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BMI correlates with pollutant-induced interleukin-1 beta in sputum and blood

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Capsule Summary

Murine studies suggest a role for the NLRP3 inflammasome in obesity-driven airway inflammation; human studies are lacking. Our findings demonstrate that human *in vivo* ozone-induced airway and systemic inflammation, measured by IL-1 β , correlates with BMI.

Keywords

BMI; obesity; adiposity; asthma; inflammation; pulmonary inflammation; NLRP3; inflammasome; IL-1 β ; ozone

Introduction

Increasing BMI has been proposed to be associated with asthma development and with airway hyperresponsiveness that is harder to treat with conventional therapies^{1,2}. Increased

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Author Contributions:

- Conception and design of study – Todoric, Hernandez
- Data generation – Todoric, Mills, Hernandez
- Analysis and interpretation of data – Todoric, Zhou, Zhang, Hernandez
- Preparation or critical review of manuscript -- Todoric, Zhou, Zhang, Mills, Peden, Hernandez

BMI has been associated with ozone-induced decrements in spirometry³ in humans as well as with increased pulmonary inflammation following ozone (O₃) exposure in murine models of obese mice.^{4,5}

Murine studies have recently demonstrated that the NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome, responsible for release of mature IL-1 β from a cell, played an important role in obesity-induced airway hyperresponsiveness through the induction of IL-17a-producing innate lymphoid cells type 3 (ILC3)⁶. Furthermore, IL-1 β blockade was able to mitigate obesity-induced airway hyperreactivity⁶. Addition of the saturated fatty acid palmitate to *in vitro* cultures (macrophage) after LPS-priming was sufficient to induce IL-1 β release through NLRP3 activation.⁷ These two studies add to a growing body of evidence that the NLRP3 inflammasome may represent a central figure in BMI-driven pulmonary inflammation and enhanced susceptibility to environmental stimuli.

Previous work has shown that increased BMI influences O₃-induced decrements in spirometry in otherwise healthy human volunteers³. However, studies examining the relationship between pollutant-induced pulmonary inflammation in overweight and obese humans are still lacking. We hypothesized that increased BMI would be associated with increased airway inflammation after an environmental challenge with O₃.

Methods

In this retrospective study, we examined the data from a total of 40 individuals (see **Table I** for subject characteristics) who underwent a 0.4 part per million (ppm) O₃ exposure. These included healthy volunteers and mild, intermittent allergic asthmatics who required no daily controller medications. Subjects underwent a baseline screening visit 2 days to 2 weeks before O₃ exposure, with spirometry, sputum induction, and blood collection. Spirometry (Forced Vital Capacity, FVC and Forced Expiratory Volume, FEV1) was obtained immediately after the subject exited the O₃ chamber and sputum induction 4 hours after the end of O₃ exposure. Twenty-four hours after O₃ exposure, each subject returned for repeat sputum induction and blood collection. 18/40 individuals were overweight, defined by a BMI ≥ 25 .

Results

%Neutrophils (%PMNs), %Eosinophils (%Eos) and cytokine assessments for IL-1 β , IL-6, and IL-8 were reviewed from the 24 hour sputum and blood samples, comparing 24 post-O₃ to baseline values (See **Table 1**). Cytokine levels from sputum supernatants and from plasma were measured using multiplex technology (MesoScaleDiscovery/MSD, Gaithersburg, MD).⁸

Mann-Whitney tests were used to compare 24 hour post-O₃ %PMNS, %Eos, and cytokine levels to baseline levels. Spearman correlation analyses were performed to assess the impact of BMI on change in spirometry measures immediately after O₃ exposure, and on change in %PMNs, %Eos, and in cytokine levels at 24 hours. Linear regression analysis was first performed to assess the impact of BMI on changes (24 hour post-O₃ minus pre-O₃) in sputum %PMNs and sputum cytokine levels after adjusting for gender alone. A subsequent

linear regression analysis was then performed to adjust for gender, age, ethnicity, and asthma status.

FVC and FEV1 were significantly diminished immediately after exiting the O₃ chamber ($p<0.0001$ for both). Sputum %PMNs were significantly increased 24 hours after O₃ exposure ($p<0.0001$) as were levels of IL-6 ($p<0.001$) and IL-8 ($p<0.001$). Sputum %Eos increased after O₃ exposure, though not this did not reach significance ($p=0.06$). IL-1 β was not significantly elevated at 24 hours compared to baseline.

There was no significant correlation between BMI and change in FVC or FEV1. Spearman correlation analysis showed that increased BMI was positively and significantly correlated with 24 hour post-O₃ sputum IL-1 β levels ($r=0.5$, $p=0.004$) but not with IL-6 or IL-8. BMI was weakly correlated with change in sputum IL-1 β after O₃ exposure ($r=0.4$, $p=0.03$, Table I). Linear regression analysis adjusting for gender alone revealed that increased BMI was positively correlated with O₃-induced increase in sputum IL-1 β in females only ($p=0.0095$). After adjusting for gender, age, ethnicity, and asthma status, this positive and significant correlation held true for all individuals ($p=0.02$). In this analysis of all subjects, there was a 20 pg/ml increase in sputum IL-1 β for each 1 unit increase in BMI. BMI was not correlated with change in IL-6 or IL-8 (Table I).

There was no correlation noted with BMI and change in sputum %PMNs ($p=0.30$ or %Eos ($p=0.09$) at 24 hours. Furthermore, linear regression analysis adjusting for gender alone, and for gender, age, ethnicity, and asthma status did not demonstrate a significant correlation between BMI and change in sputum %PMNs.

Serum %PMNs significantly increased ($n=22$, $p<0.01$) while %Eos significantly decreased ($p=0.01$) 24 hours after O₃ exposure compared to baseline values. There was no significant change in serum IL-1 β , IL-6, or IL-8 ($n=14$; $p=0.7$, $p=0.8$, $p=0.8$, respectively). Spearman correlation analysis demonstrated that BMI was significantly correlated with change in serum IL-1 β after O₃ exposure ($r=0.7$, $p=0.003$) but not with change in %PMNs, %Eos, IL-6, or IL-8 (Table I).

Discussion

Our work is the first to show that *in vivo* airway and systemic inflammation, measured by IL-1 β , following O₃-exposure is positively correlated with BMI. Here, BMI is significantly correlated with change in IL-1 β but not with other measured indicators of inflammation including neutrophilia, eosinophilia, IL-6, and IL-8, after O₃-exposure. These findings suggest that NLRP3 inflammasome activation or priming with IL-1 β release as a unique feature of BMI-related airway inflammation in humans after pollutant exposure. Underlying mechanisms for this increased activity have been proposed in animal models^{6,7} but have not yet been explored in humans. We feel that these findings point to an important area of future study and could inform new strategies for therapeutic interventions in this population.

Limitations to our study include the retrospective nature, lack of information regarding other measures of adiposity, and fat content of the meal preceding O₃ exposure. In addition, we did not evaluate the effect of BMI on airway hyperreactivity as measured by methacholine

challenge. These are important factors to consider for future study as inflammatory-mediated effects of adiposity may differ at extremes of BMI⁹ and could be affected by lifestyle and medication use.

This is the first study to suggest that IL-1 β is uniquely involved in pollutant-induced BMI-related human inflammation.

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Abbreviations

BMI	body mass index
Eos	eosinophil
FEV1	forced expiratory volume in one second
FVC	forced vital capacity
ILC3	innate lymphoid cells type 3
IL-1β	interleukin 1 beta
IL-6	interleukin 6
IL-8	interleukin 8
LPS	lipopolysaccharide
NLRP3	NOD-like receptor family pyrin domain containing 3
PMN	polymorphonuclear leukocyte

REFERENCES

1. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. *Nat Med.* 2012; 18:716–725. [PubMed: 22561835]
2. Juel CT, Ulrik CS. Obesity and asthma: impact on severity, asthma control, and response to therapy. *Respir Care.* 2013; 58(5):867–73. [PubMed: 23258582]
3. Bennett WD, Hazucha MJ, Folinsbee LJ, et al. Acute pulmonary function response to ozone in young adults as a function of body mass index. *Inhal Toxicol.* 2007; 19(14):1147–54. [PubMed: 17987466]
4. Shore SA, Rivera-Sanchez YM, Schwartzman IN, et al. Responses to ozone are increased in obese mice. *J Appl Physiol.* 2003; 95:938–945. [PubMed: 12794034]
5. Johnston RA, Theman TA, Lu FL, et al. Diet-induced obesity causes innate airway hyperresponsiveness to methacholine and enhances ozone-induced pulmonary inflammation. *J Appl Physiol.* 2008; 104(6):1727–35. [PubMed: 18323466]
6. Kim HY, Lee HJ, Chang YJ, et al. Interleukin-17-producing innate lymphoid cells and the NLRP3 inflammasome facilitate obesity-associated airway hyperreactivity. *Nat Med.* 2014; 20(1):54–61. [PubMed: 24336249]
7. Wen H, Gris D, Lei Y, et al. Fatty acid-induced NLRP3-ASC inflammasome activation interferes with insulin signaling. *Nat Immunol.* 2011; 12(5):408–15. [PubMed: 21478880]

8. Hernandez M, Brickey WJ, Alexis N, et al. Airway cells from atopic asthmatic patients exposed to ozone display an enhanced innate immune gene profile. *J Allergy Clin Immunol.* 2012; 129(1):259–261. [PubMed: 22196529]
9. Celedon JC, Palmer LJ, Litonjua AA, et al. Body mass index and asthma in adults in families of subjects with asthma in Anqing, China. *Am J Respir Crit Care Med.* 2001; 164:1835–1840. [PubMed: 11734432]

Table 1

Subject characteristics, variable means before and after ozone exposure, and correlations of BMI with ozone-induced inflammatory endpoints.

Subject Characteristics				
N	Median BMI (25-75%)	Age (Mean + SD)	Gender (M/F)	Ethnicity
40 Total	24.1 (21.8-27.5)	24.4±4.3	25/15	27 W, 7 AA, 4 A, 2 B
31 Healthy Volunteers	24.1 (22.2-29.8)	24.2±5.7	23/8	20 W, 6 AA, 4 A, 1 B
9 Allergic Asthmatics	23.4 (20.0-27.2)	24.5±4.0	2/7	7 W, 1 AA, 1 B

Variable Means (95% Confidence Interval) Pre/Post O ₃ Exposure				
	FVC		FEV1	
	Baseline	Post O ₃	Baseline	Post O ₃
	% Predicted	106 (103-109)	94.59 (91.69-97.49)	102.9 (99.69-106)

	Sputum		Blood	
	Baseline	Post O ₃	Baseline	Post O ₃
	%PMN	37.4 (31.56-43.25)	62.66 (56.72-68.61)	51.42 (47.01-55.83)
%Eos	0.45 (0.16-0.74)	0.98 (0.17-1.8)	3.5 (2.5-4.5)	2.9 (1.9-3.8)
IL-1β (pg/mL)	276.1 (195.4-356.8)	320.4 (192.7-448)	0.068 (0.043-0.094)	0.1291 (0.067-0.192)
IL-6 (pg/mL)	91.28 (38.96-143.6)	210.2 (106.1-314.3)	0.9189 (0.703-1.135)	0.7656 (0.575-0.958)
IL-8 (pg/mL)	7713 (4932-10494)	15606 (9739-21473)	5.324 (3.136-7.512)	8.871 (2.7-20.44)

Correlations of Subject BMI with O ₃ -induced Inflammatory Markers				
Endpoint of Interest	Sputum		Blood	
	R ^a	p-value	R	p-value
%PMNs	−0.3	0.06	−0.2	0.4
IL-1β	0.4	0.03*	0.7	0.003*
IL-6	0.04	0.8	−0.08	0.7
IL-8	0.2	0.3	0.1	0.7

A, Asian; AA, African American; B, biracial; W, white

^aSpearman Correlation