Title

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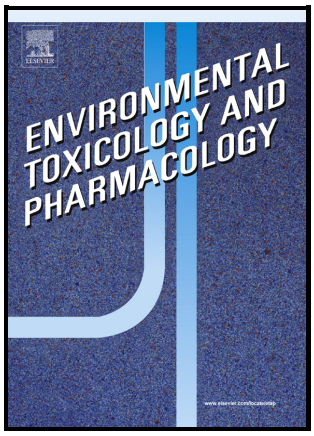

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Chronic exposure to traffic-related air pollution reduces lipid mediators of linoleic acid and soluble epoxide hydrolase in serum of female rats

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Abstract

Chronic exposure to traffic-related air pollution (TRAP) is known to promote systemic inflammation, which is thought to underlie respiratory, cardiovascular, metabolic and neurological disorders. It is not known whether chronic TRAP exposure dampens inflammation resolution, the homeostatic process for stopping inflammation and repairing damaged cells. *In vivo*, inflammation resolution is facilitated by bioactive lipid mediators known as oxylipins, which are derived from the oxidation of polyunsaturated fatty acids. To understand the effects of chronic TRAP exposure on lipid-mediated inflammation resolution pathways, we measured total (i.e. free+bound) pro-inflammatory and pro-resolving lipid mediators in serum of female rats exposed to TRAP or filtered air (FA) for 14 months. Compared to rats exposed to FA, TRAP-exposed rats showed a significant 36-48% reduction in fatty acid alcohols, specifically, 9-hydroxyoctadecadienoic acid (9-HODE), 11,12-dihydroxyeicosatetraenoic acid (11,12-DiHETE) and 16,17-dihydroxydocosapentaenoic acid (16, 17-DiHDPA). The decrease in fatty acid diols (11,12-DiHETE and 16, 17-DiHDPA) corresponded to a significant 34-39% reduction in the diol to epoxide ratio, a marker of soluble epoxide hydrolase activity; this enzyme is typically upregulated during inflammation. The findings demonstrate that 14 months exposure to TRAP reduced pro-inflammatory 9-HODE concentration and dampened soluble epoxide hydrolase activation, suggesting adaptive immune changes in lipid mediator pathways involved in inflammation resolution.

Running title

Effects of chronic air pollution on rat serum oxylipins

Keywords

Oxylipins, blood, inflammation, resolution

Abbreviations:

AA, arachidonic acid;

ALA, alpha-linolenic acid;

BHT, butylated hydroxytoluene;

CHE, Center for Health and the Environment;

COX, cyclooxygenase;

CYP, cytochromes P450;

DGLA, dihomo-γ-linolenic acid;

DHA, docosahexaenoic acid;

DiHDPA, dihydroxydocosapentaenoic acid ;

DiHETE, dihydroxyeicosatetraenoic acid;

DiHETrE, dihydroxyeicosatrienoic acid ;

EDTA, ethylenediaminetetraacetic acid;

EPA, eicosapentaenoic acid;

EpDPE, epoxydocosapentaenoic acid ;

EpETE, epoxyeicosateteaenoic acid;

EpETrE, epoxyeicosatrienoic acid;

FA, filtered air;

HODE, hydroxyoctadecadienoic acids;

LA, linoleic acid;

LOX, lipoxygenase;

Oxo-ODE, oxo-octadecadienoic acid;

PM, particulate matter;

PGH, prostaglandin dehydrogenase;

PUFAs, polyunsaturated fatty acids;

sEH, soluble epoxide hydrolase;

SPE, solid phase extraction;

TRAP, traffic-related air pollution;

TPP, triphenylphosphine;

UPLC-MS/MS, ultra-high pressure liquid chromatography coupled to tandem mass-spectrometry.

1. Introduction

Ambient air pollution is a risk factor for metabolic (Paul et al., 2020; Persson et al., 2018; Voss et al., 2021; Zhang et al., 2020; Zhao et al., 2016), respiratory (Cesaroni et al., 2008; Oftedal et al., 2003; Shima et al., 2003) and cardiovascular disease (Gan et al., 2011; Pang et al., 2021), as well as neurological impairments (Shi et al., 2020). It is one of the leading risk factors for all-cause mortality worldwide, accounting for an estimated 10.2 million annual deaths globally (Landrigan et al., 2018; Vohra et al., 2021).

Vehicles are a major source of traffic-related air pollution (TRAP), a heterogeneous mix of gases composed of CO, NOx, volatile organic compounds and particulate matter (PM) with an aerodynamic diameter <10 μm (PM10) including dominant size fractions <2.5 μm (PM2.5, fine) and <0.1 μm (ultrafine PM) (Suh et al., 2000). Concentrations of these pollutants are greatest near major roads (Karner et al., 2010). In the United States, an estimated 45 million individuals live, work or attend school within 100 m of a major transportation structure such as highways, railroads or airports (EPA, 2014).

TRAP is known to induce systemic inflammation by activating macrophages (Chen et al., 2018; Lam et al., 2020; Li et al., 2016) that increase pro-inflammatory cytokines (TNF-α, IL-1, IL-8, IL-10 and others) in both rodents (Edwards et al., 2020; Li et al., 2015; Wei et al., 2016) and humans (Han et al., 2019; Lam et al., 2020). *In vivo*, the effects of macrophage-derived cytokines are facilitated by 'lipid mediators' (i.e. oxylipins) generated from the oxidation of polyunsaturated fatty acids (PUFAs) such as omega-6 linoleic acid (LA) (Mattmiller et al., 2014) and arachidonic acid (AA) (Merched et al., 2008; Wang et al., 2021a). Multiple enzyme isoforms of the lipoxygenase (LOX), cyclooxygenase (COX), prostaglandin dehydrogenase (PGH), cytochrome P450 (CYP) and soluble-epoxide hydrolase (sEH) are involved in oxylipin synthesis

(Arnold et al., 2010b; Chang et al., 2015; Earles et al., 1991; Fer et al., 2008a; Fer et al., 2008b; Funk, 2001; Greene et al., 2000; Inceoglu et al., 2007; Laneuville et al., 1995; Lee and Levine, 1975; Murphy et al., 1995). As shown in **Figure 1**, LOX and COX hydroxylate PUFAs, PGH converts hydroxylated compounds to ketones (Lee and Levine, 1975), CYPs epoxidize PUFAs (Arnold et al., 2010a), and sEH converts epoxidized PUFAs into fatty acid diols (Moghaddam et al., 1996).

In general, COX- and LOX-derived products of LA and AA are pro-inflammatory (e.g. octadecadienoic acids, hydroxyeicosatetraenoic acids, prostaglandins), as are CYP-derived epoxides of LA. Conversely, CYP-derived epoxides of AA (i.e. epoxyeicosatrienoic acids (EpETrEs)), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), as well as 15-LOX-1 hydroxylated metabolites of EPA and DHA are pro-resolving (Hasegawa et al., 2017; Rao et al., 2019; Teixeira et al., 2020; Wagner et al., 2017; Werz et al., 2018). These pro-resolving oxylipins act via specialized receptors (Reviewed in (Chiang and Serhan, 2017)) to stop and resolve inflammation by promoting cellular repair and phagocytosis of dead cells and debris (Kohli and Levy, 2009; Lahvic et al., 2018; Serhan and Levy, 2018).

To date, the majority of studies have focused on the role of acute or sub-chronic (weeks) exposure to TRAP components on the synthesis of pro-inflammatory oxylipins in rodents and humans. Mice exposed to ultrafine particle components of TRAP for 8 weeks showed increased plasma, liver and intestinal LA- and AA-derived hydroxylated and prostanoid lipid mediators of 5-LOX and COX1/2, respectively, compared to mice exposed to filtered air (FA) (Li et al., 2015). Similar findings were reported in human plasma and lung lavage following acute diesel exposure (Gouveia-Figueira et al., 2017; Gouveia-Figueira et al., 2018). A recent observational study in humans showed that exposure to elevated TRAP over a 3-day period was associated

with higher serum concentrations of hydroxylated LOX metabolites of AA, increased sEH-derived diols of AA, and reduced COX-derived prostanoids of AA (Wang et al., 2021b).

A critical knowledge gap in the field is whether chronic TRAP exposure for many months impairs the resolution of inflammation. In humans, acute TRAP exposure for up to 3 days was associated with increased LOX-derived 17-hydroxyDHA, a precursor to multiple pro-resolving lipid mediator species (Wang et al., 2021b; Yang et al., 2015). This highlights a possible role of acute TRAP exposure in regulating resolution pathways. However, chronic effects of TRAP exposure on both inflammation and resolution pathways have not been studied.

The key objective of the present exploratory study was to investigate the chronic (14 months) effects TRAP exposure on inflammation and resolution lipid mediator pathways in serum of female rats. We hypothesized that the acute TRAP-induced elevations in pro-inflammatory oxylipins documented by several studies would be exacerbated by chronic TRAP exposure. The analysis was performed on a subset of serum samples from a prior study in which male and female rats were exposed to ambient TRAP or FA for 14 months to assess the effects of TRAP on Alzheimer's disease phenotypes (Patten et al., 2021). We focused on female rats because they exhibited more pronounced elevations in circulating pro-inflammatory cytokines (IL-1α, interferon-γ and TNF-α) compared to males following 14 months TRAP exposure (Edwards et al., 2020). TRAP was collected from a heavily trafficked freeway tunnel in Northern California and delivered unchanged to the animals in real time to recapitulate the natural intensity and variability of vehicular emissions representative of real-world exposures for those living near highways (Allen et al., 2001; Gross et al., 2000). FA control animals were exposed to background ambient air that was subjected to multiple emission control technologies to remove residual air pollutants as previously described (Edwards et al., 2020). We measured oxylipins

covering the various PUFA-derived enzymatic pathways shown in **Figure 1**, in view of prior studies showing acute or sub-acute effects of TRAP exposure on COX, LOX and sEH pathways in both rodents and humans (Gouveia-Figueira et al., 2017; Gouveia-Figueira et al., 2018; Li et al., 2015; Wang et al., 2021a).

Materials & Methods:

2. Methods

Animals

All procedures were conducted in compliance with the University of California-Davis IACUC approved protocols and in accordance with ARRIVE Guidelines 2.0. Samples consisted of archived serum from a prior study in which rats were exposed to TRAP or FA from 1 to 15 months of age (Edwards et al., 2020; Patten et al., 2021). As previously described, wildtype Fischer 344 rats used were obtained from mating Fischer 344 females (Charles River Laboratories) with hemizygous TgF344-AD male rats obtained from a colony established at UC Davis in 2016 (Patten et al., 2021). At postnatal day 28, male and female rats were randomly divided into two groups, filtered air (FA, n=45) and TRAP (n=45), and transported from the UC Davis campus to an IACUC-approved vivarium located adjacent to a freeway tunnel. Rats were exposed to FA or TRAP in this vivarium until they were 15 months old (Edwards et al., 2020; Patten et al., 2021). The analysis in the present study was performed on surplus female serum samples from Edwards et al. study (Edwards et al., 2020) of which 8 were exposed to FA and 6 to TRAP.

Details of the tunnel vivarium have been previously described (Berg et al. 2020; Edwards et al. 2020; Patten et al. 2020). The facility was maintained under controlled environmental conditions (20-26 °C, 12:12 light dark cycle) with food (Envigo Teklad Global 18% Protein Rodent Diet) and tap water provided *ad libitum*. While at UC Davis, the animals were housed in a vivarium at the UC Davis Center for Health and the Environment (CHE). The husbandry conditions and procedures at CHE were the same as those at the tunnel facility, except that rats in the CHE vivarium, there was no background traffic noise and vibration, the animals were exposed to standard vivarium air, and cages were housed within the same room/space.

TRAP exposure

The tunnel vivarium was set-up within 50 m of a heavily trafficked freeway tunnel (approximately 60,000 vehicles/day) in Northern California as previously detailed (Geller et al., 2005). The facility (44 feet x 10 feet wide) contained three rooms, including the 1st one (9 feet x 13 feet) for monitoring TRAP and FA pollutant concentrations, a 2nd one (9 feet x 18 feet) which contained exposure chambers (12.8 feet x 3 feet x 7.8 feet (LxWxH) with 6 shelves), and a 3rd one (9 feet x 12 feet) for on-site laboratory work. Each chamber accommodated 18 cages with dimensions of 18.75 inches x 10.25 inches x 8 inches. One of the chambers was used for TRAP exposure, while the other for FA exposures. For TRAP exposure, air was continuously drawn from the eastbound exit of the tunnel bores with an air flow rate of 35 cubic feet per minute in each chamber (Edwards et al., 2020; Patten et al., 2021). FA was delivered to rats in a separate exposure chamber within the second room. As previously reported, for FA, the average particle number filtration efficiency, average $PM_{2.5}$ mass filtration efficiency, and average total suspended particulate mass filtration efficiency were 97.7±0.7%, 89±5%, and 89±5% respectively (Edwards et al., 2020). This was achieved by drawing air surrounding the facility

and sequentially processing it using coarse filtration to remove large debris/dust, an activated carbon scrubber (Phresh® HGC701018 Air Carbon Filter; 8"×39"; 950 cfm max flowrate) to remove volatile organic compounds, an inline activated carbon scrubber (Phresh® HGC701180 Inline Air Carbon Filter; 8"×24"; 750 cfm max flowrate) and three-way catalytic converters (MagnaFlow® 445006; CARB-compliant) to remove nitrogen oxides, hydrocarbons and carbon monoxide, and an inline, custom-made, ultrahigh-efficiency particle filtration system to remove ultrafine, fine and coarse mode PM. Animals (8 FA and 6 TRAP) were exposed continuously 24 hours a day, 7 days a week for a total of 427 days (about14 months). Mean daily $PM_{2.5}$ in the air delivered to the chambers was 15.6±3.7 μg/m^3 in TRAP and 0.25 ± 0.11 μg/m^3 in FA (Patten et al., 2021). At the end of the exposure, animals were transported to the UC Davis campus where they went through MRI/PET imaging before being euthanized 23 days later. Rats were anesthetized using 4% isoflurane (Southmedic Inc., Barrie ON) in 2/3 medical grade air and medical grade oxygen, delivered via inhalation at a rate of 1.5 L/min. Following deep anesthesia, the chest cavity was opened and blood was obtained from the heart via cardiac puncture. Samples were collected into serum separator tubes (Becton-Dickinson, East Rutherford, NJ) which were allowed to sit at room temperature for 30 min before centrifugation at 1500 x g for 10 min. The resulting serum was transferred to microcentrifuge tubes and immediately frozen and stored at -80 C (Patten et al., 2021).

Serum total oxylipin extraction

Total oxylipins (i.e. free + esterified oxylipins) derived from the n-3 PUFAs, alpha-linoleic acid (ALA), EPA and DHA, and from the n-6 PUFAs LA, dihomo-γ-linolenic acid (DGLA) and AA were measured with targeted mass-spectrometry analysis as previously described (Zhang et

al., 2021). Oxylipins were extracted from archived rat female serum that had been previously subjected to one freeze-thaw cycle for cytokine measurements (Edwards et al., 2020). The samples were thawed on ice for approximately 2 hours, vortexed and 100 µL was transferred into an 8 mL Kimble Borosilicate glass tubes (100 x 13mm, Cat. No. 73750-13100, Thomas Scientific) with PTFE-Faced Rubber Liner caps (Cat. No. 45066C-13, Thomas Scientific). Three 100 µL water blank samples were subjected to parallel procedures. To each sample, 500 µL solution of 1 mM disodium EDTA (Cat No. E5134-50G, Sigma) and 0.9% sodium chloride (Cat. No. S7653-250G, Sigma), and 2.4 mL of chloroform and methanol (v/v, 2/1) containing 0.002% butylated hydroxytoluene (BHT) were added. Samples were vortexed and centrifuged for 15 min at 2000 rpm (SORVALL RT 6000D, rotor H1000B) at 0 ^{o}C. The lower chloroform phase was transferred into new 8 mL glass tubes using a glass pipette. Another aliquot of 1.6 mL chloroform was added to the first test-tube, vortexed and centrifuged for 15 min. The lower chloroform phase was combined with the first chloroform phase.

The combined chloroform extracts were evaporated under nitrogen and spiked with 200 µL ice-cold methanol containing 0.1% acetic acid and 0.1% BHT, 10 µL of antioxidant mixture containing 0.2 mg/mL triphenylphosphine (TPP), 0.2 mg/mL BHT and 0.2% mg/mL EDTA, and 10 µL surrogate mix containing 2 uM deuterated surrogate standard mix (d11-11(12)-EpETrE, d11-14,15-DiHETrE, d4-6-keto-PGF1a, d4-9-HODE, d4-LTB4, d4-PGE2, d4-TXB2, d6-20-HETE and d8-5-HETE) dissolved in LCMS methanol. The mixture was vortexed and 300 µL 0.4 M sodium hydroxide dissolved in methanol and water (v/v, 1:1) was added. The mixture was vortexed and heated at 60 ^{o}C for 30 min to catalyze the hydrolysis of bound oxylipins. Following hydrolysis, the samples were cooled for 5 min. Acetic acid (37.5 µL) was added to bring the pH

of the samples to 4-6. Water (1796 uL) was added to adjust the methanol content to approximately 15%.

The samples were vortexed and subjected to solid phase extraction (SPE) using 60mg Oasis HLB columns (Waters Coorp) pre-washed with 1 column volume of ethyl acetate and 2 column volumes of methanol, and equilibrated with 2 column volumes of SPE buffer containing 0.1 % acetic acid and 5% methanol in MilliQ water. The columns were washed with 2 column volumes of SPE buffer, and dried with vacuum suction (~20min, 15~20psi). Oxylipins were eluted into 2 mL centrifuge tubes with 0.5 mL methanol and 1.5 mL of ethyl acetate.

The extracts were reconstituted with 100 µL methanol, vortexed and centrifuged for 2 min at 15,871 ×g (13,000 rpm) on an Eppendorf, 5424 R centrifuge, at 0°C. The reconstituted samples were transferred to centrifugal filter unit (PVDF 0.1 µm) and centrifuged for 20 min at 15,871 ×g, 0°C. The filter was discarded, and the filtrate was transferred into LC-MS vials with inserts.

Mass-spectrometry analysis

Oxylipins were analyzed on an Agilent 1290 Infinity UPLC system coupled to an Agilent 6460 triple-quadrupole tandem mass spectrometer (UPLC-MS/MS) with electrospray ionization (ESI) (Agilent, Palo Alto, CA, USA). Analyte separation was achieved using an Agilent ZORBAX RRHD Eclipse Plus C18 column (2.1 × 150 mm, 1.8 µm Agilent Corporation). Mobile phase A consisted of MilliQ water containing 0.1% acetic acid, and mobile phase B consisted acetonitrile/methanol (v/v, 80/15) containing 0.1% acetic acid. Samples were maintained at 4 °C during the analysis. The column temperature was 45 °C. The injection volume was 10 µL and the run time was 20 min. The mobile phase gradient and flow rate were set as follows: 1) 0-2 min, 65% A, 0.25 mL/min; 2) 2-12 min, 65 to 15% A, 0.25 mL/min; 3) 12-

15min, 15% A, 0.25 mL/min; 4) 15.1-17 min, 0% A, 0.4 mL/min; 5) 17.1-19 min, 65% A, 0.4 mL/min; and 6) 19-20 min, 65% A, 0.3 mL/min. The MS ion source parameters were set as follows: gas temperature 300 °C, gas flow 10 L/min, nebulizer gas 35 psi, sheath gas heater temperature 350 °C, and capillary voltage 4000 V. Oxylipins were captured using optimized dynamic multiple reaction monitoring parameters shown in **Supplementary Table 1**.

Effects of freeze-thawing

Because the oxylipin measurements were done using previously thawed samples, we tested whether one freeze-thaw cycle alters total oxylipin concentrations. Three randomly selected samples that were thawed and assayed for oxylipins (as described above) were thawed and re-extracted one month later. Oxylipins were quantified with UPLC-MS/MS as described above.

Data and Statistical analysis

There were no animal or data exclusions for this study, meaning that all available samples from 8 FA (control) and 6 TRAP rats were analyzed with mass-spectrometry and corresponding data reported below. The sample size is similar to a prior study which reported significant effects of TRAP exposure during the prenatal period until 8 weeks postpartum on circulating lipid, inflammation and oxidative stress markers in rats (Wei et al., 2016). Sample extractions and UPLC-MS/MS peak analysis was performed in a blinded manner. Oxylipin quantitation was achieved using a standard curve for each analyte to correct for the response factor on the mass-spec detector, and deuterated surrogate standards to account for losses during the extraction.

Compounds that used d4-PGE2 as a surrogate standard were not analyzed due to degradation of the deuterated standard during hydrolysis, consistent with previous reports (Emami et al., 2020; Ostermann et al., 2020). The ratio of PUFA diol to precursor epoxides (x 100) was calculated to estimate in vivo sEH activity (Stefanovski et al., 2020). Only detected oxylipins in all rats were subjected to statistical analysis. An unpaired t-test (two-tailed distribution) was performed using Microsoft Excel to compare the effects of TRAP vs FA on oxylpin concentrations. A paired t-test was used to test the effects of freeze-thawing on oxylipins. Statistical significance was set at $P<0.05$.

3. Results

9-Hydroxyoctadecadienic acid (9-HODE), an LA metabolite formed by autooxidation or 5-LOX, was significantly reduced by 36% (P=0.038) in TRAP-exposed rats compared to FA controls (**Figure 2**). The concentration of 9-oxo-octadecadienoic acid (9-oxo-ODE), a ketone metabolite of 9-HODE formed by PGH (Earles et al., 1991), was reduced by 36% in TRAP-exposed rats relative to FA rats, but this difference did not reach statistical significance (P=0.060; **Supplementary Table 2**). EPA-derived 11,12-dihydroxy-eicosatetraenoic acid (11,12-DiHETE) and DHA-derived 16,17-dihydroxy-docosapentaenoic acid (16, 17- DiHDPA), two products of sEH, were reduced by 48% (P=0.049) and 39% (P=0.012), respectively, in TRAP-exposed rats compared to FA controls (**Figure 2**). Similar reductions of 38-39% were observed in AA-derived 8,9- dihydroxyeicosatrienoic (DiHETrE) and 11,12-DiHETrE, but these changes were not statistically significant (P=0.062 and 0.084). Precursor PUFA epoxides including 11(12)- wpoxydocosapentaenoic acid (EpETE), 16(17)-epoxydocosapentaenoic acid (EpDPE), 8(9)-EpETrE and 11(12)-EpETrE did not change significantly.

The diol to epoxide ratio, an *in vivo* marker of sEH activity (Stefanovski et al., 2020), was reduced by 34% and 39% for the ratios of 16,17-DiHDPA/16(17)-EpDPE (P=0.005) and 11,12-DiHETrE/11(12)-EpETrE (P=0.026), respectively, in TRAP compared to FA rats (**Figure 3**). There were no significant changes in COX, CYP or other LOX/PGH metabolites (**Supplementary Table 2**) or diol to epoxide ratios of other PUFAs (**Supplementary Table 3**).

Freeze-thawing did not have a major impact on total (free+esterified) oxylipin concentrations. As shown in **Supplementary Table 4**, freeze-thawing significantly altered the concentration of only 6 out of 66 detected oxylipins in serum (P<0.05 by paired t-test). None of these oxylipins were altered by TRAP exposure.

4. Discussion

Chronic exposure of female rats to ambient TRAP for 14 months reduced serum concentrations of LA-derived 9-HODE, EPA-derived 11,12-DiHETE and DHA-derived 16,17-DiHDPA compared to FA controls. The diol to epoxide ratio, a marker of sEH activity, was also reduced by TRAP compared to FA exposure. 9-HODE can be formed by LOX catalysis or autooxidation of LA, whereas 11,12-DiHETE and 16, 17-DiHDPA are synthesized by sEH from their corresponding epoxides. Thus, our findings demonstrate that chronic TRAP exposure reduced LOX/autooxidation and sEH pathways.

LOX/autoxidation and sEH pathways are typically upregulated during inflammation (Patwardhan et al., 2010; Trindade-da-Silva et al., 2020; Warner et al., 2017), and inhibition of 5-LOX and sEH has been shown to halt and resolve inflammation (Fredman et al., 2014; Hiesinger et al., 2020; Liu et al., 2021; Teixeira et al., 2020; Yang et al., 2015). In this study, TRAP exposure dampened LOX/autoxidation and sEH pathways despite increasing pro-inflammatory cytokines (Edwards et al., 2020). This may reflect adaptive changes to chronic

TRAP exposure, potentially aimed at counteracting systemic inflammation, as observed in one study showing a downregulation of Th1 immune cells following acute and sub-chronic intranasal exposure to PM in an autoimmune encephalomyelitis mouse model of immune activation (O'Driscoll et al., 2019). Thus, based on the observed reduction in pro-inflammatory 9-HODE and sEH-derived EPA/DHA diols, chronic TRAP exposure appears to dually suppress inflammation and upregulate resolution by sparing pro-resolving PUFA-epoxide breakdown into less active PUFA diols.

Our results are opposite to prior studies which reported an increase in circulating pro-inflammatory mono- and di-hydroxylated oxylipins of LOX and sEH following acute TRAP exposure. Mice exposed to ultrafine particles for 8 weeks, and humans exposed to diesel exhaust acutely or TRAP for up to 3 days exhibited elevations in LA or AA mono- and di-hydroxylated metabolites of LOX and sEH (Gouveia-Figueira et al., 2017; Gouveia-Figueira et al., 2018; Li et al., 2015; Wang et al., 2021b). The observed reductions in mono- and di-hydroxylated lipid mediators in this study suggest differing effects of acute versus chronic TRAP exposure, and point to temporal changes in oxylipin metabolism depending on the duration of exposure.

One factor potentially confounding our interpretation is that the animals were moved from the exposure tunnel to the UC Davis main campus vivarium for 23 days (for MRI/PET imaging) prior to euthanasia. It is possible, therefore, that the observed lipid mediator changes reflect an adaptive response to withdrawal from TRAP exposure, rather than a direct effect of TRAP. This is unlikely, however, for several reasons. First, PM and various dust elements are known to accumulate in tissues (Leffler et al., 1984; Patten et al., 2020), and reside there for up to 6 months (e.g. lungs) after the initial exposure is removed (Parkhomchuk et al., 2016; Parkhomchuk et al., 2019). This means that TRAP-related effects are likely to persist even after

the exposure is removed, consistent with studies showing that acute exposure to PM causes long-lasting (order of weeks) effects on lung inflammatory pathways in rats (Kodavanti et al., 2002). Second, there is evidence of transfer of leukocytes and myeloperoxidase from the lungs to blood after pulmonary PM exposure, suggesting that systemic changes in lipid mediator levels observed in this study reflect PM-induced effects in the lungs and possibly other tissues, followed by transfer to the blood (Nurkiewicz et al., 2006). Regardless, it is acknowledged that the current experimental design cannot establish whether the observed lipid mediator changes are due to chronic effect of TRAP or withdrawal from TRAP exposure. Future studies are needed to address this limitation, and to better understand the time-course of lipid-mediated changes following cessation of TRAP exposure.

There are other limitations worth acknowledging. The small sample size (6 to 8 per group), although similar to other rodent TRAP studies, may have increased the risk of a type I statistical error. However, significant changes within an oxylipin pathway were corroborated by changes in other lipid mediator(s) within the same network, thus strengthening our findings. For instance, the significant reduction in 9-HODE in TRAP-exposed rats was corroborated by the 36% reduction in 9-oxo-ODE (a metabolite of 9-HODE), which was not significant but had a large effect size ($d > 1.0$). Changes in fatty acid diols were also confirmed across multiple compounds, thus strengthening the evidence. Another limitation is that the present analysis was confined to females; future studies should explore sex differences in lipid mediator response to TRAP exposure. Although measurements were done in previously freeze-thawed serum samples, we confirmed that this process does not impact the majority of oxylipins, particularly lipid mediators that were altered by TRAP exposure. An important strength of the study is the long-

term exposure model involving real-world TRAP, which makes our findings translationally relevant to humans.

In summary, this preliminary study provides new evidence of dampened inflammatory signaling and enhanced resolution pathways following chronic TRAP exposure in female rats. This points to adaptive lipid-mediated mechanisms that may mitigate the effects of pro-inflammatory cytokines. Our findings also point to clear differences in lipid mediator response to acute versus chronic TRAP exposure; acute exposure increases pro-inflammatory lipid mediators (Gouveia-Figueira et al., 2017; Gouveia-Figueira et al., 2018; Li et al., 2015; Wang et al., 2021b), whereas chronic exposure reduces them and decreases sEH-mediated breakdown of pro-resolving oxylipins. These distinct oxylipin changes may potentially serve as biomarkers of acute versus chronic exposure to TRAP. Future studies should evaluate the time-course of lipid-mediated inflammation resolution in this real-world exposure model to better characterize windows of opportunity for interventions targeting inflammation and resolution pathways.

Declaration of interest statement:

There are no competing interests to declare.

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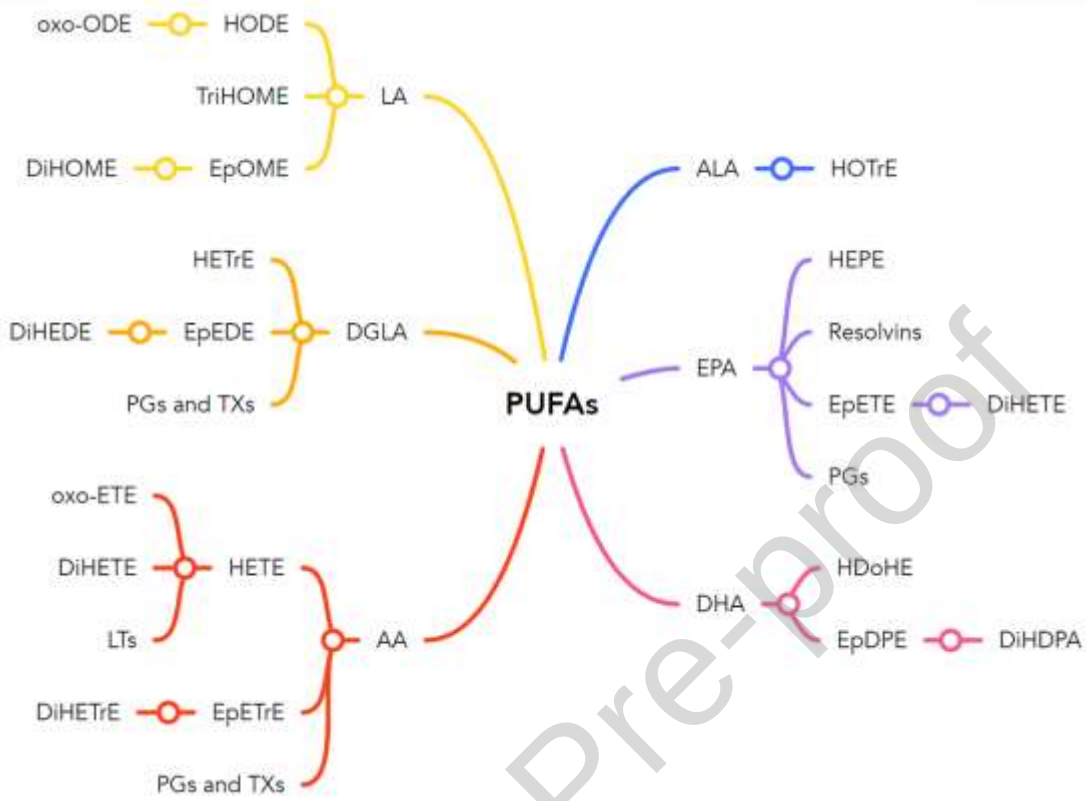
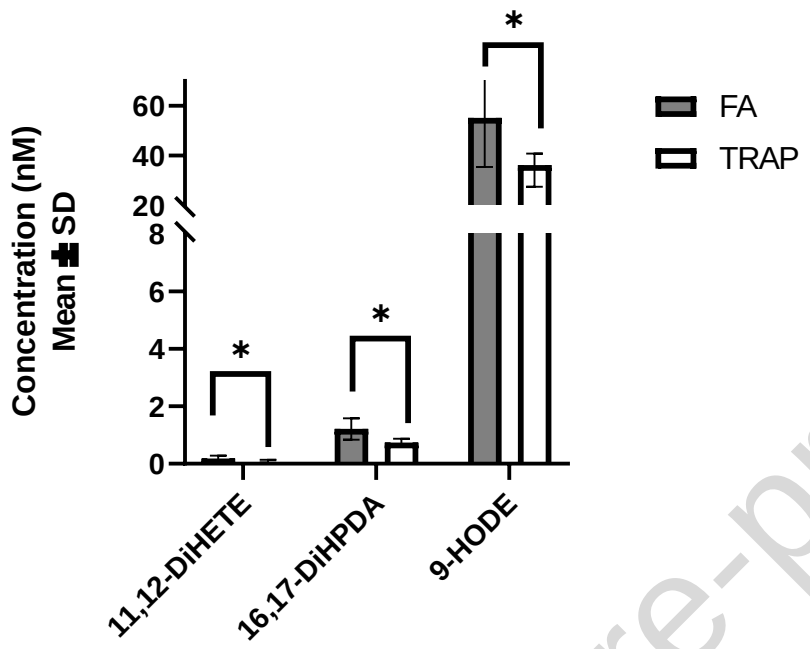
Figure 1: Biosynthesis of oxylipins from polyunsaturated fatty acids.



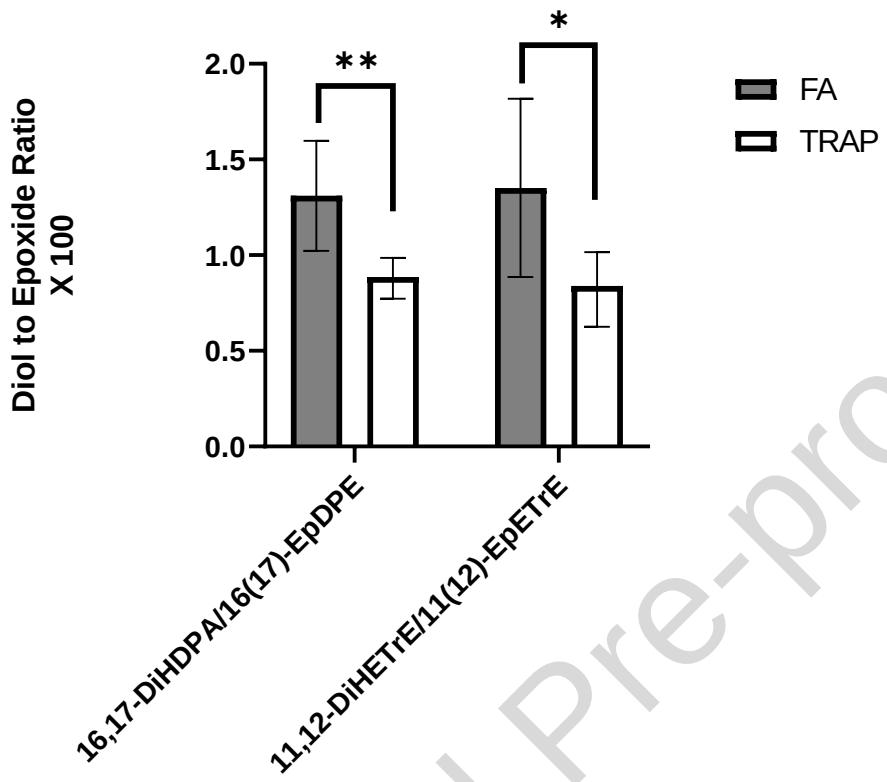
Biosynthesis of oxylipins from polyunsaturated fatty acids (PUFAs) via lipoxygenease (LOX), prostaglandin dehydrogenase (PGH), cyclooxygenease (COX), cytochrome P45 (CYP) and soluble epoxide hydrolase (sEH). PUFAs that serve as precursors to aoxylipins include linoleic acid (LA), dihomo-gamma-linolenic acid (DGLA), arachidonic acid (AA), alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). LOX enzymes are involved in the synthesis of LA-derived hydroxyoctadecadienoic acid (HODE), DGLA-derived hydroxyeicosatrienoic acid (HETrE); AA-derived hydroxyeicosatetraenoic (HETE) and leukotrienes (LTs), ALA-derived hydroxyoctadecatrienoic acid (HOTrE), EPA-derived hydroxyeicosapentaenoic acid (HEPE) and DHA-derived hydroxydocosahexaenoic acid. PGH concerts hydroxyl fatty acids into ketones such as oxo-octadecadienoic acid (oxo-ODE) and oxo-eicosatetraenoic acid (oxo-ETE). COX enzymes are involved in the synthesis of DGLA- and AA-derived prostaglandins (PGs) and thromboxanes (TXs), and EPA-derived PGs. Both COX and LOX are involved in resolvin synthesis. CYPs are involved in epoxide synthesis; e.g. epoxyoctadecenoic acid (EpOME), epoxyeicosadienoic acid (EpEDE), epoxyeicosatrienoic acid (EpETrE), epoxyeicosateteaenoic acid (EpETE) and epoxydocosapentaenoic acid (EpDPE). sEH converts PUFA epoxides into diols including dihydroxyoctadecenoic acid (DiHOME), dihydroxyeicosadienoic acid (DiHEDE), dihydroxyeicosatrienoic acid (DiHETrE), dihydroxyeicosatetraenoic acid (diHETE) and dihydroxydocosapentaenoic acid (DiHDPA). The exact mechanism of for LA-derived trihydroxyoctadecamonoenoic acid (TriHOME) synthesis is not known.

Figure 2: Serum concentrations (nM) of total (i.e. free+bound) oxylipins in serum of rats exposed to filtered air (FA) or Traffic related air pollution (TRAP) for 14 months.



Data are mean ± SD of n=8 FA and 6 TRAP. (*) denotes significantly different means by unpaired t-test at $p<0.05$.

Figure 3. Fatty acid diol to epoxide ratios in serum of rats exposed to filtered air (FA) or Traffic related air pollution (TRAP) for 14 months.



Data are mean ± SD of n=8 FA and 6 TRAP. (*) denotes significantly different means by unpaired t-test at $p<0.05$.

CRediT authorship contribution statement

Nuanyi Liang: Investigation, Data curation, Formal Analysis, Writing – Original Draft; **Shiva Emami:** Investigation, Writing – Review and Editing; **Kelley T. Patten:** Investigation, Resources, Writing – Review and Editing; **Anthony E. Valenzuela:** Resources, Writing –

Review and Editing; **Christopher D. Wallis:** Resources, Writing –Review and Editing; **Anthony S. Wexler:** Resources, Writing –Review and Editing; **Keith J. Bein:** Resources, Writing –Review and Editing; **Pamela J. Lein:** Conceptualization, Resources, Writing –Review and Editing; **Ameer Y. Taha:** Supervision, Conceptualization, Funding Acquisition, Formal Analysis, Writing –Original Draft, Review and Editing

Declaration of Competing Interest

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights:

- Rats were exposed to traffic-related air pollution (TRAP) or filtered air for 14 months.
- Serum was analyzed for inflammation and resolution lipid mediators.
- TRAP reduced pro-inflammatory lipid mediators.
- TRAP reduced the degradation of pro-resolving lipid mediators.
- The data suggest adaptive immune changes to chronic TRAP exposure.