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Understanding HPA Axis Regulation and *ARG-1* Expression in a Young Mouse Model of Developmental Asthma

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ABSTRACT

Asthma is one of the most common noncommunicable diseases affecting over 300 million people worldwide (To et al., 2012; Vos et al., 2017). Asthma is particularly common among children aged 0 to 17 with a global prevalence of approximately 8% (Akinbami, Simon, & Rossen, 2016). However, studies of asthma in very young children are limited, and few pharmacotherapy options are available for children under the age of 5. To better understand asthma development in young children, we utilized a model of allergic asthma in mice at post-natal day 21 with the goal of examining hypothalamic-pituitary-adrenal (HPA) axis regulation and arginase-1 (*ARG-1)* gene expression in the lungs. We created asthma symptoms in male and female BALB/cJ mice (n=95) with intranasal house dust mite protein (HDM) to produce chronic airway inflammation and aerosolized methacholine (MCH) to produce acute bronchoconstriction. At three timepoints (immediate, 4-hours and 24-hours) following final HDM and MCH exposure, lung and blood samples were collected and analyzed for *ARG-1* expression changes and serum corticosterone levels. Results indicated that serum corticosterone concentrations were highest in all treatment groups at the immediate timepoints. Experimental groups that received HDM or MCH had higher serum corticosterone levels compared to controls at the immediate timepoint. HDM-exposure was associated with a significant increase in *ARG-1* expression in lungs immediately and at 24-hours after HDM exposure, with marginal changes at 4-hours. No sex-dependent differences in gene expression or serum corticosterone data were observed. These results help validate our mouse model of developmental asthma while also providing a foundation for future work investigating asthma mechanisms and treatment options in very young children.

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Chapter 1

Introduction

Asthma is one of the most common noncommunicable diseases affecting over 300 million people worldwide (To et al., 2012; Vos et al., 2017). Among children aged 0 to 17, asthma is the most common chronic disease with a global prevalence of approximately 8% (Akinbami et al., 2016). While the global prevalence of asthma has remained relatively stable over the last few years, there are significant disparities in asthma's prevalence and severity among select racial, ethnic and socioeconomic groups (Guilbert et al., 2019; Hughes, Matsui, Tschudy, Pollack, & Keet, 2017). For example, a higher prevalence of morbidity and mortality have been observed in Black and Latino children living in low socioeconomic societies compared to White children (Akinbami et al., 2016; Hill, Graham, & Divgi, 2011; Loftus & Wise, 2016). In addition, research suggests that the incidences of asthma in males and females vary throughout development. Adult females observe a higher incidence of asthma and higher severity of symptoms compared to adult males with these findings being reversed between males and females in childhood (Barbro N. Melgert et al., 2010; Barbro N. Melgert, Ray, Hylkema, Timens, & Postma, 2007). Not only does asthma impact human health and quality of life disproportionately, but it also poses a significant economic burden. In 2013 alone, the total cost of asthma in the United States surpassed $81 billion (Nurmagambetov, Kuwahara, & Garbe, 2018). Asthma's extensive prevalence among children, racial and sexual disparities, and significant economic impacts make asthma and its underlying mechanisms an important area of research.

Characteristics of Allergic Asthma

The National Institute of Allergy and Infectious Diseases defines asthma as a chronic inflammatory condition of the lungs with common symptoms of wheezing, breathlessness, chest tightness and coughing (NIAID, 2020). In general, asthma diagnoses can be classified as either allergic or nonallergic asthma. Nonallergic asthma symptoms develop in response to irritants like cold weather or exercise (Raedler et al., 2015). In contrast, allergic asthma is characterized by mucus production, lung influx of eosinophils (key white blood cells of the immune system), and airway hyperreactivity in response to an allergen (Hirose, Iwata, Tamachi, & Nakajima, 2017; Raedler et al., 2015). Allergic asthma is more common in the United States than nonallergic asthma representing approximately 60% of asthma cases (AAFA, 2015; Pakkasela et al., 2020). While the prevalence of nonallergic asthma has not changed much throughout the last decade, the prevalence of allergic asthma has increased with the highest incidence of allergic asthma found in early childhood compared to adulthood (Backman et al., 2017; Pakkasela et al., 2020). Prevalence of allergic asthma also varies by sex with males having a higher prevalence of asthma before puberty and females observing a higher prevalence than males later in life (Fuseini & Newcomb, 2017; Senna et al., 2021; Vink, Postma, Schouten, Rosmalen, & Boezen, 2010). This variation is likely explained in part by differences in allergic immune responses in males versus females, and it is a core reason why data from both male and female mice are analyzed in this study.

Two hallmarks of allergic asthma include airway inflammation and labored breathing. Airway inflammation can be classified as an early- or late-phase response to allergen exposure (Galli, Tsai, & Piliponsky, 2008). Within minutes following exposure to an allergen, an early-phase reaction occurs and involves the synthesis of Immunoglobulin E (IgE), an immune system

antibody, which activates the inflammatory response (Galli et al., 2008). IgE recruits inflammatory cells like mast cells, T cells and eosinophils, which can release inflammatory mediators including cytokines (Galli et al., 2008; Hirose et al., 2017). Allergic asthma is generally considered a T-helper-2 (T_H2) cell response, recruiting cytokines, like IL-4, IL-5, IL-10 and IL-13 (Wong, Ho, & Ko, 2001). While T_H2 cytokines have been frequently studied in relation to allergic asthma (Komai et al., 2003; Leung et al., 1995; Smart, Horak, Kemp, Robertson, & Tang, 2002), there is evidence that T_H1 cells (responsible for cell-mediated immunity) may also play a role in the immune response in allergic asthma. Several studies have found an imbalance of T_H1 and T_H2 cytokines in asthmatics with T_H1 cytokine production significantly decreased in asthma (e.g. IFN-g and TNFα, Chung, 2001; Hirose et al., 2017). In response to allergens, patients with asthma will respond with a heightened T_H2 compared to T_H1 response (Hirose et al., 2017). The early-phase response, which is elicited within seconds to minutes after allergen challenge, can cause mucus secretion, bronchoconstriction and further activate mediators that lead to late-phase reactions (Galli et al., 2008). Late-phase allergic reactions develop in the hours following allergen exposure, peaking up to 9 hours after exposure (Galli et al., 2008). Animal studies of allergic asthma have observed this early- and late- phase response to allergen (Caulfield, Schopf, & Cavigelli, 2021; Smith & Johnson, 2005; Tonelli et al., 2009).

In addition to airway inflammation, labored breathing is another hallmark of allergic asthma that can result from bronchoconstriction, or the narrowing of airways (Bosnjak, Stelzmueller, Erb, & Epstein, 2011; Grainge, Lau, Ward, & Dulay, 2011). Previous studies have identified increased hypoxia, or oxygen deprivation, in lung samples from patients with asthma (Ahmad et al., 2012). Furthermore, increased hypoxia can contribute to airway remodeling

through repeated epithelial stress (Ahmad et al., 2012; Grainge et al., 2011). Irreversible airway remodeling can occur in chronic and severe cases of asthma and can begin to develop early in life, even in preschool children aged 1 to 3 (Halwani, Al-Muhsen, & Hamid, 2010; Saglani et al., 2007).

While labored breathing, airway inflammation, and airway remodeling have been studied in adolescents and adults with asthma, there are limited studies on these mechanisms early in life. Previous research has suggested that asthma diagnosis in very young children can be challenging with limited diagnostic and pharmacotherapy options available for children under the age of 5 (Bacharier et al., 2008; Hafkamp-de Groen et al., 2013). Preschool-aged children have also shown a higher prevalence of asthma exacerbations compared to asthma patients aged 7 to 18 (Sonnappa, Bastardo, Saglani, Bush, & Aurora, 2011; Yu, Huang, & Wang, 2019). In general, females have more severe asthma symptoms leading to a greater number of hospitalizations compared to males later in life (Senna et al., 2021). Due to our limited understanding of asthma development early in life and limited available treatment options for young patients, further research on early-life asthma development is needed.

HPA Axis Dysregulation in Asthma

Not only does asthma present symptoms such as labored breathing and airway inflammation, but it can also be associated with comorbid disorders like anxiety and depression (Dudeney, Sharpe, Jaffe, Jones, & Hunt, 2017; Guerry & Hastings, 2011; Katon et al., 2007). For example, a meta-analysis on anxiety in youth with asthma concluded that asthmatic youth under the age of 18 have three times higher the prevalence rate for anxiety than what is seen in

nonasthmatic children (Dudeney et al., 2017). Given the association between asthma and mental health comorbidities, hypothalamic-pituitary-adrenal (HPA) axis dysregulation is a potential mechanism of interest, as HPA axis irregularities and adrenal insufficiency can be observed in both asthma patients and those with anxiety (Caulfield & Cavigelli, 2020; Kroll, Brown, & Ritz, 2019). HPA axis dysregulation, specifically irregularities in glucocorticoid levels, are examined here, and the mechanism is outlined in Figure 1 below.

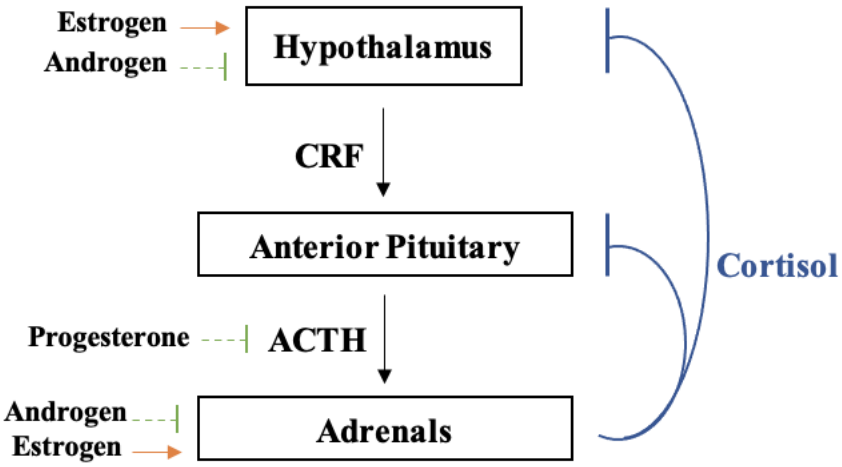


Figure 1 Hypothalamic-Pituitary-Adrenal Axis Overview.

Corticotropin releasing factor (CRF) is released from the hypothalamus to initiate the HPA axis response. Signals from the hypothalamus are transmitted to the anterior pituitary via the bloodstream to stimulate the production and secretion of adrenocorticotropic hormone (ACTH). ACTH then causes the production of cortisol, which provides negative feedback to the pituitary and hypothalamus. Sex hormones also play an influential role in either stimulating or inhibiting the various parts of the axis (Panagiotakopoulos & Neigh, 2014). Arrowhead indicates stimulation while bar indicates inhibition.

Cortisol and corticosterone are both glucocorticoids involved in modulating the HPA axis and regulating a variety of physiological and behavioral processes, including regulating responses to stress (Smith & Vale, 2006). Previous research has shown that asthmatic children have lower cortisol levels, including lower levels in response to recurrent stress early in life, and

that serum cortisol levels in asthma patients are diminished during asthma attacks (Dreger, Kozyrskyj, HayGlass, Becker, & MacNeil, 2010; Fujitaka et al., 2000; Kamps et al., 2014). HPA axis function also varies by sex possibly due to changes in sex steroid hormones, including estrogen and androgens, which vary in males and females throughout development (Handa & Weiser, 2014; Panagiotakopoulos & Neigh, 2014). Analyzing serum corticosterone levels in male and female mice at post-natal day 21 will not only allow for further validation of this murine model of asthma but also provide a greater understanding of asthma mechanisms and associated comorbidities early in life.

Arginase-1 and Allergic Asthma

This research will also analyze arginase-1 (*ARG-1)* gene expression in the lungs of mice at the beginning of allergic asthma development. Previous research has identified an important role of *ARG-1* in airway inflammation, airway hyperresponsiveness and airway remodeling associated with allergic asthma (Kenyon, Bratt, Linderholm, Last, & Last, 2008; Mabalirajan et al., 2010; North et al., 2013). Other research has indicated that expression of the gene that encodes for arginase-1 (*ARG-1*) is upregulated in patients with allergic asthma, making arginase-1 an area of interest in this study (Benson, Hardy, & Morris, 2011; North, Khanna, Marsden, Grasemann, & Scott, 2009; Zimmermann et al., 2003).

Arginase-1 is an enzyme primarily expressed in the liver and plays a role in the urea cycle, breaking down the amino acid L-arginine into nitric oxide (King, Rothenberg, & Zimmermann, 2004; Morris, 2002). Despite its primary role in the liver, arginase-1 is also associated with modulating symptoms of allergic asthma in the lungs through the arginase

pathway (Grasemann & Holguin, 2021; North et al., 2013). L-arginine is an amino acid that plays a critical role in several enzymatic pathways, including in the creation of nitric oxide (NO), L-ornithine, L-proline, and polyamines (Durante, Johnson, & Johnson, 2007; Felix & Kuschnir, 2020). Increased expression of *ARG-1* in the lungs reduces the concentration of nitric oxide (NO) and contributes to disordered NO metabolism, which can be associated with allergic airway inflammation (Benson et al., 2011; Mabalirajan et al., 2010). *ARG-1* expression has also been linked to the T_H2 inflammatory response with arginase-1 gene expression being induced by IL-4 and IL-13 cytokines in the lungs (Hesse et al., 2001; King et al., 2004; Lindemann & Racké, 2003).

In addition to its involvement in airway inflammation, arginase-1 may also contribute to the airway hyperresponsiveness, and airway remodeling seen in allergic asthma. Increased expression of *ARG-1* decreases the availability of L-arginine and contributes to the creation of polyamines, which are essential components in cell growth and normal cell functioning (Durante et al., 2007; North et al., 2013). Previous studies in mice and human subjects have shown that increased concentrations of these polyamines, specifically L-ornithine-derived polyamines, correlate with increased airway hyperresponsiveness (North et al., 2013). Allergen exposure, including ovalbumin and environmental pollutants, is associated with higher L-ornithine-derived polyamine levels as well as augmented arginase activity in the lungs (Kenyon et al., 2008; North et al., 2011, 2013). The production of polyamines and the amino acid L-proline is important for cell growth and collagen synthesis, indicating that the arginase-1 pathway may also play a role in airway remodeling commonly seen in allergic asthma (Benson et al., 2011; Durante et al., 2007).

Arginase-1 and its connection to allergic asthma has already been established. A prior research study also suggests a possible connection between arginine and cortisol levels, making

correlations between arginase-1 and HPA axis dysregulation a research area of interest (Flynn & Wu, 1997). Specifically, the study conducted in cortisol-treated pigs indicated that cortisol exposure enhances arginine metabolism through a glucocorticoid receptor-mediated mechanism (Flynn & Wu, 1997). In addition to physiological effects of altered *ARG-1* expression, research into L-arginine and behavior has indicated that daily administration of the amino acid L-arginine diminishes behavioral depression that was measured with the tail suspension behavioral test in mice (Pervin, Unno, Konishi, & Nakamura, 2021). Additional studies in humans have also shown connections between arginase-1, L-arginine and major depression, providing further evidence that the arginase pathway may play a role in behavioral responses as well as physiological outcomes (Ormonde do Carmo et al., 2015; Raw, Gallaher, & Powers, 2014).

Studies focusing on the connections between arginase-1, L-arginine, glucocorticoid mechanisms and behavioral outcomes in young subjects and within our specific murine model of developmental asthma are limited. In addition, previous studies examining arginase-1 are also limited in that they have only analyzed BALB/c mice aged 6 to 12 weeks (Kenyon et al., 2008; North et al., 2009). In the current study, I analyzed *ARG-1* expression in mice at post-natal day 21 (P21, P: post-natal day, i.e. weaning age) when allergic asthma symptoms are just beginning to develop and which correlates to approximately the age of 2, or infancy, in humans (Dutta & Sengupta, 2016). A greater understanding of mechanisms underlying allergic asthma very early in life will help inform potential treatment options for young patients.

Murine Models of Asthma

Experimental studies of allergic asthma typically use mice to model asthma mechanisms in response to chronic allergen exposure. Repeated allergen exposure is required to induce asthma symptoms because mice do not spontaneously develop asthma (Kips et al., 2003; Temelkovski, Hogan, Shepherd, Foster, & Kumar, 1998). Penn State's Behavioral Neuroendocrinology Lab (BNEL) used a mouse model of allergic asthma, utilizing house dust mite (HDM), one of the most common human aeroallergens, to induce airway inflammation and methacholine (MCH) to produce labored breathing (Caulfield et al., 2018, 2017; Saglani et al., 2009). Prior research using this model has found that intranasal HDM exposure in neonatal mice beginning at P3 resulted in heightened inflammation, airway hyperresponsiveness and airway remodeling that lasted for weeks post-allergen exposure (Saglani et al., 2009). In addition to intranasal HDM-exposure, MCH, a non-selective muscarinic agonist that mirrors the action of acetylcholine, was used here to bind to airway smooth muscle and cause bronchoconstriction (Zlotos, Bender, & Holzgrabe, 1999). MCH has been shown to cause airway remodeling without modulating significant inflammation in the lungs (Grainge et al., 2011; Matsumoto et al., 2009). In contrast, more recent research into the effects of MCH in an ovalbumin-induced asthma model indicates that MCH promotes allergic airway inflammation as well as airway remodeling (Miyata et al., 2020). The goal of HDM and MCH administration in mice is to create the symptoms of asthma—airway inflammation and labored breathing—independently. These treatments have also been shown to affect circulating glucocorticoid levels in a manner that mimics what is seen in humans. Previous research using this model has also looked at behavioral effects of these treatments on mice. In multiple studies using this murine model of asthma, mice exposed to MCH observed fewer entries into open arms in the elevated plus maze indicative of higher

levels of anxiety (Caulfield et al., 2018, 2017; Saglani et al., 2009). With this model of developmental asthma, we can study the physiological and behavioral impacts of asthma symptoms in developing mice.

Hypothesis and Research Goals

The purpose of this research is two-fold. The first goal is to determine if airway inflammation or acute bronchoconstriction leads to altered HPA regulation as early as P21 in a mouse model. Prior research has determined that chronic airway inflammation (induced by HDM exposure) during development is associated with diminished serum corticosterone and persisting lung inflammation in adult mice that continues months after exposure (Caulfield et al., 2017, 2018). In prior work, female mice exposed to HDM had lower corticosterone levels immediately and 24 hours after exposure, while males showed lower corticosterone levels 1 hour and 4 hours after exposure to allergen (Caulfield et al., 2021). In addition, the study found that females exposed to acute bronchoconstriction (induced by MCH) had higher corticosterone levels immediately after MCH exposure compared to females exposed to HDM (Caulfield et al., 2021). Our *a priori* hypothesis for this experiment mirrors the findings from the P56 data: we expect mice exposed to HDM to have lower serum corticosterone concentrations compared to control mice, and for females to have a greater corticosterone response to MCH than HDM. Overall higher serum corticosterone levels are expected in females compared to males based on results from mice at P56 (Caulfield et al., 2021).

The second objective of this research is to analyze *ARG-1* expression in the lungs. This study is a continuation of previous research that analyzed lung and brain gene expression

changes in a mouse model of allergic asthma at post-natal day 56 (Caulfield et al., 2021). By analyzing mice at P21, we can explore the impacts of asthma in the lungs during early-life development. The results will also focus on sex- and timepoint- differences to highlight how responses in males and females may vary at an early age and how the early- and late-phase immune response may influence mechanisms of allergic asthma. For the current study, the *a priori* hypothesis is that mice that received HDM will have upregulated *ARG-1* compared to control mice that do not receive HDM or MCH. Studies in murine models of adult asthma have shown that exposure to ovalbumin, another common allergen, is associated with higher arginase-1 content in the lungs (Kenyon et al., 2008; King et al., 2004). In the group receiving MCH, we expected an upregulation of *ARG-1* compared to controls, but to a lesser extent than that seen in the HDM group. Previous research indicates that MCH can promote an allergy-specific inflammatory response as seen in heightened *IL-5* expression in mice 2-hours after MCH exposure (Caulfield et al., 2021; Jones, Caulfield, & Cavigelli, 2020). *ARG-1* transcription is induced by T_H2 cytokines, like IL-5, that are associated with allergic asthma (King et al., 2004). Therefore, it is expected that the group receiving MCH will also show heightened *ARG-1* expression due to the heightened inflammatory response. In addition to treatment-specific changes, it is expected that sex will significantly affect gene expression results with females observing greater changes in gene expression compared to males. Previous research has shown that females have a heightened T_H2 inflammatory response to allergen-challenge compared to males (Bekhbat & Neigh, 2018; Caulfield et al., 2017; Masuda et al., 2018).

This study examined gene expression at multiple timepoints (immediate, 4-hours and 24-hours) following final exposure to HDM or MCH. The study in mice aged P56 analyzed lung gene expression at immediate, 1-hour, 2-hour, 4-hour, 8-hour and 24-hour timepoints following

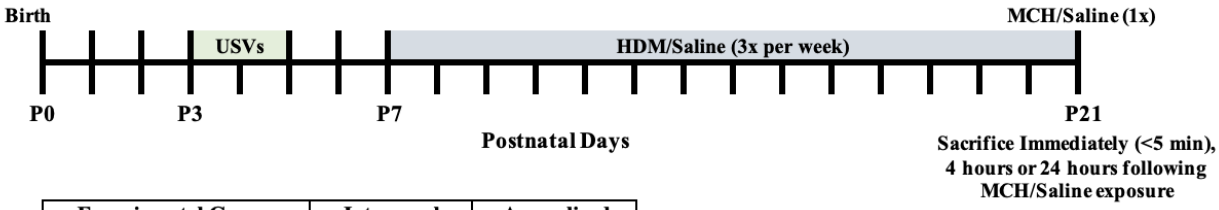
exposure to HDM or MCH with peak cytokine gene expression in the lungs occurring 4 hours after HDM exposure (Caulfield et al., 2021). As was seen in P56 mice, it is expected that peak lung gene expression will occur 4 hours following final exposure to HDM in mice at P21. Studying gene expression at 0-, 4- and 24-hours following exposure will allow us to document temporal changes in *ARG-1* expression over a 24-hour timeframe. Overall, the results of this study will illuminate early-life mechanisms of allergic asthma and may help inform potential treatment options, particularly for very young children.

Chapter 2
Materials and Methods

General Study Design

This study represented a component of a larger long-term project completed by Penn State's Behavioral Neuroendocrinology Lab. Mouse breeding, housing, corticosterone analyses and tissue collection was completed under the direction of Dr. Jasmine Caulfield, a former PhD candidate at Penn State. Mice included in this study were bred and tested through 2016, 2017 and 2019 across eight consecutive study waves. RNA extraction and gene expression analyses occurred between September 2020 through March 2021.

We used three experimental groups: airway inflammation (AI), bronchoconstriction (BR), and control (CON). Intranasal house dust mite extract (HDM) was administered to the AI group, while the BR group received aerosolized methacholine (MCH). The CON group received saline in place of HDM or MCH. Lung gene expression and serum corticosterone were analyzed following euthanasia. The timeline of administrations and experimental groups are outlined in Figure 2 below. The Pennsylvania State University IACUC committee approved all tests and procedures.



Experimental Groups	Intranasal HDM or saline (P7-P21)	Aerosolized MCH or saline (P21)
Control (CON)	saline	saline
Airway Inflammation (AI)	HDM	saline
Bronchoconstriction (BR)	saline	MCH

Figure 2 Experimental Timeline

Time points for house dust mite (HDM)/saline and methacholine (MCH)/saline administration. Intranasal HDM/saline administration occurred three times per week starting at P7. Aerosolized MCH/saline administration occurred one time at P21. On P21, mice were sacrificed at immediate (<5 mins), 4 hours, and 24 hours following the final intranasal and aerosolized doses, and lung tissue and a blood sample collected. USVs: ultra-sonic vocalizations, P: postnatal day.

Animals and Housing

BALB/cJ breeders purchased from Jackson Laboratories (Bar Harbor, ME) were bred in an animal facility at Penn State. Male and female mice were used in this study (N=95, 51 females, 44 males, see Table 1). Mice had unrestricted access to food and water and were housed in cages containing corn-cob bedding and a red polypropylene tube. A non-toxic Sharpie marker was used to identify individual pups from P3 to P9. On P10, each mouse received an identifying ear notch which replaced the ink markings. All procedures began at 10:00h, and lights for the lab were on a 12-hour light:12-hour dark cycle with lights off at 10:00h EDT. To control for natural variations in anxiety among pups prior to experimental exposures, ultra-sonic vocalizations (USVs) were measured once daily during post-natal days 3 through 5 and pups with high versus low USVs were distributed equally among experimental groups (Caulfield et al., 2017, 2018, 2021). As best as possible, mice from the same litter were evenly distributed across the three

experimental conditions. To determine acute responses to allergen and bronchoconstriction, mice were sacrificed at three timepoints (Immediate, 4-hours and 24-hours) following their final intranasal and aerosolized exposures on P21. Blood and lung tissue samples were collected.

Table 1 Experimental Subjects.

This study included 95 total subjects. Male and female subjects were distributed across 3 different timepoints after final treatment and 3 different treatment groups. CON: control. AI: airway inflammation. BR: bronchoconstriction.

Time After Treatment	Sex	Treatment Group	N
0 (Immediate)	Female	CON	6
		AI	5
		BR	6
		Total	17
	Male	CON	5
		AI	5
		BR	5
		Total	15
4	Female	CON	5
		AI	6
		BR	6
		Total	17
	Male	CON	5
		AI	5
		BR	4
		Total	14
24	Female	CON	5
		AI	6
		BR	6
		Total	17
	Male	CON	5
		AI	5
		BR	5
		Total	15
TOTAL			95

HDM and MCH Administration

Mice in the airway inflammation (AI) group were exposed to house dust mite extract (HDM) to induce lung inflammation. HDM extract was obtained from Greer Labs (*Dermatophagoides Pteronyssinus*, Lenoir, NC). HDM administration was performed following procedures previously outlined (Caulfield et al., 2017, 2018, 2020, 2021). The AI experimental group was exposed to HDM extract intranasally three times per week starting at P7. From P7-16, mice received a dose of 10 μg of HDM extract diluted in 10 μL of saline. From P17-21, mice received a dose of 15 μg extract diluted in 15 μL of saline, which was administered under brief isoflurane anesthesia. The CON and BR groups received intranasal saline solution in the same volume and on the same schedule as the AI group.

Methacholine (MCH) was used to induce bronchoconstriction according to procedures previously outlined (Caulfield et al., 2017, 2018, 2021). Once, on P21, immediately after intranasal HDM or saline exposure, mice were placed in a plethysmograph chamber (diameter 7.5cm and height 7cm) to administer aerosolized MCH. In the chamber, the mice were given 3 minutes to adapt to the chamber, 3 minutes to measure baseline breathing, and then five 3 minute-sessions of exposure to MCH in the case of the BR group or saline in the case of the CON and AI groups. The BR experimental groups received an initial saline dose and then four phases of MCH exposure at increasing doses of 6.25, 12.5, 25, 50 ng/mL in 100 μL of saline. While in the chamber, enhanced pause (Penh) values were recorded via FinePointe software and incidences of labored breathing were tallied to quantify bronchoconstriction.

Corticosterone Analysis

Following euthanasia, cardiac puncture was used to collect a blood sample into ice-cold vials. The samples were centrifuged at 4°C for 15 minutes at 12,000*g*. Serum was stored at -80 °C. Corticosterone was measured in serum using a radioimmunoassay kit (MP Biomedicals, Solon, OH, USA) following a slightly altered guideline (Caulfield et al., 2017, 2018, 2021).

Gene Expression

Lung tissue was dissected and samples from the left lobe and the inferior right lobe were immediately put into RNA Later and stored at -80 °C. The samples were processed following procedures previously utilized by the lab (Caulfield et al., 2017, 2018, 2020, 2021). 600μL of TRIzol™ (Invitrogen, USA) was used to extract RNA from lung tissue and the lungs were promptly homogenized. 240μL of chloroform was added, tubes were incubated, then centrifuged at 12,000*g* for 15 minutes at 4°C. The clear aqueous phase was removed from the tubes and mixed with 600μL of isopropanol. Samples were incubated for 10 minutes and then centrifuged for 10 minutes at 12,000*g* at 4°C. Supernatant was removed from the tubes and 1200μL of 75% ethanol added to the remaining sample. Samples were centrifuged once again at 7,500*g* for 5 minutes at 4°C. Supernatant was again removed and the remaining RNA pellet was left to dry until the pellet turned from white to translucent. Once dried, 50μL of RNase-free water was added to each tube and then incubated in a heat block for 12 minutes at 55°C. Samples were stored at -80°C until cDNA was made.

RNA concentrations, 260/280 values and 260/230 values were determined with a NanoDrop™ spectrophotometer (Thermo Fisher Scientific, USA). RNA concentrations between

200 and 500 ng/μL were used. If the RNA concentration was above 500, samples were diluted. A High-Capacity cDNA Reverse Transcription kit was used to reverse transcribe cDNA from RNA (Applied Biosystems, USA). cDNA was stored at -20°C until polymerase chain reaction (PCR) was conducted.

PerfeCTa SYBR Green SuperMix with ROX (Quantabio, USA) was used to conduct real-time polymerase chain reaction (PCR). An Applied Biosystems StepOnePlus Real-Time PCR system was used to determine relative abundances of the Arginase-1 gene using the $2^{-\Delta\Delta CT}$ method. Each sample was run in triplicate, for 45 cycles, at 55°C on 96-well PCR plate. Beta-actin was used as the reference gene to calculate ΔCT values. The reference group for calculating $\Delta\Delta CT$ was the average male control ΔCT value at the immediate timepoint following treatment. NCBI GenBank was used to determine forward and reverse primers for PCR. Primers were obtained from Integrated DNA Technologies (Coralville, Iowa, USA). For beta-actin, the forward sequence was 5'-GCCCTGAGGCTCTTTTCC-3' and the reverse sequence was 5'-TGCCACAGGATTCCA-3'. For arginase-1, the forward sequence was 5'-ACACTCCCCTGACAACCAGC-3' and the reverse sequence was 5'-GGTCTACGTCTCGCAAGCCA-3'.

Statistical Analyses

IBM SPSS Statistics Version 27 was used to conduct statistical analyses. Data that did not follow a normal distribution were natural log transformed. Skewness and kurtosis values were used to determine normal distributions. The following variables were natural log-transformed for analysis: gene expression in the lungs and serum corticosterone data at the 24-

hour timepoint. The remaining outcome variables, including serum corticosterone data at the immediate and 4-hour timepoints, were not natural log-transformed. An initial 4-way analysis of variance (ANOVA) was run with treatment group (CON, AI, BR), sex (male, female), time after treatment (immediate, 4-, 24-hour timepoints) and wave (2, 6, 7, 8, 9, 11, 15, 16) as factors for the outcome variable of log-transformed *ARG-1* expression data ($\ln(2^{-\Delta\Delta CT})$). Once significant factors were determined, multiple ANOVAs were conducted to determine the temporal effect of each experimental condition and to determine the influence of experimental condition at each timepoint. To determine the influence of time within each experimental condition, time after treatment, sex and wave number were used as factors for the outcome variables of *ARG-1* data or serum corticosterone measures. Factors, excluding time after treatment, that were not significant in the initial ANOVA were eliminated and the ANOVA re-run without them. Significance was set as a p-value of less than 0.05. The same process was used to determine the influence of experimental condition at each timepoint. Experimental condition (CON, AI, BR), sex (male, female) and wave (2, 6, 7, 8, 9, 11, 15, 16) were used as factors for the outcome variables of *ARG-1* expression data ($\ln(2^{-\Delta\Delta CT})$) and serum corticosterone measures separately for each timepoint following treatment. For all ANOVAs with a significant experimental condition effect, I ran post-hoc pairwise comparisons between all three experimental groups. I reported which pairs were significantly different from one another with a p-value of less than 0.05. Because significant wave effects are not germane to the research questions, the factor was included in ANOVA to control for its effect but was not reported in the results section. Non-log transformed averages and measures of standard error were calculated using SPSS and graphed using Excel. No significant main or interaction effects of sex were observed in any of the analyses, so males and females were graphed together. Because some research suggests a connection between

cortisol and arginine metabolism, a bivariate correlation analysis with variables of serum corticosterone and log-transformed arginase-1 gene expression data ($\ln(2^{-\Delta\Delta CT})$) was also conducted within each timepoint.

Chapter 3

Results

Effect of Time on Circulating Corticosterone Concentration

Time after HDM or MCH exposure had a significant effect on serum corticosterone levels in all three treatment groups. Within the control group, there was a significant main effect of time post treatment on serum corticosterone levels ($F_{2,27}$=6.267, p=0.006, Figure 3). The control group at the immediate timepoint had a corticosterone level that was on average 2 times greater than the level at the 4- and 24-hour timepoints. We previously predicted that the immediate timepoint would have the greatest serum corticosterone levels, so this part of our *a priori* hypothesis was supported.

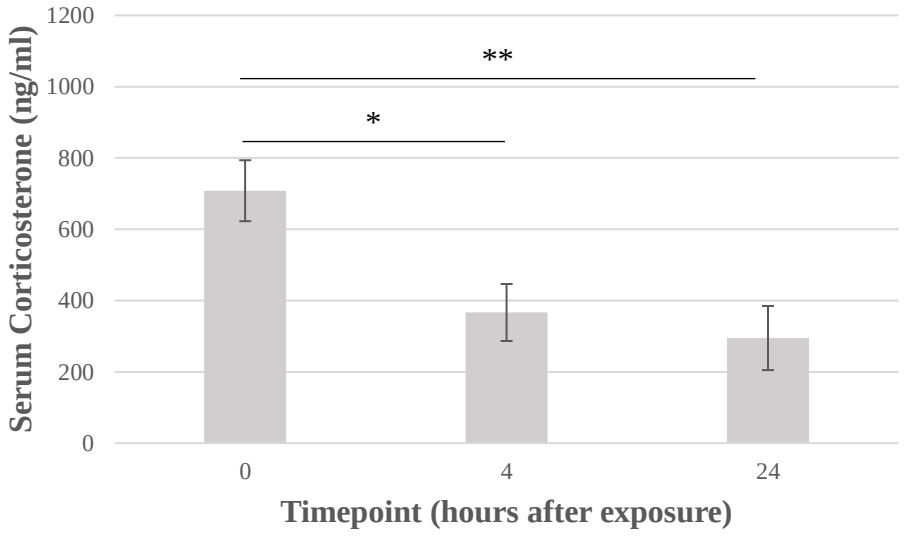


Figure 3 Serum Corticosterone Levels in Control Group.

Within the control (CON) group, significant differences were seen between the immediate timepoint and the 4-hour timepoint (p=0.013) and the 24-hour timepoint (p=0.003). n= 10-11 mice per group. *p ≤ 0.05, **p≤ 0.01

Within the airway inflammation (AI) group, there was a significant main effect of time after treatment on serum corticosterone levels ($F_{2,29}$=20.527, p=<0.001, Figure 4). The immediate timepoint in the airway inflammation group had significantly higher serum corticosterone. Serum corticosterone at the immediate timepoint was twice as high as concentrations at the 4- and 24-hour timepoints.

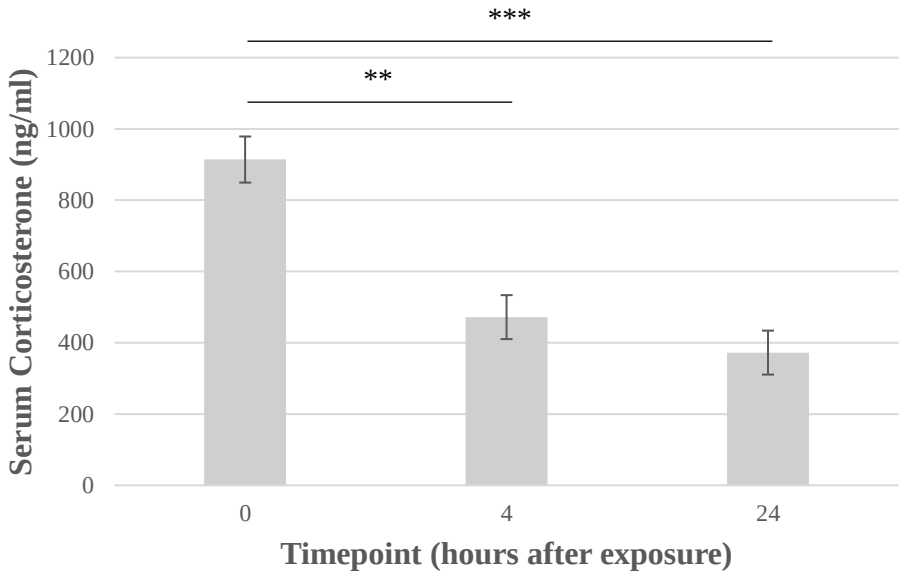


Figure 4 Serum Corticosterone Levels in Airway Inflammation Group.

Within the airway inflammation (AI) group, significant differences were seen between the immediate timepoint and the 4-hour timepoint (p=0.002) and the 24-hour timepoint (p<0.001). n= 10-11 mice per group. **p≤ 0.01, ***p≤ 0.001

Within the bronchoconstriction group, there was a significant main effect of time after treatment on serum corticosterone levels ($F_{2,30}$=6.626, p=0.004, Figure 5). Again, serum corticosterone in the bronchoconstriction group was approximately twice as high at the immediate timepoint compared to the 4- and 24-hour timepoints.

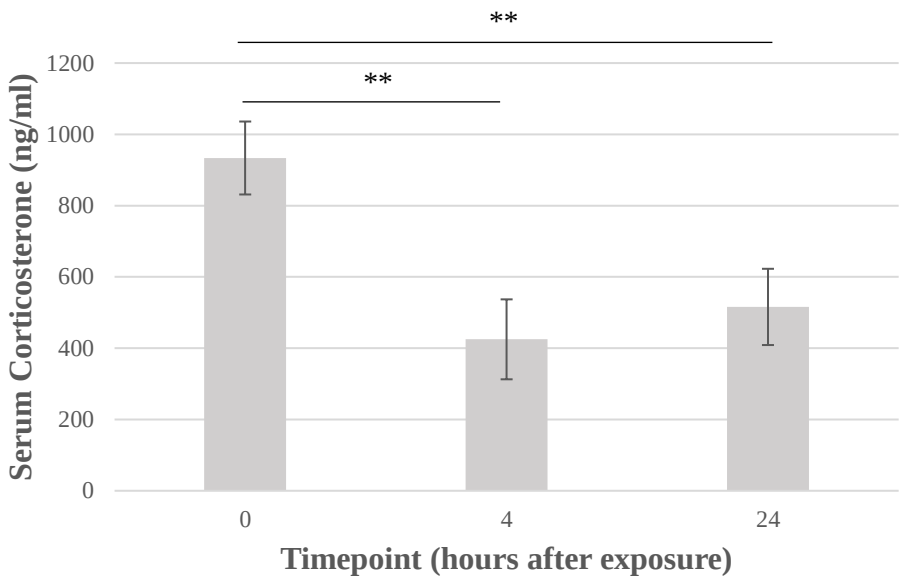


Figure 5 **Serum Corticosterone Levels in Bronchoconstriction Group.**

Within the bronchoconstriction (BR) group, significant differences were seen between the immediate timepoint and the 4-hour timepoint (p=0.002) and the 24-hour timepoint (p=0.008). n= 10-11 mice per group. **p≤ 0.01

Effect of Time on *ARG-1* Expression within each Treatment Group

Unexpectedly, when each treatment group was analyzed individually to test for effects of time following treatment, no significant differences in *ARG-1* expression were observed across time for the CON ($F_{2,28}$=1.123, Figure 6), AI ($F_{2,29}$=1.454, Figure 7) or BR ($F_{2,20}$=1.484, Figure 8) groups.

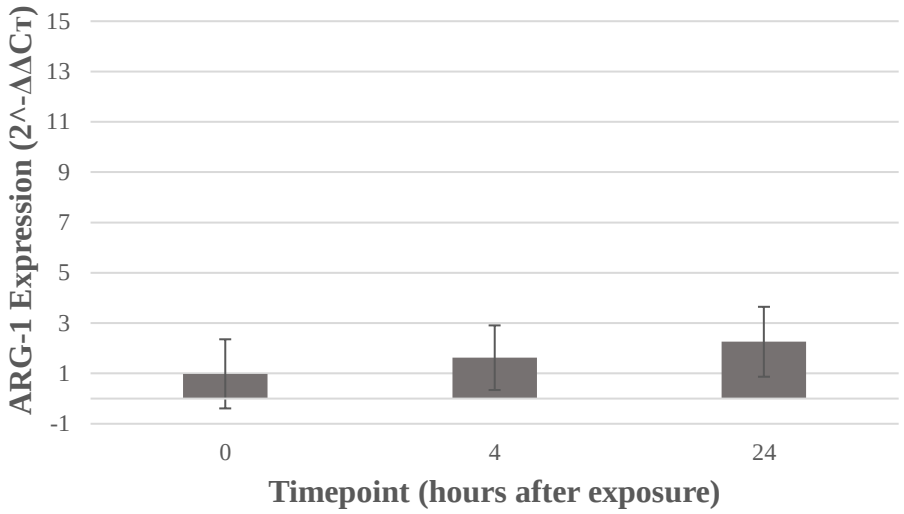


Figure 6 Arginase-1 Gene Expression in the Lungs of Control Group.

Within the control group, there were no significant differences in *ARG-1* expression across time (Immediate <5 minutes, 4-hours, 24-hours). p-value for time after treatment = 0.34. n= 10-11 mice per group.

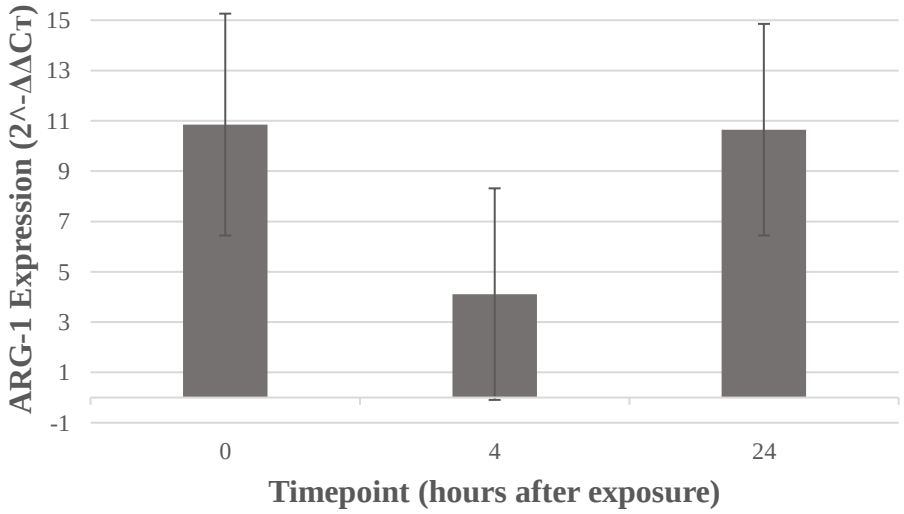


Figure 7 Arginase-1 Gene Expression in the Lungs of Airway Inflammation Group.

Within the airway inflammation (AI) group, there were no significant differences in *ARG-1* expression across time (Immediate <5 minutes, 4-hours, 24-hours). p-value for time after treatment = 0.25. n= 10-11 mice per group.

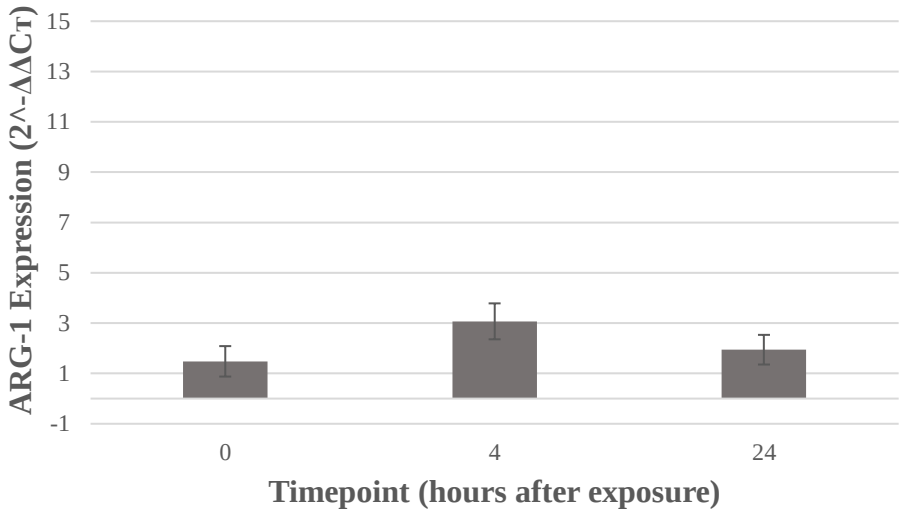


Figure 8 Arginase-1 Gene Expression in the Lungs of Bronchoconstriction Group.

Within the bronchoconstriction (BR) group, there were no significant differences in *ARG-1* expression across time (Immediate <5 minutes, 4-hours, 24-hours). p-value for time after treatment = 0.25. n= 10-11 mice per group.

Effect of Treatment Group on Circulating Corticosterone Concentration

At the immediate timepoint, treatment group was found to have a significant main effect on serum corticosterone levels ($F_{2,30}$=3.441, p=0.049, Figure 9) with the AI group and BR group showing higher serum corticosterone levels compared to the CON group according to pairwise comparisons.

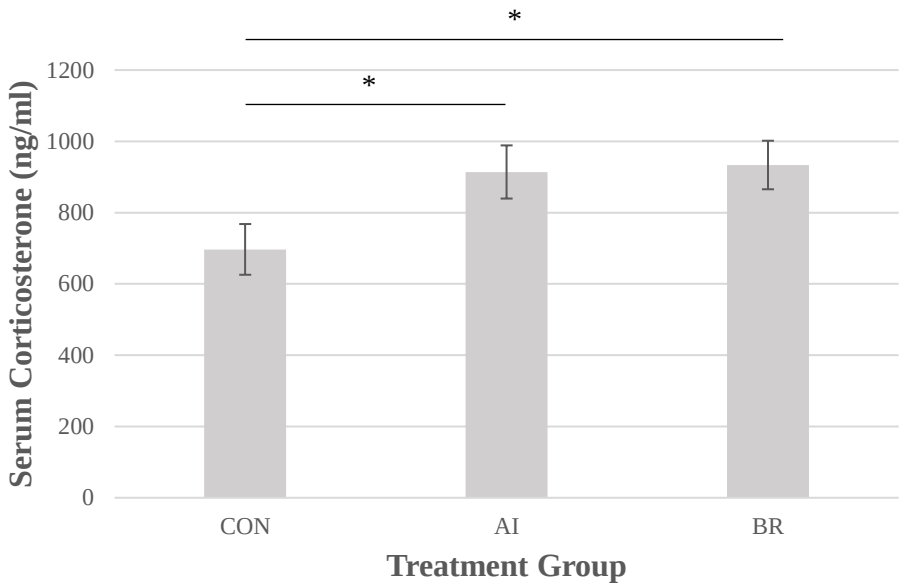


Figure 9 Serum Corticosterone Levels at Immediate Timepoint.

Immediately (<5 minutes) following exposure to HDM, MCH or saline, the AI group had significantly higher corticosterone than the CON group (p=0.044) and BR group (p=0.023). n= 10-11 mice per group. *$p \leq 0.05$

Treatment group did not have a significant main effect on serum corticosterone levels at 4-hours ($F_{2,28}$=0.155, Figure 10) or 24-hours ($F_{2,28}$=0.089, Figure 11).

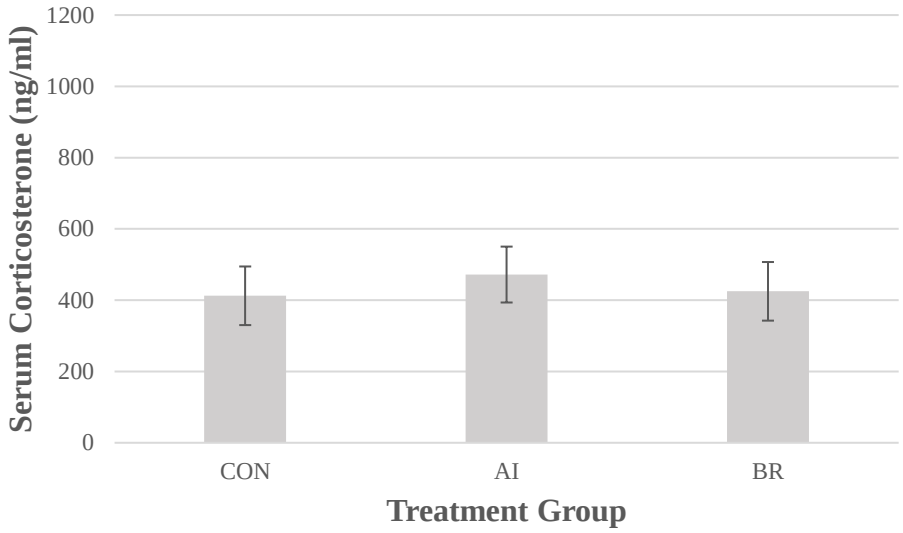


Figure 10 Serum Corticosterone Levels at 4-hour Timepoint.

Four hours following exposure to MCH or saline, no significant differences were determined across experimental groups. P-value for experimental group= 0.857. n= 10-11 mice per group.

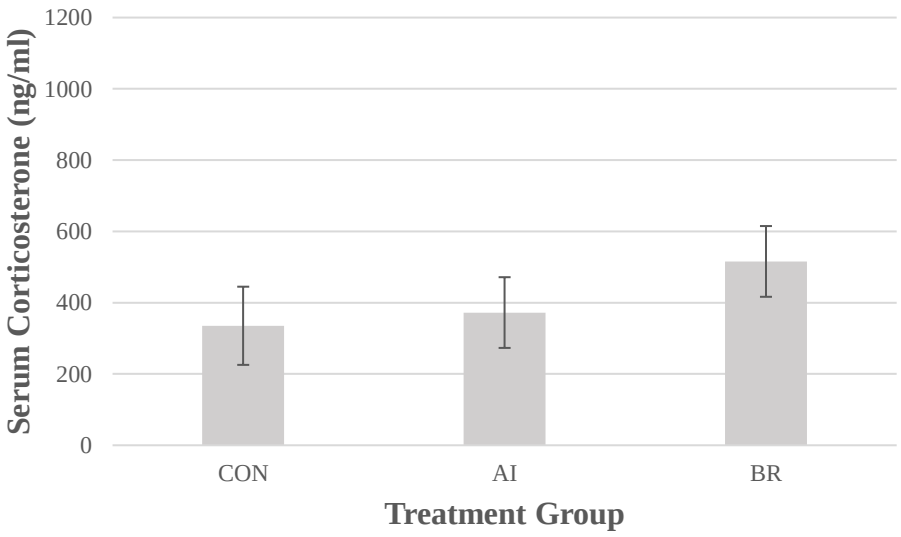


Figure 11 Serum Corticosterone Levels at 24-hour Timepoint.

24 hours following exposure to MCH or saline, no significant differences were determined across experimental groups. P-value for experimental group= 0.266. n= 10-11 mice per group.

Effect of Treatment Group on *ARG-1* Expression within each Timepoint

When each timepoint was analyzed separately, treatment group had a significant main effect on *ARG-1* expression in the lungs at the immediate ($F_{2,29}$=10.563, p<0.001, Figure 12) and 24-hour timepoints ($F_{2,29}$=7.266, p=0.015, Figure 14). At the immediate timepoint, the AI group had *ARG-1* expression in the lungs that was 11 times greater than the CON group and roughly 8 times higher than the BR group.

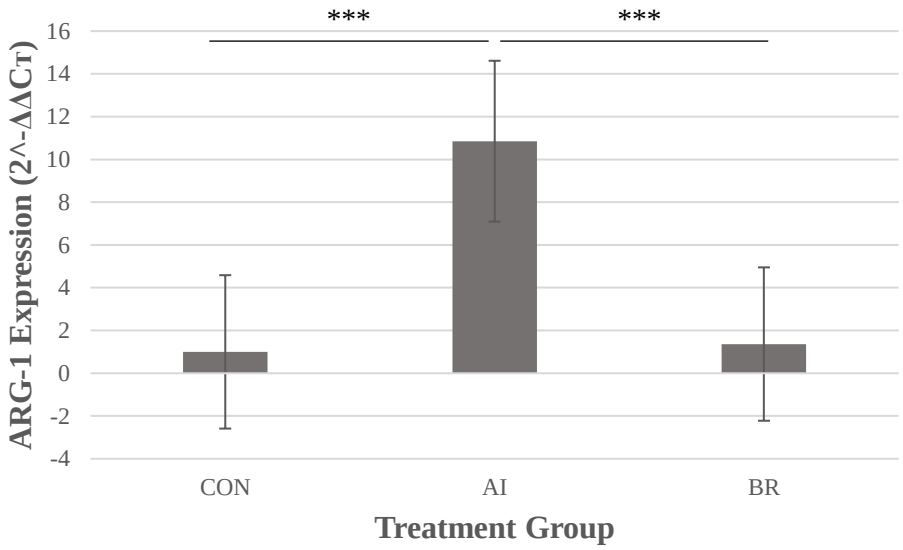


Figure 12 Arginase-1 Gene Expression in the Lungs at Immediate Timepoint.

Immediately (<5 minutes) following exposure to MCH or saline, *ARG-1* expression in the lungs of the AI group was significantly upregulated compared to the CON group (p<0.001) and BR group (p=0.001). n=10-11 mice per group. ***p≤ 0.001

At the 4-hour timepoint, treatment group was found to have a marginally significant main effect on *ARG-1* expression in the lungs ($F_{2,28}$=2.777, p=0.079, Figure 13). Pairwise comparisons indicated that the AI group has 2.5 times higher *ARG-1* expression in the lungs compared to the BR group, but significant differences were not observed between the AI and CON groups at the 4-hour timepoint.

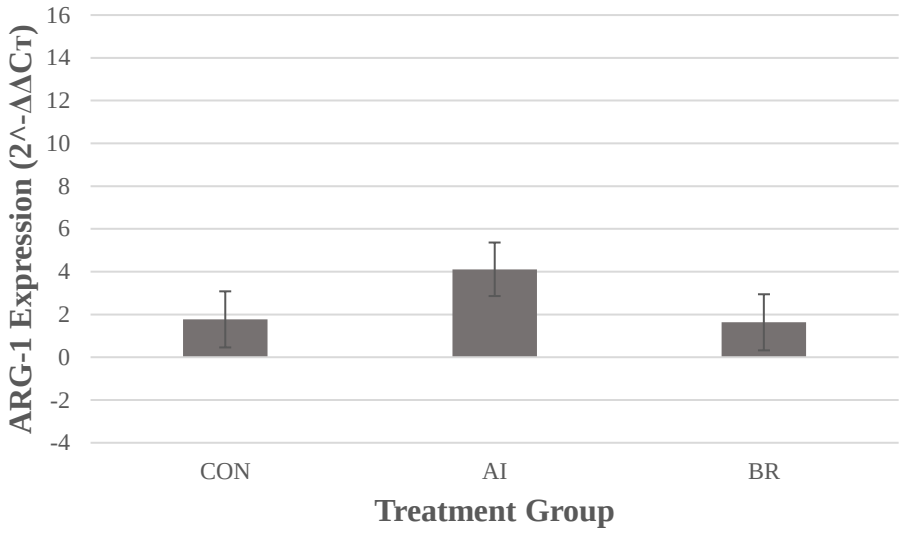


Figure 13 Arginase-1 Gene Expression in the Lungs at 4-hour Timepoint.

Four hours following exposure to MCH or saline, *ARG-1* expression in the lungs of the AI group was significantly upregulated compared to the BR group (p=0.039). n=10-11 mice per group. *p ≤ 0.05

At the 24-hour timepoint, treatment group had a significant main effect on *ARG-1* expression in the lungs ($F_{2,29}$=7.266, p=0.015, Figure 14). Pairwise comparisons indicated that the AI group had roughly 5 times higher *ARG-1* expression in the lungs compared to the BR group and 3 times higher *ARG-1* expression in the lungs compared to the CON group.

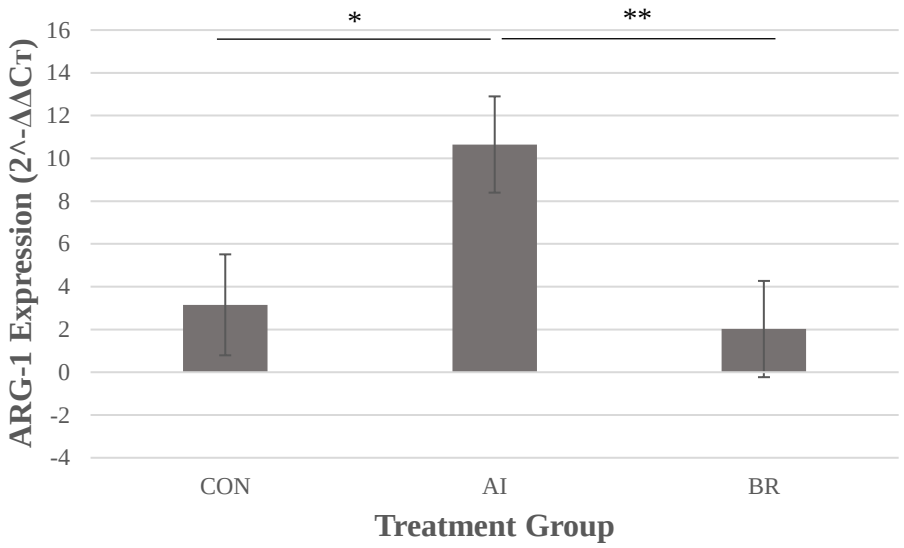


Figure 14 Arginase-1 Gene Expression in the Lungs at 24-hour Timepoint.

24 hours following exposure to HDM, MCH or saline, *ARG-1* expression in the lungs was significantly upregulated in the HDM-exposed (AI) group compared to the CON group (p=0.031) and MCH-exposed (BR) group (p=0.006). n=10-11 mice per group. *p ≤ 0.05, **p≤ 0.01

The results support our *a priori* hypothesis that the group receiving HDM, or the airway inflammation (AI) group would have higher expression of *ARG-1*. However, these results do not support the hypothesis that MCH exposure will also be associated with higher *ARG-1* expression or that the 4-hour timepoint would show peak changes in gene expression.

Correlation Between Serum Corticosterone and *ARG-1* Measurements

Results from the bivariate correlation analysis indicated no correlation between serum corticosterone and *ARG-1* expression data. Pearson correlation coefficient between the natural log of serum corticosterone concentrations (ng/mL) and the *ARG-1* expression data (ln(2^-ΔΔCT)) was 0.073 with a two-tailed significance value of 0.487 (N=94). Within each timepoint,

correlations between *ARG-1* expression data and serum corticosterone concentrations were also calculated with values shown in Table 2 below.

Table 2 Pearson Correlation Coefficients between Serum Corticosterone and *ARG-1* Expression Data.

Within each timepoint, a univariate correlation between natural log-transformed *ARG-1* and serum corticosterone data was determined. 0 hours following final treatment, the Pearson correlation coefficient was 0.217 with a significance value of 0.233. 4 hours following final treatment, the Pearson correlation coefficient was 0.273 with a significance value of 0.138. 24 hours following final treatment, the Pearson correlation coefficient was -0.049 with a significance value of 0.793. A p-value of less than 0.05 was considered statistically significant. No statistically significant correlations were determined.

***ARG-1* ln(2^-ΔΔCт)**	**LN Adjusted Serum Corticosterone Dose**		
	Hour 0	Hour 4	Hour 24
Hour 0	0.217		
Hour 4		0.273	
Hour 24			-0.049

Physiological Results

In addition to analyses on serum corticosterone and *ARG-1* expression in the lungs, labored breathing and enhanced pauses (Penh) were measured during MCH administration to verify its effectiveness in eliciting bronchoconstriction. Results recorded during the third and fourth doses of MCH at P21 indicated that treatment group had a significant effect on the number of labored breathing events ($F_{2,92}$=92.423, p<0.001, Figure 15 top) and average Penh values ($F_{2,73}$=6.657, p=0.002, Figure 15 bottom). Mice in the BR group experienced a greater number of labored breathing events and had increased average Penh values compared to the CON group and AI group at P21. These results confirm that MCH-induced bronchoconstriction is apparent in mice receiving a single bout of MCH administration at P21.

To compare the effects of a single MCH treatment to repeated MCH treatments throughout development, I compared my results to those from a prior study, in which mice received additional HDM (3x/week) and MCH administrations (1x/week) until P56 (Caulfield et al., 2018). Data from P21 and P56 are included in Figure 15 to illustrate how mice at P21 that received fewer treatments showed a pattern comparable to the labored breathing and Penh data from mice that received treatments until P56.

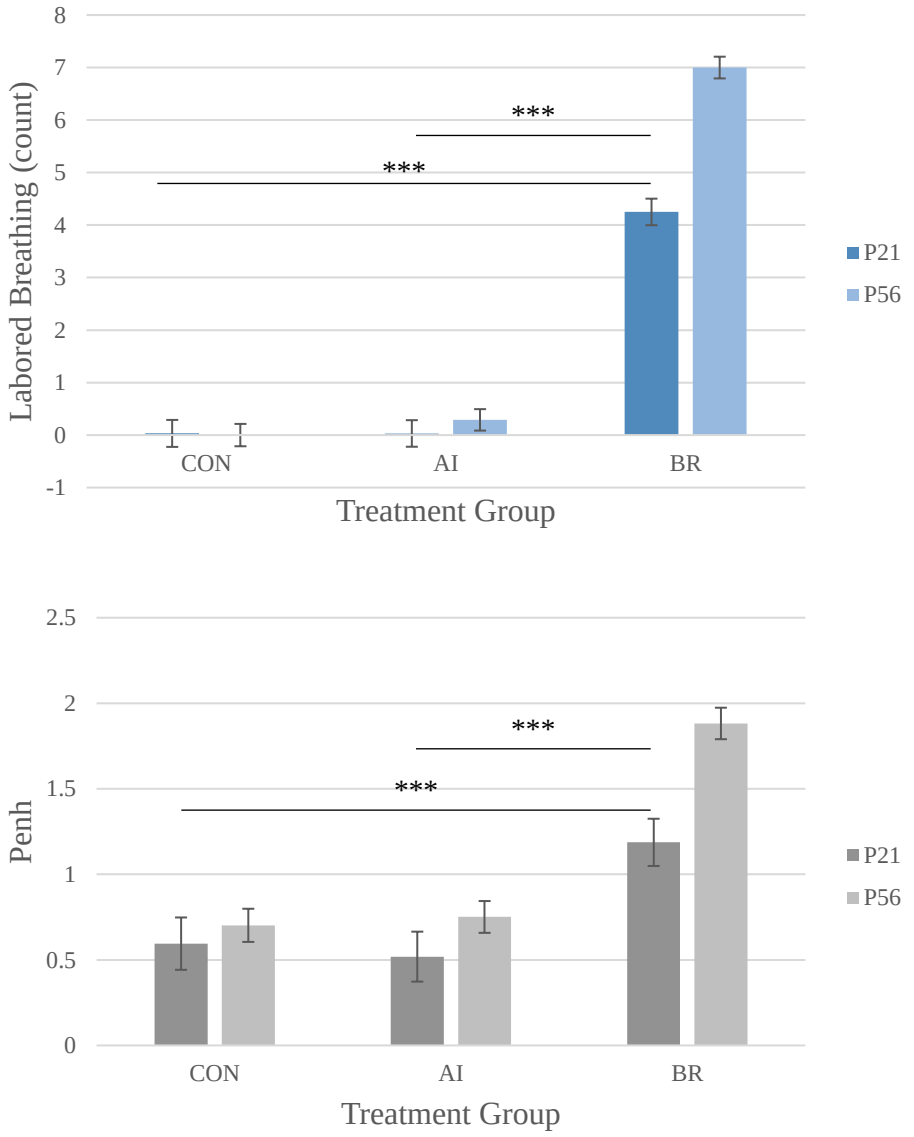


Figure 15 Labored Breathing Events and Enhanced Pause (Penh) Values at P21 and P56.

Top: The total number of labored breathing events during the 3rd and 4th doses of methacholine (25, 50 ng/mL in 100 μL of saline respectively) at P21 and P56 for CON, AI, and BR groups. Mice that received MCH experienced a higher number of labored breathing events compared to the CON group and the AI group at P21 with a similar pattern observed at P56. **Bottom**: The average Penh values for the 3rd and 4th doses of methacholine (25, 50 ng/mL in 100 μL of saline respectively) at P21 and P56 for CON, AI, and BR group. Mice that received MCH experienced higher Penh values compared to the CON group and AI group at P21 with a similar pattern observed at P56. ***$p \leq 0.001$

Chapter 4

Discussion

In this current study, house dust mite (HDM) and methacholine (MCH) were used to induce airway inflammation (AI) and bronchoconstriction (BR), two hallmarks of allergic asthma, in young male and female BALB/cJ mice. Mice received intranasal HDM or saline (in the case of controls) three times per week from P7 through P21. On P21, mice received either aerosolized MCH or saline for the controls. Either immediately (<5 minutes), 4-hours, or 24-hours following final intranasal and aerosol treatments, lung tissue and blood samples were collected. Lung RNA was analyzed to determine how HDM or MCH exposure influenced *ARG-1* expression. The two major objectives of this study were first to determine if HPA regulation is altered as early as P21 and second to determine if short-term allergic asthma symptoms early in life change *ARG-1* expression in the lungs. Our *a priori* hypothesis for this experiment was that serum corticosterone levels would be blunted in mice exposed to HDM with significant changes expected at the immediate and 4-hour timepoints, and with overall higher levels in females compared to males. The second major *a priori* hypothesis was that mice exposed to HDM or MCH will observe an upregulation of *ARG-1* in the lungs compared to the control group and these changes will occur in a time- and sex-dependent manner.

Single administration event of methacholine induced labored breathing and heightened Penh values.

Although methacholine is typically used to diagnose asthma and assess airway hyperresponsiveness, it was used in this study to induce labored breathing in mice to mimic the allergic asthma symptom seen in humans (Ahmad et al., 2012; Davis & Cockcroft, 2012).

Results from this study found that very young mice (P21) receiving methacholine had an increased number of labored breathing events and heightened Penh values during the 3rd and 4th dose of methacholine compared to mice receiving saline. These results from P21 showed a similar pattern as results from mice at P56, indicating that a single administration of methacholine at P21 can induce labored breathing and bronchoconstriction mimicking allergic asthma symptoms seen in humans (Caulfield et al., 2018). It is important to note that mice at P56 received MCH exposure once per week from P21 through P56. Similar findings about methacholine's effectiveness in inducing labored breathing have been reported in previous studies using this mouse model of adolescent asthma (Caulfield et al., 2017, 2021). This finding provides further validation of this mouse model of allergic asthma, particularly in very young mice with minimal exposure to experimental conditions.

No sex differences in serum corticosterone levels or lung ARG-1 expression at post-natal day 21.

Unexpectedly, sex was not a significant factor in influencing serum corticosterone or *ARG-1* expression in the lungs, likely due to the young age of the mice in this study. We anticipated that serum corticosterone levels in female mice would be higher than males and that more significant changes in *ARG-1* expression would be apparent in female mice. Previous research has identified that female rodents have stronger responses to acute stress including increased corticosterone and adrenocorticotropic hormone levels (Heck & Handa, 2019). In addition, a prior study using the same mouse model of allergic asthma determined that female mice elicit a greater T_H2 inflammatory response and greater signs of lung inflammation than male mice, including higher IL-4 and IL-5 expression (Caulfield et al., 2018). This has been shown by others using different methods and in young humans as well (Almqvist, Worm, &

Leynaert, 2008; Carey et al., 2007; Melgert et al., 2005; Wada, Okuyama, Ohkawara, Takayanagi, & Ohno, 2010). However, sex-dependent differences in corticosterone and *ARG-1* were not observable in this study in mice at P21. Although these findings did not support our initial hypothesis, the results make sense when we consider the very young age of mice in this study. Gonadal steroid hormones play a large role in modulating HPA activity and underlie the sex differences in HPA axis function (Handa & Weiser, 2014). Significant changes in HPA function in rodents occur during puberty (which begins on average at post-natal day 42 in mice) when significant increases in sex steroid concentrations are seen (Dutta & Sengupta, 2016; Handa & Weiser, 2014; Panagiotakopoulos & Neigh, 2014). Mice in this study were well-before this timepoint in development, which could explain why no significant sex-dependent changes in serum corticosterone levels were observed here. Translating these findings to humans, there have been mixed reports on sex differences in human HPA axis function prior to puberty with some studies concluding that the HPA axis functioning in male and female children does not differ prior to puberty while others have documented sex-dependent HPA axis differences as early as the neonatal period (Panagiotakopoulos & Neigh, 2014).

A similar explanation may help clarify why no sex differences were found in *ARG-1* expression. Initially we predicted that females would observe higher upregulation in *ARG-1* due to the heightened T_H2 immune response in females compared to males (Bekhbat & Neigh, 2018; Caulfield et al., 2017; Masuda et al., 2018). In contrast to our findings, prior research has shown that adult males and females differ in arginine-metabolism and expression levels in response to mild stress (Cloots et al., 2017; Liu, Li, Su, Wang, & Jiang, 2019). However, studies specifically focused on sex-differences in *ARG-1* expression during development are limited. Males and females may have shown no statistically signifcant differences in expression data due to the

young age of mice in the study. Many sex-differences in immune responses, particularly pro-inflammatory responses, begin at puberty (Klein & Flanagan, 2016). Mice in this study may not have been far enough along in development to show sex-differences in *ARG-1* expression. Moving forward, these results help elucidate the development of HPA mechanisms and *ARG-1* expression in male and female mice and have applications for future asthma studies using this murine model.

Mice in all experimental groups had pronounced deviations in serum corticosterone levels immediately after intranasal/aerosol exposures compared to other timepoints. The AI and BR groups had significantly higher serum corticosterone levels compared to the control group.

Mice in this study had elevated serum corticosterone levels immediately following intranasal and aerosolized exposures. We hypothesized that serum corticosterone levels would be blunted in mice receiving HDM based on results from prior research in mice at P56 (Caulfield et al., 2021). In humans, repeated stress early in life can result in lower cortisol levels later in childhood (Dreger et al., 2010). These findings have been replicated in rodents with evidence to suggest that early life or adolescent stress can be associated with blunted corticosterone levels and dysregulated HPA axis later in life (Caruso, Kamens, & Cavigelli, 2017; Caulfield et al., 2021; Pohl, Olmstead, Wynne-Edwards, Harkness, & Menard, 2007; Sotnikov et al., 2014). In this study, however, corticosterone levels were elevated, potentially because the mice here received minimal exposures to HDM and MCH, meaning that stress exposure was not recurrent enough to produce the typical blunted cortisol responses we initially expected. The elevated serum corticosterone showed that exposure procedures, whether that be handling or the intranasal/aerosol treatments themselves, provided significant stress to the mice.

Higher serum corticosterone in the AI group at the immediate timepoint compared to the BR group may indicate an allergen-specific stress response in mice at P21. Overall, these results validate that the experimental procedures produce a significant stress response. However, at P21, the repetitive aspect of the asthma model may not be fully developed enough to produce the blunted corticosterone levels that have been validated in prior research.

HDM-exposure leads to upregulated ARG-1 expression in the lungs at the immediate and 24-hour timepoints with marginal differences at the 4-hour timepoint.

We initially hypothesized that *ARG-1* expression in the lungs would be upregulated following HDM and MCH exposure. *ARG-1* expression upregulation in the lungs was confirmed with the results from this study at the immediate and 24-hour timepoints following final intranasal HDM treatments. The upregulation of *ARG-1* expression following HDM exposure is aligned with previous research that showed allergen exposure caused higher ARG-1 protein content and activity in the lungs one to three hours following exposure to ovalbumin (Kenyon et al., 2008; King et al., 2004; North et al., 2009). Ovalbumin and house dust mite are both common allergens that can induce airway inflammation and asthma symptoms in murine models.

We additionally hypothesized that *ARG-1* expression in the lungs would be upregulated following MCH exposure, which was not supported by the results of this study. This was likely due to limited MCH exposure. Mice at P21 only received a single aerosolized dose of MCH. Although MCH did induce higher counts of labored breathing and heightened Penh values compared to HDM or saline exposure, these measures of bronchoconstriction at P21 were not as elevated as seen at P56. This indicates that younger mice may not experience the same extent of bronchoconstriction as older, more developed mice. For reference, mice at P56 received MCH once a week for six weeks (Caulfield et al., 2021). Therefore, it is possible that the minimal

MCH exposure in mice at P21 may explain why *ARG-1* expression in the lungs did not change like we initially hypothesized. Additional studies that examine *ARG-1* expression in the lungs in mice at P56 would be helpful to clarify the connection between MCH, bronchoconstriction and *ARG-1* expression in the lungs.

Our *a priori* hypothesis also predicted that peak gene expression changes would occur 4 hours following final intranasal or aerosolized exposures. However, time after treatment did not influence *ARG-1* expression in any of the treatment groups. In addition, at the 4-hour timepoint, the AI group had significantly heightened expression compared to the BR group, but not compared to the CON group.

A possible explanation for the pattern in *ARG-1* expression in the lungs with significant upregulation at immediate and 24-hour timepoints and marginal differences at the 4-hour timepoint may be circadian rhythms in arginase activity. Prior research reports a circadian rhythm in arginase activity with maximum enzyme activity in the liver prior to periods of darkness, or the active phase for nocturnal animals, and prior to a scheduled meal (Davidson, Castañón-Cervantes, & Stephan, 2004). Although we were focused on *ARG-1* expression in the lungs, not the liver, circadian rhythms directly related to the inflammatory processes in the lungs have been reported (Nosal, Ehlers, & Haspel, 2020). If the circadian rhythm reported in arginase activity in the liver also applies to arginase-1 activity in the lungs, then circadian rhythms in arginase activity may help explain the pattern seen here. Experimental procedures in this study started around the time the lights went off in the animal facilities based on the 12-hour light: 12-hour dark cycle. The immediate and 24-hours timepoints in this study occurred closest to the beginning of the dark period, which could, in part, explain the significant upregulation of *ARG-1* expression at these two timepoints. Therefore, circadian rhythms cannot be ruled out as a

possible explanation for the trend in arginase expression seen here. However, the marginal differences between the BR and AI group at the 4-hour timepoint following treatment may indicate a treatment-specific effect independent of circadian rhythms in arginase activity.

General Conclusions and Future Directions

Results from this study validate this murine model of developmental asthma at post-natal day 21 while also confirming that allergen exposure may alter *ARG-1* expression in the lungs. However, a repeated study with a larger sample size would be helpful to substantiate these findings. In addition, it would be valuable to expand the number of timepoints included beyond immediate, 4-hours and 24-hours following intranasal and aerosolized exposures. With increased timepoints, we could better illustrate the temporal effects of these exposures on gene expression and HPA axis regulation. There is potential to study *ARG-1* expression in the lungs in mice at P56 using this same asthma model to determine how arginase activity can be altered with increased HDM and MCH exposures. Future studies should also attempt to research more specific HPA axis mechanisms beyond serum corticosterone. There is also an opportunity to analyze connections between arginase dysregulation and behavioral measures in mice. Because prior research has shown connections between arginase activity and depression in mice (Pervin et al., 2021), and our results show upregulated *ARG-1* in response to HDM exposure, there is an opportunity to further explore depression behaviors in a mouse model this young.

These findings have applications not only for future studies investigating asthma mechanisms using this mouse model but also for studies on asthma treatment options for very young children. Arginase inhibitors have already been targeted as a potential new treatment for

asthma and allergic rhinitis with the possibility of offering anti-inflammatory and bronchoprotective effects (Felix & Kuschnir, 2020). L-arginine administration to stabilize nitric oxide metabolism and alleviate allergic airway inflammation has also been explored as a potential treatment mechanism for asthma (Mabalirajan et al., 2010). Currently, one of the most common asthma treatments are inhaled corticosteroids (Beasley et al., 2019). An additional area for future research should focus on the interactions of supplemental glucocorticoids on patients with asthma who already may have a dysregulated HPA axis due to their symptoms. These results provide a foundation for future research into the roles of HPA axis mechanisms and *ARG-1* expression in asthma development among youth.

Chapter 5

Works Cited

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ACADEMIC VITA

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EDUCATION

The Pennsylvania State University Fall 2017-Fall 2021
Schreyer Honors College
Science BS/MBA Program
Bachelor of Science in General Science
Minors in Biology and French
Honors in Biobehavioral Health

Penn State Smeal College of Business Fall 2021-Spring 2023
Master of Business Administration

ACADEMIC AWARDS AND HONORS

President's Freshman Award
The Evan Pugh Scholar Senior Award
Schreyer Academic Excellence Scholar Award
Balog Science BS/MBA Program Award

RESEARCH EXPERIENCE

Behavioral Neuroendocrinology Lab Spring 2018-Summer 2021
Undergraduate Research Assistant under Dr. Sonia Cavigelli

Collaborated on project to determine neurobiological links between adolescent asthma and internalizing disorders like anxiety and depression

Presented results at 2019 BBH Research Forum and 2019 Penn State Undergraduate Research Exhibition with project titled, "Chronic house dust mite exposure causes lasting inflammatory impacts in lungs of mouse model"

Awarded 1st place research award in Health and Life Sciences Category at 2020 Penn State Undergraduate Research Exhibition with project titled, "Methacholine induces an acute inflammatory response in a mouse model of adolescent asthma"

PROFESSIONAL EXPERIENCE

Janssen Pharmaceuticals R&D Procurement Intern June 2020-August 2020
Johnson & Johnson Supply Chain Co-op July 2019-January 2020

VOLUNTEER ACTIVITIES

Science LionPride Ambassador to the Eberly College of Science
Science LionPride THON co-chair for Explorers Program
Science BS/MBA Mentoring Initiative Student Lead
English Tutor at Mid-State Literacy Council
Lecture Assistant for Introductory Chemistry Course (CHEM110B)