1   **Pro-resolving lipid mediators within brain esterified lipid pools are reduced in female rats**

2   **chronically exposed to traffic-related air pollution or genetically susceptible to Alzheimer's**

3   **Disease phenotype**

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28   **Running title**: Air pollution and AD modify brain esterified lipid mediators

Abstract:

Traffic-related air pollution (TRAP) is a risk factor for Alzheimer's disease (AD) where neuroinflammation underlies disease progression and pathogenesis. Unresolved inflammation in AD is known to be exacerbated by brain deficits in unesterified pro-resolving lipid mediators enzymatically synthesized from polyunsaturated fatty acids. Recently, we reported that in the brain, unesterified pro-resolving lipid mediators which are bioactive, can also be supplied from less bioactive esterified lipid pools such as neutral lipids (NLs) and phospholipids (PLs). It is not known whether esterified pro-resolving lipid mediators are affected by AD pathology and exacerbated by TRAP exposure. In the present study we addressed this data gap using TgF344-AD male and female rats that express human AD risk genes and their wildtype littermates exposed to filtered air (FA) or TRAP from 1 to 15 months of age. Esterified lipid mediators within NLs and PLs were quantified by mass-spectrometry. We observed a significant reduction in pro-resolving lipid mediators in both NLs and PLs of female TgF344-AD rats compared to wildtype controls. TRAP exposure also reduced pro-resolving lipids in the female brain, mainly in PL pools, but did not exacerbate changes observed in TgF344-AD rats. Minimal changes were observed in males. Our findings indicate that AD genotype and chronic TRAP exposure result in sex-specific deficits in brain esterified pro-resolving lipid mediators, the pool that supplies free and bioactive lipid mediators. These data provide new information on lipid-mediated mechanisms regulating impaired inflammation resolution in AD, and show for the first time that chronic TRAP exposure targets the same lipid network implicated in AD.

Keywords:

oxylipin, Alzheimer's Disease, air pollution, rat brain, lipid mediator, phospholipid, neutral lipid,

mass-spectrometry, inflammatory resolution

Abbreviations:

AA: arachidonic acid

AD: Alzheimer's Disease

ALA: alpha-linolenic acid

ANOVA: analysis of variance

BHT: butylated hydroxytoluene

COX: cyclooxygenase

CYP: cytochrome P450

DGLA: dihomo-gamma-linoleic acid

DHA: docosahexaenoic acid

DiHETE: dihydroxy-eicosatetraenoic acid

DiHETrE: dihydroxy-eicosatrienoic acid

DiHOME: dihydroxy-octadecenoic acid

DiHPDA: dihydroxy-docosapentaenoic acid

EDTA: ethylenediaminetetraacetic acid

EPA: eicosapentaenoic acid

EpDPE: epoxy-docosapentaenoic acid

EpETE: epoxy-eicosatetraenoic acid

EpETrE: epoxy-eicosatrienoic acid

EpOME: epoxy-octadecenoic acid

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73   FA: filtered air

74   HDoHE: hydroxy-docosahexaenoic acid

75   HEPE: hydroxy-eicosapentaenoic acid

76   HETE: hydroxy-eicosatetraenoic acid

77   HETrE: hydroxy-eicosatrienoic acid

78   HODE: hydroxy-octadecadienoic acid

79   HOTrE: hydroxy-octadecatrienoic acid

80   LA: linoleic acid

81   LOX: lipoxygenase

82   LT: leukotriene

83   LX: lipoxin

84   NO_2: nitrogen dioxide

85   NL: neutral lipid

86   oxo-ETE: oxo-eicosatetraenoic acid

87   oxo-ODE: oxo-octadecadienoic acid

88   PM: particulate matter

89   PG: prostaglandin

90   PGDH: hydroxy-prostaglandin dehydrogenasePL: phospholipid

91   PUFA: polyunsaturated fatty acid

92   sEH: soluble epoxide hydrolase

93   SPE: solid phase extraction

94   TPP: triphenyl phosphine

95   TRAP: traffic-related air pollution

96   TriHOME: trihydroxy-octadecenoic acid

97   TX: tromboxane

98   UPLC-MS/MS: ultra high-pressure liquid chromatography-tandem mass spectrometry

1. Introduction

Alzheimer's disease (AD), the main cause of age-related dementia, affects 6.2 million Americans aged 65 or older [1], and is the fifth-leading cause of death among the elderly [2]. At present there is no therapy for AD, which is why considerable efforts have been made to understand modifiable risk factors such as environmental exposures (reviewed in [3]).

One environmental factor strongly associated with increased risk of AD is chronic exposure to traffic-related air pollution (TRAP) [4, 5]. TRAP is a complex and heterogeneous mixture of vehicle emissions, road dust and secondary air pollutants including gases and particles [6]. Evidence from epidemiological studies suggests that individuals who live less than 50 meters from a major roadway have a 7% increased risk of dementia compared to individuals living 200 meters away [7]. Consistent with these observations, increased exposure to TRAP components (i.e., ozone and particulate matter 2.5 ($PM_{2.5}$) [8], nitrogen dioxide (NO_2) and $PM_{2.5}$ [9]) has been shown to increase the risk of AD.

Both AD and TRAP exposure are associated with immune activation characterized by an elevation in circulating and tissue (lung and brain) cytokines [10-12]. In vivo, the effects of cytokines are mediated by short-lived bioactive lipid mediators (i.e. oxylipins) derived from the oxidation of polyunsaturated fatty acids via cyclooxygenase (COX) [13, 14], lipoxygenase (LOX) [15, 16], cytochrome P450 (CYP) [17], 15-hydroxyprostaglandin dehydrogenase (15-PGDH) [18] and soluble epoxide hydrolase (sEH) enzymes [19-21]. Pro-inflammatory oxylipins are elevated in the brain of transgenic animal models of AD [21, 22] and in post-mortem brain of patients with AD pathology [15, 23, 24]. Similarly, TRAP exposure has been shown to increase the concentration of pro-inflammatory oxylipins in human serum/plasma [25, 26].

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121   Oxylipins are also involved in inflammation resolution, the process of halting inflammation,

122   and repairing or replacing damaged cells [27]. Resolution pathways are impaired in AD, as

123   evidenced by the marked reduction of pro-resolving oxylipins of docosahexaenoic acid (DHA),

124   including 10,17S-docosatriene (neuroprotectin D1) and maresin 1, as well as arachidonic acid

125   (AA)-derived lipoxin A4 (LXA4) in cerebrospinal fluid, hippocampus, and entorhinal cortex of

126   AD patients compared to non-AD controls [24, 28, 29]. Brain concentrations of pro-resolving

127   DHA-derived neuroprotectin D1[30] and DHA-epoxides (epoxydocosapentaenoic acids,

128   EpDPEs)[31], as well as AA- derived epoxides (epoxyeicosatrienoic acids, EpETrEs) [21, 31, 32]

129   and 15-hydroxy-eicosatetraenoic acid (15-HETE) [22], were also shown to be lower in

130   transgenic mouse models of AD compared to genetically unaltered controls. It is not known

131   whether TRAP exposure alters these inflammation resolution oxylipin pathways in the brain.

132   To date, all studies have characterized lipid mediator disturbances in AD by measuring the

133   concentration of free (i.e., unesterified) oxylipins. Although oxylipins are enzymatically

134   synthesized in the brain by COX, LOX, 15-PGDH, CYP450 and sEH, they can also be released

135   from or sequestered (i.e. re-esterified) to the more abundant esterified lipid pool within the brain

136   as shown in the pathway illustrated in **Figure 1**. In this regard, we reported that approximately

137   90% of oxylipins in the rat brain are bound to phospholipids (PLs) and neutral lipids (NLs)

138   consisting of traiacylglycerides and cholesteryl esters [33, 34]. We also showed, in vivo, that

139   esterified oxylipins can both release or sequester free oxylipins through a turnover pathway that

140   regulates the bioavailability of the free oxylipin pool (**Figure 1**) [34]. Free oxylipins are

141   bioactive [35, 36], whereas oxylipins bound to PLs or NLs are inactive [37, 38].

142   It is not known whether *esterified* oxylipins involved in inflammation or resolution are

143   altered in AD or by TRAP exposure. This knowledge gap is important to address because

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144   changes in the esterified oxylipin pool might mechanistically explain why free pro-resolving

145   oxylipins are reduced in animal models of AD and in human post-mortem brain of subjects with

146   AD pathology. Also, knowing whether TRAP exposure targets the same pathways might help

147   understand convergent biochemical networks that underlie AD etiology.

148   The purpose of this study was three-fold. First, we aimed to test whether bound (i.e.

149   esterified) oxylipins involved in inflammation and inflammation resolution are altered by AD

150   phenotype in rats. Second, we wished to understand whether chronic TRAP exposure, a

151   significant risk factor for AD, also alters bound oxylipins in a manner similar to AD. Third,

152   because AD disproportionally affects more females than males [39, 40], we explored whether

153   females would be more impacted than males by the effects of AD and TRAP.

154   We hypothesized that AD genotype and TRAP exposure alter rat brain NL- and PL-bound

155   oxylipins in a sex-dependent manner. The TgF344-AD rat, a transgenic rat model of AD

156   expressing mutations in the human Swedish amyloid precursor protein (APPswe) and Δexon 9

157   presenilin-1 (PS1ΔE9), was used to test this hypothesis. The TgF344-AD rat develops cognitive

158   impairment and neuropathological features of AD including microglial activation, beta amyloid

159   plaques and neurofibrillary tangles in the brain, unlike other transgenic models of AD which

160   develop only a subset of these hallmark AD phenotypes [41]. TRAP exposure was recently

161   shown to promote AD phenotypes in the TgF344-AD rat and their WT littermates [42] Thus, in

162   this study, we exposed male and female TgF344 and wildtype littermate rats for 14 consecutive

163   months to filtered air (FA) or TRAP captured from a heavily trafficked freeway tunnel in

164   Northern California, to test whether AD genotype or chronic TRAP exposure alters esterified

165   oxylipins in the brain.

Figure 1. Pathway of detectable oxylipins that were measured in the present study. Frames surrounding each oxylipin classify whether it is pro- or anti-inflammatory based on the literature: 1) oxylipins with red frames have pro-inflammatory effects; 2) oxylipins with blue frames have anti-inflammatory / pro-resolving effects; and 3) oxylipins with yellow frames have both pro- and anti-inflammatory effects. Esterified oxylipins within phospholipids (PL) or neutral lipids (NL) pool can be released via lipase enzymes to generate free (unesterified) oxylipins. Free oxylipins can also be sequestered into NL and PL pools via acyl-CoA synthetase and acyltransferase enzymes. Both lipase-mediated release and acyl-CoA synthetase/acyltraferase-mediated sequestration regulate the availability of free oxylipins. Abbreviations: PUFA, polyunsaturated fatty acid; LA, linoleic acid; DGLA, dihomo-gamma-linoleic acid; AA, arachidonic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; COX, cyclooxygenase; CYP, cytochrome P450; LOX, lipoxygenase; sEH, soluble epoxide hydrolase; 15-PGDH, 15-hydroxyprostaglandin dehydrogenase; DiHETE, dihydroxy-eicosatetraenoic acid; DiHETrE, dihydroxy-eicosatrienoic acid; DiHOME, dihydroxy-octadecenoic acid; DiHPDA, dihydroxy-docosapentaenoic acid; EpDPE, epoxy-docosapentaenoic acid; EpETE, epoxy-eicosatetraenoic acid; EpETrE, epoxy-eicosatrienoic acid; EpOME, epoxy-octadecenoic acid; HDoHE, hydroxy-docosahexaenoic acid; HEPE, hydroxy-eicosapentaenoic acid; HETE, hydroxy-eicosatetraenoic acid; HETrE, hydroxy-eicosatrienoic acid; HODE, hydroxy-octadecadienoic acid; HOTrE, hydroxy-octadecatrienoic acid; oxo-ETE, oxo-eicosatetraenoic acid; oxo-ODE, oxo-octadecadienoic acid; TriHOME, trihydroxy-octadecenoic acid; LX, lipoxin; PG, prostaglandin; LT, leukotriene.
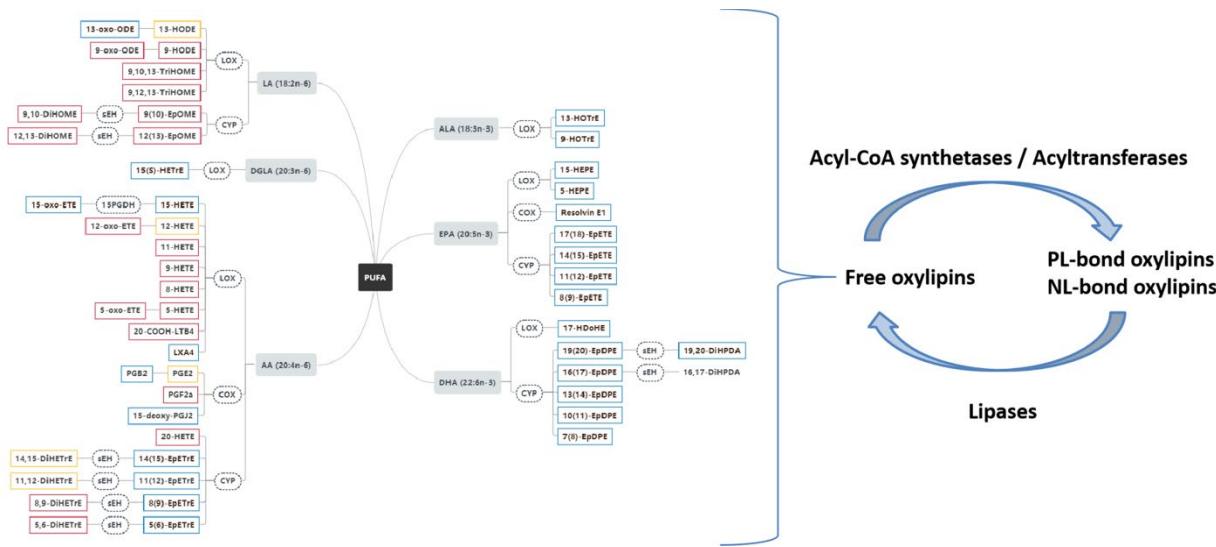
2. Methods

2.1 Chemicals and reagents

Ethylenediaminetetraacetic acid (EDTA; Cat #EDS-100G), butylated hydroxytoluene (BHT; Cat #W218405-SAMPLE-K) and triphenyl phosphine (TPP; Cat #3T84409) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Oxylipin standards were purchased from Cayman Chemical (Ann Arbor, MI, USA) or Loradan Biomedical (Davis, CA, USA). Deuterated surrogate standards used for oxylipin quantitation were obtained from Cayman Chemical. These include d11-11(12)-epoxyeicosatrienoic acid (d11-11(12)-EpETrE, Cat # 10006413), d11-14,15-dihydroxyeicosatrienoic acid (d11-14,15-DiHETrE, Cat # 1008040), d4-6-keto-Prostaglandin F1 alpha (d4-6-keto-PGF1a, Cat # 315210), d4-9-hydroxyoctadecadienoic acid (d4-9-HODE, Cat # 338410), d4-Leukotriene B4 (d4-LTB4, Cat # 320110), d4-Prostaglandin E2 (d4-PGE2, Cat # 314010), d4-Tromboxane B2 (d4-TXB2, Cat # 319030), d6-20-hydroxyeicosatetraenoic acid (d6-20-HETE, Cat # 390030), and d8-5-hydroxyeicosatetraenoic acid (d8-5-HETE, Cat # 334230).

2.2 Animals and traffic-related air pollution exposure

Animal experiments were conducted according to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the UC Davis Institutional Animal Care and Use Committee (IACUC). Male TgF344-AD transgenic rats expressing Swedish" mutant human APP (APPsw) and Δ exon 9 mutant human presenilin-1 (PS1ΔE9) genes were obtained from Emory University [41]. Female wildtype Fischer 344 (WTF344) rats were purchased from Charles River Laboratories. Male TgF344-AD and female WTF344 rats were bred at UC Davis vivarium, and the resulting offspring was genotyped [42]. On postnatal day 28 (approximately 1 month of age),

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214   54 rats (27 males and 27 females consisting of TgF344-AD and WTF344 rats each) were

215   transferred to a tunnel facility situated near a heavily trafficked freeway tunnel system in

216   Northern California (see next paragraph for details) [43]. Half of the rats per genotype and per

217   sex were were randomly assigned to the FA vs. TRAP groups and exposed continuously for up

218   to 14 months as previously described [42]. Thus, there were 8 groups in total as shown in the

219   overall study design depicted in **Figure 2** (n=54 rats in total, 8 groups, 6 or 7 rats per group). The

220   animals were euthanized at 15 months of age.

221   The tunnel facility was built to capture gaseous and particulate components of real-world

222   TRAP [44, 45]. It had a filtering system that provided FA to exposure chambers housing the FA

223   group or TRAP collected from the traffic tunnel and delivered unchanged in real-time to

224   exposure changers housing the TRAP group. During the 14 month-long exposure period, total

225   particle numbers and mean 24 h $PM_{2.5}$ levels in the TRAP chambers were 10-100 and ~62 fold

226   higher than in FA chambers, respectively [42]. At the end of the exposure period, rats were

227   transported to the UC Davis vivarium, where they were anesthetized with 2-3% isoflurane for a

228   single MRI/PET imaging session before being euthanized 23 days later with 4% isoflurane

229   (Southmedic Inc., Barrie ON) in medical-grade air/oxygen (2:1 v/v) mixture delivered at a rate of

230   1.5 L/min followed by exsanguination via perfusion of ice-cold saline as previously described

231   [42]. Brains were dissected and cut in half using a stainless-steel rat brain matrix (Zivic

232   Instruments, Pittsburgh, PA). The left hemisphere was microdissected to obtain brain regions for

233   cytokine assays. The right hemisphere was used for lipidomic measurements as detailed below.

234   Samples were immediately collected in centrifuge tubes, snap frozen in liquid nitrogen and

235   stored at -80 °C until they were analyzed.
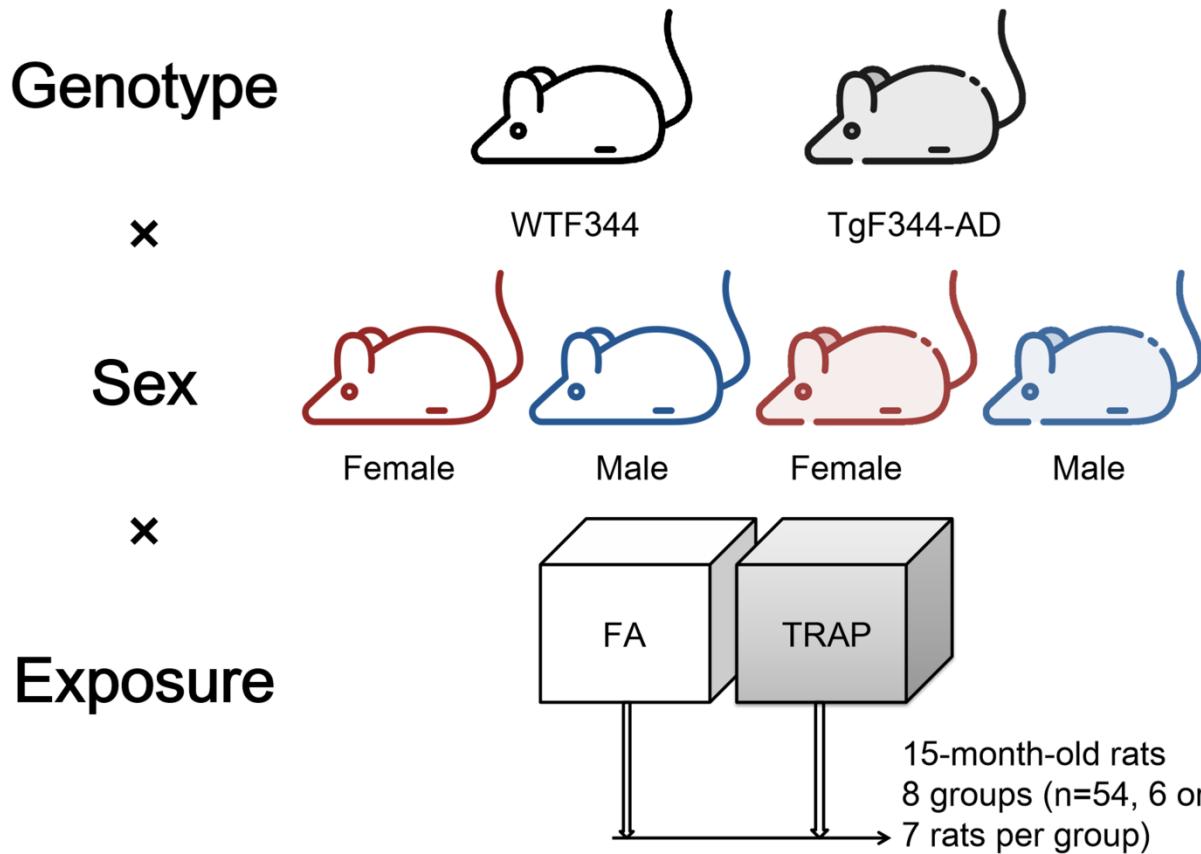
Figure 2. Study design. A total of 54 male and female TgF344-AD and WTF344 rats were randomly distributed to filtered air (FA) or traffic-related air pollution (TRAP) exposure beginning at approximately one month of age (at postnatal day 28) until they were 15-months old (total exposure period of 14 months). There were 8 groups in total, each composed of 6 or 7 rats per group.

2.3 Brain lipid extraction

Brain total lipids were extracted from the right hemisphere using a modified Folch method [46, 47]. Brains were weighed and transferred into new 2 mL centrifuge tubes pre-cooled and maintained on dry ice. The average brain weight was ~800 mg. Three zirconia beads and approximately 700 µL solution of 1 mM Na_2EDTA and 0.9% NaCl dissolved in MilliQ water (kept at 4 °C before use) were then added into each centrifuge tube containing the brain samples. Because rat brain contains ~90% water [48], the total volume of the aqueous phase was

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250   approximately ~1420 µL (700 µL added + ~720 uL coming from the brain). The brain was

251   homogenized in a Bullet Blender (Next Advance Storm 24, Averill Park, NY, USA) for 30 s

252   twice, and the resulting homogenate was transferred into 8 mL glass tubes containing 4 mL

253   chloroform. The centrifuge tubes were then washed with 1 mL of 0.006% BHT methanol

254   solution (pre-cooled in a 4 °C fridge before use) and vortexed for 30 s. The mixture in the

255   centrifuge tubes was transferred into the above 8 mL glass tubes. This step was repeated one

256   more time to ensure that all lipids in the 2 mL centrifuge tubes were completely transferred to the

257   8 mL glass tubes. The 8 mL glass tubes containing brain total lipid extracts were vortexed and

258   centrifuged at 920×g for 15 min at 0 °C in a Sorvall RT 6000 centrifuge (Bio Surplus, San

259   Diego, CA, USA). The bottom chloroform layer from each extraction was transferred into a new

260   8 mL glass tube. 4 mL chloroform were added to the remaining upper layer and the samples were

261   vortexed and centrifuged again at 920×g for 15 min at 0 °C. The bottom chloroform layer was

262   transferred and combined with the first chloroform extract in the 8 mL glass tube.

263   The total brain lipid extract was dried under nitrogen and reconstituted in 8 mL of

264   chloroform/isopropanol (2:1 v/v). Samples were stored in a -80 °C freezer.Every 19 brain

265   samples were accompanied by an additional method blank consisting of 800 µL of MilliQ water

266   (instead of 800 mg of rat brain), that underwent the same extraction procedures outlined above.

267   *2.4 Separation of neutral lipids (NL) and phospholipids (PL)*

268   Waters silica solid phase extraction (SPE) columns (Sep-Pak Silica, 1 cc, 100 mg, Waters

269   Corporation, Milford, MA; Cat #WAT023595) were used to separate NLs from polar lipids

270   including PLs and any residual free oxylipins that were not removed during Folch extraction [49].

271   Methanol (1.5 mL) and 2:1 v/v chloroform/isopropanol (1.5 mL) were loaded onto each silica

272   SPE column to activate and equilibrate the column. The column was loaded with 300 µL of brain

14

273 total lipid extract (containing ~ 3 mg of total lipids) dissolved in chloroform/isopropanol (2:1

274 v/v), and eluted with 1.5 mL of chloroform/isopropanol (2:1 v/v). The eluent containing NLs was

275 collected in 2 mL centrifuge tubes. The column was then loaded with 1.5 mL of 95% methanol,

276 and the eluent containing polar lipids (e.g. phospholipids) was collected in another 2 mL

277 centrifuge tube.

278   The eluent containing polar lipids in 95% methanol was adjusted to 80% methanol by

279 adding 281 µL of MilliQ water to the 1.5 mL extract. The entire mixture was loaded onto Waters

280 tC18 columns (Sep-Pak tC18, 1 cc, 100 mg, Waters Corporation, Milford, MA; Cat

281 #WAT036820) pre-rinsed with one column volume of methanol and 1.5 mL of 80% methanol.

282 The column was washed with 2 mL of 80% methanol to remove free fatty acids and free

283 oxylipins, followed by 2 mL methanol to elute PLs which were collected in 2 mL centrifuge

284 tubes and stored in -80 °C until further use. The efficiency of separation of PLs from free

285 oxylipins was confirmed using free oxylipin surrogate standards subjected to the same separation

286 method; here though, both the PL and free oxylipin fractions were collected and analyzed by

287 mass-spectrometry to measure recoveries. As shown in **Supplementary Table 1**, 97.8%-99.3%

288 of the free deuterated surrogate standards were recovered in the free fraction, suggesting that free

289 oxylipins were well-separated from PLs. The only exception was free d6-20-HETE which had a

290 recovery of 66.9% in the free fraction. This means that approximately 33% of free HETEs are

291 likely to co-elute with PLs, leading to overestimation of their concentrations in PLs.

292 *2.5 Hydrolysis of neutral lipid (NL) and phospholipid (PL)*

293   The collected NL and PL fractions were dried under nitrogen and dissolved in 200 µL of

294 ice-cold extraction solvent containing 0.1 % acetic acid and 0.1% of BHT in methanol. Each

295 sample was spiked with 10 µL of antioxidant solution containing 0.2 mg/mL BHT, EDTA and

296   TPP in water/methanol (1:1 v/v) and 10 µL of surrogate mix standard solution containing 2 µM

297   of d11-11(12)-EpETrE, d11-14,15-DiHETrE, d4-6-keto-PGF1a, d4-9-HODE, d4-LTB4, d4-

298   PGE2, d4-TXB2, d6-20-HETE, and d8-5-HETE in LC-MS grade methanol (i.e. 20 picomole per

299   sample). Then, 200 µL of 0.25 M sodium hydroxide in water/methanol (1:1 v/v) was added to

300   each sample. The mixture was vortexed and heated for 30 minutes at 60°C on a heating block to

301   hydrolyze esterified oxylipins. After cooling it for 5 min, 25 µL of acetic acid and 1575 µL of

302   MilliQ water were added. The samples were vortexed and stored at -20 °C (for ~1 h) for further

303   purification of the hydrolyzed oxylipins by SPE as described in the following section.

304   *2.6 Oxylipin separation by SPE*

305   Free oxylipins were isolated using Waters Oasis HLB SPE columns (3 cc, 60 mg, 30 µm

306   particle size; Waters Corporation, Milford, CA, USA; Cat #WAT094226) as previously

307   described [49]. The SPE columns were washed with one column volume of ethyl acetate and two

308   column volumes of methanol, and pre-conditioned with two column volumes of SPE buffer

309   containing 0.1% acetic acid and 5% methanol in MilliQ water. The hydrolyzed samples were

310   loaded onto the columns, which were then washed with two column volumes of SPE buffer and

311   dried under vacuum (≈15-20 psi) for 20 min. Oxylipins were then eluted from the columns with

312   0.5 mL methanol and 1.5 mL ethyl acetate, and collected in 2 mL centrifuge tubes. The samples

313   were dried under nitrogen, reconstituted in 100 µL LCMS grade methanol, vortexed for 2 min,

314   and centrifuged at $15,871 \times g$ (0°C; 5424 R Centrifuge; Eppendorf AG, Hamburg, Germany) for 2

315   min. The samples were transferred to centrifuge tubes containing a filter unit (Ultrafree-MC VV

316   Centrifugal Filter, 0.1 µm; Millipore Sigma, Burlington, MA, USA; Cat # UFC30VV00) and

317   centrifuged at $15,871 \times g$ (0°C) for 20 min. The filtered samples were transferred into 2 mL

318   amber LC-MS vials (Phenomenex, Torrance, CA, USA; Cat #AR0-3911-13) with pre-slit caps

319 (Phenomenex, Torrance, CA, USA; Cat #AR0-8972-13-B) and inserts (Waters Corporation,

320 Milford, CA, USA; Cat #WAT094171). Samples were stored in a -80 °C freezer for further ultra

321 high-pressure liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) analysis.

322 *2.7 Oxylipins analysis by UPLC-MS/MS*

323 A total of 76 oxylipins were measured with UPLC-MS/MS, using an Agilent 1290 Infinity

324 UPLC system coupled to an Agilent 6460 Triple Quadropole mass-spectrometer (Agilent

325 Technologies, Santa Clara, CA, USA). The ULC was equipped with an Agilent ZORBAX

326 Eclipse Plus C18 column (2.1 × 150 mm, 1.8 µm particle size; Agilent Technologies, Santa Clara,

327 CA, USA; Cat #959759-902) to separate oxylipins. The column was kept at 45 °C. The system

328 was operated in a negative electrospray ionization mode with optimized dynamic Multiple

329 Reaction Monitoring (dMRM) conditions. Optimized MRM parameters for each oxylipin are

330 shown in **Supplementary Table 2**.

331 The temperature of the auto-sampler was set at 4 °C and the sample injection volume was

332 10 µL. Mobile phase A contained 0.1% acetic acid in MilliQ water and Mobile phase B

333 consisted of acetonitrile/methanol (80:15 v/v) containing 0.1% acetic acid. The mobile phase

334 gradient and pressure program was as follows: 1) 0-2 min, 35% B, 0.25 mL/min (this was

335 diverted into a waste bottle and not injected into the mass-spec); 2) 2-12 min, 35 to 85% B, 0.25

336 mL/min; 3) 12-15min, 85% B, 0.25 mL/min; 4) 15.1-17 min, 85% to 100% B, 0.4 mL/min; 5)

337 17.1-19 min, 100 to 35% B, 0.4 mL/min; and 6) 19-20 min, 35% A, 0.3 mL/min. The total run

338 time was 20 minutes.

339 *2.8 Data and statistical analysis*

340 Data were analyzed on GraphPad Prism v.8.02 (La Jolla, CA, USA) or SPSS 20.0 (SPSS

341 Inc., Chicago, IL, USA). Data are presented as mean ± standard deviation (SD). Missing

342   oxylipins values in 1, 2 or 3 subjects per group were imputed by dividing the lowest observable

343   concentration on the standard curve by the square root of 2. The number of imputed values for

344   each group are shown in **Supplementary Table 3**. The effects of sex, genotype and exposure on

345   brain NL and PL oxylipins were compared by three-way analysis of variance (ANOVA); the

346   effects of genotype and exposure on brain NL or PL oxylipins per sex were compared by one-

347   way ANOVA followed by Duncan's post-hoc test. Statistical significance was accepted at $p <$

348   0.05.

349   **3. Results**

350   *3.1 Effects of AD genotype and TRAP exposure on NL-bound oxylipins in brain of 15-month-old*

351   *rats*

352   Three-way ANOVA showed that sex and AD genotype were the main factors affecting

353   oxylipins in NLs; in contrast, TRAP was not a main factor affecting oxylipins (**Supplementary**

354   **Table 4**).

355   Sex effects were statistically significant for dihomo-gamma-linoleic acid (DGLA)-derived

356   15(S)-hydroxy-eicosatrienoic acid (15(S)-HETrE), AA-derived 12-oxo-eicosatetraenoic acid

357   (12-oxo-ETE), 5(6)-EpETrE, and LXA4, eicosapentaenoic acid (EPA)-derived 11(12)-epoxy-

358   eicosatetraenoic acid (11(12)-EpETE) and Resolvin E1, and DHA-derived oxylipins including

359   19(20)-epoxy-docosapentaenoic acid (19(20)-EpDPE), 16(17)-EpDPE, 13(14)-EpDPE, 10(11)-

360   EpDPE, 7(8)-EpDPE, and 16,17-dihydroxy-docosapentaenoic acid (16,17-DiHPDA). All of

361   these oxylipins were significantly higher by 16% to 65% in brain NLs of females compared to

362   males.

363   AD genotype significantly impacted DGLA-derived 15(S)-HETrE, AA-derived 15-HETE,

364   11-HETE, 11(12)-EpETrE, 14,15-DiHETrE, 11,12-DiHETrE, and 8,9-DiHETrE, EPA-derived

18

365   11(12)-EpETE, and DHA-derived 19(20)-EpDPE, 19,20-DiHPDA and 16,17-DiHPDA within

366   NLs ($p < 0.05$; **Supplementary Table 4**).

367   To better visualize AD-specific changes per sex, a one-way ANOVA was applied in male

368   and female wildtype and TgF344-AD rats exposed to FA or TRAP. The analysis revealed

369   significant changes in NL-bound oxylipins in TgF344-AD females exposed to either FA or

370   TRAP (**Figure 3**), and a few changes in males (**Supplementary Table 5**).

371   In females (**Figure 3),** DGLA-derived 15(S)-HETrE (**3-a**), AA-derived 20-HETE, 15-

372   HETE, 11-HETE, 11(12)-EpETrE and 14,15-DiHETrE (**3-b**), EPA-derived 17(18)-EpETE and

373   11(12)-EpETE (**3-d**), and DHA-derived 19(20)-EpDPE, 16(17)-EpDPE, 13(14)-EpDPE, 10(11)-

374   EpDPE, 7(8)-EpDPE and 19,20-DiHPDA (**3-e)**, were significantly lower by 22%-43% in Tg-FA

375   rats compared to WT-FA controls ($p < 0.05$). The majority of these oxylipins (AA-derived

376   11(12)-EpETrE and 14,15-DiHETrE, and DHA-derived 19(20)-EpDPE, 16(17)-EpDPE, 13(14)-

377   EpDPE, 7(8)-EpDPE and 19,20-DiHPDA), as well AA-derived 5-oxo-ETE and 11,12-

378   DiHETrE, and DHA-derived 16,17-DiHPDA, were also lower by 8%-43% in Tg-TRAP rats

379   compared to WT-FA or WT-TRAP, suggesting an AD-effect, independent of TRAP exposure on

380   these oxylipins. Alpha-linolenic acid (ALA)-derived 13-hydroxy-octadecatrienoic acid (13-

381   HOTrE) was 4-fold higher in Tg-TRAP compared to WT-TRAP (**Figure 3-c**, $p < 0.05$), but

382   neither groups differed significantly from WT-FA controls. Overall, the data suggest that AD-

383   genotype reduced multiple oxylipins in NLs of female rats, and that TRAP exposure did not

384   further exacerbate the effects of AD genotype on NL oxylipin concentrations.

385   TRAP exposure minimally affected NL oxylipins in WT rats. The few observed changes

386   included a significant increase in AA-derived 12-oxo-ETE by ~2-fold in WT-TRAP rats

387   compared to WT-FA, Tg-FA and Tg-TRAP rats (**Figure 3-b**), and a significant 22% decrease in

388   DHA-derived 7(8)-EpDPE in WT-TRAP rats compared to WT-FA controls (**Figure 3-e**).

(a) DGLA-derived oxylipins
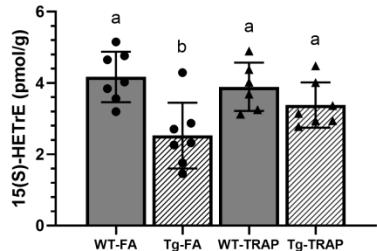
389

(b) AA-derived oxylipins

390
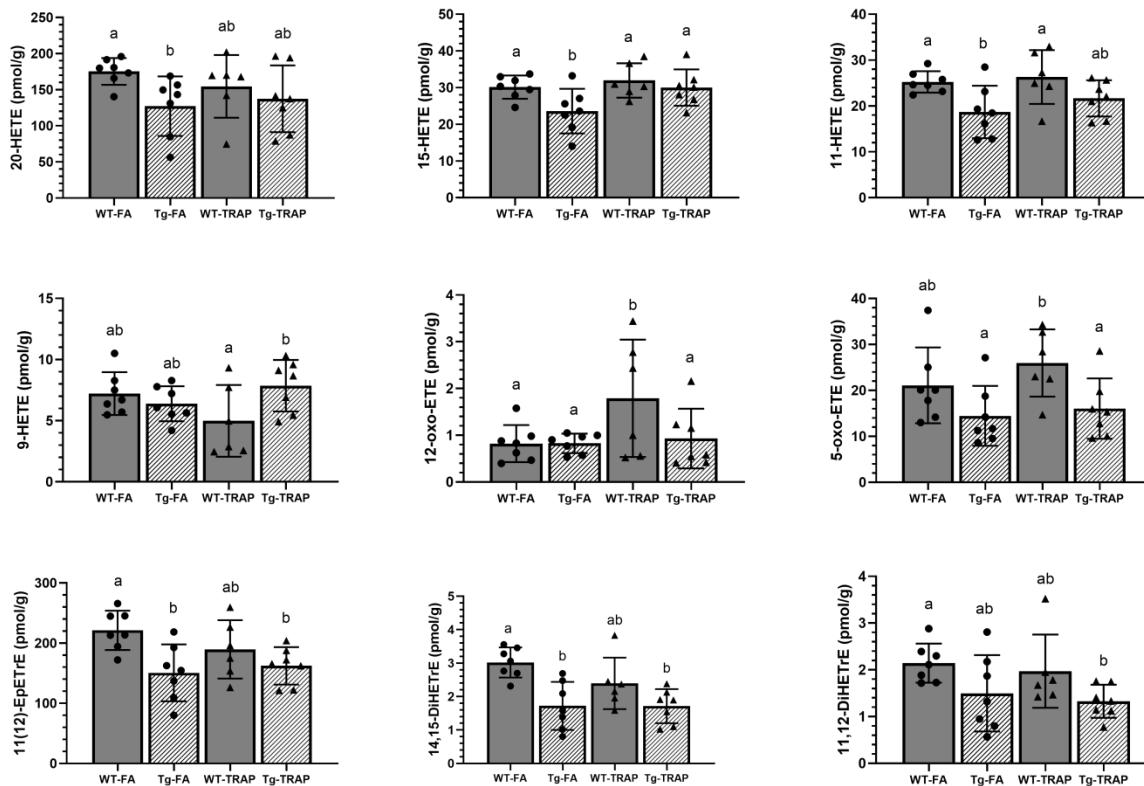
20

(c) ALA-derived oxylipins
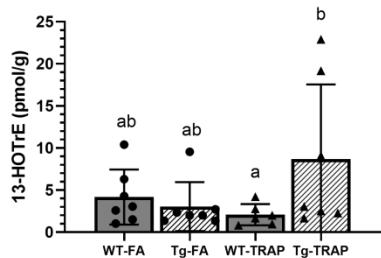
391

(d) EPA-derived oxylipins
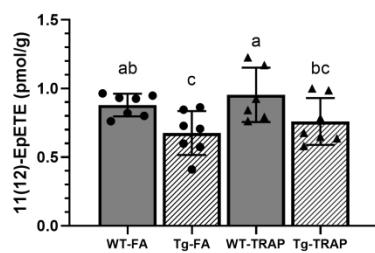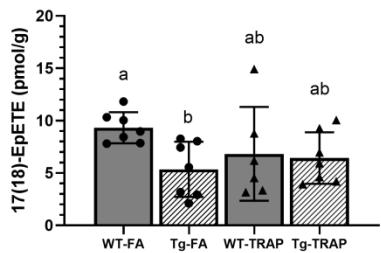
392

(e) DHA-derived oxylipins
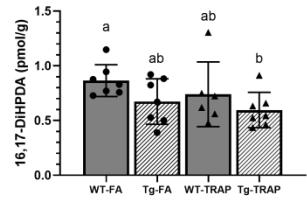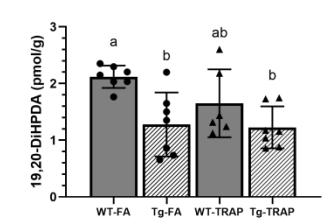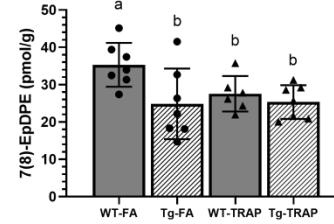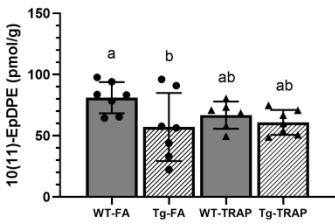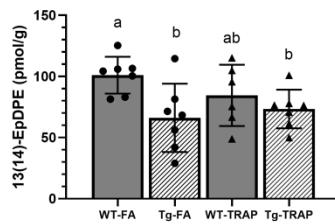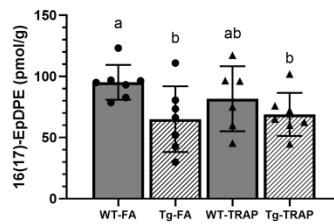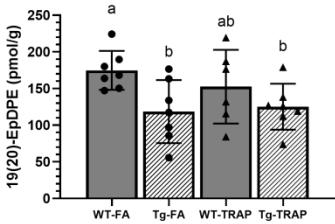

393

394 **Figure 3**. Oxylipin concentrations in brain neutral lipids (NLs) of 15-month-old wildtype (WT)
395 or TgF344-AD (Tg) female rats exposed to filtered air (FA) or traffic-related air pollution (TRAP)
396 for 14 months (n=27). Bar graphs represent mean ± SD of n = 7 WT-FA, n = 7 Tg-FA, n = 6
397 WT-TRAP, and n=7 Tg-TRAP. (a) dihomo-gamma-linoleic acid (DGLA)-derived oxylipins; (b)
398 arachidonic acid (AA)-derived oxylipins; (c) alpha-linolenic acid (ALA)-derived oxylipins; (d)
399 eicosapentaenoic acid (EPA)-derived oxylipins; (e) docosahexaenoic acid (DHA)-derived
400 oxylipins. Oxylipin abbreviations: DiHETE, dihydroxy-eicosatetraenoic acid; DiHETrE,
401 dihydroxy-eicosatrienoic acid; DiHOME, dihydroxy-octadecenoic acid; DiHPDA, dihydroxy-
402 docosapentaenoic acid; EpDPE, epoxy-docosapentaenoic acid; EpETE, epoxy-eicosatetraenoic
403 acid; EpETrE, epoxy-eicosatrienoic acid; EpOME, epoxy-octadecenoic acid; HDoHE, hydroxy-
404 docosahexaenoic acid; HEPE, hydroxy-eicosapentaenoic acid; HETE, hydroxy-eicosatetraenoic
405 acid; HETrE, hydroxy-eicosatrienoic acid; HODE, hydroxy-octadecadienoic acid; HOTrE,
406 hydroxy-octadecatrienoic acid; oxo-ETE, oxo-eicosatetraenoic acid; oxo-ODE, oxo-
407 octadecadienoic acid; TriHOME, trihydroxy-octadecenoic acid.
408

409   In males, no significant differences in brain NL-bound oxylipins of LA, DGLA, ALA, EPA

410 and DHA were observed (**Supplementary Table 5**); however, a few AA-derived oxylipins were

411 altered (**Supplementary Table 5**). 9-HETE was 40% lower in Tg-TRAP versus Tg-FA rats ($p <$

412 0.05), 8-HETE was lower by 45% in Tg-TRAP compared to WT-TRAP, and 15-deoxy-PGJ2

413 was 49% lower in Tg-TRAP than WT-FA ($p < 0.05$). These minimal changes are difficult to

414 interpret.

415 *3.2 Effects of AD genotype and TRAP exposure on PL-bound oxylipins in brain of 15-month-old*

416 *rats*

417   Three-way ANOVA showed significant main effects of sex, TRAP exposure and AD

418 genotype on brain PL oxylipins (**Supplementary Table 6**). Sex significantly altered LA-derived

419 13-oxo-octadecadienoic acid (13-oxo-ODE) and 12(13)-epoxy-octadecenoic acid (12(13)-

420 EpOME), which were higher by 21% and 19% in brain PLs of females than males, respectively

421 ($p < 0.05$). TRAP significantly altered PL-bound AA-derived 11,12-DiHETrE and LXA4, and

422 DHA-derived 19,20-DiHPDA ($p < 0.05$). Genotype significantly altered AA-derived 20-HETE,

22

423 8-HETE, 8(9)-EpETrE, 14,15-DiHETrE, 11,12-DiHETrE and 8,9-DiHETrE, and DHA-derived

424 19,20-DiHPDA ($p < 0.05$).

425 A one-way ANOVA followed by Duncan's post-hoc test was used to examine the effects of

426 genotype and exposure within female and male rats. **Supplementary Table 7** shows all oxylipin

427 concentration values in brain PLs of males and females. As shown, there were no significant

428 effects of AD genotype or TRAP exposure in males. However, significant changes were

429 observed in females as depicted in **Figure 4**. AD genotype was associated with significant

430 changes in PL-bound oxylipins. Compared to WT-FA controls, the Tg-FA group had

431 significantly lower concentrations of LA-derived 13-oxo-ODE, 9-oxo-ODE and 9(10)-EpOME

432 (by 31%-40%, **Figure 4-a**), AA-derived HETEs, 15-oxo-ETE, 12-oxo-ETE, 8(9)-EpETrE,

433 DiHETrEs, PGE2 and PGB2 (by 27%-63%, **Figure 4-b**), EPA-derived 15-hydroxy-

434 eicosapentaenoic acid (15-HEPE) (by ~42%, **Figure 4-c**), and DHA-derived 17-hydroxy-

435 docosahexaenoic acid (17-HDoHE), 19(20)-EpDPE, 19,20-DiHPDA and 16,17-DiHPDA (by

436 24%-42%, **Figure 4-d**). Similar reductions in PL-bound oxylipins were observed in Tg-TRAP

437 rats compared to WT-FA controls.

438 TRAP exposure alone resulted in significant reductions in AA, EPA and DHA-derived PL-

439 bound oxylipins in wildtype rats. Compared to WT-FA controls, WT-TRAP rats showed

440 significant reductions in AA-derived 15-HETE, 11-HETE, 9-HETE, 5-HETE, 14,15-DiHETrE,

441 11,12-DiHETrE and 5,6-DiHETrE by 23%-40% (**Figure 4-b**, $p < 0.05$), EPA-derived 15-HEPE

442 by 27% (**Figure 4-c**, $p < 0.05$), and DHA-derived 17-HDoHEE, 19,20-DiHPDA and 16,17-

443 DiHPDA by 29% to 37 % (**Figure 4-d**, $p < 0.05$).

(a) LA-derived oxylipins
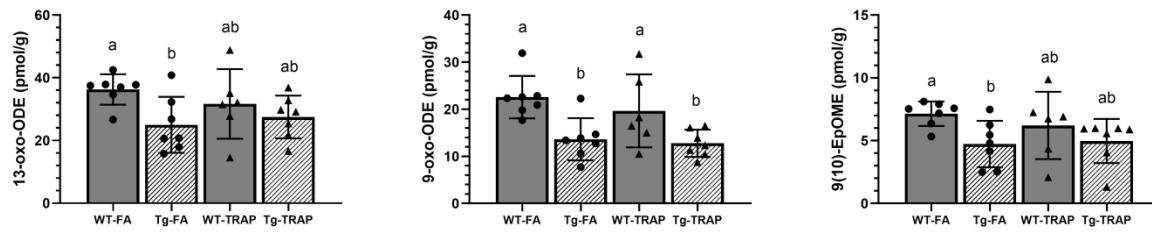
444

(b) AA-derived oxylipins

445
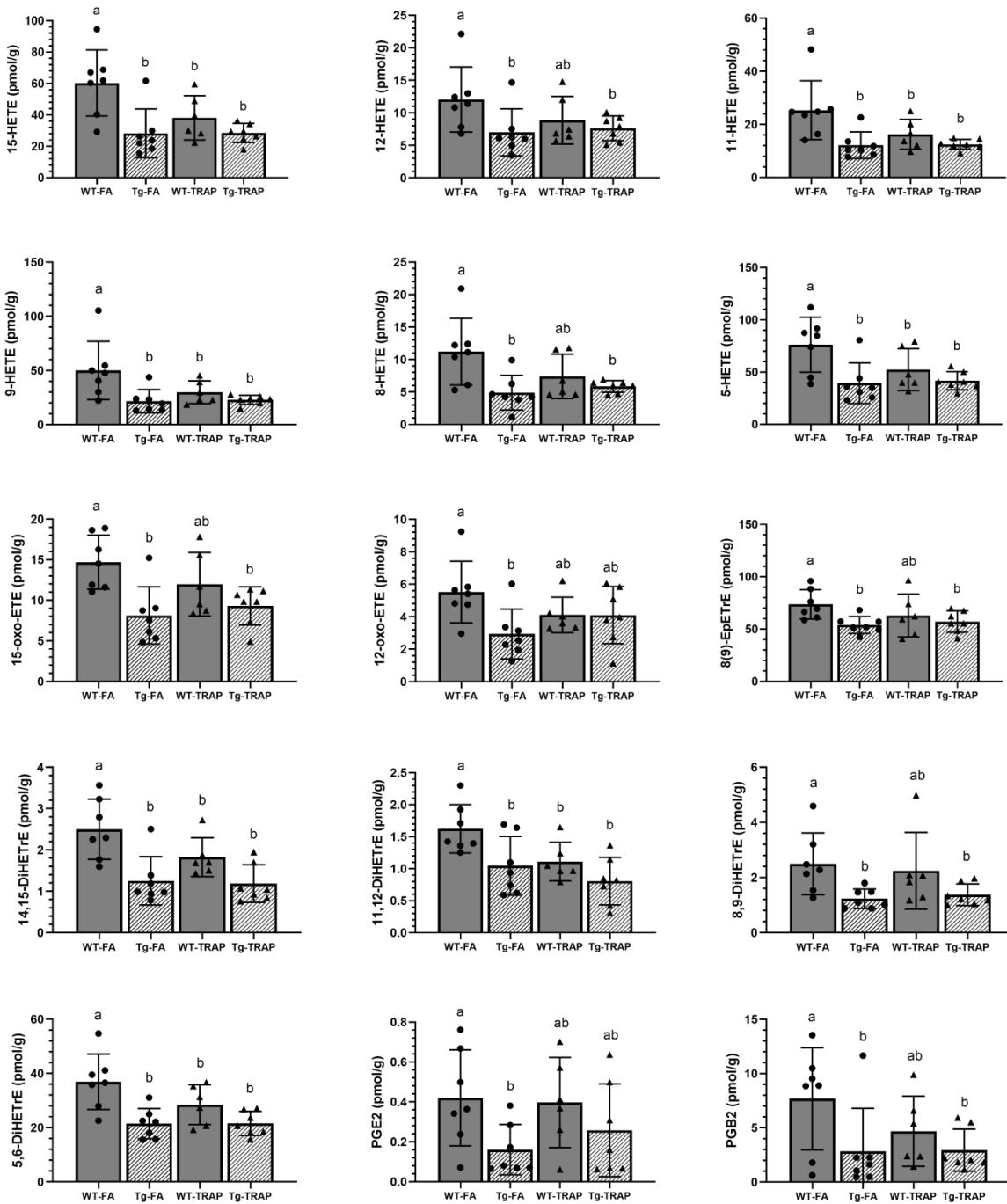
(c) EPA-derived oxylipins
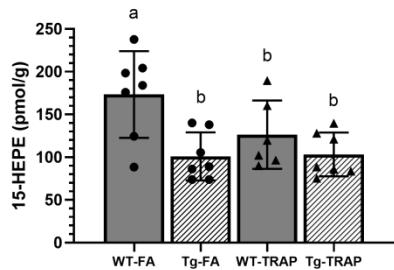

(d) DHA-derived oxylipins
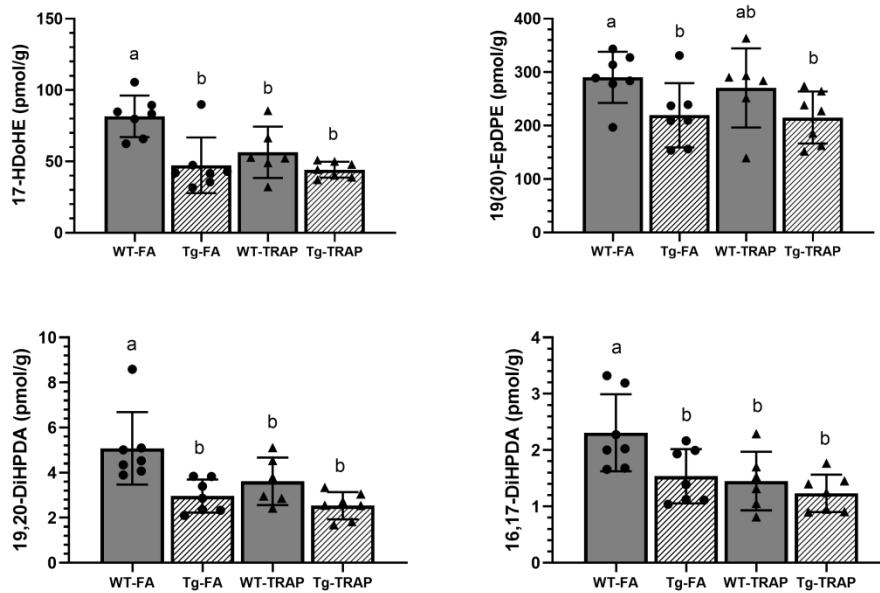
Figure 4. Oxylipins concentrations in brain phospholipids (PLs) of 15-month-old wildtype (WT) or TgF344-AD (Tg) female rats exposed to filtered air (FA) or traffic-related air pollution (TRAP) for 14 months (n=27). Bar graphs represent mean ± SD of n = 7 WT-FA, n = 7 Tg-FA, n = 6 WT-TRAP, and n=7 Tg-TRAP. (a) linoleic acid (LA)-derived oxylipins, (b) arachidonic acid (AA)-derived oxylipins, (c) eicosapentaenoic acid (EPA)-dedrived oxylipins, (d) docosahexaenoic acid (DHA)-derived oxylipins. Oxylipin abbreviations: DiHETE, dihydroxy-eicosatetraenoic acid; DiHETrE, dihydroxy-eicosatrienoic acid; DiHOME, dihydroxy-octadecenoic acid; DiHPDA, dihydroxy-docosapentaenoic acid; EpDPE, epoxy-docosapentaenoic acid; EpETE, epoxy-eicosatetraenoic acid; EpETrE, epoxy-eicosatrienoic acid; EpOME, epoxy-octadecenoic acid; HDoHE, hydroxy-docosahexaenoic acid; HEPE, hydroxy-eicosapentaenoic acid; HETE, hydroxy-eicosatetraenoic acid; HETrE, hydroxy-eicosatrienoic acid; HODE, hydroxy-octadecadienoic acid; HOTrE, hydroxy-octadecatrienoic acid; oxo-ETE,

460 oxo-eicosatetraenoic acid; oxo-ODE, oxo-octadecadienoic acid; TriHOME, trihydroxy-
461 octadecenoic acid; PG: Prostaglandin.
462

Summary of findings:

464 More pro-resolving than pro-inflammatory oxylipins were changed in AD and TRAP-

465 exposed female rats. Of the 20 significantly altered oxylipins in brain NLs of female TgF344-AD

466 rats or WT/ TgF344-AD female rats exposed to TRAP, 75% (or 15 oxylipins) have pro-resolving

467 effects in vivo (**Figure 3** or **Supplementary Table 5**). Similarly, in PLs, of the 23 significantly

468 altered oxylipin , 61% (14 oxylipins) are considered pro-resolving (**Figure 4** or **Supplementary**

469 **Table 7**). Specifically, the following anti-inflammatory oxylipins were significantly lower in

470 female brain NLs of TgF344-AD rats by 23%-42% relative to WT controls: DGLA-derived

471 15(S)-HETrE, AA-derived 11(12)-EpETrE, EPA-derived 17(18)-EpETE and 11(12)-EpETE, and

472 DHA-derived 16(17)-EpDPE, 13(14)-EpDPE, 10(11)-EpDPE and 7(8)-EpDPE). In PLs, the

473 following species were reduced in TgF344-AD rats relative to WT controls by 27%-63%: LA-

474 derived 13-oxo-ODE, AA-derived 15-oxo-ETE, 8(9)-EpETrE and PGB2, EPA-derived 15-HEPE,

475 and DHA-derived 17-HDoHE.

476 In TRAP-exposed WT female rats, anti-inflammatory DHA-derived 7(8)-EpDPE was

477 significantly lower by 22% in brain NLs pool compared to WT-FA controls, and in PLs, WT-

478 TRAP females exhibited significant reductions 23%-37% in pro-resolving AA-derived 15-HETE

479 and 5,6-DiHETrE, EPA-derived 15-HEPE, and DHA-derived 17-HDoHEE and 19,20-DiHPDA.

4. Discussion

481 The main finding of this study is that AD genotype or TRAP exposure for 14 months

482 reduced the concentration of esterified lipid mediators in the brain of 15-month old female rats.

483 Most of the changes were seen in pro-resolving lipid mediators. The effects of AD genotype

27

484   were seen in both NL and PL pools, whereas the effects of TRAP exposure were mainly seen in

485   PLs. Changes were mainly seen in females but not males, suggesting sex-specific effects.

486   Together, our findings reflect a sex-dependent deficit in PL- and/or NL-bound pro-resolving

487   lipid mediators in rats genetically pre-disposed to AD or exposed to TRAP for 14 months.

488   Prior studies have shown a reduction in free pro-resolving lipid mediators in the brain of

489   transgenic mouse models of AD (DHA-derived EpDPEs [31] and AA-derived EpETrEs [21, 31,

490   32]) and in the post-mortem brain of AD patients (resolvin D5, maresin 1 and protectin D1 [24,

491   28], and LXA4 [50]), reflecting impaired resolution pathways. Our present findings point to

492   marked reductions in esterified lipid precursors to free pro-resolving lipid mediators, in TgF344-

493   AD and TRAP-exposed WT female rats. As noted earlier, free oxylipins are bioactive, whereas

494   esterified oxylipins have minimal bioactivity. Thus, a deficit in the esterified oxylipin pool,

495   which serves as a major source of free oxylipins [34], may explain why free pro-resolving

496   oxylpins are reduced in AD, where inflammation resolution is impaired. In this study, we also

497   extend these findings to TRAP exposure, a significant risk factor for AD dementia [4, 5].

498   The observed reduction in esterified pro-resolving lipid mediators in AD and TRAP-

499   exposed rats may be attributed to changes in brain oxylipin turnover, involving the release of

500   bound oxylipins and re-esterification of free oxylipins. Oxylipin release is enabled by lipase

501   enzymes [51], whereas re-esterification is enzymatically facilitated by the acylation of free

502   oxylipins via fatty acyl-CoA synthetases [52] and esterification of acylated oxylipins (i.e.

503   oxylipin-CoA) into NLs or PLs by one of 9 sn-glycerol-3-phosphate acyltransferase isoforms in

504   the brain (also known as lysophosphatidic acid acyltransferases) [53]. Thus, a decrease in

505   esterified oxylipins could be due to an increase in lipase-mediated release, decreased acyl-CoA

506   synthetase/ transferase-mediated esterification or a combination of both of these pathways as

507   shown in **Figure 1**.

508   There is limited information on the specific lipase, acyl-CoA synthetase and acyltransferase

509   isoforms involved in brain oxylipin turnover. Brain lipase enzymes, including calcium-dependent

510   phospholipase A2, have been shown to be upregulated in transgenic models of AD and in

511   humans with AD dementia [28, 54], although it is not known whether these isoforms release

512   bound oxylipins. Klett et al. showed that recombinant acyl-Co synthetase 4 preferentially

513   incorporates AA-derived epoxides into PLs in vitro [52], potentially implicating this particular

514   isoform in the observed reduction in PL-bound AA-epoxides. To our knowledge, acyltransferase

515   enzymes involvement in oxylipin turnover have not been studied. Identifying the specific lipase,

516   acyl-CoA synthetase and acyltransferase isoforms involved in pro-resolving oxylipin turnover in

517   AD and TRAP-exposed rats may inform on new targets that control the bioavailability of free

518   pro-resolving lipid mediators in the brain.

519   If indeed lipase-mediated release of oxylipins is increased, and their esterification decreased

520   as we propose above, one would expect an increase in free pro-resolving mediators in AD and

521   TRAP-exposed brains. Although literature on brain lipidomic changes following TRAP exposure

522   is lacking, in AD, marked reductions (not increases) in free pro-resolving lipid mediators were

523   observed in transgenic mouse models and human brain [19, 24, 28, 30, 50]. This could be due to

524   increased degradation of free pro-resolving lipid mediators upon synthesis, as supported by

525   studies showing elevated levels of sEH in transgenic mouse models of AD [21]; sEH converts

526   CYP-derived pro-resolving AA and DHA epoxides into less active fatty acid diols [55]. We did

527   not measure free pro-resolving oxylipins in this study, because their concentrations change by up

528   to 150-fold due to post-mortem ischemia and brain dissection compared to esterified oxylipins,

529   which only change by 27-112% [34, 56]. Thus, accurately capturing changes in the free oxylipin

530   pool ought to be conducted after head-focused microwave irradiation, to stop post-mortem

531   changes in free oxylipin metabolism.

532   It is also unlikely that the observed reductions in esterified oxylipin concentrations in AD

533   and TRAP-exposed rats were due to decreases in free oxylipin synthesis via LOX, COX, CYP,

534   15-PDGH and sEH, a process which would decrease the availability of free oxylipins available

535   for esterification into NLs and PLs. This is because some of these enzymes (12/15-LOX, 5-LOX

536   and sEH) were shown to increase in both animal model of AD [21, 32] and in human AD post-

537   mortem brain [15, 16], suggesting increased capacity to make free pro-resolving oxylipins. The

538   fact that the pro-resolving free lipids are reportedly reduced in AD suggests that they are

539   degraded faster than they are synthesized or released from esterified oxylipin pools.

540   Most of the reductions caused by AD or TRAP exposure were observed in pro-resolving

541   lipid mediators, with only a few reductions seen in pro-inflammatory lipid mediators. Pro-

542   inflammatory lipid mediators that changed include LA-derived 9-oxo-ODE and 9(10)-EpOME

543   and AA-derived DiHETrEs, which were reduced by 34-51% in PLs pool of female TgF344-AD

544   rats, and AA-derived HETEs and epoxyketones (oxo-ETEs) were reduced by 26%-57% in both

545   NLs and PLs of female TgF344-AD rats or TRAP-exposed WT rats. This is both an interesting

546   and peculiar finding, because it suggests that AD and TRAP exposure almost selectively impact

547   pro-resolving lipid pathways versus pro-inflammatory pathways. These observations may be in

548   response to pro-inflammatory cytokines shown to be elevated in the brain, heart and plasma of

549   AD transgenic and TRAP-exposed rats (brain data are currently under peer review whereas heart

550   and plasma data are reported here: [43]). Our findings demonstrate a deliberate attempt by the

551 brain to resolve AD- or TRAP-induced inflammation, likely by utilizing the esterified pro-

552 resolving lipid pool to generate more free pro-resolving lipid mediators.

553   TRAP exposure reduced esterified oxylipin concentrations in the brains of WT female rats

554 similar to what we observed in TgF344-AD female rats, suggesting that both environmental and

555 genetic predispositions to AD target the same lipid esterification pathways. A notable distinction,

556 however, is that AD genotype reduced pro-resolving lipids in both NLs and PLs, whereas TRAP

557 exposure reduced them almost exclusively within PLs. It is not entirely clear why different lipid

558 pools are affected by the two conditions, when neuroinflammation plays a role in both. It is

559 possible that prolonged exposure to TRAP might alter NL-bound oxylipins. If so, this would

560 mean that PL-bound oxylipins are more vulnerable to the effects of brain inflammation than NL-

561 bound oxylipins. In other words, the brain might utilize PL-bound oxylipins first before utilizing

562 NL-bound oxylipins. This remains to be confirmed with longer exposure studies.

563   There were no additive effects between AD genotype and TRAP exposure, meaning that

564 TRAP exposure did not further exacerbate the deficits in esterified oxylipin concentrations in

565 AD transgenic rats, compared to FA exposure. This could be because both AD genes and TRAP

566 act on a common target (e.g. enzyme or receptor), that release esterified oxylipins or re-esterify

567 free oxylipins. The net effect, based on this study, is a reduction in esterified pro-resolving

568 oxylipins. However, further studies are needed to understand the molecular mechanisms

569 involved.

570   Interestingly, two pro-inflammatory oxylipins were significantly increased in brain NLs of

571 AD and TRAP-exposed rats. AA-derived 9-HETE was 58% higher in Tg-TRAP females than

572 WT-TRAP females, and AA-derived 12-oxo-ODE was 2-fold higher in WT-TRAP females than

573 WT-FA females. This is consistent with studies showing increased free HETEs in AD transgenic

574   mouse brains [22] and human post-mortem brain [15, 23], possibly due to increased

575   esterification as a mechanism to deactivate their pro-inflammatory free form.

576   The effects of AD-genotype and TRAP were mainly seen in female rats, suggesting greater

577   vulnerability of females to AD and TRAP exposure. This is consistent with epidemiological data

578   showing that the risk of AD is about twice greater in females than in males [39, 57]. TRAP

579   exposure may also contribute to sex vulnerabilities to dementia as a recent study found that,

580   compared to men, women had a significantly higher risk for cognitive function decline

581   associated with increased exposure to air pollution (i.e., PM_{10}, $PM_{2.5-10}$, and NO_2) [58]. This is

582   mechanistically aligned with findings of this study showing sex-specific changes in esterified

583   lipid mediators, and with our previous study showing that TRAP-exposed females had more

584   amyloid plaque deposition compared to TRAP-exposed males at early ages [42].

585   One limitation of this study is that unesterified oxylipins were not measured. This is

586   because they are more affected by the effects of post-mortem ischemia compared to esterified

587   oxylipins as discussed above [34, 56]. High-energy microwave-irradiation is necessary to

588   prevent the artefacts of post-mortem ischemia on the free oxylipin pool, and should be

589   considered in future studies (Reviewed in [59]). Another limitation is that the animals were

590   moved from the exposure tunnel to the UC Davis main campus vivarium for 23 days (for

591   MRI/PET imaging) prior to euthanasia. This exposure-free period is unlikely to change the

592   outcome of the present study as it is known that PM and various dust elements accumulate and

593   reside in the brain for a few months post-exposure [60-63]. A third limitation is that we did not

594   assess vulnerabilities in esterified oxylipins in different brain regions and at earlier time-points.

595   Doing so would allow us to track age-dependent changes in resolution pathways and to see

596 whether they start in brain structures known to be involved in AD pathogenesis (e.g.

597 hippocampus).

598 In summary, the present study found significant reductions in pro-resolving lipid mediators

599 in brain esterified lipid pools of female rats expressing an AD phenotype or exposed to TRAP.

600 Esterified oxylipins within PLs and NLs were impacted by AD, whereas PL-bound oxylipins

601 were impacted by TRAP exposure. Our study shows disturbances in major lipid pools regulating

602 the in vivo availability of free pro-resolving lipid mediators in brain. This may explain why

603 inflammation resolution pathways are impaired in AD, and why chronic TRAP exposure

604 increases the risk of AD dementia (i.e. by impairing resolution pathways involving esterified

605 lipids). Targeting pro-resolving oxylipin release or esterification may have therapeutic benefits

606 in AD caused by genetic vulnerabilities or chronic TRAP exposure.

607

Acknowledgements:

613

Conflicts of interest:

615 The authors declare no conflict of interest.

616

617 **Supplementary information:**

618 **Supplementary Table 1.** Percentage of free surrogates in phospholipids (PL) and free lipids

619 fractions (n=3). Data are expressed as mean ± SD.

620 **Supplementary Table 2**. Retention time, parent ion, product ion, and internal standards used in

621 neutral lipids (NL) and phospholipids (PL) of the 76 quantified oxylipins in rat brain samples.

622 **Supplementary Table 3.** Number of imputed oxylipins values in each group of 15-month-old

623 rats that were missing 1, 2, or 3 values.

624 **Supplementary Table 4.** Three-way ANOVA *p* value results of brain oxylipins in neutral lipids

625 fraction of 15-month-old rats (n=54)

626 **Supplementary Table 5.** Oxylipins concentrations in brain neutral lipids of 15-month-old rats

627 (n=54). Data within female or male groups are analyzed by one-way ANOVA followed by

628 Duncan's post-hoc test. Data are expressed as mean ± SD. WT: wildtype gene; Tg: Alzheimer's

629 Disease transgenic gene; TRAP: traffic-related air pollution exposure; FA: filtered air exposure.

630 **Supplementary Table 6.** Three-way ANOVA *p* value results of brain oxylipins in phospholipids

631 fraction of 15-month-old rats (n=54)

632 **Supplementary Table 7.** Oxylipins concentrations in phospholipids fraction of 15-month-old

633 rats (n=54). Data within female or male groups are analyzed by one-way ANOVA followed by

634 Duncan's post-hoc test. Data are expressed as mean ± SD. WT: wildtype gene; Tg: Alzheimer's

635 Disease transgenic gene; TRAP: traffic-related air pollution exposure; FA: filtered air exposure.

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References:

[1] 2021 Alzheimer's disease facts and figures, Alzheimer's & dementia : the journal of the Alzheimer's Association, 17 (2021) 327-406.

[2] K.D. Kochanek, J. Xu, E. Arias, Mortality in the United States, 2019, NCHS data brief, (2020) 1-8.

[3] R.H. Mir, G. Sawhney, F.H. Pottoo, R. Mohi-Ud-Din, S. Madishetti, S.M. Jachak, Z. Ahmed, M.H. Masoodi, Role of environmental pollutants in Alzheimer's disease: a review, Environmental Science and Pollution Research, 27 (2020) 44724-44742.

[4] P. Fu, K.K.L. Yung, Air Pollution and Alzheimer's Disease: A Systematic Review and Meta-Analysis, Journal of Alzheimer's disease : JAD, 77 (2020) 701-714.

[5] R. Peters, N. Ee, J. Peters, A. Booth, I. Mudway, K.J. Anstey, Air Pollution and Dementia: A Systematic Review, Journal of Alzheimer's Disease, 70 (2019) S145-S163.

[6] C.J. Matz, M. Egyed, R. Hocking, S. Seenundun, N. Charman, N. Edmonds, Human health effects of traffic-related air pollution (TRAP): a scoping review protocol, Systematic Reviews, 8 (2019) 223.

[7] H. Chen, J.C. Kwong, R. Copes, K. Tu, P.J. Villeneuve, A. van Donkelaar, P. Hystad, R.V. Martin, B.J. Murray, B. Jessiman, A.S. Wilton, A. Kopp, R.T. Burnett, Living near major roads and the incidence of dementia, Parkinson's disease, and multiple sclerosis: a population-based cohort study, The Lancet, 389 (2017) 718-726.

[8] C.R. Jung, Y.T. Lin, B.F. Hwang, Ozone, particulate matter, and newly diagnosed Alzheimer's disease: a population-based cohort study in Taiwan, Journal of Alzheimer's disease : JAD, 44 (2015) 573-584.

[9] I.M. Carey, H.R. Anderson, R.W. Atkinson, S.D. Beevers, D.G. Cook, D.P. Strachan, D. Dajnak, J. Gulliver, F.J. Kelly, Are noise and air pollution related to the incidence of dementia? A cohort study in London, England, BMJ open, 8 (2018) e022404.

[10] V.K. Singh, P. Guthikonda, Circulating cytokines in Alzheimer's disease, Journal of Psychiatric Research, 31 (1997) 657-660.

[11] O. Gruzieva, S.K. Merid, A. Gref, A. Gajulapuri, N. Lemonnier, S. Ballereau, B. Gigante, J. Kere, C. Auffray, E. Melén, G. Pershagen, Exposure to Traffic-Related Air Pollution and Serum Inflammatory Cytokines in Children, Environmental health perspectives, 125 (2017) 067007.

[12] L.K. Fonken, X. Xu, Z.M. Weil, G. Chen, Q. Sun, S. Rajagopalan, R.J. Nelson, Air pollution impairs cognition, provokes depressive-like behaviors and alters hippocampal cytokine expression and morphology, Molecular Psychiatry, 16 (2011) 987-995.

[13] K. Yasojima, C. Schwab, E.G. McGeer, P.L. McGeer, Distribution of cyclooxygenase-1 and cyclooxygenase-2 mRNAs and proteins in human brain and peripheral organs, Brain Res, 830 (1999) 226-236.

[14] G.M. Pasinetti, P.S. Aisen, Cyclooxygenase-2 expression is increased in frontal cortex of Alzheimer's disease brain, Neuroscience, 87 (1998) 319-324.

[15] D. Praticò, V. Zhukareva, Y. Yao, K. Uryu, C.D. Funk, J.A. Lawson, J.Q. Trojanowski, V.M.Y. Lee, 12/15-Lipoxygenase Is Increased in Alzheimer's Disease: Possible Involvement in Brain Oxidative Stress, The American Journal of Pathology, 164 (2004) 1655-1662.

[16] M.D. Ikonomovic, E.E. Abrahamson, T. Uz, H. Manev, S.T. Dekosky, Increased 5-lipoxygenase immunoreactivity in the hippocampus of patients with Alzheimer's disease, The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society, 56 (2008) 1065-1073.

[17] P. Sarkar, J. Narayanan, D.R. Harder, Differential effect of amyloid β on the cytochrome P450 epoxygenase activity in rat brain, Neuroscience, 194 (2011) 241-249.

[18] Y.J. Koh, M.-k. Shin, E. Vázquez-Rosa, C. Cintrón-Pérez, K. Franke, E. Miller, P. Sridharan, S. Barker, S. Fink, H. Li, D. Dawson, H. Fujioka, M. Flanagan, J. Ready, S. Markowitz, A. Pieper, 15-Hydroxyprostaglandin Dehydrogenase Inhibition Protects a Mouse Model of Alzheimer's Disease, Biological Psychiatry, 89 (2021) S309.

684 [19] A. Ghosh, M.M. Comerota, D. Wan, F. Chen, N.E. Propson, S.H. Hwang, B.D. Hammock, H. Zheng, An
685 epoxide hydrolase inhibitor reduces neuroinflammation in a mouse model of Alzheimer's disease,
686 Science translational medicine, 12 (2020).
687 [20] C. Griñán-Ferré, S. Codony, E. Pujol, J. Yang, R. Leiva, C. Escolano, D. Puigoriol-Illamola, J. Companys-
688 Alemany, R. Corpas, C. Sanfeliu, B. Pérez, M.I. Loza, J. Brea, C. Morisseau, B.D. Hammock, S. Vázquez, M.
689 Pallàs, C. Galdeano, Pharmacological Inhibition of Soluble Epoxide Hydrolase as a New Therapy for
690 Alzheimer's Disease, Neurotherapeutics : the journal of the American Society for Experimental
691 NeuroTherapeutics, 17 (2020) 1825-1835.
692 [21] C.P. Sun, X.Y. Zhang, J.J. Zhou, X.K. Huo, Z.L. Yu, C. Morisseau, B.D. Hammock, X.C. Ma, Inhibition of
693 sEH via stabilizing the level of EETs alleviated Alzheimer's disease through GSK3β signaling pathway,
694 Food and chemical toxicology : an international journal published for the British Industrial Biological
695 Research Association, 156 (2021) 112516.
696 [22] Y. Tajima, M. Ishikawa, K. Maekawa, M. Murayama, Y. Senoo, T. Nishimaki-Mogami, H. Nakanishi, K.
697 Ikeda, M. Arita, R. Taguchi, A. Okuno, R. Mikawa, S. Niida, O. Takikawa, Y. Saito, Lipidomic analysis of
698 brain tissues and plasma in a mouse model expressing mutated human amyloid precursor protein/tau
699 for Alzheimer's disease, Lipids in Health and Disease, 12 (2013) 68.
700 [23] Y. Yao, C.M. Clark, J.Q. Trojanowski, V.M.-Y. Lee, D. Praticò, Elevation of 12/15 lipoxygenase
701 products in AD and mild cognitive impairment, Annals of Neurology, 58 (2005) 623-626.
702 [24] M. Zhu, X. Wang, E. Hjorth, R.A. Colas, L. Schroeder, A.C. Granholm, C.N. Serhan, M. Schultzberg,
703 Pro-Resolving Lipid Mediators Improve Neuronal Survival and Increase Aβ42 Phagocytosis, Mol
704 Neurobiol, 53 (2016) 2733-2749.
705 [25] T. Wang, Y. Han, H. Li, Y. Wang, T. Xue, X. Chen, W. Chen, Y. Fan, X. Qiu, J. Gong, Y. Xu, J. Wang, W.
706 Li, T. Zhu, Changes in bioactive lipid mediators in response to short-term exposure to ambient air
707 particulate matter: A targeted lipidomic analysis of oxylipin signaling pathways, Environment
708 International, 147 (2021) 106314.
709 [26] S. Gouveia-Figueira, M. Karimpour, J.A. Bosson, A. Blomberg, J. Unosson, M. Sehlstedt, J. Pourazar, T.
710 Sandström, A.F. Behndig, M.L. Nording, Mass spectrometry profiling reveals altered plasma levels of
711 monohydroxy fatty acids and related lipids in healthy humans after controlled exposure to biodiesel
712 exhaust, Analytica Chimica Acta, 1018 (2018) 62-69.
713 [27] C.N. Serhan, N. Chiang, T.E. Van Dyke, Resolving inflammation: dual anti-inflammatory and pro-
714 resolution lipid mediators, Nature Reviews Immunology, 8 (2008) 349-361.
715 [28] W.J. Lukiw, J.-G. Cui, V.L. Marcheselli, M. Bodker, A. Botkjaer, K. Gotlinger, C.N. Serhan, N.G. Bazan,
716 A role for docosahexaenoic acid-derived neuroprotectin D1 in neural cell survival and Alzheimer disease,
717 J Clin Invest, 115 (2005) 2774-2783.
718 [29] Y. Wang, F. Li, H. Zhuang, L. Li, X. Chen, J. Zhang, Effects of plant polyphenols and alpha-tocopherol
719 on lipid oxidation, microbiological characteristics, and biogenic amines formation in dry-cured bacons, J
720 Food Sci, 80 (2015) C547-555.
721 [30] Y. Zhao, F. Calon, C. Julien, J.W. Winkler, N.A. Petasis, W.J. Lukiw, N.G. Bazan, Docosahexaenoic
722 Acid-Derived Neuroprotectin D1 Induces Neuronal Survival via Secretase- and PPARγ-Mediated
723 Mechanisms in Alzheimer's Disease Models, PLOS ONE, 6 (2011) e15816.
724 [31] A. Ghosh, M.M. Comerota, D. Wan, F. Chen, N.E. Propson, S.H. Hwang, B.D. Hammock, H. Zheng, An
725 epoxide hydrolase inhibitor reduces neuroinflammation in a mouse model of Alzheimer's disease,
726 Science translational medicine, 12 (2020).
727 [32] W. Chen, M. Wang, M. Zhu, W. Xiong, X. Qin, X. Zhu, 14,15-Epoxyeicosatrienoic Acid Alleviates
728 Pathology in a Mouse Model of Alzheimer's Disease, The Journal of Neuroscience, 40 (2020) 8188-8203.
729 [33] A.Y. Taha, M. Hennebelle, J. Yang, D. Zamora, S.I. Rapoport, B.D. Hammock, C.E. Ramsden,
730 Regulation of rat plasma and cerebral cortex oxylipin concentrations with increasing levels of dietary
731 linoleic acid, Prostaglandins Leukot Essent Fatty Acids, 138 (2018) 71-80.

732   [34] Y. Otoki, A.H. Metherel, T. Pedersen, J. Yang, B.D. Hammock, R.P. Bazinet, J.W. Newman, A.Y. Taha,
733   Acute Hypercapnia/Ischemia Alters the Esterification of Arachidonic Acid and Docosahexaenoic Acid
734   Epoxide Metabolites in Rat Brain Neutral Lipids, Lipids, 55 (2020) 7-22.
735   [35] J.S.B. Shaik, M. Ahmad, W. Li, M.E. Rose, L.M. Foley, T.K. Hitchens, S.H. Graham, S.H. Hwang, B.D.
736   Hammock, S.M. Poloyac, Soluble epoxide hydrolase inhibitor trans-4-[4-(3-adamantan-1-yl-ureido)-
737   cyclohexyloxy]-benzoic acid is neuroprotective in rat model of ischemic stroke, Am J Physiol Heart Circ
738   Physiol, 305 (2013) H1605-H1613.
739   [36] S.K. Orr, S. Palumbo, F. Bosetti, H.T. Mount, J.X. Kang, C.E. Greenwood, D.W.L. Ma, C.N. Serhan, R.P.
740   Bazinet, Unesterified docosahexaenoic acid is protective in neuroinflammation, Journal of
741   Neurochemistry, 127 (2013) 378-393.
742   [37] H. Obinata, T. Hattori, S. Nakane, K. Tatei, T. Izumi, Identification of 9-Hydroxyoctadecadienoic Acid
743   and Other Oxidized Free Fatty Acids as Ligands of the G Protein-coupled Receptor G2A *, J Biol Chem,
744   280 (2005) 40676-40683.
745   [38] J.L. Lahvic, M. Ammerman, P. Li, M.C. Blair, E.R. Stillman, E.M. Fast, A.L. Robertson, C. Christodoulou,
746   J.R. Perlin, S. Yang, N. Chiang, P.C. Norris, M.L. Daily, S.E. Redfield, I.T. Chan, M. Chatrizeh, M.E. Chase, O.
747   Weis, Y. Zhou, C.N. Serhan, L.I. Zon, Specific oxylipins enhance vertebrate hematopoiesis via the
748   receptor GPR132, Proceedings of the National Academy of Sciences, 115 (2018) 9252-9257.
749   [39] K. Andersen, L.J. Launer, M.E. Dewey, L. Letenneur, A. Ott, J.R. Copeland, J.F. Dartigues, P. Kragh-
750   Sorensen, M. Baldereschi, C. Brayne, A. Lobo, J.M. Martinez-Lage, T. Stijnen, A. Hofman, Gender
751   differences in the incidence of AD and vascular dementia: The EURODEM Studies. EURODEM Incidence
752   Research Group, Neurology, 53 (1999) 1992-1997.
753   [40] R.M. Cohen, K. Rezai-Zadeh, T.M. Weitz, A. Rentsendorj, D. Gate, I. Spivak, Y. Bholat, V. Vasilevko,
754   C.G. Glabe, J.J. Breunig, P. Rakic, H. Davtyan, M.G. Agadjanyan, V. Kepe, J.R. Barrio, S. Bannykh, C.A.
755   Szekely, R.N. Pechnick, T. Town, A Transgenic Alzheimer Rat with Plaques, Tau Pathology, Behavioral
756   Impairment, Oligomeric Aβ, and Frank Neuronal Loss, The Journal of Neuroscience, 33 (2013) 6245-6256.
757   [41] R.M. Cohen, K. Rezai-Zadeh, T.M. Weitz, A. Rentsendorj, D. Gate, I. Spivak, Y. Bholat, V. Vasilevko,
758   C.G. Glabe, J.J. Breunig, P. Rakic, H. Davtyan, M.G. Agadjanyan, V. Kepe, J.R. Barrio, S. Bannykh, C.A.
759   Szekely, R.N. Pechnick, T. Town, A transgenic Alzheimer rat with plaques, tau pathology, behavioral
760   impairment, oligomeric aβ, and frank neuronal loss, J Neurosci, 33 (2013) 6245-6256.
761   [42] K.T. Patten, A.E. Valenzuela, C. Wallis, E.L. Berg, J.L. Silverman, K.J. Bein, A.S. Wexler, P.J. Lein, The
762   Effects of Chronic Exposure to Ambient Traffic-Related Air Pollution on Alzheimer’s Disease
763   Phenotypes in Wildtype and Genetically Predisposed Male and Female Rats, Environmental health
764   perspectives, 129 (2021) 057005.
765   [43] S. Edwards, G. Zhao, J. Tran, K.T. Patten, A. Valenzuela, C. Wallis, K.J. Bein, A.S. Wexler, P.J. Lein, X.
766   Rao, Pathological Cardiopulmonary Evaluation of Rats Chronically Exposed to Traffic-Related Air
767   Pollution, Environmental health perspectives, 128 (2020) 127003.
768   [44] E.L. Berg, L.R. Pedersen, M.C. Pride, S.P. Petkova, K.T. Patten, A.E. Valenzuela, C. Wallis, K.J. Bein, A.
769   Wexler, P.J. Lein, J.L. Silverman, Developmental exposure to near roadway pollution produces behavioral
770   phenotypes relevant to neurodevelopmental disorders in juvenile rats, Translational Psychiatry, 10
771   (2020) 289.
772   [45] K.T. Patten, E.A. González, A. Valenzuela, E. Berg, C. Wallis, J.R. Garbow, J.L. Silverman, K.J. Bein, A.S.
773   Wexler, P.J. Lein, Effects of early life exposure to traffic-related air pollution on brain development in
774   juvenile Sprague-Dawley rats, Translational Psychiatry, 10 (2020) 166.
775   [46] J. Folch, M. Lees, G.S. Stanley, A simple method for the isolation and purification of total lipides
776   from animal tissues, J Biol Chem, 226 (1957) 497-509.
777   [47] Y. Otoki, S. Kato, K. Nakagawa, D.J. Harvey, L.-W. Jin, B.N. Dugger, A.Y. Taha, Lipidomic Analysis of
778   Postmortem Prefrontal Cortex Phospholipids Reveals Changes in Choline Plasmalogen Containing

779   Docosahexaenoic Acid and Stearic Acid Between Cases With and Without Alzheimer's Disease,
780   NeuroMolecular Medicine, 23 (2021) 161-175.
781   [48] J.E. Rice, R.C. Vannucci, J.B. Brierley, The influence of immaturity on hypoxic-ischemic brain damage
782   in the rat, Annals of Neurology, 9 (1981) 131-141.
783   [49] Q. Shen, Z. Zhang, S. Emami, J. Chen, J.M. Leite Nobrega de Moura Bell, A.Y. Taha, Triacylglycerols
784   are preferentially oxidized over free fatty acids in heated soybean oil, npj Science of Food, 5 (2021) 7.
785   [50] X. Wang, M. Zhu, E. Hjorth, V. Cortés-Toro, H. Eyjolfsdottir, C. Graff, I. Nennesmo, J. Palmblad, M.
786   Eriksdotter, K. Sambamurti, J.M. Fitzgerald, C.N. Serhan, A.C. Granholm, M. Schultzberg, Resolution of
787   inflammation is altered in Alzheimer's disease, Alzheimer's & dementia : the journal of the Alzheimer's
788   Association, 11 (2015) 40-50.e41-42.
789   [51] P. Chaitidis, T. Schewe, M. Sutherland, H. Kühn, S. Nigam, 15-Lipoxygenation of phospholipids may
790   precede the sn-2 cleavage by phospholipases A2: reaction specificities of secretory and cytosolic
791   phospholipases A2 towards native and 15-lipoxygenated arachidonoyl phospholipids, FEBS Letters, 434
792   (1998) 437-441.
793   [52] E.L. Klett, S. Chen, A. Yechoor, F.B. Lih, R.A. Coleman, Long-chain acyl-CoA synthetase isoforms
794   differ in preferences for eicosanoid species and long-chain fatty acids, J Lipid Res, 58 (2017) 884-894.
795   [53] R.M. Bradley, R.E. Duncan, The lysophosphatidic acid acyltransferases (acylglycerophosphate
796   acyltransferases) family: one reaction, five enzymes, many roles, Current opinion in lipidology, 29 (2018)
797   110-115.
798   [54] G. Esposito, G. Giovacchini, J.S. Liow, A.K. Bhattacharjee, D. Greenstein, M. Schapiro, M. Hallett, P.
799   Herscovitch, W.C. Eckelman, R.E. Carson, S.I. Rapoport, Imaging neuroinflammation in Alzheimer's
800   disease with radiolabeled arachidonic acid and PET, J Nucl Med, 49 (2008) 1414-1421.
801   [55] D. Stefanovski, P.-a.B. Shih, B.D. Hammock, R.M. Watanabe, J.H. Youn, Assessment of soluble
802   epoxide hydrolase activity in vivo: A metabolomic approach, Prostaglandins & Other Lipid Mediators,
803   148 (2020) 106410.
804   [56] M. Hennebelle, A.H. Metherel, A.P. Kitson, Y. Otoki, J. Yang, K.S.S. Lee, B.D. Hammock, R.P. Bazinet,
805   A.Y. Taha, Brain oxylipin concentrations following hypercapnia/ischemia: effects of brain dissection and
806   dissection time, J Lipid Res, 60 (2019) 671-682.
807   [57] A. Ott, M.M.B. Breteler, F.v. Harskamp, T. Stijnen, A. Hofman, Incidence and Risk of Dementia: The
808   Rotterdam study, American Journal of Epidemiology, 147 (1998) 574-580.
809   [58] H. Kim, J. Noh, Y. Noh, S.S. Oh, S.-B. Koh, C. Kim, Gender Difference in the Effects of Outdoor Air
810   Pollution on Cognitive Function Among Elderly in Korea, Frontiers in Public Health, 7 (2019).
811   [59] E.J. Murphy, Brain fixation for analysis of brain lipid-mediators of signal transduction and brain
812   eicosanoids requires head-focused microwave irradiation: an historical perspective, Prostaglandins
813   Other Lipid Mediat, 91 (2010) 63-67.
814   [60] K.T. Patten, E.A. Gonzalez, A. Valenzuela, E. Berg, C. Wallis, J.R. Garbow, J.L. Silverman, K.J. Bein, A.S.
815   Wexler, P.J. Lein, Effects of early life exposure to traffic-related air pollution on brain development in
816   juvenile Sprague-Dawley rats, Transl Psychiatry, 10 (2020) 166.
817   [61] P. Leffler, L. Gerhardsson, D. Brune, G.F. Nordberg, Lung retention of antimony and arsenic in
818   hamsters after the intratracheal instillation of industrial dust, Scand J Work Environ Health, 10 (1984)
819   245-251.
820   [62] E.V. Parkhomchuk, D.G. Gulevich, A.I. Taratayko, A.M. Baklanov, A.V. Selivanova, T.A. Trubitsyna, I.V.
821   Voronova, P.N. Kalinkin, A.G. Okunev, S.A. Rastigeev, V.A. Reznikov, V.S. Semeykina, K.A. Sashkina, V.V.
822   Parkhomchuk, Ultrasensitive detection of inhaled organic aerosol particles by accelerator mass
823   spectrometry, Chemosphere, 159 (2016) 80-88.
824   [63] E.V. Parkhomchuk, E.A. Prokopyeva, D.G. Gulevich, A.I. Taratayko, A.M. Baklanov, P.N. Kalinkin, S.A.
825   Rastigeev, D.V. Kuleshov, K.A. Sashkina, V.V. Parkhomchuk, Ultrafine organic aerosol particles inhaled by

826   mice at low doses remain in lungs more than half a year, J Labelled Comp Radiopharm, 62 (2019) 785-
827   793.
828