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The Effects of a Blueberry-enriched Diet on Particulate Matter-Induced Lung Injury, Oxidative Stress, and Inflammation in an Adult Mouse Model

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The Effects of a Blueberry-enriched Diet on Particulate Matter-Induced Lung Injury, Oxidative Stress, and Inflammation in an Adult Mouse Model

by

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Undergraduate honors thesis under the direction of

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Abstract

Ambient air pollution represents a public health issue for people across the globe. Pollution sources vary, but combustion engines and thermal hazardous waste elimination processes have both been shown to produce particularly harmful ultrafine particulate matter (PM) that acts as a scaffold for the formation of environmentally persistent free radicals (EPFRs). When inhaled, these EPFRs induce oxidative stress, airway hyperresponsiveness, and effect immune cell recruitment to the lungs. Since EPFRs are known to affect redox balance (i.e. induce oxidative stress), dietary antioxidant consumption represents a novel method for combatting PM-induced maladies. Blueberries in particular possess high levels of a class of antioxidant molecules called anthocyanins, granting them an antioxidant capacity beyond that of most other readily available berries. Consequently, they represent an accessible and potent source of dietary antioxidants for study. Using a surrogate EPFR-particle system and a 4% blueberry-enriched diet (BBD) in an adult mouse model, we showed that as little as 1 week of BBD consumption can significantly elevate reduced glutathione (GSH) levels in PM-exposed mice. Further, we showed that 1 week of BBD consumption significantly elevates reduced/oxidized glutathione (GSH/GSSG) ratio in Vehicle-exposed lung tissues and also contributes toward other positive data trends in both exposure groups. Overall, our results suggest blueberries as candidates for use as an easily implementable dietary intervention for mitigating the effects of PM exposure. This study's use of dietary antioxidant consumption as a method of bolstering resistance to PM-induced maladies represents a unique investigation into the interplay between nutrition and lung health.

Introduction

Epidemiological studies show increased prevalence of pulmonary health maladies as well as exacerbated symptoms in areas with increased ambient air pollution [1-3]. While these pollutants arise from a variety of sources, approximately 40-70% of this airborne particulate matter (PM) is the byproduct of combustion engines and thermal processes [4]. As a result, much investigative attention is devoted to characterizing the contents of pollution sources like diesel exhaust fumes but comparatively less attention is paid to particle-associated pollutant by-products of combustion and thermal treatment of hazardous waste. Examination of PM from these less-common sources revealed the presence of a class of molecules termed environmentally persistent free radicals (EPFRs) [5, 6]. Due to the known health effects of free radical exposure (i.e. oxidative stress induction), EPFRs emerged as prime candidates for the role of causative agents in PM-associated adverse health effects.

In the present study, we elected to use a common by-product of waste combustion, known as 2-monochlorophenol (2-MCP), which is associated with a transition metal–containing fly ash. This pollutant-particle system serves as our model PM and is a known source for formation of radicals. Thus, to be clear, we are using the specific EPFR of 2-MCP that is formed by reaction with fly ash containing Cu(II)O at 230°C (referred to as MCP230). Earlier Cormier lab studies confirm *in vitro* MCP230 exposure's ability to induce cellular oxidative stress and cytotoxicity [4] as well as its ability to induce airway hyperresponsiveness and pulmonary inflammation in neonatal rats [7].

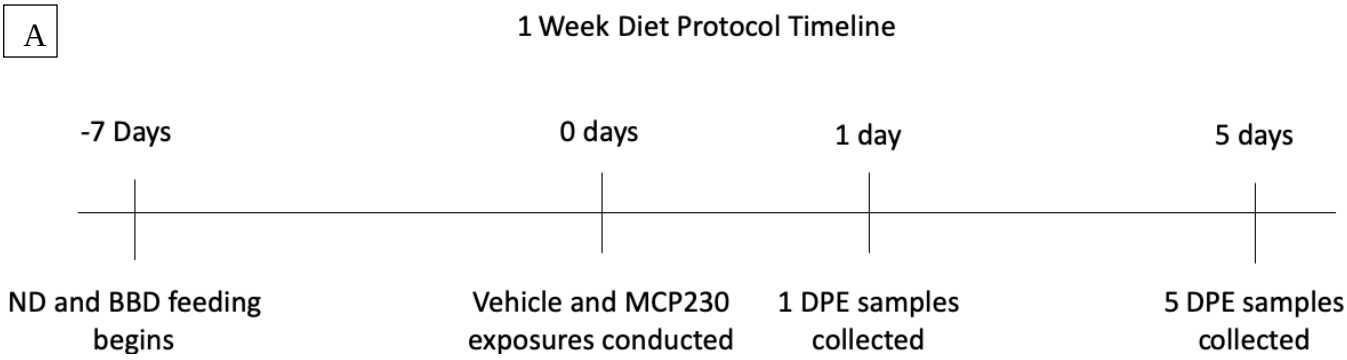
A state of cellular oxidative stress occurs when an organism's natural redox balance is skewed toward an oxidized state [8]. Common sources of these imbalances include perturbations in nutrition, pathology of inflammatory diseases, and exposure to ambient air pollution [9-11]. Regardless of the source though, the key idea here is that the body's redox system is a balance. When natural production of reactive oxygen species (ROS) exceeds the body's stored antioxidant defense, oxidative stress occurs and system-wide health is affected [12].

Given ROS's involvement in various disease states [13-16], increased dietary antioxidant consumption has arisen as an actionable and legitimate method for individuals to improve their body's ability to combat oxidative stress. While antioxidant compounds are common in most fruits, blueberry species' comparatively high concentrations of anthocyanins grants them an impressive capacity for neutralizing ROS [17-20] and minimizing oxidative stress.

Therefore, after considering the reported benefits of blueberries and their consumption, our aim was to determine if a blueberry-enriched diet could prevent PM-induced lung inflammation and injury in a murine model. This study represents the novel application of a well-studied relationship (i.e. how blueberry consumption effects oxidative stress-related conditions) and helps form our understanding of how we can use natural dietary methods to defend against the adverse health effects of PM pollution. We hypothesized that a blueberry-enriched diet would attenuate PM-induced lung injury (total protein level in bronchoalveolar lavage fluid), oxidative stress (ROS production rate and reduced/oxidized glutathione ratio), and inflammation (immune cell recruitment to the lungs) in an adult mouse model.

Materials and Methods

A



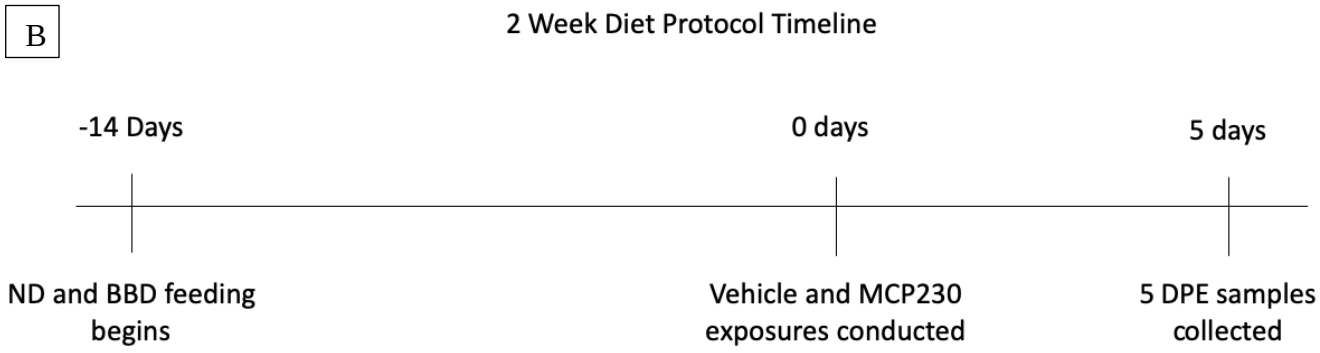


Figure 1: Experimental timeline for both the 1 week (A) and 2 week (B) diet protocols. *We continued to provide the respective diets after exposures and until sample collection.*

Mice
Male C57BL/6 mice were purchased from Jackson Labs (Bar Harbor, ME) and kept in ventilated cages with *ad libitum* access to tap water and feed. Normal diet (ND) mice received standard rodent chow and blueberry-enriched diet (BBD) mice received the blueberry-enriched diet acquired from the Francis lab. All animal protocols were prepared in accordance with the Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee at Louisiana State University.

Particulate matter (PM)
EPFR-containing particles in the form of MCP230 (MCP) were synthesized as previously described by the Cormier lab [4].

Particulate matter (PM) exposure
MCP particles were suspended and sonicated in sterile saline containing 0.02% Tween 80 to a final concentration of 1 mg/ml. Mice were administered particles by oropharyngeal aspiration. Mice were anesthetized (isoflurane (5% induction; 2% maintenance)) and placed in a holder physically supporting the mice in an upright position. The tongue was gently extended to the mouse's left to keep the mouse from swallowing the material using forceps. A 50 µl suspension of particles was pipetted into the trachea while the nose was held closed, thus forcing the mice to breathe through the mouth. The nose and tongue were released after two breaths had been completed.
Mice in the vehicle group received 50 µl of sterile saline containing 0.02% Tween 80 in the same manner as described above.

Blueberry-enriched diet (BBD)
The blueberry-enriched diet used in these experiments was acquired from the Francis lab and was prepared according to the specifications they previously described [20]. In regard to feeding, all mice, except for one cohort, were fed the BBD for 7 days (or 14 days) prior to and throughout the particle exposures. See Figure 1 for an outline of the protocol timeline.

Bronchoalveolar lavage fluid (BALF) protein content quantification
Total protein content was quantified from BALF that had been isolated in 1 ml phosphate-buffered saline (PBS) on days 1 and 5 post exposure. A Pierce™ BCA Protein Assay Kit was used, and all manufacturer instructions were followed.

Electro paramagnetic resonance (EPR) spectroscopy

Total ROS (*n* = 4,5/group) were measured in fresh lung tissue isolated at 1- and 5-days post exposure (DPE) via electron paramagnetic resonance (EPR). Total ROS levels were compared between all four groups. Spin probe 1-Hydroxy-3-mehoxycarbonyl-2,2,5,5-tetramethyl-pyrrolidine (CMH) was used to measure total ROS. Briefly, lung tissue was incubated at 37 °C with CMH (200 μM) for 30 min for ROS measurement, PEG-SOD (50 U/μl) for 30 min, then CMH (200 μM) for an additional 30 min for $O_2^{\bullet-}$ measurement. Aliquots of incubated probe media were then taken in 50-μl disposable glass capillary tubes for determination of $O_2^{\bullet-}$ or total ROS production. All EPR measurements were performed using an EMX ESR eScan BenchTop spectrometer and high-quality factor microwave cavity (Bruker Company, Germany).

Determination of Lung Tissue Homogenate Reduced/Oxidized Glutathione (GSH/GSSG) Ratio

Lungs were immediately isolated, flash frozen in liquid nitrogen and stored at −80°C until use. Lung lysates from vehicle and particle treated lungs were prepared by homogenization in 0.1 M phosphate buffer (pH 7.4) and sonicated on ice for 15–20 seconds. Samples were centrifuged at 12,000 g for 15 min at 4°C; supernatant was then removed and stored in −80°C to preserve for future analysis. Using these stored homogenates, an Arbor Assays Fluorescent Detection Kit for Glutathione was carried out. The supplied protocol was followed exactly and both GSH and GSSG levels were quantified according to the analysis methods described in the protocol. To determine GSH/GSSG ratio, we simply divided each group's mean GSH concentration by their mean GSSG concentration.

BALF Cellularity and Differential Cell Counts

BALF was isolated in 1 ml of PBS containing 2% heat inactivated Fetal Bovine Serum (FBS) at 5 DPE in both the 1- and 2-week diet protocols. The cells were then centrifuged onto slides and stained using a Hema-3 staining kit (Fisher). Next, a total of 400 cells were counted per slide and differential cell counts based on the morphology and staining of the cells was recorded.

Statistical Analysis

All data were plotted as mean ± SEM and analyzed using GraphPad Prism (GraphPad Software Inc., Version 5.0.0). One-way ANOVA was used to evaluate the differences between groups. Tukey's one-way analysis of variance was performed to test for significance between the groups. Differences between means were considered significant when $p < 0.05$.

Results

BBD improves lung health both in the presence and absence of PM exposure

Total protein content in the isolated BALFs of mice from each treatment group was determined at 1 DPE and 5 DPE. This data represents the health of each group's lungs, respectively, and higher or lower levels of protein present are interpreted as more or less severe lung injury. As expected, MCP exposure increased total protein in the BALF - indicative of lung injury. Neither of the BBD-fed groups showed improvements compared to their ND controls at the 1 DPE time point (Figure 2A). However, at 5 DPE, the BBD-fed groups' means were reduced compared to their ND counterparts (Figure 2B). MCP exposure again caused significant and observable increases in total protein content compared to Vehicle exposure at this extended time point. These findings support previous research by the Cormier lab documenting MCP's negative influence on pulmonary health [21]. Additionally, this data suggests that as time spent on the BBD increases, so may the BBD's ability to provide protection against PM-induced lung injury.

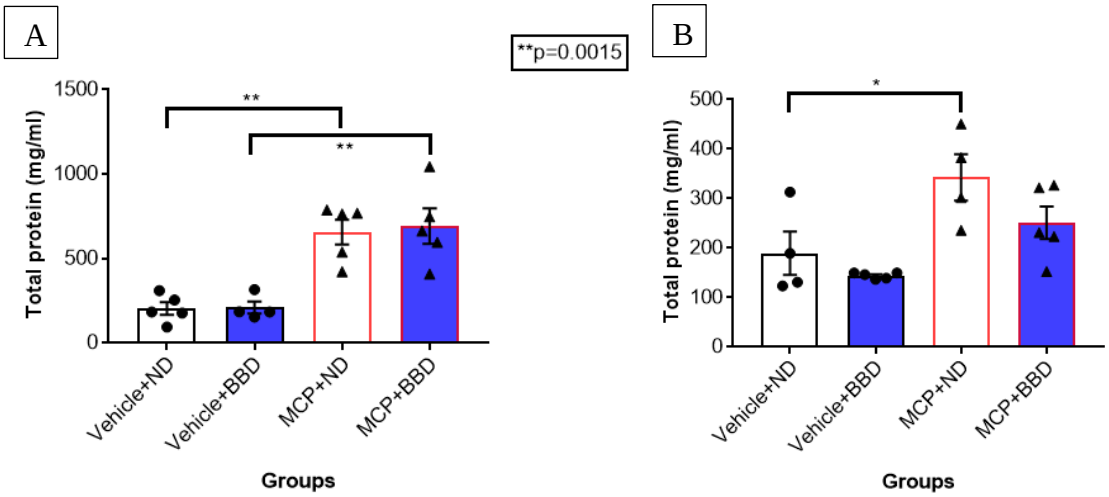
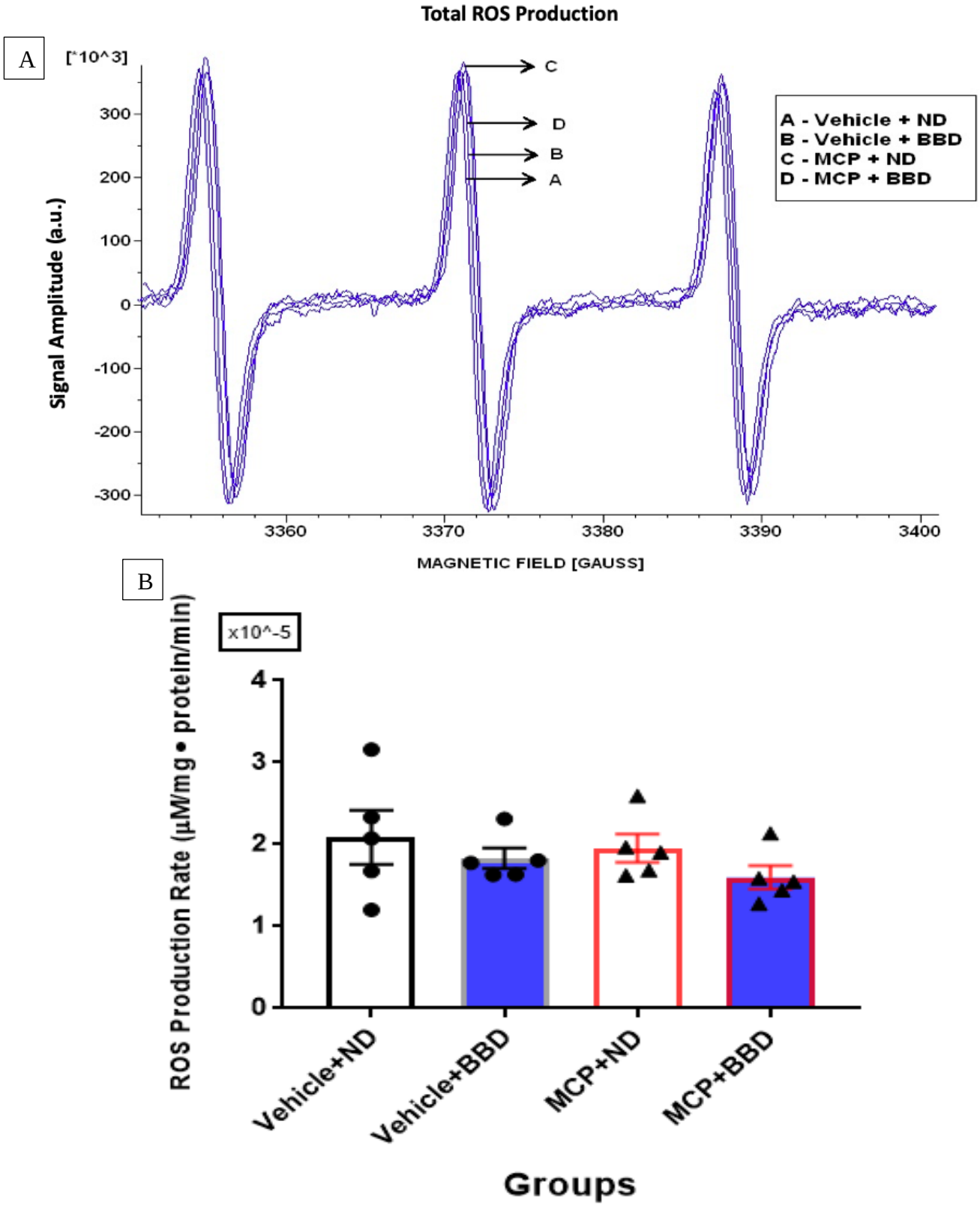


Figure 2: BALF total protein levels in adult, male mice were determined after PM exposure. *BALF was collected at 1 day (A) and 5 days (B) post PM exposure (DPE). In the 5 DPE timepoint (B), the MCP+ND vs. MCP+BBD comparison has a p value of p=0.2709.*

BBD may attenuate ROS production in lung tissue of PM exposed mice

EPR was performed to determine the respective rates of ROS production across treatment groups. Since oxidative stress is exacerbated by increased ROS levels, faster production rates are interpreted as more severe cellular oxidative stress whereas slower production rates are interpreted to be less severe. At the 1 DPE timepoint (Figure 3A, B), there are two things to note. First, the BBD groups show reduced ROS production rates compared with the ND groups, especially in the MCP-exposed cohort. Second, there was no significant or observable increase in ROS production rates in the MCP-exposed groups compared to Vehicle-exposed. Based on a range of previous research [22, 23], we had originally hypothesized that MCP exposure would cause an increased ROS production rate in lung tissues. At the 5 DPE timepoint, there were no apparent differences in ROS production rate across any of the groups (Figure 3C, D).


A
Total ROS Production
[*10^3]
Signal Amplitude (a.u.)
300
200
100
0
-100
-200
-300
3360
3370
3380
3390
3400
MAGNETIC FIELD [GAUSS]
C
D
B
A
A - Vehicle + ND
B - Vehicle + BBD
C - MCP + ND
D - MCP + BBD
B
x10^-5
ROS Production Rate (µM/mg • protein/min)
4
3
2
1
0
Vehicle+ND
Vehicle+BBD
MCP+ND
MCP+BBD
Groups

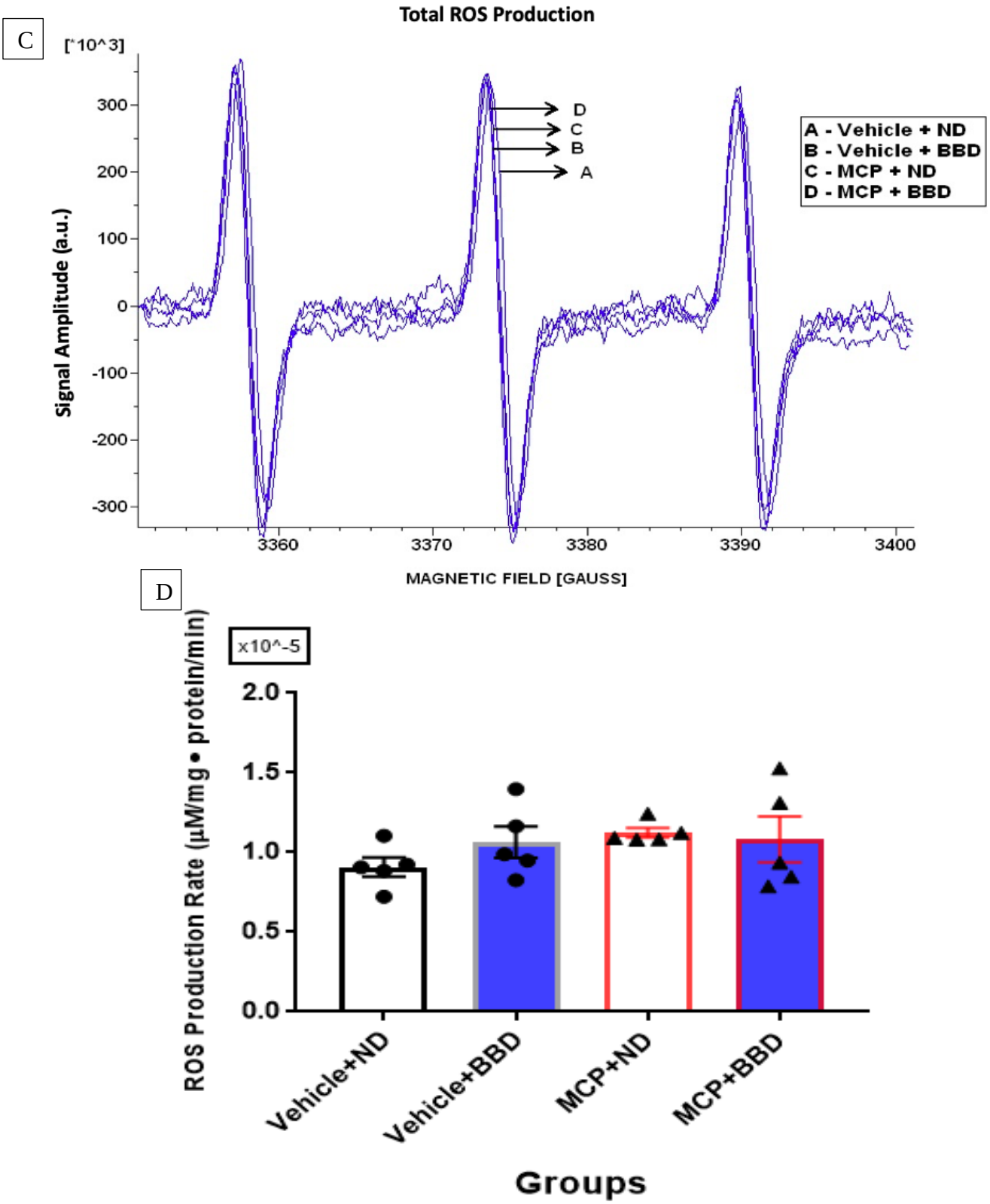
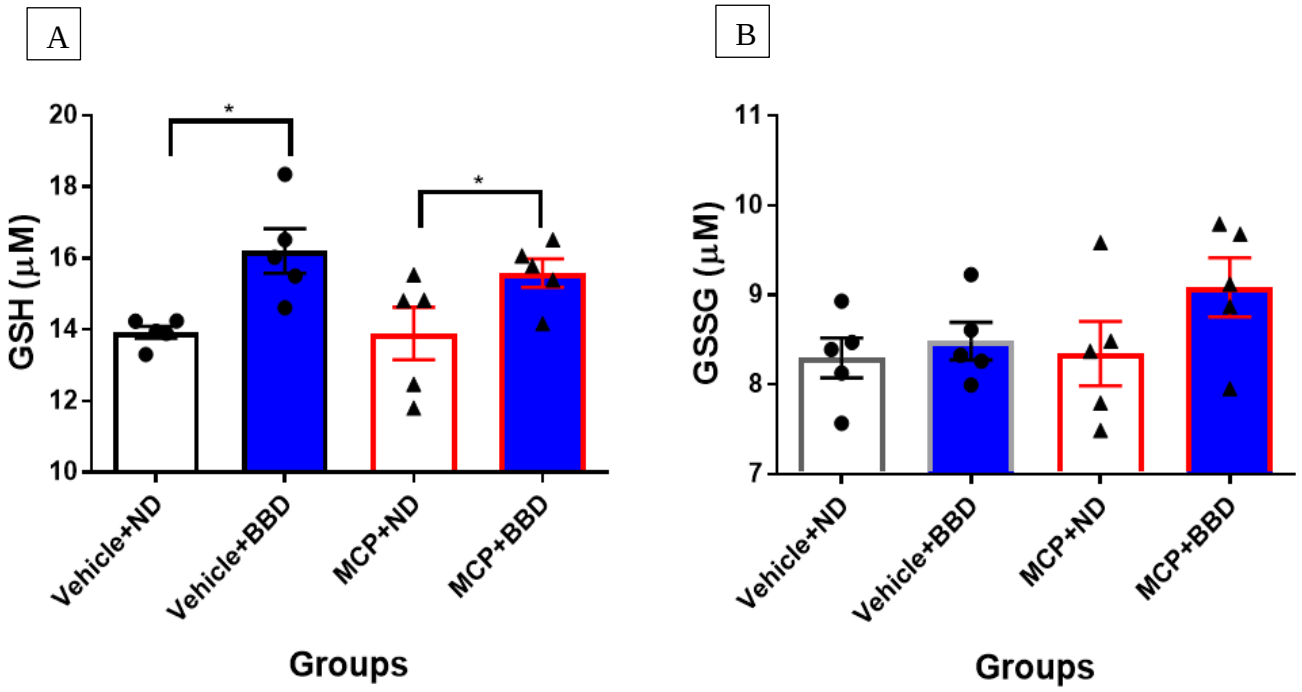


Figure 3: Reactive oxygen species prevalence in lungs. *ROS production rate was examined at 1 day (A and B) and 5 days (C and D) post PM exposure. All procedures were conducted on freshly isolated lung tissues. Differences in peak height represent differences in signal amplitude and correspond to the relative free radical concentrations of each group. Data are means ± SEM, n=4/5 per group.*

BBD is capable of significantly elevating GSH levels, thus improving GSH/GSSG ratio

The glutathione/oxidized glutathione (GSH/GSSG) ratio is a well-established measure of oxidative stress levels within a given tissue. The larger the GSH/GSSG ratio, the less oxidative stress the tissue is interpreted to be experiencing. Using lungs isolated at 5 DPE, we quantified both GSH (Figure 4A) and GSSG (Figure 4B) levels, respectively. From this, the GSH/GSSG ratio of each group was calculated and is presented in Figure 4C. In alignment with our hypothesis, both BBD-fed groups showed significant increases in GSH compared to their ND controls (Figure 4C). Beyond that, both BBD-fed groups also showed visible increases in GSH/GSSG. Most notable of these was the Vehicle+BBD group which showed a significantly higher ratio than its ND counterpart. Together, this data confirms BBD feeding is capable of improving oxidative stress levels in healthy mice but also suggests that MCP-exposed mice require longer feeding times (or higher blueberry content in the diet) to see benefits from the BBD.



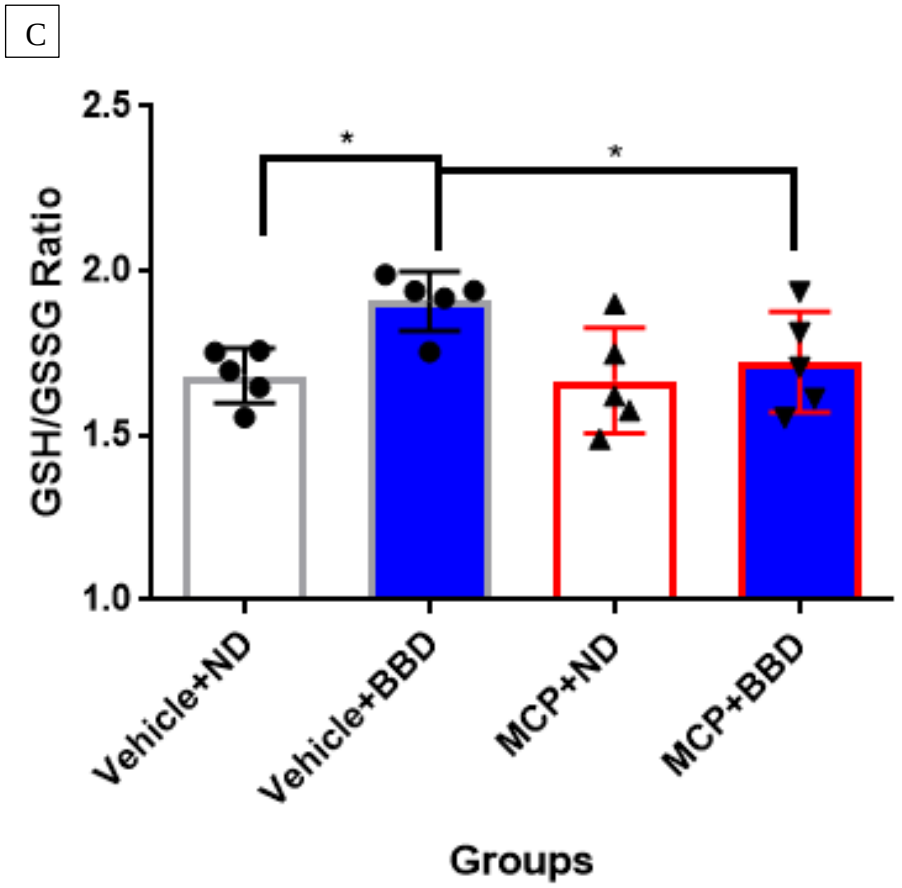


Figure 4: Effect of diet and PM exposure on GSH/GSSG ratio: an oxidative stress indicator. *At 5 DPE, GSH (A) and GSSG (B) levels in lung tissue homogenates were quantified and their respective ratio was determined for each group (C). Data are mean ± SEM, n=5. *p<0.05.*

1-week BBD feeding has little impact on inflammation and immune cell recruitment

Based on previous research [19, 24], we hypothesized that the BBD would aid the immune system in minimizing inflammation and modulating immune cell recruitment in PM-exposed mice. To test this hypothesis, total and differential cell counts were performed on BALF samples isolated at 5 DPE. Total cell counts were found to have little variance across groups, indicating similar levels of inflammation regardless of PM-exposure or feeding (Figure 5A). Differential cell counts showed some distinctions between groups but are more likely due to experimental error than meaningful changes (Figures 5B and 5D). The one point of note is that the MCP+BBD group shows an elevated lymphocyte count, indicating a possible immunomodulatory role of the BBD (Figure 5D). Overall, the lack of meaningful trends prevents us from drawing important conclusions based on this experiment.

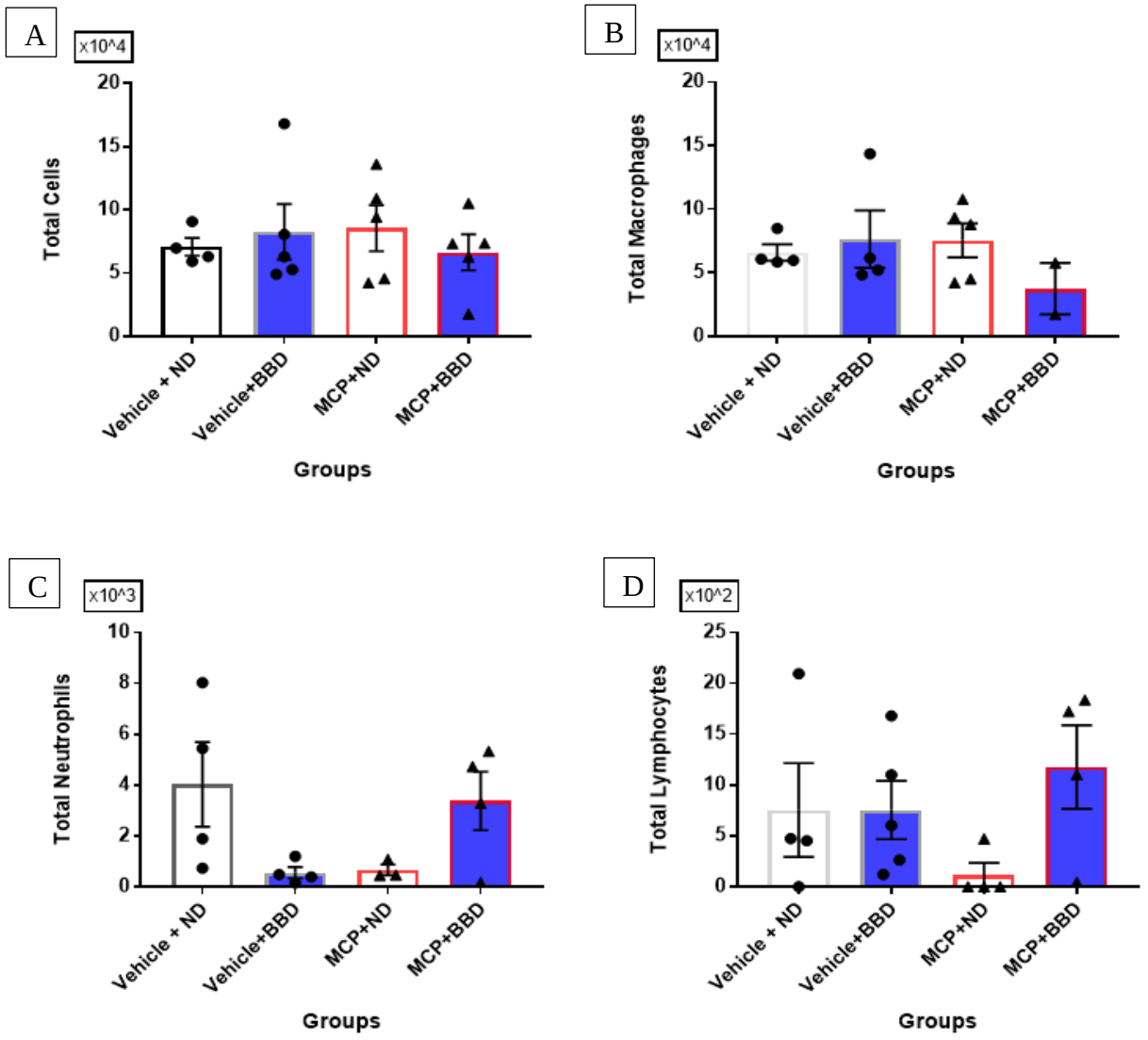


Figure 5: BALF cellularity of adult, male mic fed normal or blueberry diet for 1 week. *Differential cells counts were performed on BALF isolated 5 days after PM exposure. Total cells (A), macrophages (B), neutrophils (C), and lymphocytes (D) were all quantified. Data are means ± SEM, n=4/5 per group.*

Effects of a blueberry-enriched diet on immune cell recruitment become stronger as feeding time is extended

After seeing the results of the previous differential cell count, we decided to conduct another trial with a 2-week feeding period rather than only 1 week. In this experiment, the mice had been on the respective diets for 2 weeks at the time of exposure (see Figure 1B) and BALF was again isolated at 5 DPE. As before, total cell counts remained relatively similar (Figure 6A). However, the macrophage (Figure 6B) and neutrophil (Figure 6C) counts began to show some observable trends with regard to the BBD-fed cohorts. Both macrophages and neutrophils were notably elevated in the BBD-fed groups compared to their ND counterparts. Furthermore, the lymphocyte counts (Figure 6D) showed a notable increase in the MCP-exposed groups,

indicating a possible role for lymphocyte recruitment in managing PM-induced inflammation. Additionally, BBD feeding again increased the number of lymphocytes (Figure 6D) present compared to ND in the MCP cohorts, supporting our hypothesis that a blueberry-enriched diet may play a role in modulating immunological function.

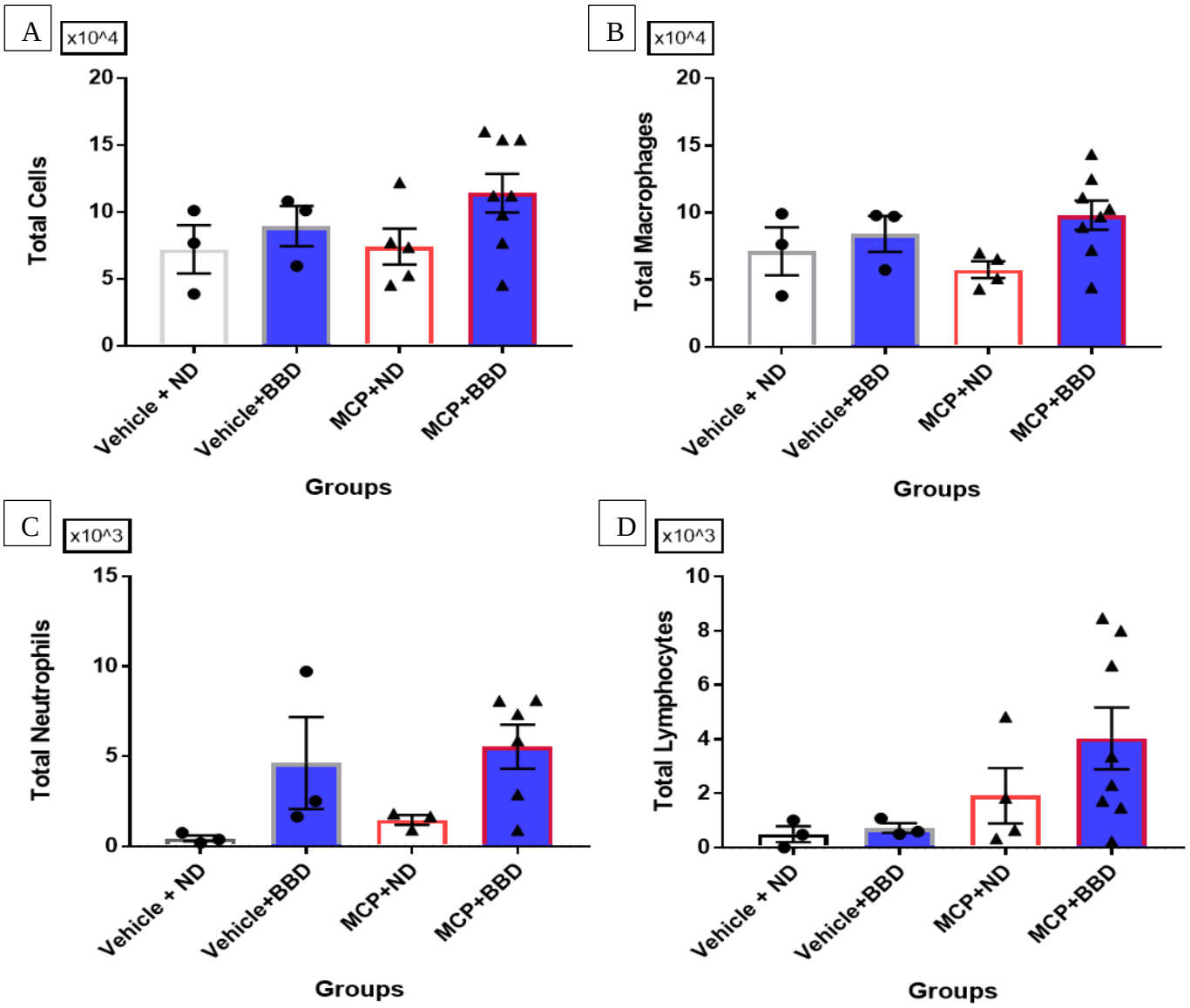


Figure 6: BALF cellularity of adult, male mice fed normal or blueberry diet for 2 weeks*. Differential cells counts were performed on BALF isolated 5 days post PM exposure. Total cells (A), macrophages (B), neutrophils (C), and lymphocytes (D) were all quantified. Data are means ± SEM, n=3,4, and 7 per group.*

Discussion

Previous studies have confirmed MCP exposure causes lung damage, increases ROS concentration leading to oxidative stress conditions, and induces inflammatory immune

responses in the lungs of adult mice [4, 25]. Yet, though these harmful effects are well-established, novel methods for combatting these maladies through the use of antioxidant-rich foods have not been thoroughly examined.

Blueberries arose as an ideal candidate for study because of their extraordinary antioxidant capacity and overall global availability [17]. Beyond that, studies have confirmed their ability to reduce ROS production rate, increase GSH levels, and even improve renal pathology in hypertensive rats [20]. Considered alongside a host of other research outlining the benefits of blueberry supplementation [19, 20, 26-31], this ability to improve redox balance at a systemic level led us to examine their prowess as a potential for-everyone method of combatting PM-induced lung maladies.

Through the use of an adult C57BL/6 mouse model, BBD-feedings, and exposure to a known pulmonary irritant (MCP230), we were able to quantify the potential for BBD consumption to prevent the adverse effects of MCP exposure in adult mouse lungs.

Our study used all adult, male mice, which allowed for direct comparisons across groups without the need to account for sex or age-related variance. With this, we sought to determine if a blueberry-enriched diet was capable of attenuating PM-induced lung damage, oxidative stress, and inflammation.

Starting with the lung injury aim, we will examine the total protein data where MCP exposure and BBD feeding both had notable effects on BALF total protein levels. In the 1 DPE groups, only MCP's effects were observed. At this early timepoint, there was a significant increase in total protein for both MCP groups compared to their Vehicle-exposed counterparts but no notable effects of BBD feeding (Figure 2A). At 5 DPE however, the results were a bit more interesting. Here, the BBD-fed groups all reported lower total protein levels than their ND counterparts. Further, BBD's ability to attenuate lung damage is most evident by the fact that the Vehicle+BBD group's protein levels were not significantly different than the MCP+BBD group's (Figure 2B), indicating that BBD feeding afforded protection to the extent that the damage caused by MCP exposure was all but negated. Overall, this data supports previous research documenting MCP exposure's ability to cause lung damage [4, 25] and our hypothesis that BBD feeding can afford some protection against PM-induced lung injury. Additionally, it serves as interesting evidence of BBD's apparent ability to "build up" protection over time.

Next, we will take a closer look at the oxidative stress markers we evaluated. Starting with the EPR data, these data points represent the total ROS present in the animals' lungs and allow us to compare ROS prevalence across all groups. Beginning at 1 DPE, all groups' means are relatively similar, but the BBD-fed groups show slight decreases in ROS presence compared to their ND-fed counterparts (Figure 3A). While these decreases do represent a potential protective effect afforded by the BBD, they are not significant enough to make sound conclusions about the BBD's effects. Moving forward to the later 5 DPE timepoint, there are no obvious effects across any of the treatment groups (Figure 3D). This lack of meaningful difference between groups may be explained by the fact that the mice had begun to return to homeostasis after exposures, but we cannot be certain. One other important note is that MCP did not have a significant or observable effect at either time point which is in contrast to previous Cormier lab research that indicated a

link between EPFR containing particle exposure and increased oxidative stress in neonatal mice and human airway epithelial cell cultures [4, 22]. Absence of an MCP-induced effect is certainly intriguing and is unclear if due to age-related differences in antioxidant capacity of the lungs [32, 33], thus requiring a longer exposure time to induce oxidative stress, or if a more sensitive indicator of oxidative stress such as 8-isoprostanes should have been evaluated. Overall, this EPR data disagrees with both earlier Cormier lab research and confounds our ability to test our hypothesis that BBD feeding would cause a decrease in ROS production.

As expected, GSH levels were found to be significantly elevated in the BBD-fed members compared to the ND-fed members of both exposure cohorts (Figure 4A). These elevated GSH levels found in the BBD-fed mice support our hypothesis that BBD may be able to mitigate oxidative stress in PM-exposed mice. In regard to the GSSG data though, the only notable difference in means across groups is the MCP+BBD group, which shows a trend toward higher GSSG levels than any other (Figure 4B). This result is in agreement with previous research detailing MCP's harmful effects [4, 21] but is in direct opposition with our hypothesis that BBD feeding would attenuate oxidative stress (i.e. decrease GSSG). The GSH/GSSG ratios demonstrate that BBD feeding exerted a significant effect on the Vehicle-exposed cohort since the Vehicle+BBD group's ratio is the largest of all (Figure 3C). However, the BBD's effects are far more subtle in the MCP-exposed group where the ratio increase is barely noticeable. This difference in magnitude of effect may be evidence that Vehicle exposure's comparative mildness allows BBD to exert an effect on antioxidant balance and suggest that increased intake of BBD or higher blueberry dose will be necessary to counter MCP's comparatively adverse effects. Just like the EPR data though, the MCP-exposed groups do not show dramatic differences in GSH/GSSG compared to the Vehicle-exposed groups. This absence of an effect is in contradiction with previous Cormier lab research showing MCP's adverse effects [4] but supports our hypothesis that the BBD can improve oxidative stress levels. This data also perpetuates the ideas that MCP exposure was performed poorly and/or that Vehicle exposure is equally effective as MCP exposure at inducing oxidative stress. Overall, while MCP exposure had no significant effect on GSH/GSSH, BBD feeding helped improve oxidative stress levels compared to ND feeding.

As a marker of pulmonary inflammation and an indication of lung injury, BALF cell counts were performed. Starting with the 1-week cell counts (Figure 5), the only real trend of note is the elevated lymphocyte counts in the BBD-fed members of the MCP cohort (Figure 5D). Beyond that, the lack of variation in total cell counts (Figure 5A) indicates relatively equal inflammation severity across exposures and diets which goes against our hypothesis that BBD would attenuate the PM-induced inflammatory response. After analyzing the data from the 1-week diet cell counts, we decided to conduct a 2-week diet trial for two main reasons: First, the data was not in line with earlier MCP exposure research showing dramatic effects on total cell counts as well as neutrophil and lymphocyte recruitment [25]. Second, we hoped that more time on the BBD would allow its effects to build over time as human data [34] and our own total protein data suggested it might. Any abnormalities or oddities in the aforementioned 1-week diet data are

most likely due to experimental errors in BALF collection techniques and lack of a more robust cell counting protocol.

Trends became more evident in the 2-week diet data (Figure 6). Most notably, BBD-fed groups reported higher total cell counts than ND-fed groups (Figure 5A), indicating BBD feeding supported the deployment of a larger magnitude immune response than ND feeding. In the macrophage counts (Figure 6B), the BBD-fed groups again showed notably higher cell numbers than their ND-fed counterparts but MCP and Vehicle exposures' effects were indistinguishable. Neutrophil counts (Figure 6C) offered a bit more insight, with MCP-exposed cohorts showing higher cell counts than their Vehicle-exposed counterparts and BBD-fed groups showing far higher neutrophil numbers than ND-fed groups. A similar trend was also evident in the lymphocyte counts (Figure 6D) as well, with MCP exposure increasing cell counts compared to Vehicle exposure and BBD amplifying this effect. These noticeable increases in neutrophil and lymphocyte counts we observed are in agreement with earlier Cormier lab research [25] reporting neutrophilic and lymphocytic inflammation following MCP exposure. Interestingly, as observed in the 1-week diet experiments, the MCP+BBD group again showed the highest lymphocyte counts out of all the groups. Overall, these finding are in alignment with previously conducted human research indicating that longer BBD feeding times were required in order for the diet to exert its immunological effects [34]. Further, the same study also reported significant increases in lymphocyte recruitment in BBD-fed individuals compared to those consuming a regular diet which also supports our findings. As a whole, the 2-week diet data indicates that BBD actually enhances the pulmonary immune response and causes notable increases in neutrophil and lymphocyte recruitment. These trends are in opposition with our hypothesis that BBD would reduce inflammation but support the idea that BBD feeding exerts some immunological effects. However, more research is needed to determine if these affects are protective or harmful.

In summary, 1-week of BBD feeding is capable of attenuating lung injury and oxidative stress in otherwise healthy mice. Additionally, BBD feeding improves some of these same parameters in MCP-exposed animals, but longer exposure periods, longer feeding times, and/or higher blueberry content is needed to properly characterize these effects. Further, the 1- and 2-week diet cell count data indicated BBD indeed does have a role in modulating immune function and that its effects are much stronger after 2 weeks of feeding.

Taken together, our results contribute to a growing field of research supporting the systemic health benefits of blueberry consumption [19, 26, 29-31]. We showed that as little as 1 week of blueberry intake is sufficient to improve pulmonary health markers in a Vehicle-exposed mouse model and data suggests longer feeding times would yield similar results for MCP-exposed mice as well. Unlike the majority of other BBD-feeding research, our study uses dietary methods to combat PM-induced maladies in the lungs. This unique method represents a widely applicable (and enjoyable) method for bolstering pulmonary immune health. Our hope is that these data will motivate future studies using extended BBD feeding periods, cytokine analyses, and more robust

oxidative stress measures to better characterize BBD's interactions with PM-induced lung maladies.

In conclusion, we believe the findings of this study contribute to the fields of both BBD and MCP research, as well as suggest the use of a blueberry-enriched diet as a method for individuals to improve their wellbeing through informed nutrition.

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