SUPPLEMENTAL MATERIAL

DETAILED METHODS

Animal

Male apolipoprotein E-null mice ($ApoE^{-/-}$) on a C57BL/6J background (Stock number: 002052) were purchased from Jackson Laboratory (Bar Harbor, ME), fed a standard mouse chow and housed in a controlled environment with a 12 hour light/dark cycle in an animal facility at St. Paul's Hospital, Vancouver, BC. Beginning at 8-12 weeks of age, the mice were switched to a Western-type diet (WD) containing 21% fat from lard and 0.15% cholesterol (TD88137, Harlan Laboratories, Madison, WI, USA) for 8 weeks to induce atheroma formation in the brachiocephalic trunk (BCT). This study was approved by the Animal Care Committee of the University of British Columbia (A06-1494; A10-0257).

Plaque Destabilisation Model

Anesthesia and Endotracheal Exposure of LPS with a Microsprayer

After completion of 8 weeks WD, mice (mean weight {± standard deviation}: 32.2 ± 2.5 g) were randomly divided into 3 groups: 1) LPS (N=28), 2) saline (N=20) or 3) WD control group (N=6). Other additional mice used in subsequent experiments are documented in the specific sections of the manuscript (Figure 1 and Supplemental Figure S1). Mice were anesthetized with inhalation of 2.0% isoflurane combined with a single intraperitoneal injection of ketamine (30 mg/kg) and xylazine (3 mg/kg). In the LPS group (N=28), a single dose of 100 µg LPS (3mg/kg) (L2630, Sigma-Aldrich, St. Louis, MO), dissolved in 50 µl of sterile saline, was sprayed in its aerosol form (16-22µm in mass median diameter) directly into the mouse lungs through the vocal cords using a microsprayer device (MSA-250-M, Penn-Century Inc., Wyndmoor, PA)[1]. In the saline

group (N=20), a single administration of 50 µl sterile saline was sprayed directly into the lungs in the same manner. To prevent dehydration, the mice were given a single administration of 1.0 ml of warm sterile saline subcutaneously in the back after the procedure and were allowed to recover by resting on a heating pad.

LPS Dosage and Timeline Determination

In order to optimize the atheroma destabilisation model, three different LPS doses and three different time-points were investigated. Mice instilled with intratracheal LPS (25µg, 50 µg, 100 µg) were sacrificed after 4 hours, 24 hours and 48 hours (N=4 each). Saline-instilled mice that were sacrificed at the same time-points were used as controls. (Supplemental Figure S1a) Experiment with LPS (100 µg) at 48 hours time-point failed due to sudden deaths and occurrence of paralysis in mice. Atheromas were evaluated based on histological evidence described.[2] Fisher exact test were used to compare plaque destabilisation rate in the right brachiocephalic trunk (BCT) between LPS and saline group. Fourteen mice were used in the saline control group.

Organ and Tissue Procurement and Bronchoalveolar Lavage (BAL)

Under anesthesia (isoflurane 5% inhalation), blood was collected from the inferior vena cava using a 25 gauge catheter needle (Protectiv® Plus, Medex®, Lancashire, UK) 24 hours after LPS challenge for cytokine measurements in serum. Immediate procurement of the heart and blood vessels was performed after blood collection. The heart, aortic arch and branching arteries containing the BCT were carefully harvested and perfused with 3ml of phosphate buffered saline (PBS) injected directly into the left ventricle at a consistent infusion rate of 1ml/minute (Harvard Apparatus, Massachusetts, USA). Bronchoalveolar lavage (BAL) fluid was collected by washing

the right lung once with 600 µl of PBS. The left lung was harvested and perfused with 400 µl of 10% formalin at a pressure of 25 cm H_2O.

Optical Projection Tomography (OPT)

Optical projection tomography (OPT; Bioptonics 3001M, MRC Technology, London, United Kingdom) was used to image the aortic tree. To enable imaging, the aortic arch containing branching arteries was carefully removed from the surrounding fat tissues. The samples were then immediately fixed in 10% formalin for 48 hours, after which, the samples were rinsed in PBS 3 times (30 minutes for each wash) and then embedded in 1% low melting point agarose. Next, the solidified agarose blocks were immersed in 100% methanol for 48 hours, which was changed five times during the procedure to ensure complete removal of water from the blocks. After dehydration, the samples were stored in Benzyl Alcohol Benzyl Benzoate (BABB) solution for 72 hours. The samples were then imaged using an OPT scanner. UV light in the OPT scanner (metal-halide 120W UV source) was filtered to enable an excitation wavelength of 425 nm/40 nm with an emission wavelength of LP475 nm. The projection images were acquired by a charge-coupled device (CCD) camera every 0.9 degrees while the samples were rotated. In total, 400 projection images with a pixel size of 6.4 µm were captured per sample. The projection images were then reconstructed using the SkyScan NRecon software (Bruker microCT, Kontich, Belgium), resulting in a three-dimensional (3D) picture with an isotropic voxel size of 6.4 µm in all directions. All volume rendering and quantification was performed using the Amira visualization software (Amira V6.0, FEI Visualization Sciences Group, Burlington, MA, USA). Blood clots and hemorrhage in samples were detected via autofluorescence of red blood cells

that occur at wavelength of 425 nm and were segmented and quantified three dimensionally (in volume) using Amira V6.0 visualization software.

Serial Cross-Sectioning of BCT: Frozen Sections

BCT was carefully removed from the surrounding fat tissues then embedded with optimum cutting temperature compound (OCT) (Sakura Finetek, Torrance, CA) on dry ice, followed by cryopreservation in liquid nitrogen and storage at -80°C. Using a cryostat (Leica, UK), serial 5 µm sections of vessels perpendicular to the long axis were obtained at approximately 50 µm intervals starting from the arch (proximal end) to the site of the BCT bifurcation. For each specimen, light microscopy was performed to confirm the quality of the cross-sections. Serial cross-sections (approximately 18 cross-sections per mouse) were stained with hematoxylin and eosin (H&E) and Movat's pentachrome for morphological analysis.

Serial Cross-Sectioning of BCT: Formalin Perfusion and Paraffin Embedment

In order to further preserve the histological features of plaque rupture and validate the thrombus and intraplaque hemorrhage observed in this model, we also performed immediate formalin perfusion. (Figure 1a) LPS was microsprayed as described previously[1] into the lungs of 9 mice, and saline was microsprayed into 3 mice as saline controls After 24 hours, the aortic tree was harvested and immediately perfused with 3.0 ml of 10% formalin at constant perfusion rate (1ml per minute) following complete blood withdrawal. After 48 hour of formalin fixation, the aortic tree was paraffin embedded and continuous sections were obtained for H&E and Movat's pentachrome staining.

Morphometric Analyses of Plaque Parameters

To phenotype the plaques in detail, we chose the cross-section of the frozen BCT that contained the largest plaque area and performed morphometric analysis. BCTs with formalin fixation may not be adequate for this purpose due to possible vessel shrinkage. All measurements were performed on H&E stained images using Image Pro software (Image-Pro Plus, Media Cybernetics) at 200X magnification. From these images, plaque size was determined by ascertaining the area enclosed by the internal elastic lamina (IEL) minus the area enclosed by the endothelial layer (lumen area). The necrotic core was defined as the component of the plaque that did not contain any matrix material (either collagen or elastin) or cells. This area was quantified by using color segmentation (illustrated in Supplemental Figure S1b). The percent (%) necrotic core was calculated by dividing the necrotic core area by the total plaque size. The cap-to-plaque thickness ratio was determined by first generating linear rays from the midpoint of IEL of the plaque and then measuring the distance from the endothelium to necrotic core and dividing this value by the distance from endothelium to the origin of the rays (illustrated in Supplemental Figure S1c).

Immunofluorescent Staining and Signal Quantitation

***Myeloperoxidase (Peripheral Neutrophil Marker) and CD68 Co-staining*:**

The frozen mouse BCT cross-sections were fixed in 10% formalin for 10 minutes and then rinsed in running water for two minutes. The sections were incubated with 10% chicken serum (Gene Tex Inc., USA) and donkey serum (Sigma-Aldrich, USA) for 30 minutes and then incubated with the mouse MPO affinity purified polyclonal antibody goat IgG (5µg/ml; R&D, USA) and the mouse macrophage/smooth muscle CD68 monoclonal antibody rabbit IgG

(2.5µg/ml; Abcam, Toronto, ON, Canada) for 2 hours at 4^{o}C. After PBS washes (4X of PBS incubation for 5 minutes each), the BCT sections were incubated with the secondary antibodies: donkey anti-goat 488 (400X dilution, Invitrogen, USA) and chicken anti-rabbit 594 (400 X dilutions; Invitrogen, USA) for another 2 hours at room temperature. After washes (4X of PBS incubation for 5 minutes each), the sections were air dried for 30 minutes and mounted using the Vectashield mounting medium with DAPI (Vector Lab Inc, USA). Immunofluorescence images of the 3 serial sections containing the largest plaque areas were captured under a fixed computerized setting with Nikon immunofluorescence light microscope Eclipse TE300 (Nikon, Japan) at 100X, 200X and 600X magnification. The MPO signal in given plaques were quantified by color segmentation in pixels. The value of %MPO was obtained by the MPO signal divided by the plaque size using Image-Pro Plus software. The values for the 3 serial cross sections with the largest plaque size were averaged to represent the center plaque region and values for the serial sections with the smallest 3 plaque size were averaged to represent the shoulder region. The arithmetic mean was used for statistical analysis. Normal rabbit and goat IgG were used for negative controls. The frozen lungs of LPS treated mice were used for positive controls.

Thrombin and Endothelial Co-staining

The same procedure was followed as described above, except sections were fixed in methanol for 10 minutes instead of 10% formalin. The anti-mouse CD31 monoclonal antibody rat IgG (5ug/ml; Cedarlane, ON, Canada) and the anti-mouse thrombin polyclonal antibody rabbit IgG (2.5mg/ml; Abcam, Toronto, ON, Canada) were used as primary antibodies. Donkey anti-rat 488 (400X dilution; Invitrogen, USA) and Chicken anti-rabbit 594 (400X dilution; Invitrogen, USA)

were used as the secondary antibodies. Normal rabbit and rat IgG were used as the primary antibodies for negative controls.

MMP9 Staining

The standard staining procedure was followed according to manufactures' recommendation with primary antibody: goat anti-MMP9 polyclonal IgG (sc6840; Santa Cruz Biotechnology, Dallas, Texas USA); and secondary antibody: donkey anti-goat 488 (400X dilution, Invitrogen, USA)

Investigation of the Intratracheal (IT) LPS versus Intraperitoneal (IP) LPS in causing Atheroma Destabilisation at 8 Hours

In order to investigate the early stages of atheroma destablisation, the eight hour time point was chosen. 40 mice were randomly divided into 4 groups, LPS-IT (N=16), Saline-IT (N=14), LPS-IP (N=5) and Saline-IP (N=5). The same dosage (100µg) of LPS or sterile saline was sprayed into the mouse lungs of IT groups using a microspray technique described previously[1]; the same dosage of LPS or sterile saline was injected into mouse intraperitoneal space of the IP groups using a 28G needle. The same anesthetic procedures were followed for the IT and IP groups. 8 hours post-instillation, the BCTs, blood, and organs were collected.

Neutrophil Depletion by Anti-Ly6G Clone 1A8 Antibody against Neutrophil

After 8 weeks of high fat diet, 7 mice were injected with rat anti-mouse Ly6G clone 1A8 antibodies (Bio X cell, New Hampshire, USA) and another 7 mice were injected with rat IgG2A isotype clone 2A3 antibodies (Bio X cell, New Hampshire, USA) twice, first at 24 hours prior to LPS lung challenge and a second dose immediately following LPS challenge (each time 400µg

diluted in 400µl sterile PBS). 24 hours following LPS lung challenge, aortic and lung tissues were collected as described for OPT scanning and immunofluorescence staining. Neutrophil depletion was verified by performing cytospin of cells collected from BAL (Supplemental Figure 2c and d). White blood cell counts were determined by hemocytometry of blood cells. (Supplemental Table S2).

Investigation of Intraperitoneal Injections of Antibodies on Plaque Phenotype

In order to investigate the reproducibility of this model and investigate the effect of antibody injection on plaque phenotype, the same neutrophil depletion experiment was repeated as described above with additional antibody control groups added: 1) additional LPS-ND (N=8), 2) additional LPS-Ctrl (N=7), 3) Ctrl group (N=4), and 4) ND group (N=5). The Ctrl group (N=4) included three saline-exposed mice and one WD mouse that were sacrificed 24 hours after injections of rat IgG2A isotype clone 2A3 antibodies (Bio X cell, New Hampshire, USA); ND group (N=5) were five WD mice sacrificed 24 hours after injections of rat anti-mouse Ly6G clone 1A8 antibodies (Bio X cell, New Hampshire, USA). BCT of these mice were analyzed with OPT. These data were pooled together with the neutrophil depletion experiment for plaque characterization (Figure 1b). An additional PBS control group for LPS exposed mice (LPS-PBS; N=7) was also included.

Evaluation of Pulmonary Inflammation

Histology of the Left Lung

To evaluate the histological changes of the lungs, mouse left lungs were harvested and inflated at a pressure of 25 cmH_2O using 400 µl 10% phosphate-buffered formalin. Left lungs were fixed in

10% phosphate-buffered formalin over 24 hours and embedded in paraffin. Paraffin-embedded left lungs were coronally sectioned in 4 μm slice thickness and were stained with H&E. The H&E slides were scanned with Aperio ScanScope XT (Aperio Technology Inc, USA) at 400X.

Cell Counts in Bronchoalveolar Lavage (BAL)

Cells in the BAL were gently re-suspended with PBS and cytospun down to a glass slide and fixed with methanol before staining with H&E and Giemsa-Wright stain. PMN (neutrophil) counts were obtained by dividing the cell pellet into 4 equal quarters and then taking 4 random fields from each quarter using Image Pro software at 400X magnification. PMNs were morphologically identified by evaluating size, shape, and presence of granules and multinucleation. Monocytes and alveolar macrophages were also determined by morphology. The arithmetic mean was used for statistical analysis.

Evaluation of Systemic Inflammation

Serum samples were used to measure monocyte chemotactic protein-1 (MCP-1), keratinocyte chemoattractant (KC), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α) using Luminex MagPix (EMD Millipore, MA) and mouse multiplex assay kit according to the manufacturer's recommendation.

Extrapulmonary Organs Morphological Examination

The liver and kidneys of animals were also harvested and their H&E sections were examined under light microscope at 400X. There was no inflammatory cell infiltration in these organs and

there was no evidence of vascular structural change or disturbance of morphological features with intratracheal LPS or saline exposure.

Myeloperoxidase Enzymatic Activity Assay

MPO activity in BAL fluid (diluted 25 times in PBS) was measured using an MPO colorimetric activity kit (Sigma-Aldrich, St. Louis, MO) following manufacturer's protocols. One unit of MPO activity was defined as the amount of enzyme that hydrolyses taurine and generates taurine chloramines which in the process consumes 1.0 micromole of 5-thio-2-nitrobenzoic acid (TNB) per minute at 25 °C to yield 5-5-dithiobis(2-nitrobenzoic acid) (DTNB). Samples were loaded in duplicate (one as a blank and one as a test sample) and assayed at 60 minutes. A zero value was assigned when there was no detectible difference.

Statistical Analysis

All results were expressed as mean value ± standard deviation unless otherwise indicated. Data were analyzed using Fisher's exact test (for comparison of rupture incidence between two independent groups), t-tests (for comparison of two independent groups that were normally distributed), and Mann Whitney U tests (for comparison of two independent groups that were not normally distributed). Kruskal Wallis test was used to compare more than two independent groups when distributions of the variables are not normal. All analyses were conducted using Prism 5 and p-values less than 0.05 (two-tailed) were considered statistically significant.

EXPANDED RESULTS

LPS dosage and timeline determination

Mice exposed to intratracheal LPS at dosage of 50 µg and 100 µg had superimposed high rates of atheroma destabilisation at 4 hours and 24 hours, by 24 hours they were significantly different from that of saline exposed mice. The 24 hour time point was therefore chosen as the primary timepoint for investigation. (Supplemental Figure S1a)

Male ApoE$^{-/-}$ mice fed with an 8 weeks high fat diet successfully developed stable plaque in the brachiocephalic trunk (BCT)

Atheromatous plaque that developed in mouse BCT after an 8 week high fat diet contained basic features that were similar to human atheroma, including intimal thickening, lipid/necrotic core formation and presence of a cap (Supplemental Figure S1b). The plaque morphology data are shown in Supplemental Table S1.

Supplemental Table S1. Morphological quantification of atheromatous plaques developed in the BCT of ApoE$^{-/-}$ mice fed with an 8 week high fat diet. The cross-section of BCT containing maximal plaque size was stained by H&E and quantified by Image-Pro Plus. The data are shown as mean ± standard deviation.

2D Cross-sectional Parameters	**ApoE$^{-/-}$ Baseline** **N=6**
Cap-plaque thickness ratio (%)	37 ± 21
Necrotic core / Plaque size (%)	22.7 ± 10.2
Plaque size × 10^3 (μm^2)	42.3 ± 24.9

Neutrophilic lung inflammation and acute lung injury induced by LPS intratracheal exposure

LPS intratracheal exposure induced acute lung injury characterized by increased numbers of neutrophils, disruption of alveolar epithelial cells, blood cell congestion, and edema in lungs. Animals exposed to saline instillation demonstrated none of these histological features (Supplemental Figure S2 a-b).

Systemic inflammation induced by intratracheal LPS exposure

All pro-inflammatory cytokines tested showed an increasing trend 24 hours after LPS challenge compared to saline exposed controls. Inflammatory cytokine concentrations of KC and MCP-1 in mouse plasma 24 hours after LPS intratracheal administration were significantly higher than those in saline exposed animals (Supplemental Figure S3).

Thrombus and intraplaque hemorrhage detected by OPT in LPS-exposed mice

Three dimensional OPT enabled direct visualization of intraplaque hemorrhage because red blood cells auto-fluorescence nearly 10 times more intensely than components of vessel wall. Blood clot was present in the shoulders of the BCT plaque in LPS-exposed but not saline-exposed mice (N= 2 out of 3 LPS-exposed mice versus 0 out of 3 saline-exposed mice; see Video S1 and S2 for 3-D presentation). In some plaques, hemorrhage was accompanied by thrombosis, which extended from the shoulder region of the BCT plaque into the aortic arch (multiple blood clots presented at proximal end of BCT and along the aortic arch in Video S3). Intraplaque hemorrhage without luminal thrombi was also detected by OPT imaging in LPS treated mouse (N=1 out of 3 LPS treated mice) (Video S4). Plaque destabilisation phenomena was not only limited to atheroma in BCT, atheroma along the arch, even plaques in abdominal aorta, could be destabilised. However, due to inconsistent plaque formation at those branches, they are not suitable for rate quantitation and analysis.

Intra-tracheal LPS exposure induces plaque de-stabilisation

Quantitation of vulnerable plaque by frozen sections

Histological quantification using frozen contiguous cross sections revealed 10 out of the 16 (63%) mice exposed to LPS harbored atheromatous plaques that displayed morphological characteristics of plaque de-stabilization. 5 of 16 LPS treated mice (31%) contained luminal blood or thrombus in the shoulder regions of the BCT plaque. In comparison, only 2 out of the 14 (14%) mice exposed to saline demonstrated features of plaque de-stabilization ($p = 0.011$ comparing LPS vs. saline exposed animals).

Quantitation of vulnerable plaque by paraffin embedded sections

Histology of immediate formalin perfusion and paraffin embedded sections revealed consistent findings. 7 out of 9 (78%) LPS treated mice demonstrated plaque destabilisation, and 3 of 9 LPS exposed mice (33%) were associated with thrombus or luminal blood aggregation. None of the 3 (0%) saline treated animals showed any signs of plaque de-stabilization or thrombus formation in the BCT ($P=0.0455$). Please see Supplemental Figure S4 for details.

Plaque phenotype characterization of neutrophil depletion experiment and additional experiment

We have characterized the plaque phenotype of LPS-Ctrl group and LPS-ND group. The results are analyzed altogether (with additional control antibody experiment) and described in the text of result section and Figure 5a. Separately, in the first experiment, there were 5 of 7 (71.4%) LPS-Ctrl mice, which demonstrated plaque destabilization; 3/7 (43%) of LPS-Ctrl mice contained evidence of intraplaque hemorrhage and 3/7 (43%) of LPS-Ctrl mice demonstrated luminal blood clots. In contrast, only 2/7 (29%) mice in LPS-ND group showed plaque destabilisation. 1/7 (14%) mice showed intraplaque hemorrhage and 1/7 (14%) mice showed luminal blood clot.

In the additional experiment, very similar to the result obtained from the first experiment, there were 5/7 (71.4%) LPS-Ctrl mice demonstrated plaque destabilization; 5/7 (71%) of the extra LPS-Ctrl mice contained evidence of intraplaque hemorrhage; and 2/7 (29%) of mice demonstrated luminal blood clots. In contrast, only 2/8 (25%) mice in extra LPS-ND group showed plaque destabilisation. 1/7 (14%) extra LPS-Ctrl mice showed intraplaque hemorrhage and 1/7 (14%) extra LPS-Ctrl mice showed luminal blood clot. There was no vulnerable plaque observed in both ND group (0/5) and Ctrl group (0/4). These data indicated antibody injections did not induce plaque vulnerability in the BCT.

Attenuation of intraplaque MPO$^+$ signal by neutrophil depletion using in vivo anti mouse Ly6G clone 1A8 antibody.

It was found that neutrophils (MPO$^+$) were expressed at the endothelium surfaces, edges and shoulder regions, and necrotic core areas of atheroma in LPS-exposed mice (Supplemental Figure S5e), but not in the saline-exposed mice. In order to experimentally validate the role of neutrophils, we injected mice with antibody clone 1A8 that specifically bind to Ly6G on neutrophils, 1 day-prior to intratracheal LPS exposure and another dose immediately following intratracheal LPS exposure (Figure 1b). Mice treated with anti-Ly6G antibodies demonstrated reduced MPO+ neutrophils and reduced MPO deposition in the plaque (<1%) 24 hours following LPS exposure compared with mice injected with control antibody 2A3 (6%; p= 0.03, Figure 5d). There was reduced vulnerability signal in the BCT of LPS-ND mice compared to LPS-Ctrl mice (Figure 5c). Neutrophilic lung inflammation was also attenuated by administration of anti-Ly6G antibody (Supplemental Figure S2c-d) No plaque vulnerability was observed in the ND and Ctrl mice treated with the antibodies without LPS exposure (Figure 4b, Supplemental Figure S5d).

BAL cell counts and circulating white blood cell counts

The BAL cell counts are shown in Supplemental Figure S6. In the absence of LPS exposure, injections of non-specific control IgG antibodies into mice did not induce neutrophil infiltrations in lungs or change macrophage counts. (Supplemental Figure S6 a and b) Only in the presence of LPS, neutrophilic lung infiltrations were observed (Supplemental Figure S2). However, neutrophil counts in the BAL were significantly reduced by injections of neutrophil-specific antibodies (Supplemental Figure S2c and d). Macrophage counts in the BAL were similar among the antibody control groups with or without exposure to LPS intratracheally (Supplemental Figure S6b). Intraperitoneal administration of LPS (LPS-IP) or saline (Saline-IP) had no significant effect on neutrophil and macrophage counts in the BAL. Only the LPS exposure intratracheally induced an increase in BAL neutrophil but not macrophage counts (Supplemental Figure S6c and d)

The circulating white blood cell counts of the various groups are shown in Supplemental Table S2. Both circulating lymphocytes and monocytes counts in the blood were similar between LPS-ND and LPS-Ctrl group. Administrations of neutrophil-specific antibodies significantly reduced circulating neutrophil counts in the presence of LPS (LPS-ND group: 0.10 ± 0.06 $X10^9$/L vs. LPS-Ctrl group: 0.8 ± 0.5 $X10^9$/L; Mann-Whitney test $P=0.0021$). There was no significant difference in blood lymphocyte and monocyte counts between Ctrl and ND group (in the absence of LPS stimulation), though there was a slight trend towards a reduction in neutrophil count in the ND group (Mann-Whitney test, $P=0.11$.). The white blood cell counts revealed a very similar cell composition in the blood of mice given LPS via the IT route and those given LPS via the IP route (Supplemental Figure S7).

SUPPLEMENTAL TABLE S2. White blood cell counts

(X10^9/L)	WBC	Neutrophils	Lymphocytes	Monocytes
WD (N=3)	7.1 ± 1.4	3.2 ± 1.5	2.9 ± 0.3	0.9 ± 0.2
Saline (N=7)	3.9 ± 2.1	1.0 ± 0.5	2.6 ± 2.4	0.2 ± 0.1
LPS (N=7)	1.8 ± 0.5	1.6 ± 0.9	0.8 ± 1.6	0.8 ± 2.0
LPS-PBS (N=7)	1.9 ± 1.0	1.5 ± 0.9	0.3 ± 0.2	0.03 ± 0.02
Ctrl (N=4)	4.9 ± 4.2	2.4 ± 2.5	2.0 ± 1.6	0.3 ± 0.3
ND (N=4)	3.1 ± 1.4	0.6 ± 0.2	2.2 ± 1.2	0.06 ± 0.03
LPS-Ctrl (N=7)	0.9 ± 0.5[a]	0.8 ± 0.5	0.10 ± 0.03 [abf]	0.01 ± 0.01[abe]
LPS-ND (N=7)	0.27 ± 0.04[abef]	0.10 ± 0.06[acde]	0.06 ± 0.03[abef]	0.06 ± 0.07
P value	<0.0001	=0.0002	<0.0001	<0.0001

The data are shown as mean ± standard deviation. Kruskal-wallis test was used to compare all groups; Dunn's multiple comparison tests were performed to compare between all individual groups. Dunn's test shown a: Significant differences compared to WD group; b: Significantly different from Saline group; c: Significantly different from LPS group; d: Significantly different from LPS-PBS group; e: Significantly different from Ctrl group; f: Significantly different from ND group. WD: 8 weeks western-style diet control group; Saline: intra-tracheal saline exposure group; LPS: intra-tracheal lipopolysaccharide exposure group; PBS: intraperitoneal injections of phosphate buffered saline; Ctrl: intraperitoneal injection with control IgG antibody; ND: intraperitoneal injection with anti-Ly6G antibody for neutrophil depletion

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