended exposure to ozone (0.68), highest for Condition 2 with elevated RH (0.91) (which also happened to have lower ozone concentration).

Exposure in the chamber (pre-vs. post-exposure) had no significant effect on TEWL measured on dorsal or volar forearm regardless of ozone concentration (Tables S8 and S9). At both locations, TEWL decreased slightly during 3h exposures (regardless of the presence of ozone), but it increased slightly after 6h exposure with ozone present. The correlation between pre-exposure and post-exposure data was very strong and the datapoints were very close to the line of equality (Fig. S3). The relationship between MDA in skin wipes and the corresponding TEWL was

Table 1MDA concentrations in skin wipes (ng/cm²) before and after ozone exposure in the three studies.

Exposure condition	N	Pre-exposure		Post-exposure		Post/Pre ratio		p-value pre vs. post ^b	p-value ratio ^a
		mean ± SD	median	mean ± SD	median	mean ± SD	median		
DTU study									
With ozone	32	0.14 ± 0.08	0.13	0.31 ± 0.21	0.24	2.6 ± 1.9	1.7	0.000/0.000	0.009/0.07
Without ozone	8	0.14 ± 0.08	0.12	0.15 ± 0.04	0.14	1.4 ± 0.7	1.1	0.76/0.58	
ICHEAR study									
With ozone	39	0.50 ± 0.17	0.51	0.56 ± 0.20	0.56	1.17 ± 0.41	1.15	0.043/0.027	0.15/0.054
Without ozone	9	0.57 ± 0.17	0.63	0.52 ± 0.21	0.48	0.95 ± 0.38	0.78	0.53/0.17	
EPFL study									
With ozone (all)	48	0.26 ± 0.04	0.25	0.31 ± 0.09	0.29	1.22 ± 0.3	1.12	0.000/0.000	-

^a Two-sample *t*-test (with unequal variances after variance ratio test)/Two-sample Wilcoxon rank-sum (Mann-Whitney) test.^b Paired *t*-test and Wilcoxon matched-pairs signed-ranks test.**Fig. 1.** Box plots of the post-/pre-exposure ratios of MDA in skin wipes in the DTU study. They display the mean (marked with X), median (marked with a horizontal line) and interquartile range (IQR). The whiskers denote minimum and maximum and the dots the outliers. Left: *n* = 32 with ozone (17–70 ppb), 8 without ozone; right: *n* = 8. Exposure duration 3 h, except long exposure condition with ozone 6 h. Time between end of exposure and wipe collection 30–60 min.**Table 2**MDA in skin wipes from two staff members. The wipes were either exposed or not exposed to chamber air after collection (ng/cm²).

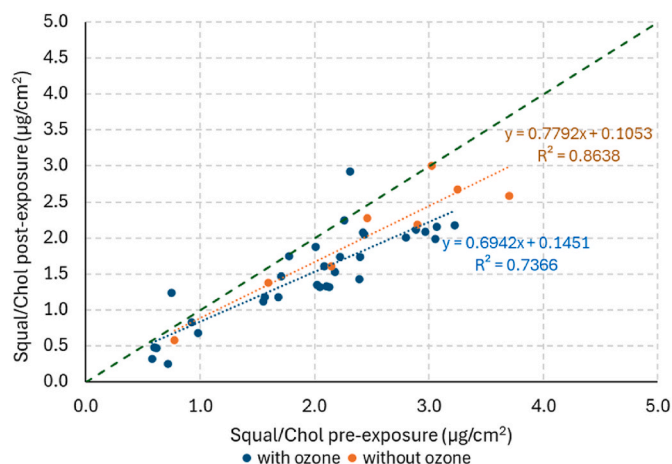
Wipe exposure condition	N	mean ± SD	median	min.	max.	p-value ^a
1. No chamber exposure	12	0.18 ± 0.07	0.16	0.07	0.31	1 vs 2: 0.000/0.000
2. Chamber with ozone	9	1.63 ± 0.51	1.92	0.89	2.32	1 vs 3: 0.006/0.021
3. Chamber without ozone	3	0.36 ± 0.09	0.34	0.25	0.47	2 vs 3: 0.003/0.013

^a Two-sample *t*-test (with equal or unequal variances after variance ratio test)/Two-sample Wilcoxon rank-sum (Mann-Whitney) test.

very weak (Fig. S4).

3.2. ICHEAR study

MDA concentrations in skin wipes collected in the ICHEAR study, stratified by ozone exposure, temperature and relative humidity, clothing type, and subject age can be found in Table S10. These data correspond to those presented for squalene in the skin wipes by Langer et al. (2024). Significantly higher MDA concentrations were measured after chamber exposure with ozone than before (Table 1). When ozone was absent in the chamber air, MDA concentrations in the wipes tended to be lower after exposure than before. The post-/pre-exposure ratio of skin MDA was higher with ozone than without ($p > 0.05$) (Fig. 3, left). MDA concentrations and the post-/pre-exposure ratio were higher when the subjects wore short sleeve clothing than with long sleeve clothing

**Fig. 2.** Pre- and post-exposure squalene concentrations normalized by concentration of cholesterol in skin wipes in the DTU study. The green dashed line is the line of equality. *N* = 32 with ozone (17–70 ppb, 3–6 h exposure), 8 without ozone (3 h exposure). Time between end of exposure and wipe collection 30–60 min. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

($p < 0.05$), likely reflecting the fact that unclothed forearm is more exposed to ozone in the air. Age, temperature and RH had no significant effect on the MDA concentrations on skin. The results are presented in Table S10, but we do not draw any conclusions due to the small sample size.

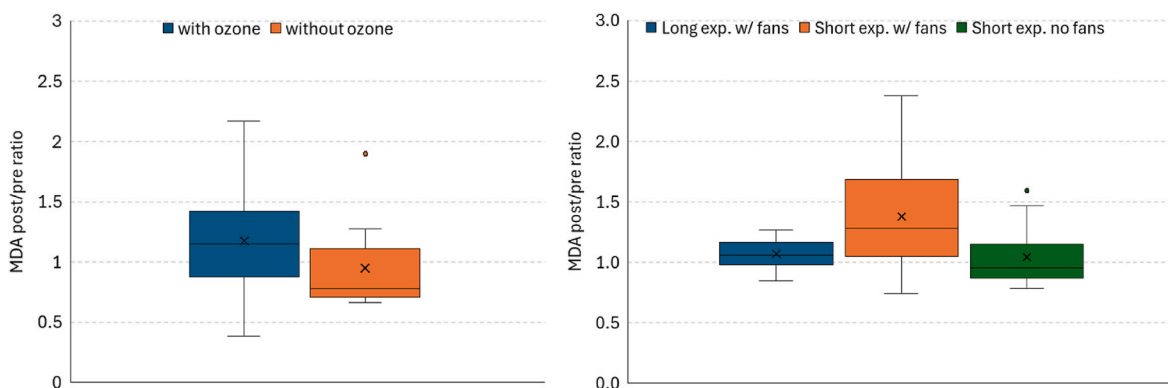


Fig. 3. Box plots of the post-/pre-exposure ratios of MDA in skin wipes in the ICHEAR study (left) and EPFL study (right). ICHEAR: $n = 39$ with ozone (35–40 ppb), 9 without ozone; exposure duration 2.5–3 h. EPFL: $n = 12$ long exp. with fans, 24 short exp. with fans, 12 short exp. without fans; exposure duration 5 h, 3 h, 3 h, respectively; ozone 25–35 ppb. Time between end of exposure and wipe collection always <10 min.

3.3. EPFL study

In the EPFL study, all experimental conditions involved exposure to ozone, which significantly increased MDA concentration on skin. Post-exposure MDA levels in skin wipes were significantly higher than pre-exposure levels (Table 1). Compared to when the mixing fans were on (short exposure), the post-/pre-exposure MDA ratios were significantly lower ($p < 0.05$) when the mixing fans in the chamber were off (short exposure) and when the exposure was longer (fans on; $p < 0.01$) (Fig. 3, right; Table S11).

4. Discussion

4.1. MDA in skin wipes

Post-exposure MDA concentrations on skin wipes were consistently significantly higher in all three studies than pre-exposure concentrations when ozone was present in the chamber, but not when ozone was absent. Post-/pre-exposure ratios were also higher with ozone than without ozone in both studies which allowed for this comparison. Taking all data from the three studies together, pre-exposure MDA was strongly correlated with post-exposure MDA (identical slopes of 0.75 with and without ozone; $R^2 = 0.45$ and $R^2 = 0.62$, respectively; Fig. S5). In skin wipes directly exposed to ozone in the chamber, MDA levels were also elevated. This suggests that ozone-initiated chemistry may generate MDA in the absence of an inflammatory process. Some MDA may be generated on the skin also without ozone exposure. This indicates that lipid peroxidation may occur to a smaller extent also in the absence of ozone, likely due to the presence of other oxidants, as well as the possibility of autooxidation (Morrison et al., 2023; Wang et al., 2025). It is important to note, however, that the dataset with ozone absent was much smaller than that with ozone present.

Our results are consistent with the literature on lipid peroxidation resulting from ozone exposure. He et al. (2006) reported a 2.3-fold increase in lipid hydroperoxides in the stratum corneum measured by tape stripping on forearms exposed for 2 h to 800 ppm ozone. Curpen et al. (2020) found a 49% increase in the concentrations of MDA in sebum samples collected from a small skin area on the back of 8 subjects exposed to ozone for 2 h to 100 to 1000 ppb ozone. To the best of our knowledge, our study is the first to demonstrate this through controlled full-body exposure to low levels of ozone routinely encountered in indoor and outdoor environments.

The order of magnitude higher MDA levels on skin wipes directly exposed to ozone compared to wipes collected from volunteers who were exposed to ozone may indicate the occurrence of MDA removal processes on skin which are not available to MDA formed after ozone reacts with skin wipes placed on aluminum foil. MDA is a reactive

substance (Janero, 1990, and references therein). MDA readily forms Schiff base adducts with proteins, and smaller species such as lysine and ammonia, and may bind within the topmost layers of the stratum corneum. This mechanism limits absorption into blood, but also lowers extractable MDA in skin wipes. On the surface of skin, non-ionized MDA may be dermally absorbed, penetrating into deeper layers of the skin. This has been suggested for ozone skin lipid reaction products such as monocarbonyls, geranyl acetone, and 6-MHO (Lakey et al., 2017). That is, dermal uptake may be a sink for MDA formed on the surface of the skin. Relatively high dermal permeability has been demonstrated for certain environmental chemicals (Weschler et al., 2015; Bekö et al., 2018). We were unable to find estimates of a dermal permeability coefficient for MDA in the literature. MDA's molecular weight is 72 g/mol, and we estimate that the log of its octanol-air partition coefficient (K_{oa}) is between 4 and 5. Taken together, these values suggest that MDA's dermal permeability coefficient is moderate (see Fig. 1 of Weschler and Nazaroff, 2014). Neither dermal absorption nor adduct formation are processes that are anticipated to occur after ozonolysis generates MDA in skin wipes resting on aluminum foil.

4.2. Effects of environmental and personal factors

Elevated temperature and RH did not result in significantly different MDA generation in the ICHEAR study. In the DTU study, we observed lower MDA generation at higher RH, but we cannot draw conclusions about the effects of RH due to the lower ozone concentration during this condition. This likely occurred because higher relative humidity may result in elevated ozone deposition velocity, and thus lower ozone concentration in the chamber, but this depends on the surface material properties (Grøntoft et al., 2004; Rim et al., 2016; Downey et al., 2024). It is, however, noteworthy, that, in the ICHEAR study, temperature and humidity did not affect squalene concentrations significantly either (Langer et al., 2024). Similarly, Qu et al. (2025) observed that air temperature and RH had little influence on ozone-driven VOC emissions from skin due to the body's regulation of these factors. On the other hand, changes in temperature and relative humidity can impact emission rates of VOCs from volunteers and reaction kinetics responsible for nanocluster aerosols and ozone-derived products formation rates (Wang et al., 2022; Yang et al., 2021; Arata et al., 2019; Zhou et al., 2016; Lakey et al., 2023). High humidity may also increase skin permeability for certain compounds (Iikura et al., 2019; Ishida et al., 2015). The effect of RH on MDA generation on skin warrants further investigation.

Substantially more MDA was measured on the skin after 6 h ozone exposure than after 3 h ozone exposure in the DTU study. On the other hand, in the EPFL study, 5 h ozone exposure led to significantly lower post-/pre-exposure MDA ratio than 3 h ozone exposure. The ACR was much lower during the longer exposure in the EPFL study. Ozone uptake

on surfaces, including human skin, is strongly influenced by boundary layer thickness which is influenced by air velocity and thus possibly by air change rate. Other factors, such as differences in ozone concentration, environmental conditions and especially personal factors (including skin properties), which may influence the peroxidation environment and be responsible for the inter-and intrapersonal variability in MDA concentrations on skin wipes, may have contributed to this observation.

The significantly higher MDA production rate with short sleeve clothing is consistent with other results from the ICHEAR study. With ozone present, emission rates of skin lipid ozonolysis products, the total OH reactivity of the chamber air and the concentration of resulting nanocluster aerosols were higher with short clothing (Wang et al., 2022; Zannoni et al., 2021; Yang et al., 2021). Squalene was depleted to a larger extent from the skin of participants wearing short sleeved clothing (Langer et al., 2024).

The results discussed above are central tendencies. Looking at individual variability, MDA on skin can both increase and decrease, regardless of ozone exposure. This can also occur throughout the day. It's noteworthy that the afternoon/morning MDA ratio among the three reference persons ranged between 0.3 and 3.4 over five days. Thus, differences in MDA on skin between individuals and between days for the same individual can be substantial. This is not surprising given that MDA yields vary with the nature of the polyunsaturated fatty acids being peroxidized and the lipid oxidation products that decompose to MDA. The peroxidation environment may change with skin properties and environmental variables. It is worth noting, in line with this, that ozone-driven skin emission rates were shown to vary with thickness of the lipid layer and abundance of fresh and oxidized skin lipid constituents and exogenous compounds (Pandurangi and Morrison, 2008; Morrison et al., 2021).

We found no relationship between MDA on skin and TEWL, the skin's water barrier integrity. The effect of ozone exposure on TEWL has not been previously studied. The literature is conflicting on the effect of temperature and humidity on TEWL, (Green et al., 2022; Goad and Gawkrödger, 2016; Engebretsen et al., 2016). Exposure to NO₂ and particulate matter has been shown to increase TEWL, possibly through oxidative damage to epithelial cells (Shamsipour et al., 2020; Kim et al., 2016; Huang et al., 2019; Oh et al., 2021). In the DTU study, pre- and post-exposure TEWL were highly correlated, providing a solid foundation for observing changes due to exposure. However, no significant differences were found between the reference condition and the other environmental conditions. The post-/pre-exposure TEWL ratio was above unity for the 6h ozone exposure, and below unity for all other experimental conditions. The effect of ozone on TEWL was, however,

not statistically significant.

4.3. Relationship between MDA and squalene

As shown in Fig. 4 (left), the slope for the relationship between normalized pre-exposure and post-exposure squalene concentration in the DTU and ICHEAR studies combined was higher with ozone absent from the chamber (1.3) than with ozone present (0.7). This was the trend also when the two studies were analyzed individually, although we judge that the combined dataset provides larger sample size and more robust results. This is in line with our earlier results from the ICHEAR study alone (Langer et al., 2024) showing depletion of squalene in the presence of ozone. However, for the DTU study alone, this slope was below unity both with and without ozone, indicating a larger loss than replenishment of squalene by secretion from the sebaceous glands. Other loss processes than squalene ozonolysis may be at play. It should be noted that squalene levels in the wipes from the DTU study were much lower than those from the ICHEAR study. The data from the two studies shows that ozone exposure clearly depletes squalene from the skin and produces MDA on the skin, with the post-exposure values of both strongly dependent on the pre-exposure level (Fig. 4). The post-/pre-exposure normalized squalene ratio was lower with ozone present (t -test $p = 0.004$, Wilcoxon $p = 0.002$), while post-/pre-exposure MDA ratio was higher with ozone present (t -test $p = 0.08$, Wilcoxon $p = 0.02$). Fig. 5 (left) indicates that the MDA/squalene ratio was more than two times higher post-exposure than pre-exposure when ozone was present (slope 2.2), but not when ozone was absent (slope 0.79). The strong effect of ozone on the MDA/squalene ratio was especially pronounced in the skin wipes directly exposed in the chamber (Figs. S6 and S7).

We found only one human study that measured squalene and MDA levels simultaneously (Curpen et al., 2020). The authors reported 42% decrease in the concentration of squalene and 75% and 49% increase in the concentrations of SQOOH and MDA, respectively, in sebum samples, after 2h ozone exposure. The relationship between squalene and MDA was not explored.

There was no relationship between post-/pre-exposure MDA ratio and post-/pre-exposure squalene ratio in skin wipes from the DTU and ICHEAR studies (combined, Fig. 5 right, or individually). There was also no relationship between squalene normalized by cholesterol and MDA in all skin wipes from the two studies (Fig. S8), nor between the pre- and post-exposure delta MDA and delta squalene. The four regression models showed no significant effect of squalene on MDA on the skin wipes. Squalene in skin oils is thus likely not a direct driver of MDA generation on the skin. Parallel, interconnected processes are probably responsible for the concentrations of squalene and MDA on the surface of the skin.

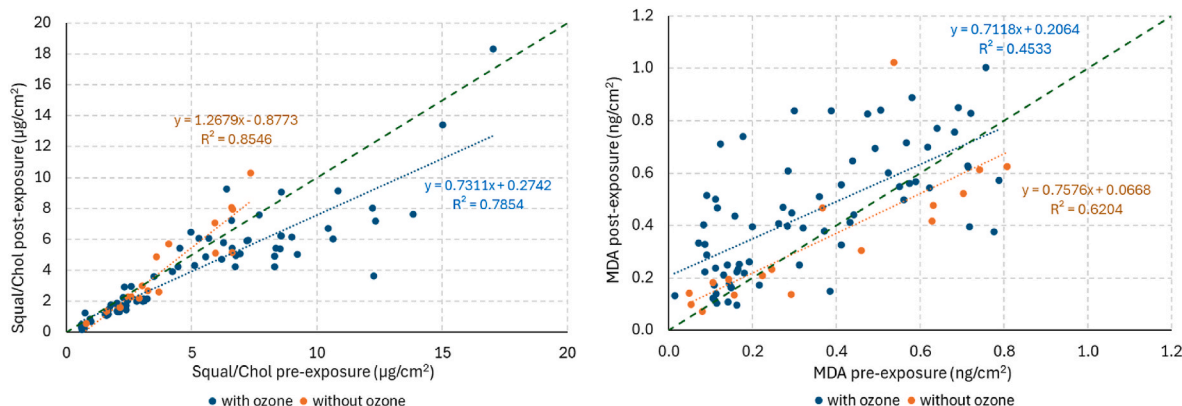


Fig. 4. Pre- and post-exposure normalized squalene (left) and MDA concentrations (right) in skin wipes from the DTU and ICHEAR studies combined, with ozone present or absent in the chamber air. The green dashed line is the line of equality. $N = 71$ with ozone, 17 without ozone. DTU: ozone 17–70 ppb; exposure duration 3–6 h; time between end of exposure and wipe collection 30–60 min. ICHEAR: ozone 35–40 ppb; exposure duration 2.5–3 h; time between end of exposure and wipe collection <10 min. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

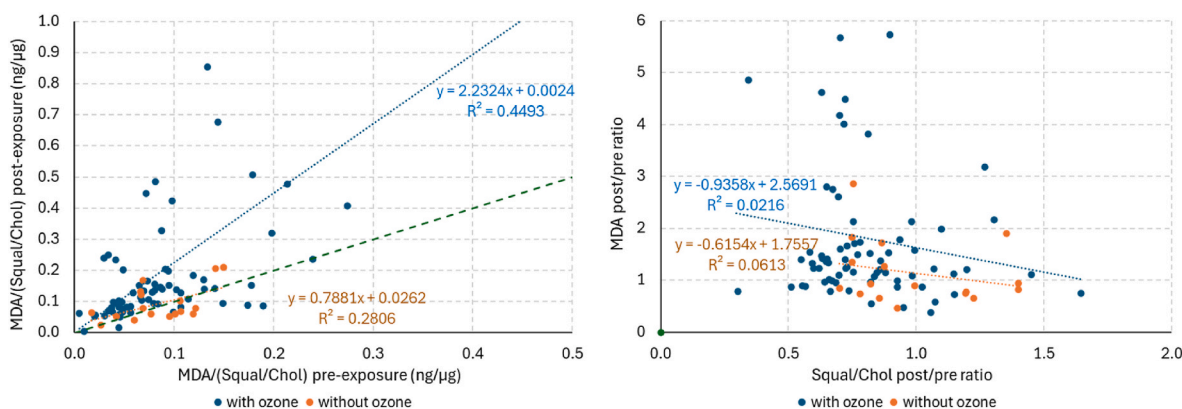


Fig. 5. Left: Relationship between pre-and post-exposure MDA/Squalene (normalized) ratio; right: Relationship between post-/pre-exposure MDA ratio and post-/pre-exposure squalene ratio (normalized by cholesterol) in the skin wipes, from the DTU and ICHEAR studies. N = 71 with ozone, 17 without ozone. DTU: ozone 35–70 ppb; exposure duration 3–6 h; time between end of exposure and wipe collection 30–60 min. ICHEAR: ozone 35–40 ppb; exposure duration 2.5–3 h; time between end of exposure and wipe collection <10 min.

Sebum is a continuous source of squalene (the major ozone sink) and of unsaturated fatty acids and triglycerides (sources of MDA). Ozonation of carbon-carbon double bonds in unsaturated compounds results first in primary ozonides, then Criegee intermediates, which can recombine to form semistable secondary ozonides and other stable products such as aldehydes, or decompose to ROS (e.g., hydroxyl radical) (Wisthaler and Weschler, 2010; Zhou et al., 2016). ROS can then initiate the peroxidation of fatty acids and triglycerides to form MDA (Yin et al., 2011). MDA is also a second generation product of ozone reacting with certain skin oil constituents (e.g., sebales and, to a lesser extent, linoleates (See Supplementary Material for details)). Squalene can react with ROS, acting as a free-radical sink, but without forming MDA. MDA may be removed from the skin surface by forming Schiff base adducts with DNA (Marnett, 1999), lysine, and ammonia and possibly by transdermal uptake, which can vary among participants. Therefore, we believe there exists a balance among processes that control the MDA concentration on the skin, including sebum emission rates, MDA generation via ozone reactions with sebales and linoleates acids present in sebum (Nicolaidis, 1974), ROS formation mechanisms, MDA generation via lipid peroxidation, competition between squalene and fatty acids for ozone and ROS, and MDA loss to various Schiff base adducts (e.g., with lysine from the outermost corneocyte layers) and perhaps dermal absorption. Individual factors can further complicate this balance. For example, temperature, moisture, and pH of the skin surface could all influence the relative reaction rates, product yields, and adduct formation, while differences in skin structure could influence MDA absorption rates. The interconnected chemical mechanism, variability among participants and low ozone exposure conditions (relative to Curpen et al., 2020) likely obscure any relationship between squalene, a marker of skin oil, and MDA concentrations.

4.4. Limitations

This study is the first to investigate the effect of ozone on human lipid peroxidation in the skin using full-scale controlled human exposure to ozone levels common in indoor and outdoor environments. Our approach has, however, limitations that should be addressed in future studies. The data were collected in three distinct studies with some differences in exposure conditions, environmental parameters, personal characteristics of the volunteers, and sampling and analytical methodologies. Therefore, the main results are presented separately for each study. ICHEAR wipe extracts were analyzed for MDA after a longer storage period (2+ years) compared to the other studies, but the impact on the presented results that rely on more than one study is expected to be minimal. While the difference in storage duration and other experimental conditions may lead to batch differences, all samples from the

same participants were analyzed in the same batch. Additionally, the post-/pre-exposure ratios of MDA levels allow subjects to be their own controls.

The data available for statistical comparisons of environmental and personal factors, the data for inter- and intrapersonal comparisons (reference persons), and the data from the *in vitro* tests exposing skin oil-containing wipes relied on a relatively small sample size with limited statistical power. The daily activities and personal habits of the 3 reference persons were not recorded. Thus, the results cannot be reliably extrapolated for the general population. Studies are required to further investigate abiotic MDA generation on skin and the effects of environmental factors such as temperature and relative humidity. Additionally, TEWL was only measured before and after ozone exposure outside the chamber, which prevents the capture of possible real-time effects of ozone exposure. The environmental effects on TEWL may differ with the anatomical region (Liu et al., 2017). We only measured TEWL on the forearm, and we did so before the volunteers entered the chamber and after they exited, in order not to disturb the steady-state ozone concentration in the chamber. Measurements on more anatomical sites during the actual exposure inside the chamber are warranted in order to capture any quickly occurring skin barrier changes, if present.

5. Conclusions

Skin MDA can serve as an indicator for skin cell membrane lipid damage as a consequence of ozone exposure commonly encountered in indoor and outdoor settings. The high rate of MDA generation observed when wipes containing skin oils were exposed to ozone indicated that ozone-driven chemistry generates MDA in the absence of an inflammatory process that generates reactive oxygen species *in vivo*. The inter- and intra-person variability observed among reference persons can be attributed to the combined influence of environmental and personal factors, which affect the production and transformation/uptake of MDA on skin. Different, although possibly interconnected, production and loss mechanisms are responsible for the measured concentrations of MDA and squalene on skin. These results may have implications for interpretation of skin biomarkers in exposure studies, as well as for indoor chemistry, personal exposure, and product evaluation. For example, certain personal care products, often marketed as anti-pollution products, have been shown to reduce ozone flux to skin and the yields of squalene oxidation products (Morrison et al., 2022). The results of this study should be replicated in future human exposure studies with realistic ozone levels using a larger number of volunteers across varied personal and environmental conditions. Such studies would benefit from added measurements of the squalene monohydroperoxide SQOOH. Finally, the potential for ozone-driven skin lipid

damage to increase the skin permeability of chemicals and biological agents (e.g., bacteria and viruses) deserves future research.

CRedit authorship contribution statement

Gabriel Bekö: Writing – review & editing, Writing – original draft, Visualization, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Yan Lin:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Sarka Langer:** Writing – review & editing, Resources, Methodology, Investigation, Formal analysis. **Glenn Morrison:** Writing – review & editing, Resources, Methodology, Investigation, Formal analysis, Conceptualization. **Meixia Zhang:** Writing – review & editing, Investigation. **Dusan Licina:** Writing – review & editing, Resources, Project administration, Methodology, Investigation, Funding acquisition. **Charles J. Weschler:** Writing – review & editing, Methodology, Conceptualization. **Junfeng (Jim) Zhang:** Writing – review & editing, Resources, Methodology, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Gabriel Bekö reports financial support was provided by Alfred P. Sloan Foundation. Gabriel Bekö reports financial support was provided by Fonden af 20. December. Dusan Licina reports financial support was provided by Swiss National Science Foundation. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envres.2026.125624>.

Data availability

Data will be made available on request.

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