Polyphenol-Rich Potato Extracts Exert Sex-Dimorphic Protective Effects on the Ozone-Induced Pulmonary Inflammatory Response in C57BL/6 Mice

by

Manyan Fung

A thesis submitted to McGill University in partial fulfilment of the requirements of the degree of Masters of Science

Faculty of Agricultural and Environmental Sciences
School of Dietetics and Human Nutrition
McGill University
Saint-Anne-de-Bellevue, Québec, Canada

Too many people dream of places they'll never go,
wish for things they'll never have,
instead of paying adequate attention
to their real lives.

Odo, Star Trek: Deep Space Nine

▪ TABLE OF CONTENTS ▪

ABSTRACT

Dietary polyphenols have been shown to improve diet-induced metabolic symptoms and pollution-related airway inflammation, yet very few studies have examined both contexts together. The current study investigated the efficacy of a polyphenolic-rich potato extract (PE) in ozone-exposed male (M) and female (F) C57BL/6 mice fed a high-fat Western-style diet (WD) based adiposity and glucose intolerance, and lung health. Mice were fed *ad libitum* with WD supplemented with either a 20 PE (chlorogenic acid, 40 mg/kg diet; ferulic acid, 1.2 mg/kg diet) or 100 PE (chlorogenic acid, 200 mg/kg diet; ferulic acid, 6 mg/kg diet). After 4 wk on the diets, animals were exposed to 0.8 ppm ozone or air by inhalation for 4 h and euthanized 24 h post exposure. Independent of ozone exposure, supplementation with 100 PE attenuated weight gain (37% M and 48% F) and significantly reduced ($p < 0.01$) adiposity and total fat mass relative to the untreated controls in both sexes. Both 20 and 100 PE treatments restored fasting blood glucose levels ($p < 0.001$) and improved insulin sensitivity ($p < 0.05$). Based on cellular evaluation from bronchoalveolar lavage fluid (BALF), ozone-induced pulmonary inflammation/injury was suppressed by 100 PE treatment as indicated by a reduction in alveolar macrophage cell counts (464 ± 12.1 F and 457 ± 16.7 M) below than the respective air-exposed group under the same treatment condition (490 ± 5.3 M and 477 ± 7.4 F). Additionally, a decrease in neutrophil cell counts (9 ± 0.7 M and 7 ± 0.9 F) relative to the ozone-exposed untreated group (13 ± 1.7 M and 11 ± 1.9 F) was observed. The 100 PE dose also reduced total protein concentrations in BALF ($p < 0.001$) in the ozone-exposed groups (106.09 ± 1.10 vs. 95.61 ± 0.83 μg/mL M and 82.45 ± 1.47 vs. 74.56 ± 0.68 μg/mL F). Similarly, epithelial counts in BALF were decreased due to 100 PE in the ozone-exposed groups (129 ± 20.5 vs. 75 ± 14.3 M and 137 ± 28.2 vs. 67 ± 18.2 F). All these markers signify acute lung injury. The lung inflammatory markers (IL-4, IL-6, IL-13, KC, MCP-1, MIP-1β, RANTES, and TNF-α) measured from BALF exhibited some changes as well. Although the BALF cytokines were in general increased with ozone exposure, these levels were lowered consistently in male mice with 20 PE treatment without reaching statistical significance. Pro-inflammatory and antioxidant gene expression profiles of the air- and ozone-exposed groups were compared to evaluate the extent of transcriptional changes based on stratification of the PE dietary treatments. A sex dimorphic response, particularly evident in the changes in mRNA abundance of *ET1*, *GPX1*, *GSTM1*, *HMOX1*, and *SOD2* where males varied in their response to

PE treatment and ozone exposure, while females displayed consistent response to PE treatment regardless of ozone exposure. For *MT2*, there appeared to be two opposing effects between the sexes, with 100 PE group in males showing significant suppression ($p = 0.05$) relative to air-exposed group yet the opposite effect occurred among the females in both treatments ($p = 0.05$ and $p < 0.05$, respectively). With *IL6* both sexes displayed significant elevation ($p < 0.05$) compared to air control in both PE-treated groups. These findings ascertain that dietary PE supplementation improves adiposity and glucose intolerance associated with consumption of WD, and protects against lung injury. However, there is sex dimorphism in the PE-mediated protection against ozone-induced lung inflammation. The work presented in this thesis is the first to document the inhibitory effects of PE on pulmonary inflammation in relation to their ability to mitigate ozone-induced toxicity in a sex-dependent manner.

RÉSUMÉ

Les polyphénols alimentaires ont démontré des capacités à améliorer les symptômes métaboliques causées par l'alimentation et l'inflammation des voies respiratoires liées à la pollution, mais très peu d'études ont examiné les deux contextes ensemble. L'étude actuelle a étudié l'efficacité d'un extrait de pomme de terre riche en polyphénol (PE) dans des souris C57BL/6 mâle (M) et femelle (F) exposées à de l'ozone et soumises à un régime de type occidental qui est riche en matières grasses (WD) l'adiposité et une intolérance au glucose, ainsi que la santé pulmonaire. Les souris ont été nourris *ad libitum* avec le WD supplémenté avec soit 20 PE (acide chlorogénique, 40 mg/kg d'aliment, l'acide férulique, 1,2 mg/kg d'aliment) ou 100 PE (acide chlorogénique, 200 mg /kg d'aliment, l'acide férulique, 6 mg/kg d'aliments). Après 4 semaines sur le régime alimentaire, les animaux ont été exposés à l'ozone de 0,8 ppm ou à l'air pendant 4 h et euthanasiés 24 h après l'exposition. Indépendamment de l'exposition à l'ozone, la supplémentation avec 100 PE a atténué le gain de poids (37% M et 48% F) et a réduit de manière significative ($p < 0,01$) l'adiposité et la masse graisseuse totale par rapport aux témoins non traités dans les deux sexes. Les deux traitements 20 et 100 PE ont restaurés les niveaux de glucose sanguin à jeun ($p < 0,001$) et amélioré la sensibilité à l'insuline ($p < 0,05$). Sur la base de l'évaluation cellulaire du liquide de lavage bronchoalvéolaire (BALF), l'inflammation pulmonaire/lésion induite par l'ozone a été supprimée par le traitement 100 PE, comme indiqué par la réduction du nombre de cellules de macrophages alvéolaires (464 ± 12,1 F et 457 ± 16,7 M) au-dessous du groupe respectif exposé à l'air dans les mêmes conditions de traitement (490 ± 5,3 M et 477 ± 7,4 F). En outre, une diminution du nombre de neutrophiles (9 ± 0,7 M et 7 ± 0,9 F) par rapport au groupe exposée à l'ozone non traité (13 ± 1,7 M et 11 ± 1,9 F) a été observée. Les doses de 100 PE ont aussi réduites les concentrations de protéines totales dans BALF ($p < 0,001$) dans les groupes exposés à l'ozone (106,09 ± 1,10 vs. 95,61 ± 0,83 µg/mL M et 82,45 M ± 1,47 vs. 74,56 ± 0,68 µg/mL F). De même, le nombre de cellules épithéliales dans BALF ont diminué à cause du traitement 100 PE dans les groupes exposés à l'ozone (129 ± 20,5 vs. 75 ± 14,3 M et 137 ± 28,2 vs. 67 ± 18,2 F). Tous ces marqueurs signifient une lésion pulmonaire aiguë. Les marqueurs d'inflammation du poumon (IL-4, IL-6, IL-13, KC, MCP-1, MIP-1β, RANTES, et TNF-α) mesurés à partir de BALF montrent des changements. Bien que les cytokines de BALF aient en général augmenté avec l'ozone, avec les traitements 20 PE ces niveaux ont baissés régulièrement chez les souris mâles sans différence

statistique. Les profils d'expression des gènes pro-inflammatoires et anti-oxydants des groupes exposés à l'air et à la couche d'ozone ont été comparés pour évaluer l'ampleur des changements de transcription basés sur la stratification des traitements alimentaires PE. Un dimorphisme sexuel, particulièrement évident dans les changements de la quantité d'ARNm de *ET1*, *GPX1*, *GSTM1*, *HMOX1*, et *SOD2* où les réponses au traitement PE et à l'ozone étaient variés chez les mâles, tandis que les femelles affichés une réponse cohérente au traitement PE indépendamment de l'exposition à l'ozone. Pour *MT2*, il semble y avoir deux effets opposés entre les sexes, avec le groupe de 100 PE chez les males présentant une suppression significative ($p = 0,05$) par rapport au groupe exposé à l'air tandis que l'effet inverse s'est produit chez les femelles dans les deux traitements ($p = 0,05$ et $p < 0,05$, respectivement). Avec *IL6* les deux sexes affichaient une élévation significative ($p < 0,05$) par rapport à l'air dans les deux groupes traités avec PE. Ces résultats montrent que la supplémentation PE améliore l'adiposité et l'intolérance au glucose associées à la consommation de WD, et protège contre les lésions pulmonaires. Cependant, il y a un dimorphisme sexuel dans la protection médié PE contre l'inflammation pulmonaire induite par l'ozone. Le travail présenté dans cette thèse est le premier à documenter les effets inhibiteurs de PE sur l'inflammation pulmonaire par rapport à leur capacité à atténuer la toxicité induite par l'ozone d'une manière dépendante du sexe.

ACKNOWLEDGEMENTS

First and foremost, I would like to acknowledge the Faculty of Agricultural and Environmental Sciences for awarding me the Walter M. Stewart Postgraduate Scholarship during the time when funding was integral for the completion of my remaining analyses in Environmental Health Canada, Ottawa. I would also like to thank the School of Dietetics and Human Nutrition for awarding me the Graduate Research Enhancement and Travel Award, without which I would not have been able to spend two amazing weeks at the Erasmus Medical Centre University in the Netherlands. Kudos to Lise for always being so helpful and showing so much patience whenever I dropped by her cubicle to ask about silly administrative questions. By and large, I am extremely humbled by my two-year graduate student experience because I managed to do/learn everything I was hoping to get out of from a Master's degree.

The greatest thanks must go to my supervisor, Dr. Stan Kubow, for allowing me to undertake such an interesting project from the get-go. Without his patient guidance, I would have been lost. His enthusiasm in pursuing scientific knowledge is unparalleled which made discussions enjoyable whenever I became stumped by my results (that happened more than I would like to admit). I must acknowledge my committee members, which included Drs. Luis Agellon, Renaud Vincent and Prem Kumarathasan. Thank you all for your insightful input and unwavering support throughout my Master's degree! There are so many people at the Inhalation Toxicology and Proteomic Laboratory in Environmental Health Canada who deserve my gratitude for making the second half of my project possible. Thank you Dr. Prem Kumarathasan for lending me those two Bio-Plex assay kits and, despite having such a hectic workload, she always had the time to offer me her scientific two-cents. Thank you Dr. Renaud Vincent for introducing me to Dr. Errol Thomson, together they provided me the guidance to conduct gene analyses. Dr. Thomson, thank you for the collaborative research efforts that have undoubtedly enabled me to think on my feet so to speak. My time in Health Canada was definitely not without struggles, but hey, now I know how to calculate concentrations properly and I will never forget about Dr. Thomson's 80-year-old uncle. Prior taking on this project, I had zero experience in any sort of animal necropsy, but Erica and Alain from Health Canada took time off from their busy schedule and trained me in how to collect bronchoalveolar lavage fluid properly which was a pretty delicate procedure on its own.

Last but not least from the Health Canada bunch is Julie. She brought over this amazing ozone chamber apparatus which she designed herself. Special acknowledgement to Katrin who assisted me in the preparation of both the Bio-Plex and the BCA experiments until the wee hours of the night. Another special person to thank for is Sidonie. Her know-how in calming down the Zephyr Workstation and Twister II made the entire qPCR procedure seem so "effortless".

To the past and present members of the Kubow lab, I don't think I could ever properly express my gratitude. Many thanks to Michèle, Behnam, and Kebba for helping me jump start my project at the beginning from making sure the lab was running smoothly to taking time answering my never-ending questions. Without their presence and assistance during the necropsy part, I would not have been able to get everything completed on time. To all the other lab members over the years, Shima and Noor, much laughter and unwinding in the Ph.D. room with you two. Thank you for your friendship it has been more than a pleasure and privilege to share these days with you. I couldn't have asked for better labmates. To all the past volunteers in our lab, Chloe, Sara, Marion, Keenan, and Laura, I haven't forgotten about you guys! Thank you Luc for showing me how to blend the animal diet properly. Oops, before I forget, thank you Dr. Duggavathi for allowing me to sit in your lab while I painstakingly count cells from the 100× microscope. Daya from his lab also helped me quite a bit when I desperately needed people for the necropsy. An honorable mention to Johanne for meeting up with me and helping me with statistics during the time when I was very overwhelmed by the amount of data I got. Lastly, I am grateful for the statistical discussions and SAS codes given to me by Dr. Correa.

Of course, I am saving the best acknowledgement for my family and friends. Thank you everyone for the unconditional support and encouragement which got me out of all sorts of pickles through life. Kudos to Zhe who treats statistics like he is solving a Rubik's cube while blindfolded. I was fortunate enough to have met Natalie and Kevin in Ottawa, who each welcomed me with open arms. Words cannot describe how much I appreciated for all the time they let me stay in their apartments. Jenny, even though you're the complete opposite of me, but your presence was enough to cheer me up during difficult times and your company always made everything seem so amusing. Mittens, are you there? You made my summer 2014 bearable. #nobeats #everythingbagels

CONTRIBUTIONS OF THE AUTHORS

Tentative title of manuscript: "Polyphenol-Rich Potato Extracts Exert Sex-Dimorphic Protective Effects on the Ozone-Induced Pulmonary Inflammatory Response in C57BL/6 Mice"

Manyan Fung (M.Sc. Candidate) was responsible for formulating the dietary treatment, animal handling, animal ozone exposure, animal necropsy procedure (BALF collection), counting the immunological cells derived from BALF, and all the experiments conducted in the Inhalation Toxicology and Proteomic Laboratory in Environmental Health Canada (i.e., BCA assay, Bio-Plex assay, and RT-qPCR). In addition, the candidate analyzed all of the resulting data, performed all the statistical analyses, and prepared all the figures and tables in this thesis.

Dr. Stan Kubow (Supervisor of candidate, Associate Professor, School of Dietetics and Human Nutrition, McGill University) provided the original concept for the study, ongoing guidance and feedback in every aspect of the project including the protocol and methodology development. He provided valuable input during frequent meetings which was integral to the progress of the study. Dr. Kubow also edited and critiqued all the chapters in this thesis.

Dr. Michèle Iskandar (Post-doctoral Fellow, School of Dietetics and Human Nutrition, McGill University) assisted in the coordination of the animal necropsy procedure including ozone exposure, BALF processing, and leukocyte count using the hemocytometer. Dr. Iskandar prepared a summary report on the preliminary data obtained early on in the study. In addition, she provided many insights/suggestions for the overall study design and analysis of results.

Dr. Renaud Vincent (Committee member of candidate, Research Scientist, Inhalation Toxicology and Proteomic Laboratory Hazard Identification Division, Environmental Health Canada) provided the necessary laboratory equipment, space, protocols, and training for the animal necropsy aspect of the project. He gave feedback/suggestions periodically corresponding to the direction of the study.

Dr. Prem Kumarathasan (Committee member of candidate, Research Scientist, Inhalation Toxicology and Proteomic Laboratory Hazard Identification Division, Environmental Health Canada) provided the laboratory equipment, space, protocols, and training for the BCA and Bio-Plex assays. She gave feedback/suggestions periodically corresponding to the direction of the study.

Dr. Luis Agellon (Committee member of candidate, Professor, School of Dietetics and Human Nutrition, McGill University) provided assistance in the analysis of RT-qPCR data.

Dr. Errol Thomson (Research Scientist, Inhalation Toxicology and Proteomic Laboratory Hazard Identification Division, Environmental Health Canada) provided the laboratory equipment, space, protocols, data analysis template, and training for the RT-qPCR aspect of the project. He gave valuable input concerning the analysis of the RT-qPCR data.

Dr. Kebba Sabally (Research Assistant, School of Dietetics and Human Nutrition, McGill University) performed the plasma insulin ELISA experiment and assisted in the animal necropsy procedure.

Behnam Azadi (Research Assistant, School of Dietetics and Human Nutrition, McGill University) assisted/participated in the coordination of the animal necropsy procedure including diet preparation, ozone exposure, exsanguination, tissue collection, and BALF/blood slide preparation. He also provided instructions on the immunocytology differentiation.

LIST OF ABBREVIATIONS

8-iso-PGF	8-isoprostane
AP-1	activator protein-1
ARE	antioxidant response element
ANOVA	analysis of variance
BALF	bronchoalveolar lavage fluid
BCA	bicinchoninic acid
BHR	bronchial hyperresponsiveness
CA	caffeic acid
CCAC	Canadian Council on Animal Care
cDNA	complementary deoxyribonucleic acid
CGA	chlorogenic acid
COPD	chronic obstructive pulmonary disease
C_t	cycle threshold
CUL3	cullin 3
DNA	deoxyribonucleic acid
E2	17β-estradiol
EDTA	ethylenediaminetetraacetic acid
EGCG	epigallocatechin gallate
ELF	epithelial lining fluid
EPI	epithelial cells
EPA	Environmental Protection Agency
ET1	endothelin 1
FA	ferulic acid
FEV1	forced expiratory volume in 1 s
FVC	forced vital capacity
GA	gallic acid
GCL	glutamate-cysteine ligase
GI	gastrointestinal
GPX1	glutathione peroxidase 1
GSH	reduced glutathione
GSTM1	glutathione S-transferase Mu 1
HEPA	high efficiency particulate air
HFD	high-fat diet
HMOX1	heme oxygenase (decycling) 1
H_2O_2	hydrogen peroxide
IFN-γ	gamma interferon
IL-1	interleukin 1
IL-1β	interleukin 1β
IL-4	interleukin 4
IL-6	interleukin 6
IL-6	interleukin 8
IL-13	interleukin 13

IκB	inhibitor of nuclear factor-kappa B
IKK	inhibitor of nuclear factor-kappa B kinase
iNOS	inducible nitric oxide synthase
IOM	Institute of Medicine
IPF	idiopathic pulmonary fibrosis
KC	keratinocyte chemoattractant
LYMPH	lymphocytes
MAPK	mitogen-activated protein kinases
Keap1	Kelch-like ECH-associated protein 1
mRNA	messenger ribonucleic acid
MCP-1	monocyte chemotactic protein 1
MIP-1β	macrophage inflammatory protein 1 beta
MT2	metallothionein-2
NAAQS	National Ambient Air Quality Standards
NADPH	reduced nicotinamide adenine dinucleotide phosphate
NF-κB	nuclear factor-kappa B
NMDR	non-monotonic dose response
Nrf2	NF-E2-related factor 2
O_2	molecular oxygen
$O_2•^-$	superoxide anion
O_3	ozone
$^{18}O_3$	radioactive ozone
•OH	hydroxyl radicals
PAM	pulmonary alveolar macrophages
PBS	phosphate buffered saline
PCA	protocatechuic acid
PE	polyphenolic-rich extract
PI3K	phosphoinositide-3 kinase
PMN	polymorphonuclear leukocytes (neutrophils)
PMSF	phenylmethylsulfonyl fluoride
polyUb	polyubiquitylation
ppb	parts per billion
ppm	parts per million
RANTES	regulated upon activation normal T-cell expressed
ROS	reactive oxygen species
RNA	ribonucleic acid
RT-PCR	real time-polymerase chain reaction
SE	standard error
SOD2	superoxide dismutase 2
TNF-α	tumour necrosis factor-alpha
TNFR	tumour necrosis factor receptor
WD	Western-style diet

LIST OF TABLES

LIST OF FIGURES

I. LITERATURE REVIEW AND INTRODUCTION

1. The cytotoxic effects of ozone on the respiratory system

Ozone (O_3; O–O=O) is a highly reactive molecule capable of interacting with all biological systems. Its toxic effects are widely documented in the mucous membranes of the nasal-respiratory tract. While ozone itself is not a radical species, many of its cytotoxic effects are derived from secondary products generated through free radical reactions with olefinic structures, as propagated by pro-inflammatory immune cells [1]. Some of these reactions can be generalized in the following mechanistic scheme known as the Criegee reaction (Eq. 1) [1]:

$$R{-}CH{=}CH{-}R' + O_3 + H_2O \rightarrow R{-}CH{=}O + R'{-}CH{=}O + H_2O_2 \quad \text{(Eq. 1)}$$

The respiratory system is unique such that it is directly linked to the external environment. Because of this, it is particularly susceptible to injury caused by inhaled toxicants including environmental ozone. The coordination and activation of inflammatory cells are two important factors that determine the toxicity of ozone. Experimental studies using animal and human models have examined/addressed the pulmonary consequences of direct ozone exposure at levels characterized as moderate, acute, or chronic [2-8]. Regardless of levels, the three major responses to ozone are epithelial disruption [9], diminished pulmonary function [10-13], and aberrant activation of innate immune signaling [14, 15].

Macrophages served as the initial sources of driving the inflammatory cascade that leads to neutrophil accumulation (pulmonary inflammation) and vascular permeability (pulmonary injury) (comprehensively reviewed in [16]). An influx of macrophages to the alveolar space generally takes place within 24 to 48 hours in response to ozone inhalation, replacing the larger and more mature resident alveolar macrophage population [15, 17-19]. It is this infiltration of secondary macrophages that has been identified as being most active in the generation of pro-inflammatory cytokines (e.g., tumour necrosis factor-alpha (TNF-α), IL (interleukin)-1, IL-6, and IL-8), neutrophil chemoattractants, adhesion molecules, and reactive oxygen/nitrogen species [19-22]. In the early phase of ozone challenge, IL-1 and TNF-α propagate lung inflammatory response by activation of nuclear factor-kappa B (NF-κB) at the transcriptional level (see next section). The

role of TNF-α has been well studied in the ozone model [23-26]. It is associated with the upregulation of the expression of surface adhesion molecules on pulmonary endothelial cells. In addition, it acts in a paracrine fashion by stimulating adjacent lung fibroblasts and epithelial cells to produce inflammatory cytokines. Both actions combined lead to the activation and recruitment of neutrophils.

Macrophages and neutrophils react to the ozone molecule by producing superoxide anion ($O_2^{\bullet -}$) via membrane-associated NADPH oxidases. This newly generated superoxide anion rapidly dismutates to hydrogen peroxide (H_2O_2), which later forms hydroxyl radicals (•OH), and molecular oxygen (O_2). Collectively, those lacking an electron are known as reactive oxygen species (ROS) and can react with their own kind to generate more radical species. For instance, the oxidation of cellular macromolecules by these ROS gives rise to the production of their cytotoxic, reactive counterparts such as DNA adducts, lipid peroxides, cationic proteins, as well as non-radical species like aldehydes and ozonides. Together, these secondary ozonation products ensue a continual autoxidation in the alveolar space, and promote further macrophage-induced tissue damage and injury, which are characteristic of both acute and chronic inflammatory diseases [1, 27]. The notable ones include emphysema, adult respiratory distress syndrome, hyperoxia, idiopathic pulmonary fibrosis, and asthma [28].

Since many oxidative intermediates can be produced through the aforementioned reactions with cellular components, thus it is neither possible nor practical to determine the toxicity kinetics of ozone with respect to a particular reaction pathway [29]. Once ozone is absorbed by the nasal mucosa [30], numerous reaction pathways can arise making the dose-response relationship difficult to establish with certainty because the various effects observed do not always involve the same mode of mechanisms [29]. Additionally, the effective dose of inhaled ozone absorption observed at the bronchoalveolar junction is not governed by the concentration or type of ozone exposure (e.g., acute or chronic), rather it is more accurately determined by the respiratory tidal volume [31]. The oxygen derived from $^{18}O_3$ in the bronchoalveolar lavage fluid (BALF) obtained from rats exposed to ozone while at rest was only about one fifth of that obtained from physically active male test persons [30]. This indicates that the tidal volume represents a good measurement for the effective dose in the lungs. However, the same radioactive-labelled oxygen was found in

extremely low concentrations in blood suggesting that the highly reactive ozone is bound in the lung tissue. As of now, there is not a definite dose-response level that can be assigned for the different types of ozone exposure, neither for human studies nor for experimental animals. The effective dose (not to be confused with ozone toxicity) can thus only be correlated with the observed inflammatory responses.

Despite having a broad reactivity towards hydrocarbon molecules, the physicochemical properties of ozone (high reactivity with limited aqueous solubility) constrict its target specificity to the epithelial lining fluid (ELF) [32]. Since ozone cannot cross cell membranes, all oxidative reactions happen primarily on the cell surface. The oxidative injury exerted by ozone is thus dependent on the availability of constituents within the fluid compartment, a mechanism referred to by Postlewaite as "reactive absorption" [32, 33]. In other words, cellular responses to ozone are not a result of the direct interaction with epithelial surface components/receptors, but are mediated through the interaction with a variety of ELF constituents. Therefore, parameters that have been adopted to evaluate ozone-related oxidative stress are performed by simply isolating BALF. The biomarker indicators present are characterized by the cytokine/chemokine release associated with inflammatory cell recruitment/activation, increased protein, appearance of plasma constituents, upregulation of airway and parenchymal oxidant production via oxidases/peroxidases and mitochondrial perturbations, and the release of arachidonic acid metabolites [16, 34-38].

2. The concept of reduction-oxidation (redox) equilibrium: roles of NF-κB and Nrf2

Since the lung has a large epithelial surface area, antioxidants are more highly expressed in the lung compared to all the other tissues so to compensate for its higher risk of oxidant-mediated attack [28]. To minimize oxidant damage, the lung possesses an integrated antioxidant defense system to detoxify ROS and to maintain a reduction-oxidative equilibrium. If the oxidant burden is sufficiently great, reactive species can easily distort such balance leading to tissue damage/injury as mentioned in the previous section. As such, the upregulation of stress-activated NF-κB signaling pathway in epithelial cells and macrophages is tightly balanced by the gene transcription of endogenous antioxidants induced under the Nrf2 (NF-E2-related factor 2) transcription program.

Nrf2 is a transcription factor that is ubiquitously expressed throughout the lung, but is predominantly found in epithelium and alveolar macrophages [39, 40]. In addition to its tumour suppressor functions, recent investigations have demonstrated that Nrf2 protects the lung from oxidative insults such as high oxygen tension and environmental particulates [41-43]. Under basal conditions, Nrf2 remains transcriptionally inactive through binding to its inhibitor, Kelch-like ECH-associated protein 1 (Keap1) in the cytoplasm, analogous to the IκB-NF-κB regulatory system (reviewed in [44]). A third protein in this complex is the cullin 3 (CUL3) ubiquitin ligase, which directs Nrf2 for proteasomal degradation. The ubiquitylation of NRF2 is blocked when Keap1 is rendered non-functional by the conformational change caused by the binding of an electrophile (i.e., ozone) to one of the reactive cysteine residues on Keap1. Nrf2 translocates to the nucleus and binds to an antioxidant response element (ARE) encoding two distinctive classes of antioxidant genes: (1) enzymatic (e.g., superoxide dismutases, catalase, glutathione peroxidases, glutathione-*S*-transferases, hemooxygenease 1, and metallothionein); and (2) non-enzymatic (e.g., ascorbic acid, α-tocopherol, taurine, and carotenoids). They function directly or indirectly to limit ROS-mediated pulmonary pathogenesis in ELF [45]. Thus, following ozone inhalation, rapid losses of ascorbic acid, glutathione, and superoxide dismutase are commonly observed in correlation with oxidative injury [46-48]. The oxidative effects of ozone on lung antioxidants are, however, transient. This is due to a rapid rate of gene transcription regulating antioxidant expression under Nrf2 transcription factor [49].

NF-κB represents one of the most well-characterized transcription factors in terms of its role in inflammation and stress responses. NF-κB dimers are normally sequestered in the cytoplasm of resting cells by association with inhibitor of NF-κB (IκB) proteins. Pro-inflammatory signals stimulate receptors belonging to the tumour necrosis factor receptor (TNFR) or IL-1/Toll-like receptor (TLR) superfamilies, which activate the IκB kinase (IKK) complex. The IKK complex phosphorylates IκB proteins on specific serine residues, thereby triggering their polyubiquitylation (polyUb) and proteasome-dependent degradation. Removal of IκB allows NF-κB dimers to translocate into the nucleus, where they activate gene transcription primarily of pro-inflammatory and stress response functions (the above is reviewed in [50]). Examples of genes under NF-κB regulation are *IL-6*, *IL-8*, *IL-13*, *IFN-γ*, keratinocyte chemoattractant (*KC*),

macrophage chemotactic protein 1 (*MCP-1*), macrophage inflammatory protein 1 beta (*MIP-1β*), regulated upon activation normal T-cell expressed (*RANTES*), and *TNF-α*.

3. Environmental ozone and its impact on health

In the environment, ozone is generated through a series of complex photochemical reactions and perpetuated cyclically by three main reaction mechanisms: photoactivation (solar radiation at wavelengths between 295 and 430 nm), photodecomposition and free radical chain reactions [51]. Ozone represents both a source of protection and risk. In the stratosphere, where the majority of atmospheric ozone naturally occurs, it provides protection against harmful ultraviolet radiation from reaching the surface of the earth. In contrast, tropospheric ozone can elicit deleterious physiological responses especially in direct exposure for a long period of time [51]. Similar to other commonly encountered urban air pollutants, environmental exposure of ozone engenders diverse biologic effects with significant impact on both acute and long-term public health [52, 53]. According to a provisional assessment conducted by the United States Environmental Protection Agency (EPA), children and adults older than 65 years of age are particularly susceptible to decreased pulmonary function and increased respiratory symptoms upon exposure to ozone levels well below the National Ambient Air Quality Standards (NAAQS) established in 2008 (acceptable level is 80 ppb or 0.80 ppm) [54, 55]. It is estimated that 113 million people in the United States alone are exposed to levels of ozone well exceeding the daily 8-hr exposure limit of 0.8 ppm [56]. Table 1 highlights some of the major epidemiological studies showing the correlation between ozone exposure and increased risk of cardiopulmonary-related symptoms, many of which were conducted at locations well below the NAAQS. Each year it is estimated that ozone-associated injury has led to 800 premature deaths, 4,500 hospital admissions, 900,000 school absences, and more than 1 million restricted activity days with an estimated $5 billion annual economic burden [57, 58].

TABLE 1. Epidemiological studies showing a causal relationship between acute/chronic ozone exposure (below 80 ppb) and increased risk of cardiopulmonary-related symptoms in humans.

Health effect	Author	Study parameter	Health outcome
Lung function	Alexeeff *et al.* (2007) [59]	• 24.4 ppb O_3 • 48-hr exposure	• Positive association between O_3 and FEV_1/FVC • Stronger among those with airway hyper-responsiveness and are obese
Mortality	Bell *et al.* (2008) [60]	• National Morbidity, Mortality, and Air Pollution Study • 98 urban areas (U.S.) • 26.8 ppb O_3 • Short-term exposure	• ↑ Mortality among areas of high unemployment, proportion of African-American residents, public transportation use, and lower prevalence of central air conditioning
All-cause mortality	Zanobetti & Schwartz (2008) [61]	• 48 cities (U.S.) • 15.1–62.8 ppb O_3 • 8-hr	• Positive association • Cardiovascular disease mortality, respiratory mortality, and stroke mortality
Cardiovascular morbidity	Lisabeth *et al.* (2008) [62]	• Texas, U.S. • 25.6 ppb O_3 • 24-hr exposure	• Positive association • Stroke/transient ischemic attacks
Asthma	Meng *et al.* (2007) [63]	• Asthmatic patients • Unknown ppb • Continuous exposure	• Positively associated with poorly controlled asthma among men but no association was present for women • Esp. in individuals +65 yr. of age
Oxidative stress	Chen *et al.* (2007) [64]	• California, U.S. • 30.5 ppb O_3 • 2-wk, 1-mth, and lifetime exposure	• Positive association • 8-isoprostane (8-iso-PGF) • Measure of lipid peroxidation • Chronic oxidative stress?

The few epidemiological studies conducted in the major cities of Canada were also in agreement with the studies cited by EPA. Rainham *et al*. analyzed the individual effect of various pollutants, including ozone, carbon monoxide, nitrogen dioxide, sulphur dioxide, and fine particles ($PM_{2.5}$), in association with the mortality data collected over a 19-year period in Toronto, Canada [65]. The objective of this study was to investigate whether mortality risk from air pollution differed during winter and summer seasons. Overall, their findings strengthened the case that subtle meteorological changes (e.g., temperature, dew point, components of wind, cloud cover, and sea level pressure) did play a role in altering the strength of pollutant associations with

cardiorespiratory outcomes, especially in the summer season. Similarly, Kolb *et al*. evaluated whether changes in weather conditions were associated with daily mortality among individuals at least 65 yr of age diagnosed as having congestive heart failure in Montreal, Canada, and who died in the urban area between 1984 and 1993 [66]. The authors identified a high correlation between O_3 (mean daily concentration = 15 ppb) and mortality during the warm season in Montreal with a strong exponential increase starting at about 25 °C, but no such association was found during the cold season.

Recent studies suggest that persons with diabetes and with cardiovascular disease may be at higher risk for the short-term effects of air pollution [61, 66, 67]. In a one-year mortality time-series study conducted by Goldberg and colleagues at McGill University, they determined that individuals with diabetes and concomitant diseases (i.e., cardiovascular disease, airways disease, cancer) were predisposed to an elevated risk of death when levels of air pollution, including all the major pollutants generated from combustion sources, increased [68]. In the same study, they also observed positive associations between most air pollutants and daily mortality from diabetes as well as among subjects diagnosed with diabetes 1 year before death. However, they did not find evidence of associations among individuals who only had diabetes. These data indicate that short- and long-term air pollutant exposure, with ozone falling under the same category, may influence the disease outcome in individuals with diabetes and other health morbidities.

4. What are dietary polyphenols?

Much of the ozone-derived toxicity is likely due to the depletion of endogenous antioxidant defenses resulting from an imbalance between NF-κB and Nrf2 transcription. Polyphenolic compounds such as sulforaphane from broccoli and cruciferous vegetables, curcumin, resveratrol, lycopene have been found to provide protection from inflammation-induced prostate, liver, cervical, and breast cancer in numerous *in vivo* and *in vitro* models [69-72]. Specifically, they are highlighted as selective activators of the Nrf2-Keap1-CUL3 pathway. Therefore, polyphenols may be effective in providing the exogenous antioxidant defenses to restore the redox equilibrium in ozone-mediated lung injury.

Dietary polyphenols are naturally occurring phytochemicals ubiquitously distributed in many plant-based foods, such as tea, coffee, wine, legumes, fruits and vegetables. Polyphenols are classified on the basis of their chemical structure. Despite the diverse chemical structures they seem to exhibit, these compounds all share a similar structural motif with the attachment of one or more hydroxyl groups to at least one aromatic ring structure [73]. Phenolic acids, flavanoids, hydroxycinnamic acid, lignans, and stilbenes represent the major classes of polyphenols. Of all, flavonoids are the largest class of polyphenols in plants and are considered to be the major active nutraceutical compounds [74]. The beneficial effects of polyphenols on human health are based primarily on epidemiological data. Early prospective and cross-sectional studies, including the Zutphen study, have confirmed the dietary consumption of polyphenols to a decreased incidence of cardiovascular diseases [75-77], diabetes mellitus [78, 79], cancer [75, 80, 81], and even neurodegenerative disorders [82]. Provided that a daily consumption of foods rich in polyphenols imparts many health-promoting benefits as indicated by epidemiological data and intervention studies, evidence as to which specific fruit or vegetable confer the optimal health benefit with respect to polyphenol content is lacking.

Polyphenols can act through several mechanisms of action. Their antioxidant properties are attributed to the structural availability of hydroxyl groups as well as conjugated double bonds typically required for electron donation to the surrounding free radicals [73]. Polyphenols can also exert modulatory activity by binding to proteins and enzymes to form radical scavenging complexes [83, 84]. However, the true biological properties of polyphenols are not merely governed by their ability to respond to oxidative stress, inflammation, and endothelial function as indicated by chemical assays and other *in vitro* models. *In vivo* bioavailability, biotransformation, and utilization must also be considered in order to fully understand their functionality [85]. Depending on the extent of polymerization and glycosylation pattern, a significant fraction of dietary polyphenols undergo extensive metabolic transformations (i.e., dehydroxylation and demethylation) by the intestinal and hepatic first pass metabolism, then later by the resident colonic microbiota [86]. This process through which the principle compound undergoes leads to an exponential production of secondary metabolites with equally diverse structures and distinctive biological properties. For example, the intake of cinnamic acids, chlorogenic and ferulic acids

results in the appearance of vanillic, isoferulic, phenylpropionic, hippuric, benzoic, and phenylacetic acids as detected in the human plasma [87, 88].

Due to the extensive degradation, one can only indirectly correlate/evaluate the *relative* antioxidant capacity of polyphenolic compounds and their associated metabolites by examining the colonic microbial profile using metabolomic methods. This is because the *true* antioxidant capacities of polyphenols are restricted to the oral cavity and gut lumen as the intestinal and hepatic first pass metabolism limits their bioaccessibility in the systemic circulation [89]. Despite their low bioavailability, the principle compounds and their associated metabolites either play a participatory role as signaling molecules in endogenous antioxidant defense pathways or a modulatory role in intracellular signaling processes. All of which are associated with oxidative stress and inflammation (Table 2). For example, olive mill waste water extract, which is known to contain several glycosylated polyphenols with hydroxytyrosol as the active component, was used to treat TNF-α exposed bronchial cells. The extract succeeded in inhibiting the interactions between NF-κB and DNA, thereby reducing IL-8 gene expression. Similarly, the polyphenol epigallocatechin gallate (EGCG) protected respiratory epithelial cells against IL-1β-dependent inflammation and IL-8 gene expression via NF-κB suppression [90].

TABLE 2. Proposed mechanistic properties of polyphenols associated with oxidative stress and inflammation.

Major mechanism regulated by polyphenols
• Translocation into the nucleus of Nrf2 and induction of Nrf2-ARE signaling pathway [91]
• Suppression of NF-κB and activator protein-1 (AP-1) [92]
• Induction of glutathione conjugates [93]
• Induction of glutathione-*S*-transferase [94]
• Caspase 3 activation [95]
• c-jun N-terminal kinase [96] and P38 activation [97]
• Modulation of phosphoinositide-3 kinase (PI3K)/Akt protein kinase B pathway [98]
• Modulation of mitogen-activated protein kinases (MAPK) [99]

5. Potatoes: a source of polyphenols

Potato (*Solanum tuberosum*) has long been recognized as a dietary staple in many parts of the world, including Canada, with nutritional attributes comparable to cereals and legumes [100]. This vegetable crop contains a balanced source of vitamin C, potassium, and phosphorus as well as several classes of polyphenolic compounds, including phenolics, flavonoids, polyamines, and carotenoids [100]. Specifically, derivatives of hydroxycinnamic acid in the free form and hydroxybenzoic acid in the bound form represent the most commonly found polyphenols in potatoes. Chlorogenic acid (CGA), caffeic acid (CA), and ferulic acid (FA) belong to the hydroxycinnamic acid group, while gallic acid (GA) and protocatechuic acid (PCA) belong to the hydroxybenzoic acid group [101, 102]. CGA is the major polyphenol present, it constitutes up to 90% of the total phenolic content of potato tubers [103].

The biological effects of CGA was investigated in an *in vivo* study involving genetically obese, hyperlipidemic, and insulin Zucker (fa/fa) rat using fasting plasma glucose, plasma and liver triacylglycerols and cholesterol concentration as indices. The treatment led to improved insulin sensitivity and postprandial blood glucose concentrations with similar effects observed in human subjects and Sprague-Dawley rats [104-106]. Ferulic acid, a flavonoid, exhibits similar therapeutic effects in which its addition to a 17% high-fat diet regimen in C57BL/6 mice counteracted hyperlipidemia and oxidative stress [107]. This was due to a combination of increased fecal lipid excretion and restored hepatic lipid-regulating antioxidant enzyme activities. The strong antioxidant activity of FA has been advocated to its ability to form a quinone methide intermediate, whereby this intrinsic property has been extended to explain its anti-inflammatory, anti-atherogenic, anti-diabetic, neuroprotective effects [108]. Rutin, a citrus flavonoid glycoside found in a variety of fruits and vegetables (e.g., cranberries, buckwheat, asparagus), is used in many countries as a medication for protecting and healing blood vessel. It inhibits platelet aggregation and decreases capillary permeability, hence improving the fluidity if blood and overall circulation. A 5-year prospective controlled trial evaluated the efficacy of rutin in the treatment of edema and microcirculatory disturbances in patients with venous microangiopathy (with or without concurrent diabetes mellitus) [109]. They found rutin was effective not only in normalizing venous edema, but also improving hypertension and in preventing deterioration of the

distal venous system. In addition, supplemented rutin ameliorated DSS-induced experimental colitis by suppressing the production of pro-inflammatory cytokines [110]. The health impact exerted by the individual dietary polyphenols, commonly found in potatoes, on carbohydrate metabolism is summarized in Table 3 and 4. However, most of the studies mentioned were in fact not using the potato-derived polyphenols, rather the pure constituents. A recent study led by Kubow and colleagues formulated a mixture of polyphenolic-rich potato extracts (PRPE) derived from Onaway and Russet Burbank cultivars to examine the combined effects of CGA and FA in attenuating diet-induced metabolic disturbances in male and female C57BL/6J mice [111]. They concluded that feeding a high-fat diet (HFD) supplemented with various PRPE combinations, in comparison to feeding only the individual CGA and FA constituents, was superior in suppressing weight gain by 63.2%, enhancing glucose tolerance response below 33 mmol/L, and improving plasma levels of insulin, ghrelin and leptin. The results from the study indicated that PRPE may serve as part of a preventative dietary strategy against the development of obesity and type II diabetes [111].

TABLE 3. The impact of dietary polyphenols found in potatoes on carbohydrate metabolism, as adapted from [112].

Health effect	Rutin	Chlorogenic acid	Ferulic acid	Caffeic acid	*p*-Coumaric acid	Gallic acid
(–) α–Amylase activity		•		•		
(–) GI glucose uptake		•	•	•		
Protection of β-cells (*in vitro*)	•					
(–) α–Glucosidase activity		•	•	•	•	•
(+) Hepatic glucokinase activity			•			
↑ Insulin secretion/ content in isolated islets/pancreas	•		•			

Notations: (–), inhibitory; (+), promoting; ↑, increasing

TABLE 4. Health properties pertaining to chlorogenic acid and ferulic acid, two primary polyphenolic compounds present in potatoes.

Polyphenol	Author	Study parameter	Health outcome
Chlorogenic acid (CGA)	Rodriguez de Sotillo *et al*. (2002) [104]	• *In vivo* • Genetically obese, hyperlipidemic, insulin-resistant Zucker (fa/fa)	• Improved insulin sensitivity • Improved postprandial blood [glucose]
	Johnston *et al.* (2003) [105]	• *In vivo* • Obese, dyslipidemic, insulin-resistant human subjects	
	Tunnicliffe *et al*. (2011) [106]	• *In vivo* • Obese, hyperlipidemic, insulin-resistant Sprague-Dawley rats	
Ferulic acid (FA)	Jin Son *et al*. (2010) [107]	• *In vivo* • C57BL/6	• 17% HFD regimen + FA • ↓ Lipidemia + oxidative stress • ↑ Lipid excretion • Restored hepatic antioxidant enzyme activities • Anti-diabetic

6. Dietary consumption of antioxidants in relation to pulmonary health and protection against air pollution

It has been recognized that the nutritional, physiological, and pharmacological status of the individual can markedly impact his/her sensitivity to environmental stressors. Since ozone exposure represents one of the major environmental contributors to pulmonary morbidities in industrialized countries, exogenous polyphenolic compounds could potentially modulate the response to inflammatory stimuli in an individual. Several epidemiological studies have associated the consumption of foods rich in antioxidants (e.g., fruits, vegetables, whole grains) to reducing the risk factors in populations afflicted with pulmonary conditions (non-environmentally related), including chronic obstructive pulmonary disease (COPD) [113, 114], asthma [115, 116], lung cancer [117-119], and idiopathic pulmonary fibrosis (IPF) [120]. Recent *in vitro* and *in vivo* studies have also indicated positive association ascribed to resveratrol, a polyphenolic phytoalexin mainly

found in the skin of grapes and is well known for its phytoestrogenic and antioxidant properties via indirect induction of Sirtuin (SIRT)1 activity [121]. In a population-based study, wine and resveratrol intake showed significant improvements in forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC) and FEV1/FVC in Dutch cohorts [122]. Similarly, resveratrol restored cigarette smoke-depleted glutathione (GSH) levels in human primary small airway epithelial and human alveolar epithelial (A549) cells by upregulation of glutamate-cysteine ligase (GCL) activity via activation of Nrf2 [71]. As for attenuation of ozone-induced lung injury, dietary antioxidant therapy has been proven to be effective as well. Studies on ozone inhalation toxicology show that vitamin E supplementation protects against lipid peroxidation and that levels of vitamin E in the lung tissue of animals receiving vitamin supplements increased significantly after the animals were exposed to ozone compared to controls. This observation suggests that vitamin E is mobilized toward the lung tissue in response to oxidative stress [123, 124]. Controlled studies in healthy subjects [125], asthmatic individuals with bronchial hyperresponsiveness (BHR) [126, 127], cyclists [128, 129], have also concluded that antioxidant supplementation (vitamin C and vitamin E) may protect against the acute effects of ozone on respiratory functions. It is possible that both vitamins interact to inhibit lipid peroxidation by mitigating the production of prostaglandin E2, a metabolite of arachidonic acid produced by lipid peroxidation of lung cells after ozone exposure. Antioxidant supplementation involving dietary carotenoids has proven its protectiveness against ozone-induced lung function decrements and airway inflammation by improving carotenoid concentrations in macrophages in supplemented individuals [130, 131]. Dietary consumption of antioxidants in relation to pulmonary health and protection against air pollution is strongly supported by animal and human studies, in which the compounds act through similar mechanistic pathways comparable to dietary polyphenols. Despite exerting anti-inflammatory and anti-oxidative properties similar to antioxidants, there are few and far between studies that highlighted the potential therapeutic effects of dietary polyphenols in the context of ozone-induced pulmonary injury. Resveratrol was an exception, but even that was not investigated in the context of ozone challenge [121].

7. The concept of sex differences and how it might influence concurrent ozone exposure and polyphenol metabolism

The topic of sex differences has long been an interest among the scientific community, but recommendations for including sex as an outcome variable in clinical research has only been recognized recently by the Institute of Medicine (IOM) [132]. Significant differences generally exist in the structural and functional integrity among organs, which dictate how each organ system will respond to external stressors. The respiratory system is no exception [133]. Females typically are more susceptible to develop more severe asthma [134], chronic obstructive pulmonary disease [135, 136], lung cancer [137, 138], and pulmonary arterial hypertension [139]. Experimental studies have focused mostly on the role hormones play in contributing the apparent sex difference in lung pathophysiology and incidence of diseases [140, 141]. Similar interest has also been explored in murine lung disease models to determine the potential influence of sex on clinical parameters such as immune cell trafficking, inflammatory response, and epithelial integrity [140]. However, the roles of sex and sex hormones in influencing how the lung responds to injurious stimuli such as ozone have not been extensively investigated. This is likely dependent on the concentration, timing and duration, and context of ozone exposure. Therefore, the impact of sex differences in influencing pulmonary inflammatory response and polyphenol metabolism should be considered in measuring outcome variables in relation to oxidative stress.

8. The congruencies shared by two by-products of urbanization

Questionable lifestyle choices including smoking, diet, and physical inactivity were once considered as the major environmental influences on human health, especially in relation to cardiovascular and metabolic disorders. Both diseases are no doubt highly correlated. The term metabolic syndrome has become somewhat synonymous with the western urbanization and adaptation of a lifestyle that focuses on dietary convenience rather than nutritional well-being. This unhealthy dietary pattern is commonly referred to as the Western-style diet (WD), a diet composed mainly of processed and refined foods with high contents of simple carbohydrates, salt, saturated fat, and protein from red meat. It is the major contributor to metabolic disturbances characterized by the clustering of cardiovascular risk factors, including insulin resistance, hypertension, central adiposity, and atherogenic dyslipidemia (i.e., elevated serum triacylglycerol, elevated levels of low

density lipoprotein cholesterol, and/or low levels of high density lipoprotein cholesterol) [142, 143]. The increasing consumption of calorically-dense foods and decreasing physical activity have, sadly, become the principal combined factors in escalating global obesity. A 2011 systematic analysis revealed that the number of overweight individuals has now greatly exceeded the number of malnourished individuals by—525 million [144]! Metabolic syndrome is a known precursor of type II diabetes mellitus and cardiovascular diseases [145]. Given the health impact of obesity on the cardiovascular system, it is reasonable to speculate that it too has an effect on the respiratory health. Such association is demonstrated when the anti-inflammatory properties of statins are considered for the therapeutic treatment of respiratory diseases [146-148].

Environmental pollutants, another by-product of urbanization, have increasingly been recognized as a major adverse health risk factor similar to metabolic disturbances. In addition to pulmonary-related diseases, their role in the etiology and progression of other chronic diseases in industrialized and developing nations has been assessed in multiple epidemiological studies [149-152]. Both short- and long-term exposure of ambient air pollution (e.g., $PM_{2.5}$ and PM_{10}, particulate matter with aerodynamic diameter < 2.5 μm and 10 μm, respectively) is consistently linked to increased incidence of arrhythmia, hospitalization for congestive heart failure, recurrent myocardial infarction, and cardiopulmonary mortality [153-156]. Exposure to ambient air particles is cited to enhance systemic inflammation, adipose inflammation, and insulin resistance in diet-induced obesity [157-159]. However, very few studies have addressed the biological consequences of ozone on inflammatory and oxidative stress markers in relation to metabolic disturbances and pulmonary inflammation. As mentioned throughout this dissertation, pre-clinical animal studies and observational studies involving human subjects have all indicated nutritional interventions as a promising modality to alleviate diet-induced metabolic symptoms, as well as pollution-related airway inflammation, yet very few have examined both contexts together. The safety profile of dietary polyphenols and their ubiquitous presence in the daily diet, therefore, make these compounds ideal candidates to pursue in this particular context.

II. RATIONALE AND STATEMENT OF PURPOSE

Food extracts confer a higher potency than individual polyphenolic compounds, as supported by animal and human studies; similarly, epidemiological and experimental evidence has suggested protection of fruit and vegetable intake against adverse respiratory disturbances caused by environmental pollution including ozone [91, 93, 95, 106, 107]. Thus, nutritional intervention using a combination of polyphenols extracted from selective polyphenol-rich potato cultivars, such as Onaway and Russet Burbank, may confer protection against ozone-induced inflammatory pulmonary damage. Much of our current understanding of the normal functioning and pathophysiology of the pulmonary system is derived from *in vivo* studies utilizing animal models. In that regard, the C57BL/6 mouse model would be appropriate to examine the possible anti-inflammatory benefits of dietary polyphenols as both male and female mice demonstrated improved glucose tolerance and anti-obesity effects following dietary intervention with polyphenol-rich potato extract under high dietary fat conditions. This mouse model has a well-understood immunologic system, comparable lung anatomy, and most importantly, a well-characterized genome to facilitate gene array analyses. Previous work has not tested the impact of a polyphenol-rich diet in the context of lung protection against ozone challenge. Also, there is limited knowledge regarding the effect of sex differences on the efficacy of polyphenols against inflammatory conditions such as ozone-exposed lung tissue. Hence, the concept of sex differences and how it might influence concurrent ozone exposure and polyphenol metabolism should be considered.

III. HYPOTHESIS

Supplementation of PE derived from Onaway and Russet Burbank cultivars is protective in a dose response fashion against pulmonary inflammation and disturbances in plasma glucose metabolism in ozone-exposed male and female C57BL/6 mice fed a Western-style, high-fat diet.

IV. OBJECTIVES

(1) To study the dose-related supplementation of PE in ozone-exposed male and female C57BL/6 mice fed a high-fat WD on: (a) weight gain and adiposity; (b) glucose concentrations and fasting plasma insulin; (c) indices of pulmonary inflammation in terms of BALF protein and cytokine concentrations; and (d) gene expression of antioxidant and inflammatory mediators in lung tissue.

(2) To examine the effect of sex differences on the responsiveness to the polyphenol-rich potato extract to all the measured outcomes following ozone exposure.

V. MATERIALS AND METHODS

1. Animal handling

The study adhered to the regulations of the Canadian Council on Animal Care (CCAC) for animal experiments and was approved by the Facility Animal Care Committees (FACCs) of McGill University. Eighty-four (42 males and 42 females) C57BL/6 mice at 7 weeks of age (19–24 g) were obtained from Charles River Laboratories (St-Constant, QC, Canada). Animals were housed in individual plexiglass cages on stainless-steel racks bedded with wood-chips under High Efficiency Particulate Air (HEPA). The animal facility maintained temperature and relative humidity in the target range of 22 ± 1 °C and 35 to 70% humidity, respectively, and a twelve-hour light cycle with lights starting at 06:00 h. All animals were observed twice daily for mortality and morbidity with detailed clinical observations up until the day of exposure. Upon reception, the animals were fed 2020X Teklad Global Soy Protein-Free Extruded Rodent Diet (6.5% energy derived from fat; Harlan Laboratories, IN, USA) and water *ad libitum*. After 4 days of acclimatization, the mice began the experimental diets *ad libitum* with free access to drinking water for a period of 4 weeks. They were randomly assigned to the 6 following groups according to Table 5.

TABLE 5. Study design.

Interaction	Group (Male/Female)		Diet	Exposure
Control x Air	MAC	FAC	WD	Air
Control x Ozone	MA20	FA20	WD + 20% PE	Air
20 PE x Air	MA100	FA100	WD + 100% PE	Air
20 PE x Ozone	MOC	FOC	WD	Ozone
100 PE x Air	MO20	FO20	WD + 20% PE	Ozone
100 PE x Ozone	MO100	FO100	WD + 100% PE	Ozone

Abbreviations: Western-style diet, WD; Polyphenol-rich extract, PE; 20%, 40 mg/kg CGA and 1.2 mg/kg FA; 100%, 200 mg/kg CGA and 6 mg/kg FA

The experimental diet represented a prototypical Western-style, high-fat diet based on the formulation of TD.88137 Adjusted Calories Diet (42% energy derived from fat; Harlan Laboratories, IN, USA) (Table 6).

TABLE 6. Formulation of TD.88137 Adjusted Calories Diet.

Diet composition	g/kg of diet
Anhydrous milkfat	210.0
Calcium carbonate	4.0
Casein	195.0
Cellulose	50.0
Cholesterol	1.5
Corn starch	150.0
DL-Methionine	3.0
Hydroquinone	0.04
Sucrose	341.46
Mineral Mix, AIN-76 (CA.170915)	35.0
Vitamin Mix, Teklad (CA.40060)	10.0

The polyphenolic content and profiles of 12 potato cultivars commonly grown in Canada has been previously characterized, which showed large variations in the antioxidant capacity and the content of major polyphenols, particularly CGA, CA, FA, and rutin. Further analysis among the same potato cultivars determined a remarkably high polyphenol content and total antioxidant capacity particularly in the potato cultivars of Onaway and Russet Burbank (see [160] for further details), whereby the contents of CGA and CA vary between the two cultivars. The mice were fed one of the three experimental diets, each differed in the concentration of polyphenol-rich potato

extract present: control (0% PE), 20% PE (40 mg/kg CGA and 1.2 mg/kg FA), and 100% PE (200 mg/kg CGA and 6 mg/kg FA). Food intake (on a per cage basis) and body weights were registered (to the nearest 0.1 g) once a day. Feces were collected on a weekly basis.

2. Inhalation exposure to ozone

Exposures were conducted in a 0.3-cubic-meter stainless steel horizontal laminar flow exposure chamber (provided by Inhalation Toxicology and Proteomic Laboratory Hazard Identification Division, Environmental Health Canada, Ottawa, ON, Canada) at a negative pressure of 0.5 inches of water and a HEPA flow rate of 50 mL/min (20 air changes per h; with or without ozone). During exposure mice were housed separately in individual open mesh stainless steel cages (16 cm width × 25 cm length × 17 cm height). The chambers (top: ozone; bottom: air) could each house nine cages at any given time, with ample space between cages for air flow uniformity. Air was converted to ozone using an ozone generator and diluted to the desired concentration of 0.8 ppm by mixing with filtered air. Ozone concentration in the chamber was monitored using a Dasibi Model 1003AH ozone analyzer (Dasibi Environmental Corp., Glendale, CA, USA), and the desired concentration was maintained by adjusting the intensity of the ultraviolet lamp with a rheostat. For control air-exposed mice, an identical procedure followed, but the ultraviolet lamp remained off. After a 4-h exposure, mice were returned to their cages in the animal facility with access to food and water *ad libitum* for 3 h before dietary restriction took place. They were euthanized 24 h post exposure. Previous dosimetric analysis indicated that under a 24-h exposure scenario, an experimental ozone dose at 0.8 ppm (427 ng O_3/cm^2) within the respiratory compartment of mice is comparable to that of the estimated internal dose attained by a human subject (127 ng O_3/cm^2) in an environmental setting [161].

3. Tissue sampling

At the time of euthanization, mice were anesthetized using isoflurane, followed by exsanguination via cardiac puncture. Blood (≈ 1 mL) was collected from the abdominal aorta using single-draw vacutainer needles (21 gauge) previously coated with heparin. The blood was then transferred into vacutainer tube containing the sodium salt of EDTA at 10 mg/mL and phenylmethylsulfonyl fluoride (PMSF) at 1.7 mg/mL, gently mixed and placed on ice [162].

Plasma was isolated by centrifugation using Beckman Optima LE-80K Ultracentrifuge (Beckman Coulter, Inc., Mississauga, ON, Canada) at 2000 rpm for 10 min at 4 °C no more than 30 min after collection, aliquoted, and frozen at −80 °C. Following complete exsanguination, the left and the right gonadal fat pads, renal fat pads, as well as intestinal fat pads were collected. Gonadal fat pads in male mice were dissected according to their defined proximity to the epididymis and vesicular gland, whereas in female mice, they were dissected according to their defined proximity to the ovaries and uterus. Adipose tissues attached to the dorsal body wall near the kidneys were collected as renal fat pads. Lastly, adipose tissues associated with the surrounding ileum, jejunum and duodenum regions of the intestine were collected as intestinal fat pads. Caecum was collected also for future microbiome assessment. The head was removed by decapitation at the first cervical vertebra, and the brain was taken out with both cerebral hemispheres intact. All sample specimens were individually weighed (to the nearest 0.0001 g) and stored at −80 °C until analysis.

4. Bronchoalveolar lavage fluid and processing

A tracheal cannula was inserted to about 0.5 cm above the carina in order to wash the entire lung cavities with isotonic 0.85% NaCl that had previously been pre-warmed in the water bath to 37 °C. A volume equal to 30 mL/g body weight was injected, suctioned, and re-injected three times in succession. This procedure was performed twice per animal to collect the primary and secondary bronchoalveolar lavage fluid samples. The primary and secondary BALF samples were each centrifuged at 1500 rpm for 10 min at a temperature of 8 °C. The supernatant form the primary BALF was frozen at −80 °C for subsequent protein and cytokine assays. The supernatant from the secondary BALF was discarded and the cell pellets from both the primary and secondary BALF samples were subsequently re-suspended in phosphate buffered saline (PBS) and pooled. Total leukocytes were counted using a hemocytometer, and 5×10^4 cells were spun onto a slide using Shandon CytoSpin 3 (Block Scientific, Inc., Bohemia, NY, USA) at 1500 rpm for 8 min. The slides were fixed with two sprays of Sheldon Cell-Fixx from a 20-cm distance and allowed to air dry in a flat position for 10 min prior staining with Wright's stain (Sigma-Aldrich, Oakville, ON, Canada). The differential leukocyte count was performed on 500 cells by a blinded investigator.

5. Fasting blood glucose measurements

Fasting blood glucose was measured using OneTouch UltraMini Diabetes Blood Glucose Monitoring System (LifeScan, Inc., Burnaby, BC, Canada) at the time of euthanization.

6. Insulin ELISA

Insulin levels were assessed in 10 μL of plasma using the EZRMI-13K Rat/Mouse Insulin ELISA Kit (Merck Millipore, Etobicoke, ON, Canada) according to manufacturer's instructions.

7. Cytokine quantitation

Supernatant collected from BALF was used for cytokine quantification using a commercial Bio-Plex™ Cytokine Assay Kit (Bio-Rad Laboratories (Canada) Ltd, Mississauga, Ontario, Canada) according to manufacturer's instructions.

8. Total protein quantitation

Total protein concentrations were assessed in 25 μL of BALF using the Bicinchoninic Acid (BCA) Protein Assay protocol (provided by Inhalation Toxicology and Proteomic Laboratory Hazard Identification Division, Environmental Health Canada, Ottawa, ON, Canada). BCA forms a purple-blue complex with Cu^{+1} in alkaline environments, thus providing a highly sensitive and selective colorimetric basis to monitor the reduction of alkaline Cu^{+2} by proteins [163]. The chelation of two molecules of BCA with one cuprous ion exhibits a strong absorbance at 562 nm which is linearly proportional to the increasing protein concentration. The concentration of each unknown is determined based on the standard curve of known protein concentrations. Detection range is between 25–2000 μg/mL.

9. Total lung RNA isolation

Frozen right lung lobe (45–142 mg) was homogenized in 1 mL of TRIzol reagent (Invitrogen Canada Inc., Burlington, Ontario, Canada) for every 75 mg of lung tissues, and total

RNA was isolated according to the manufacturer's instructions. Samples were aliquoted to avoid repeated freeze-thaw cycles and stored at −80 °C. RNA was quantified in duplicate using the RiboGreen RNA Quantitation Reagent and Kit (Molecular Probes, Eugene, OR, USA). Total RNA concentration was normalized for each sample and reverse transcribed using MuLV reverse transcriptase and random hexamers based on the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Mississauga, Ontario, Canada) according to the manufacturer's instructions. Reactions were prepared using master mixes of reagents to minimize variation in reagent composition, and reactions were performed on all samples for a given tissue simultaneously under uniform conditions. Negative control samples run in parallel were generated by replacing the reverse transcriptase with DNAse/RNAse-free water.

10. Primers

Primers for antioxidant and inflammatory mediators were previously designed by Health Canada to produce amplicons of less than 150 bases and an optimal annealing temperature of 60 °C using the Universal Probe Library design software (Roche Diagnostics Canada, Laval, Quebec, Canada) and Primer-BLAST software (National Center for Biotechnology Information, Bethesda, MD, USA) and are presented in Table 7. *In silico* testing was performed using mfold software to verify absence of secondary structure in the amplicon and surrounding template that might interfere with primer binding. Primers were validated for high (> 90%) efficiency using a dilution series of *Mus musculus* RNA [164].

TABLE 7. List of forward and reverse primers used in the experimental study.

Gene	Primer	Sequence - Left Primer	Sequence - Right Primer	Efficiency	NCBI Accession Number
β-actin	ACTb.3	actgctctggctcctagcac	ctggaaggtggacagtgagg	92%	NM_007393.3
Endothelin 1	ET-1.2	ggacatcatctgggtcaaca	tgggaagtaagtctttcaaggaa	92%	ENSMUST00000021796.6| ENSMUSG00000021367.6
Glutathione peroxidase 1	GPX1.3	gtccaccgtgtatgccttct	caatgagcagcaccttgc	99%	NM_008160.5
Glutathione-S-transferase m1	GSTm1.2	cgaaagcaccacctggat	ggtgtccatgacctggttct	98%	NM_010358.5
Heme oxygenase (decycling) 1	HMOX-1.1	ggtcaggtgtccagagaagg	gcttgttgcgctctatctcc	100%	NM_010442.2
Interleukin 6	IL-6.1	gctaccaaactggatataatcagga	ccaggtagctatggtactccagaa	100%	NM_031168.1
Metallothionein 2	MT2.2	ccgatctctcgtcgatcttc	caggagcaggatccatcg	98%	NM_008630.2
Superoxide dismutase 2	SOD2.2	ccgaggagaagtaccacgag	tatgtcccccaccattgaac	95%	–

11. Real time-polymerase chain reaction (RT-PCR) analysis

A robotic liquid handler (Caliper Zephyr® Compact Liquid Handling Workstation, PerkinElmer, Woodridge, Ontario, Canada) was employed to dispense and mix reagents, prepared in bulk for uniform reagent composition, with cDNA. Forty nanograms of cDNA were incubated with iQ SYBR Green Supermix (Bio-Rad Laboratories (Canada) Ltd, Mississauga, Ontario, Canada) and 46.08 µL of each primer in a total volume of 55 µL/well. All reactions were performed in duplicate on 96-well microtiter plates in a spectrofluorometric thermal cycler (Lightcycler 480, Roche Diagnostics Canada). Uniform reaction conditions and reproducibility of results across the PCR plate were verified according to the method previously described [165]. All time-matched samples from a given tissue were assessed on a single plate to eliminate any potential impact of plate-to-plate or run-to-run variability. Negative control samples from the reverse transcription reaction were included on all plates to detect for contamination of genomic DNA. PCR runs were

initiated by incubation at 95 °C for 3 min to activate the iTAQ polymerase followed by 50 cycles of denaturation at 95 °C, annealing at 60 °C, and elongation at 72 °C, each for 10 s. Fluorescence was monitored at every cycle during the elongation step. Melting/dissociation curve was conducted following each run to verify product purity. All target genes were expressed as fold increase compared to the control.

RefFinder [166] and other commonly used approaches (i.e., GeNorm [167], BestFinder [168], NormFinder [169], and delta C_t [170]) were employed to select for the best candidate reference gene. Statistical analyses (two-way and three-way ANOVAs) were then used to assess for the significant effects of treatment on the geometric mean of reference gene, followed by individual genes in the appropriate ranking order. For the lungs, β-actin was determined to be the most stable gene under ozone-exposed conditions [171]. Abundance of target mRNAs was calculated relative to the internal reference mRNA using the delta-delta C_t method [172], and expressed as fold change relative to air control samples.

12. Statistical analysis

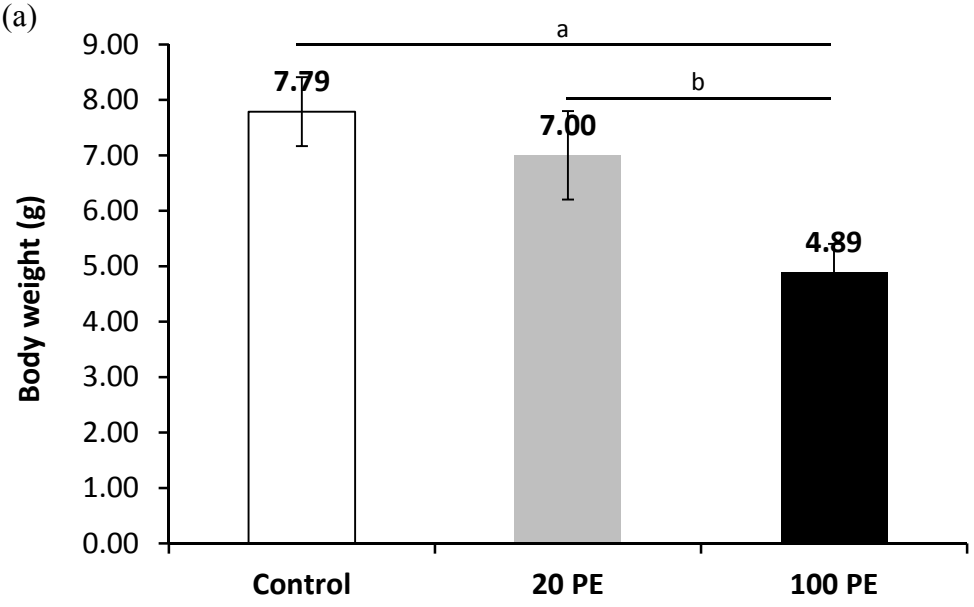
Data are expressed as mean ± SE ($p < 0.05$). Group means were compared using Student's t-test or analysis of variance (ANOVA). Data were analyzed by either two-way or three-way ANOVA with exposure (air or ozone), diet treatment (control, 20 PE, or 100 PE), and sex (male or female) as factors to elucidate the pattern of significant effects ($\alpha = 0.05$; SAS 9.3, SAS Institute Inc., Cary, NC, USA; R, 3.0.1, Open Source). Depending on the analysis in question, samples within each factor were combined to achieve the most optimized sample size (n). Multiple groups' comparisons (post-hoc analysis) were performed using Student-Newman-Keuls or Tukey-Kramer test. When necessary, results were transformed to meet the requirements of normality and equal variance. For the RT-PCR analysis, data are expressed as geometric mean ± geometric standard deviation.

VI. RESULTS

1. Effect of PE supplementation on weight gain

Obesity was induced and maintained in C57BL/6 mice following a high-fat WD (42% calories from fat). Supplementation of PE was associated with a tendency for an attenuated weight gain by 10% for the 20 PE male mice and 37% for the 100 PE male mice (both are $p < 0.001$) in relation to mice fed the control diet (Fig. 1A). The 100 PE diet group was associated with a 30% lower final body weight than 20 PE-med mice ($p < 0.05$; Fig. 1B). The female mice exhibited similar but a relatively greater effect of PE supplementation on attenuating body weight by 42% for the 20 PE mice ($p < 0.01$) and 48% for the 100 PE mice ($p < 0.01$) with respect to mice fed the control diet (Fig. 2A). In the latter case, both the 20 PE and 100 PE diets showed similar efficacy in lowering the overall final body weight in female mice (Fig. 2B).

(a)



[a] $p < 0.001$ versus respective control group

[b] $p < 0.05$ displays the difference between 20 PE and 100 PE

(b)

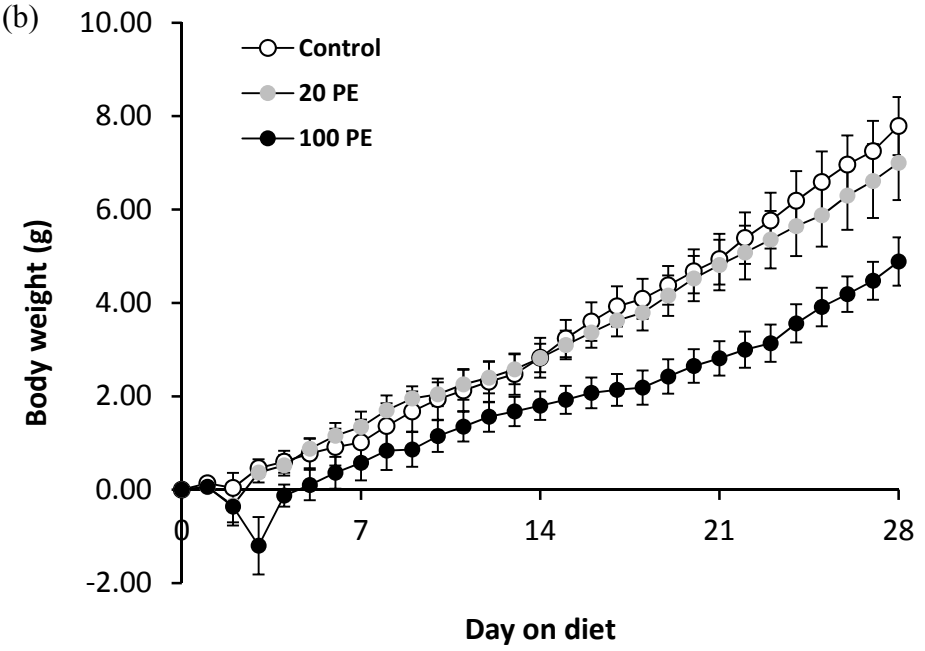
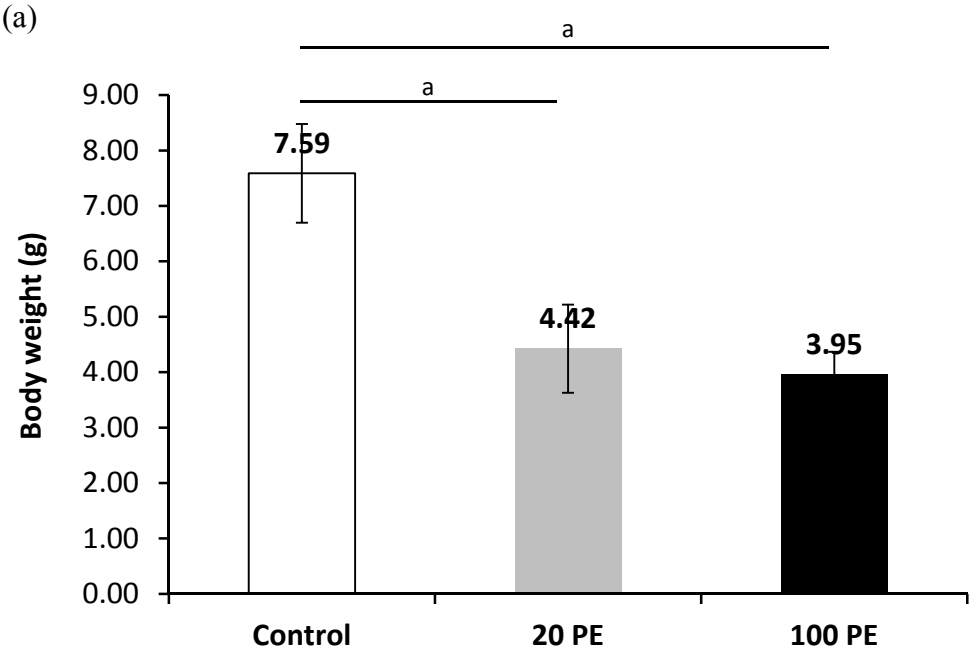


FIGURE 1. (a) Total body weight gain after a 4-wk dietary supplementation with 20 PE and 100 PE with respect to control in male mice. **(b)** Graphical representation of body weight gain over a period of 4 weeks. All data are represented as mean ± SE (n = 8–9).

(a)



[a] $p < 0.01$ versus respective control group

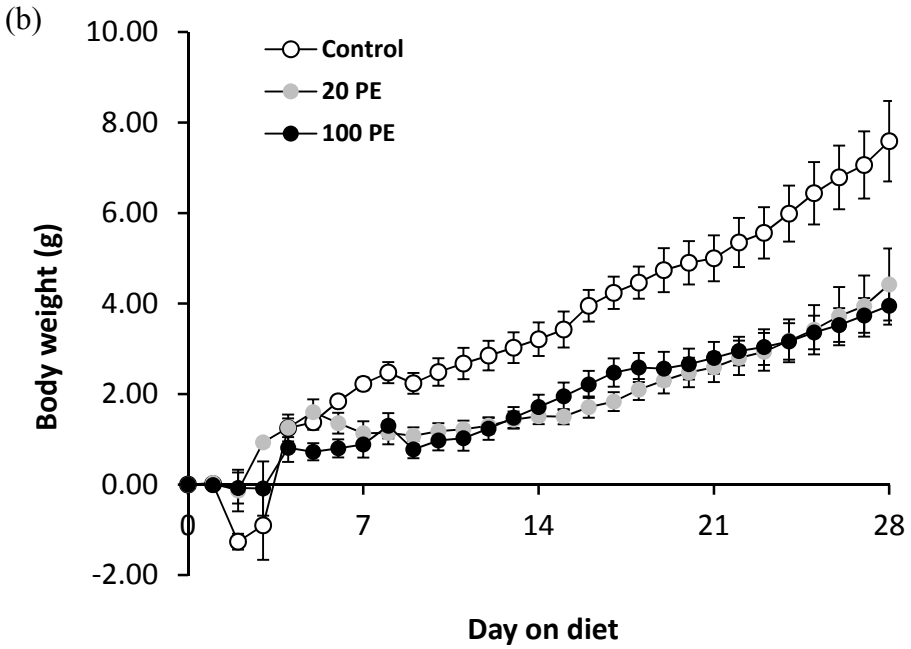


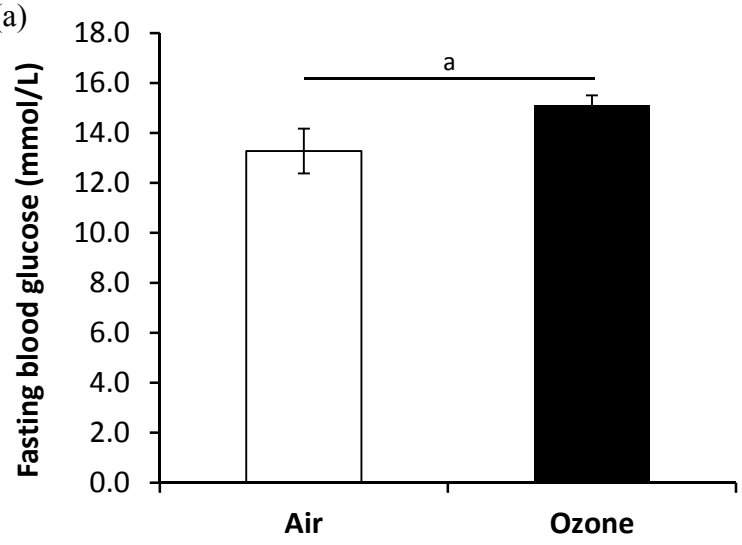
FIGURE 2. (a) Total body weight gain after a 4-week dietary supplementation with 20 PE and 100 PE with respect to control in female mice. **(b)** Graphical representation of body weight gain over a period of 4 weeks. All data are represented as mean ± SE (n = 8–9).

2. Effect of PE supplementation on fasting glucose and insulin levels

Fasting blood glucose levels were measured at the time of euthanization after 4 weeks of PE supplementation. Among the male mice, ozone exposure was associated with a tendency (trending, $p \approx 0.05$) of increased glucose levels (15.1 ± 0.4 mmol/L) relative to mice exposed to air (13.3 ± 0.9 mmol/L) (Fig. 3A). An opposite effect was observed in the ozone-exposed female mice as ozone was associated with significantly decreased fasting blood glucose levels relative to air exposed mice (12.8 ± 0.6 vs. 14.3 ± 0.6 mmol/L, respectively; $p = 0.05$) according Fig. 4A. Non-supplemented ozone-treated groups in both sexes displayed abnormal index of fasting blood glucose homeostasis as evidenced by significantly elevated fasting glucose levels versus PE-supplemented ozone exposed mice (Fig. 3B and 4B). Relative to the ozone-exposed controls, both doses of PE supplementation in male mice were associated with a 13% lower fasting blood glucose value of 14.1 ± 0.5 mmol/L ($p = 0.001$), which was significantly different than the air-exposed mice (16.2 ± 0.4 mmol/L) (Fig. 3B). In contrast, only the 20 PE dose was associated with relatively lower plasma glucose levels relative to the ozone controls in female (10.9 ± 0.7 vs. 13.9 ± 0.6

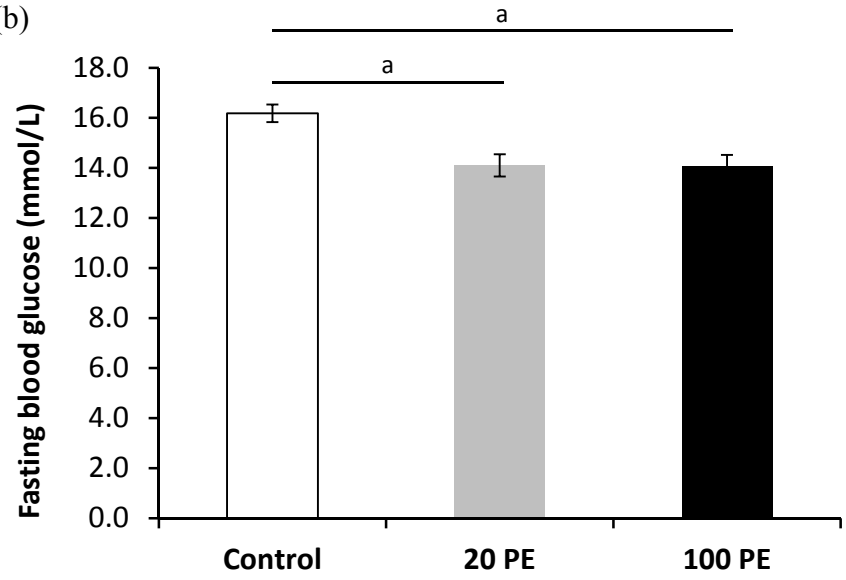
mmol/L, respectively; 21% decrease) (Fig. 4B). Similar findings were seen among male and female treatment groups in terms of plasma insulin levels. In male mice, a reduction in plasma insulin concentrations was observed in the 20 PE and 100 PE supplemented groups, which was in accordance with the results seen in fasting glucose levels as mentioned earlier. In contrast, the female counterpart differed by displaying an increase of insulin concentrations (1.29 ± 0.08 ng/mL) in the 20 PE supplemented group as opposed to a decrease in the 100 PE supplemented group (0.68 ± 0.05 ng/mL) (Fig. 5).

(a)



[a] $p \approx 0.05$ versus respective air group, trending

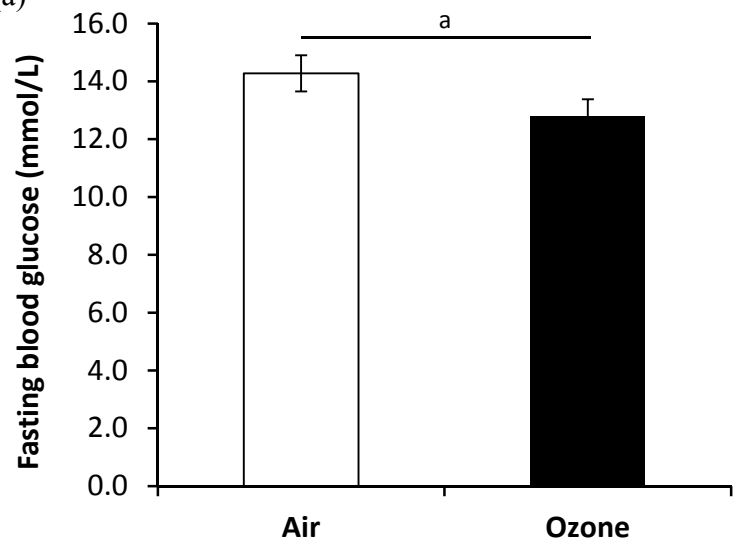
(b)



[a] p = 0.001 versus respective control group

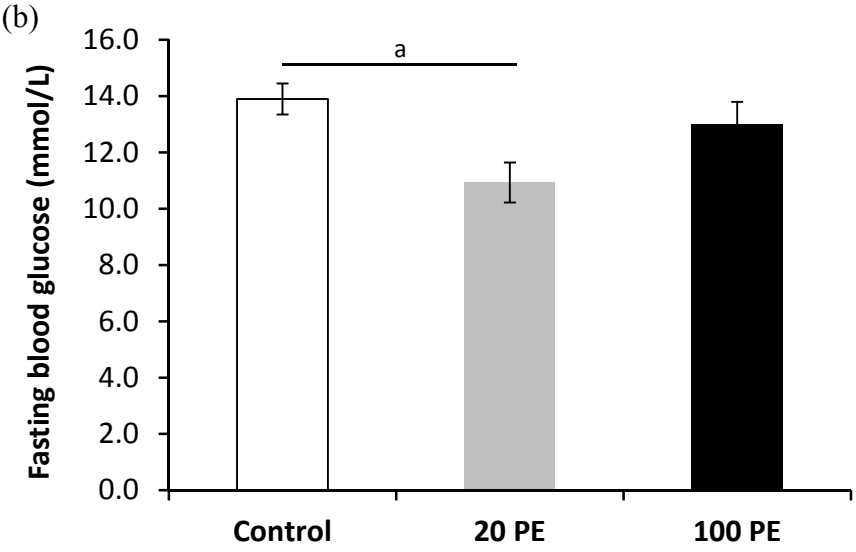
FIGURE 3. (a) The effect of ozone exposure on total fasting blood glucose levels in male mice (n = 19). **(b)** The effect of PE supplementation on total fasting blood glucose levels in air- and ozone-exposed male mice combined (n = 8–11). All data are represented as mean ± SE.

(a)



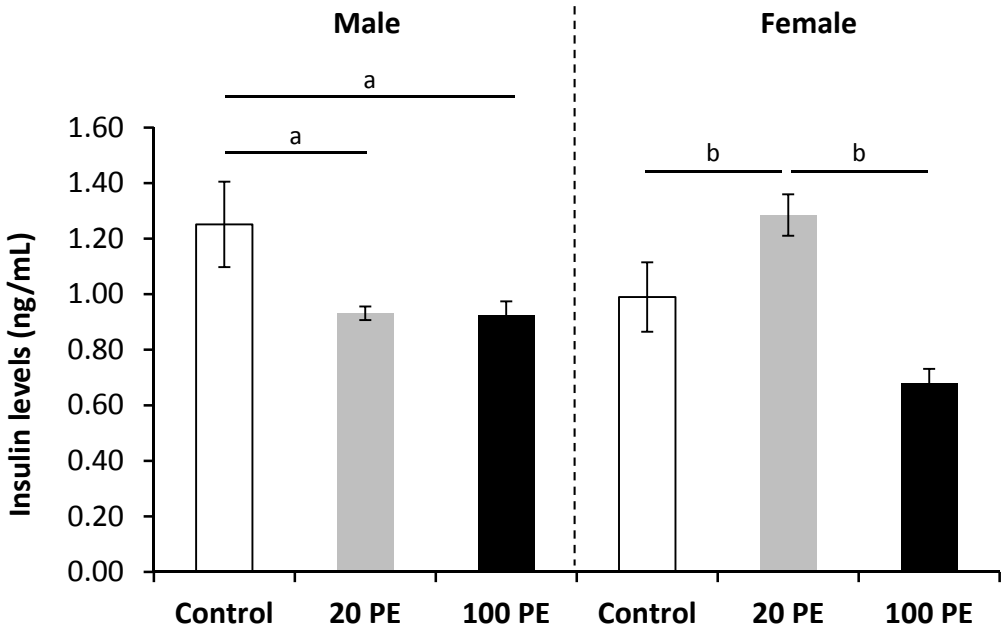
[a] p = 0.05 versus respective air group

(b)



[a] p < 0.01 versus respective control group

FIGURE 4. (a) The effect of ozone exposure on total fasting blood glucose levels in female mice (n = 16–17). **(b)** The effect of PE supplementation on total fasting blood glucose levels in air- and ozone-exposed female mice combined (n = 11–13). All data are represented as mean ± SE.



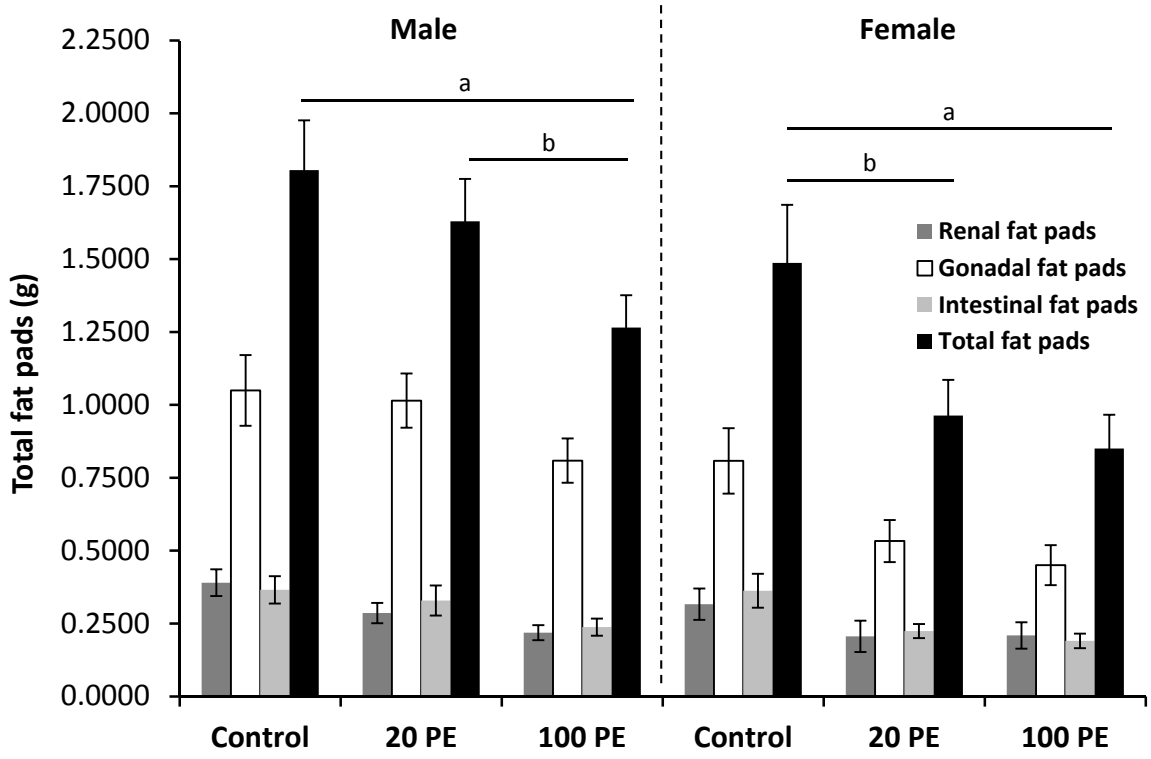
[a] p = 0.05 versus respective control group

[b] p < 0.05 versus respective control group

FIGURE 5. The effect of PE supplementation on plasma measurements of insulin concentrations for air- and ozone-exposed male/female mice combined (n = 14–15). All data are represented as mean ± SE.

3. Effect of PE supplementation on adiposity

Various fat pads including renal, gonadal, and intestinal were collected at the time of sacrifice after 4 weeks of PE supplementation. Tissue lipid profiles were consistent with the diet-induced phenotype. Regardless, both sexes exhibited an overall improved adiposity in the PE treatment groups as seen in Fig. 6, with 100 PE group showing a significant reduction ($p < 0.01$) in the total fat pads relative to the diet control group. The 20 PE-fed male mice did not show a significant reduction in total fat pads as opposed to female mice ($p < 0.05$).



[a] $p < 0.01$ versus respective control group

[b] $p < 0.05$ versus respective control or 20 PE group

FIGURE 6. The weight of fat pads for various tissues of air- and ozone-exposed male/female mice combined (n = 13–15). All data are represented as mean ± SE.

4. Effect of PE treatment on pulmonary cellular, inflammatory and total protein indices

Pulmonary injury and inflammation was evaluated using cellular and molecular inflammatory markers in BALF recovered from male and female mice 24 h after ozone challenge. Neutrophil infiltration and hyperpermeability are both undisputable characteristics of lung injury [16]. The cytology profile presented in Table 8 is in agreement with ozone-induced pulmonary injury as shown by a concurrent marked increase of neutrophils ($p < 0.05$ in males and $p \approx 0.05$ in females) and marked decrease of resident alveolar macrophages ($p < 0.05$ in males; females did not reach significance) with respect to the air-exposed group. This was not observed in the air-exposed control group of both sexes. A significant reduction of 100 PE-treated alveolar macrophage cell counts ($p < 0.05$) in ozone-exposed males indicates that a high PE dose succeeded

in suppressing the influx of macrophages into the alveolar space, thereby preventing an inflammatory cascade that can trigger neutrophil accumulation (pulmonary inflammation) and vascular permeability (pulmonary injury). This was further evidenced by a significantly decrease number of PMN ($p < 0.05$ in males and trending, $p \approx 0.05$ in females) and EPI ($p < 0.05$) cell counts under the same 100 PE condition relative to ozone-exposed control mice (Table 8). In contrast, 20 PE treatment was not as effective in lowering PAM, PMN, and EPI versus respective control group within the same exposure parameter and versus respective air- diet group within the same sex. Similarly, both 20 and 100 PE treatments were associated with significantly decreased total protein concentrations in BALF in both sexes ($p < 0.001$) (Fig. 7).

TABLE 8. Cytology measurement of BALF in air- and ozone-exposed mice (n = 4–8).

MEASUREMENT	**AIR-EXPOSED MALES**			**AIR-EXPOSED FEMALES**		
(count/μL)*	**Control**	**20 PE**	**100 PE**	**Control**	**20 PE**	**100 PE**
PAM[a]	491 (4.9)	486 (5.1)	490 (5.3)	485 (6.8)	485 (4.5)	477 (7.4)
LYMPH[b]	7 (3.4)	9 (3.1)	12 (3.7)	12 (6.2)	11 (3.9)	18 (6.6)
PMN[c]	3 (1.5)	5 (2.1)	6 (1.5)	5 (2.0)	4 (0.9)	5 (1.0)
EPI[d]	23 (8.4)	19 (7.0)	22 (10.1)	33 (4.2)	33 (5.5)	40 (10.0)

MEASUREMENT	**OZONE-EXPOSED MALES**			**OZONE-EXPOSED FEMALES**		
(count/μL)*	**Control**	**20 PE**	**100 PE**	**Control**	**20 PE**	**100 PE**
PAM[a]	476 (8.8)	462 (16.0)	457 (16.7)[g]	454 (15.1)	470 (11.2)	464 (12.1)
LYMPH[b]	24 (5.4)	28 (14.1)	35 (16.3)	21 (7.2)	18 (10.0)	25 (11.17)
PMN[c]	13 (1.7)	10 (2.1)[g]	9 (0.7)[e,g]	11 (1.9)	11 (3.8)[g]	7 (0.9)[f]
EPI[d]	129 (20.5)	85 (8.7)[f,g]	75 (14.3)[e,g]	137 (28.2)	99 (21.7)[g]	67 (18.2)[e]

* All data are represented as mean ± SE

[a] PAM, pulmonary alveolar macrophages

[b] LYMPH, lymphocytes

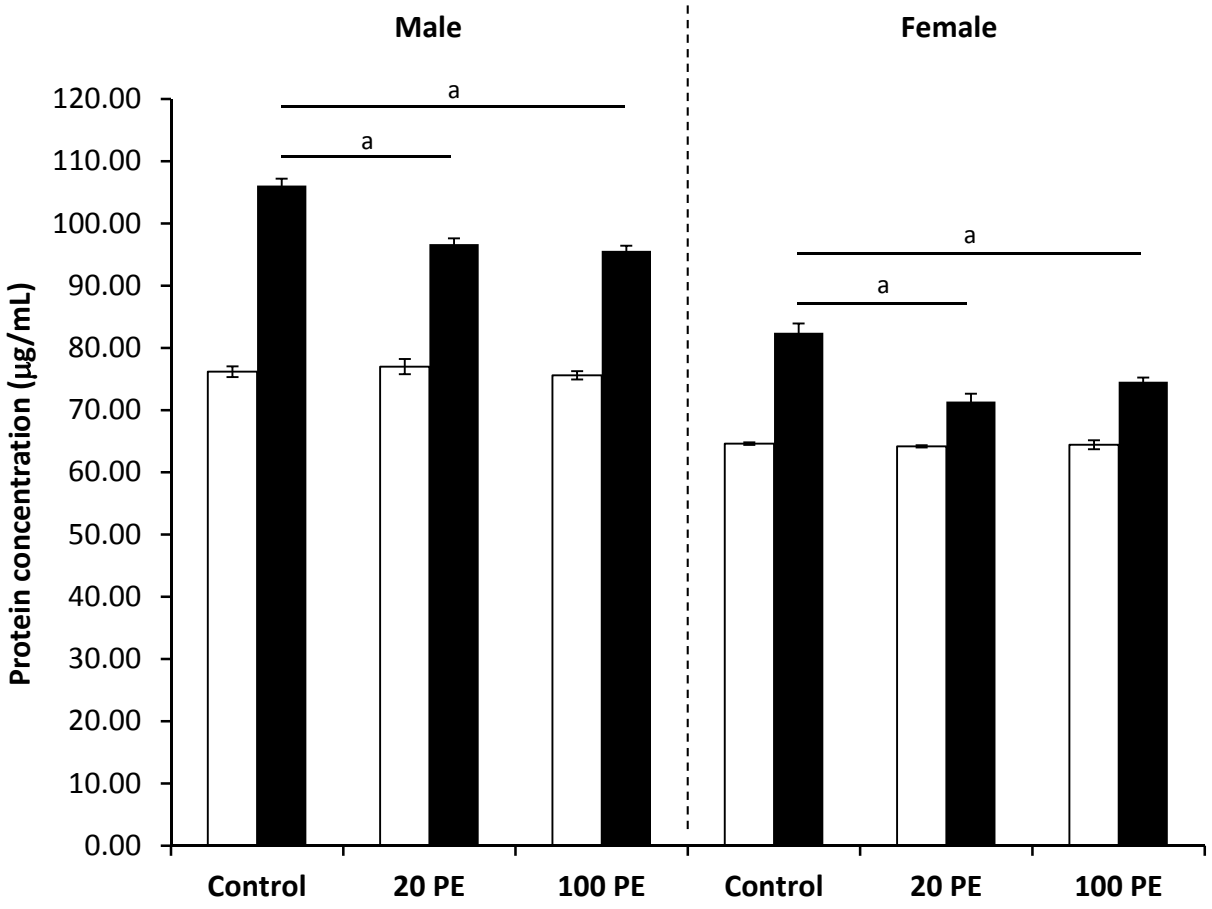
[c] PMN, polymorphonuclear leukocytes (neutrophils)

[d] EPI, epithelial cells

[e] $p < 0.05$ versus respective control group within the same exposure parameter

[f] $p \approx 0.05$ versus respective control group within the same exposure parameter, trending

[g] $p < 0.05$ versus respective air × diet group within the same sex



[a] $p < 0.001$ versus respective ozone control group

FIGURE 7. Total protein measurements of BALF in air- and ozone-exposed mice (n = 4–8). All data are represented as mean ± SE. White bars: air; black bars: ozone.

Table 9 shows the BALF concentrations of cytokines, chemokines and inflammatory biomarkers. Levels of the eight proteins assayed (IL-4, IL-6, IL-13, KC, MCP-1, MIP-1β, RANTES, and TNF-α) in both of the PE treatment groups were not significantly different from the respective control group as indicated by the lack of statistical significance between air- and ozone-exposed groups as well as between diet-ozone and diet-control group (Table 9 and Fig. 8A and B). These findings correspond to the cytology profile of the ozone-exposed PE-supplemented groups, whereby a diminished involvement/infiltration of secondary macrophages and neutrophil signifies an attenuated production of pro-inflammatory cytokines, neutrophil chemoattractants, and reactive oxygen/nitrogen species.

TABLE 9. BAL Bio-Plex™ measurements in air- and ozone-exposed mice (n = 4–8).

MEASUREMENT	AIR-EXPOSED MALES			AIR-EXPOSED FEMALES		
(ρg/mL)*	Control	20 PE	100 PE	Control	20 PE	100 PE
IL-4[a]	46.83 (3.33)	52.85 (8.72)	42.29 (7.1)	39.34 (1.11)	36.8 (3.58)	30.87 (3.75)
IL-6[b]	19.94 (4.09)	20.02 (0.3)	31.64 (24.69)	9.96 (0)	9.96 (0)	9.96 (0)
IL-13[c]	496.24 (75.84)	453.36 (60.79)	480.49 (74.53)	220.68 (28.11)	224.49 (52.05)	184.7 (16.71)
KC[d]	1137.91 (65.09)	930.66 (57.11)	956.87 (88.25)	521.97 (44.97)	563.2 (59.7)	658.74 (63.71)
MCP-1[e]	525.97 (24.05)	476.28 (31.08)	527.67 (21.23)	294.37 (71.35)	410.81 (230.64)	662.6 (180.12)
MIP-1β[f]	285.18 (45.48)	265.33 (35.42)	307.88 (21.86)	83 (10.02)	68.92 (5.88)	93.23 (6.1)
RANTES[g]	73.68 (6.32)	69.08 (5.66)	75.31 (8.91)	32.37 (4.87)	36.32 (4.11)	32.45 (3.61)
TNF-α[h]	325.96 (123.12)	141.82 (50.06)	395.18 (236.79)	127.69 (18.59)	158.83 (24.39)	163.35 (27.72)

MEASUREMENT	OZONE-EXPOSED MALES			OZONE-EXPOSED FEMALES		
(ρg/mL)*	Control	20 PE	100 PE	Control	20 PE	100 PE
IL-4[a]	72.36 (11.72)	64.39 (3.86)	80.67 (9.45)	77.83 (30.31)	184.17 (143.11)	89.95 (36.79)
IL-6[b]	171.45 (16.15)	142.2 (30.71)	148.84 (26.35)	87.92 (24.19)	87.27 (37.72)	49.63 (13.27)
IL-13[c]	681.29 (83.25)	624.35 (78.32)	822.28 (51.89)	193.62 (37.77)	249.44 (68.16)	144.33 (9.24)
KC[d]	1829.78 (114.64)	1694.26 (200.28)	1704.03 (34.81)	1716.8 (290.9)	1591.41 (398.03)	1554.39 (241.44)
MCP-1[e]	820.07 (30.91)	701.61 (20.69)	808.05 (31.13)	959.82 (259.33)	889.1 (181.46)	587.62 (71.86)
MIP-1β[f]	391.87 (13.97)	326.23 (8.83)	370.22 (27.14)	209.08 (57.85)	156.31 (53.26)	134.38 (14.78)
RANTES[g]	85.26 (6.06)	82.84 (3.69)	91.24 (3.77)	59.35 (9.76)	54.63 (8.86)	45.35 (5.81)
TNF-α[h]	161.38 (82.81)	71.17 (60.44)	413.27 (170.83)	129.38 (21)	250.95 (121.57)	162.94 (28.59)

[*] All data are represented as mean ± SE

[a] IL-4, interleukin 4

[b] IL-6, interleukin 6

[c] IL-13, interleukin 13

[d] KC, keratinocyte chemoattractant

[e] MCP-1, monocyte chemotactic protein 1

[f] MIP-1β, macrophage inflammatory protein 1 beta

[g] RANTES, regulated upon activation normal T-cell expressed

[h] TNF-α, tumour necrosis factor-alpha

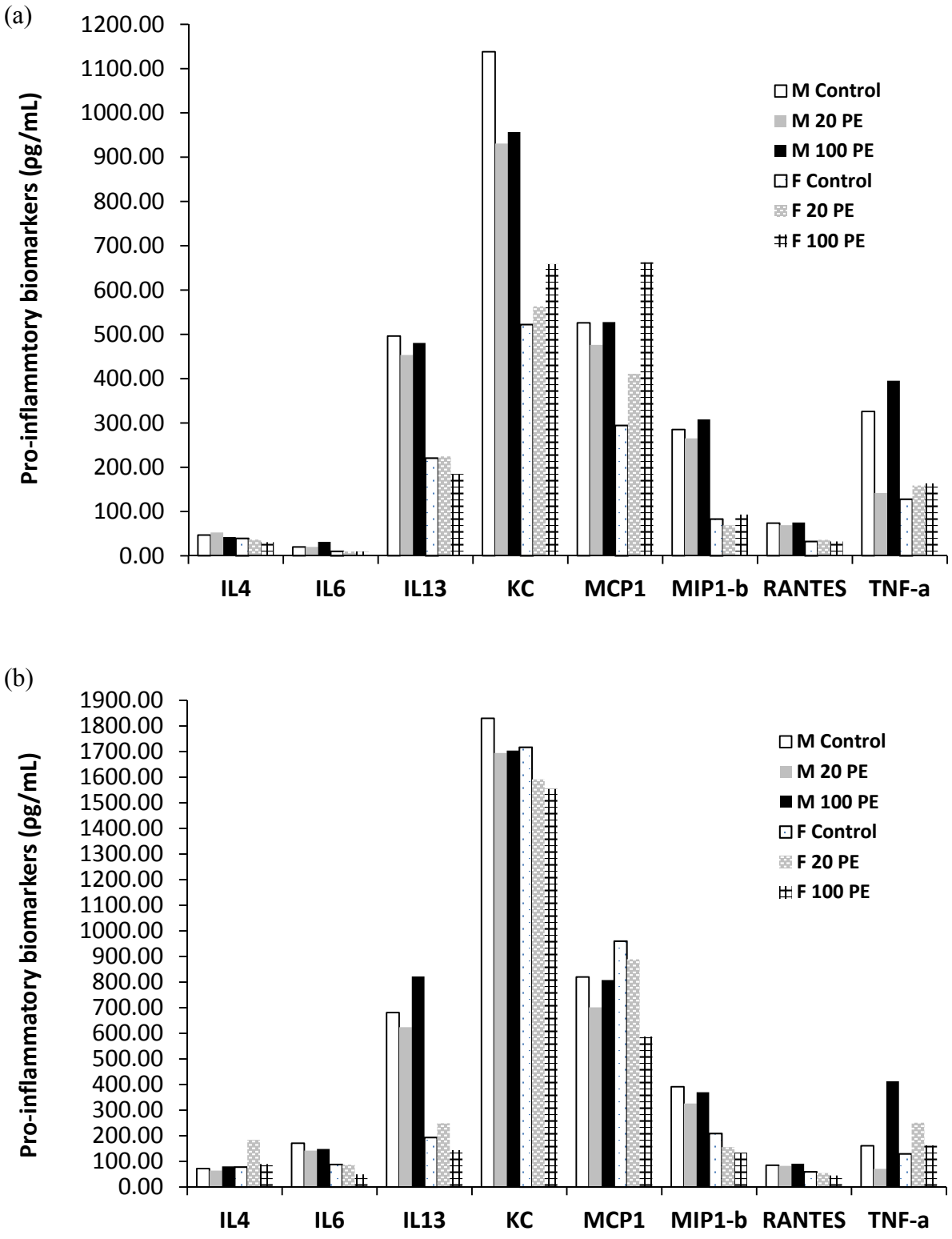


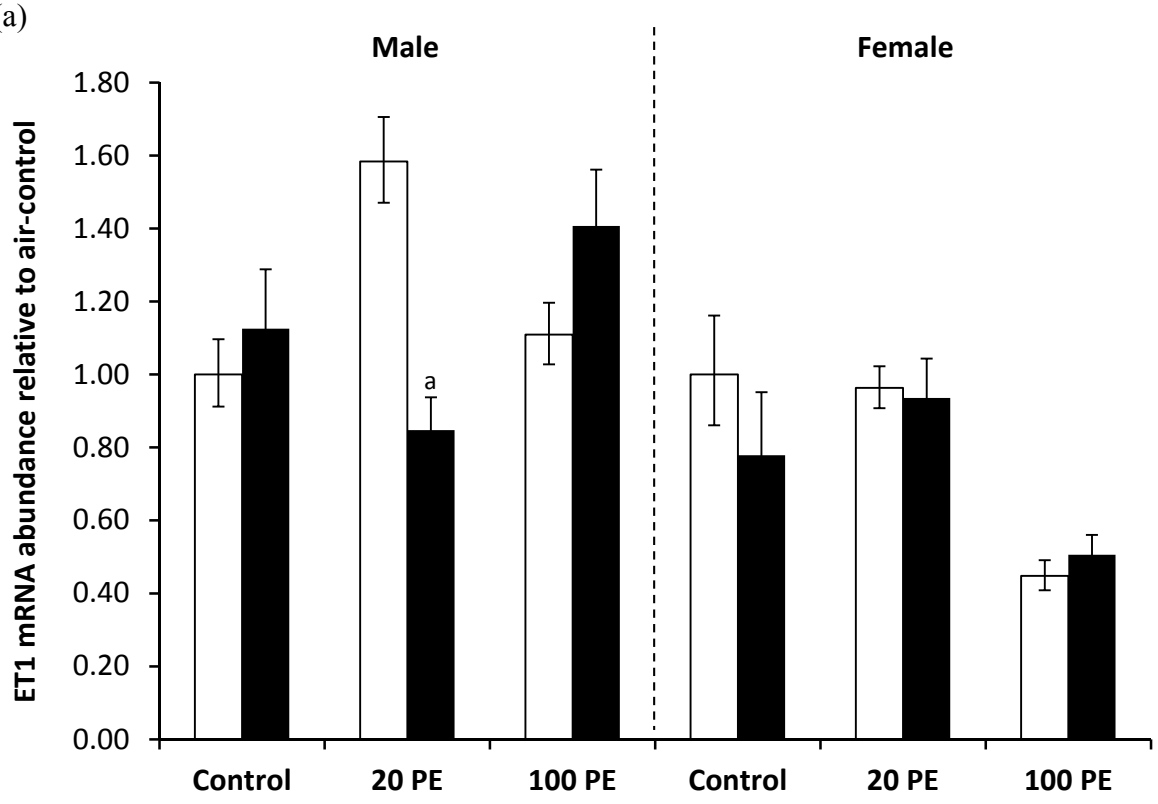
FIGURE 8. BAL Bio-Plex™ measurements in **(a)** air- and **(b)** ozone-exposed mice (n = 4–8). Error bars are omitted for clarity, refer to Table 9. All data are presented as mean ± SE.

5. Effect of PE supplementation on mRNA abundance of pro-inflammatory and antioxidant genes

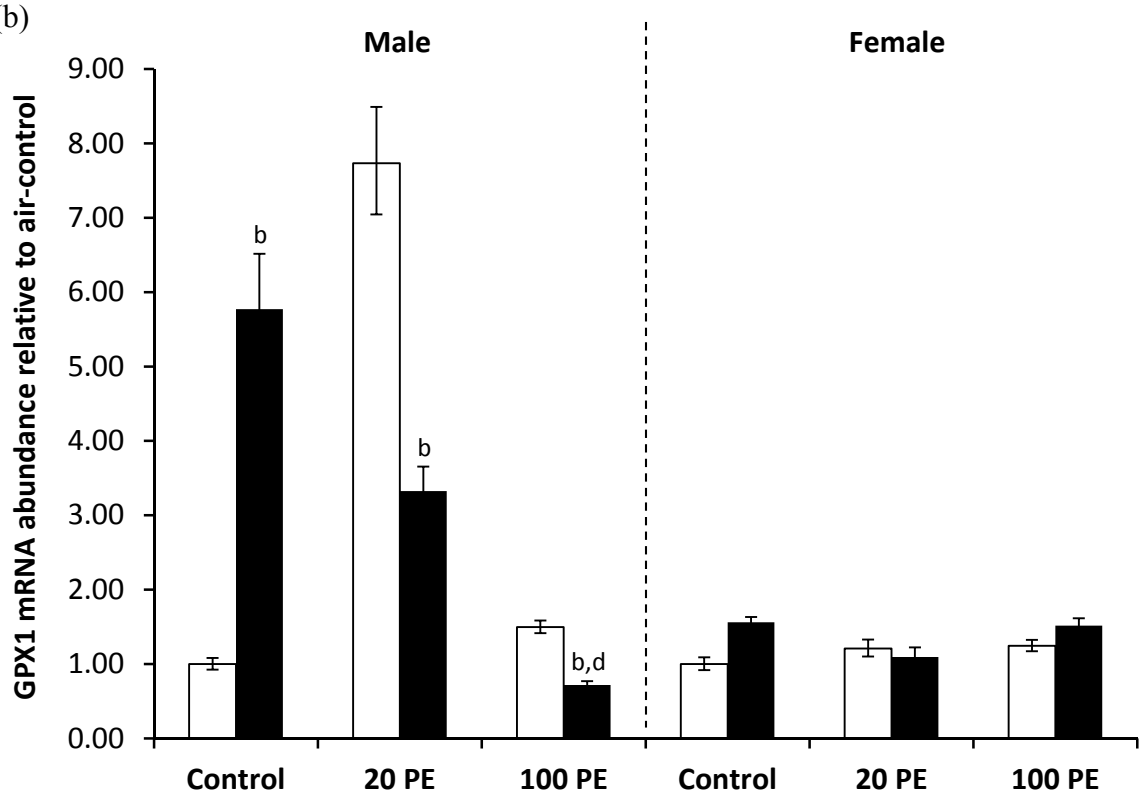
In order to further understand the altered environment and early pulmonary events associated with the acute pro-inflammatory ozone response, the transcriptional expression of various pro-inflammatory and antioxidant genes was measured in the lung tissues of male and female mice within the first 24 h. These data reflect the levels of mRNA response in alveolar cells as well as infiltrating immune cells present in the lung tissue at the time of sampling. Enhanced expression of antioxidant and inflammatory genes in response to oxidant toxicity has been previously evaluated in experimental models of mice with acute lung injury [173-175]. The gene expression profiles of the air- and ozone-exposed groups in this study describe the extent of transcriptional changes based on the stratification of the PE dietary treatments (Fig. 9A–G). According to the results, there was a clear divergent response between the two sexes in the genes analyzed. This was particularly evident in the mRNA abundance of *ET1*, *GPX1*, *GSTM1*, *HMOX1*, and *SOD2* where the male group varied in their responsiveness (lack of consistency/pattern) towards either of the PE treatments as indicated by the varying mRNA abundance levels with respect to their controls. In contrast, there was a consistent responsiveness among the female group under 100 PE treatment based on the fact that ozone exposure did not alter the mRNA abundance levels (no statistical significant differences were observed in the measured parameters). Similarly, the expression of *ET1*, *HMOX1* and *SOD2* among the female mice treated with 100 PE demonstrated even lowered activity than the air-exposed control group (although no statistical significant differences were observed in the measured parameters) indicative of PE supplementation protecting/modifying ozone-mediated inflammation through gene transcription, albeit in a dose- and sex-dependent manner. In the case of *MT2* expression, there appeared to be two opposing effects between the sexes, with 100 PE group in males showing strong trend for suppression ($p = 0.05$) relative to the air-exposed group yet the opposite effect occurred among the females in both the 20 PE and 100 PE treatments ($p = 0.05$ and $p < 0.05$, respectively). Compared to the female counterpart, neither of the PE treatments siginificantly promoted the expression of *MT2* (100 PE actually showed a significant decrease in expression by 1.0-fold with respect to its air control, $p = 0.05$). On the other hand, both 20 PE (6.10-fold, $p = 0.05$) and 100 PE (1.85-fold, $p < 0.05$) treatments promoted the mRNA abundance of *MT2* in the female group (Fig. 9F). In line with the other findings, the polyphenol-rich potato extract appeared to suppress the transcription

activation of *SOD2* even lower than the respective air group within each dietary treatment (Fig. 9G), with 100 PE extending its antioxidative benefits even without the presence of an oxidant insult (although this was not significant) in the female group. Disregarding the exposure factor, the *SOD2* abundance levels were altered to more than half of what the control group exhibited (0.40-fold vs. 1.00-fold). An exception was *IL6*, in which both sexes displayed significant elevation ($p < 0.05$) compared to air control within both PE-treated groups. Even though an overexpression of *IL6* levels was clearly observed in mice supplemented by PE, the levels were no different compared to the ozone control group (Fig. 9E). In fact, both antioxidant treatments actually suppressed *IL6* expression significantly as shown among the females, with 20 PE displaying a 2.46-fold ($p \approx 0.05$) and 100 PE a 3.11-fold ($p < 0.05$) less than the ozone controls.

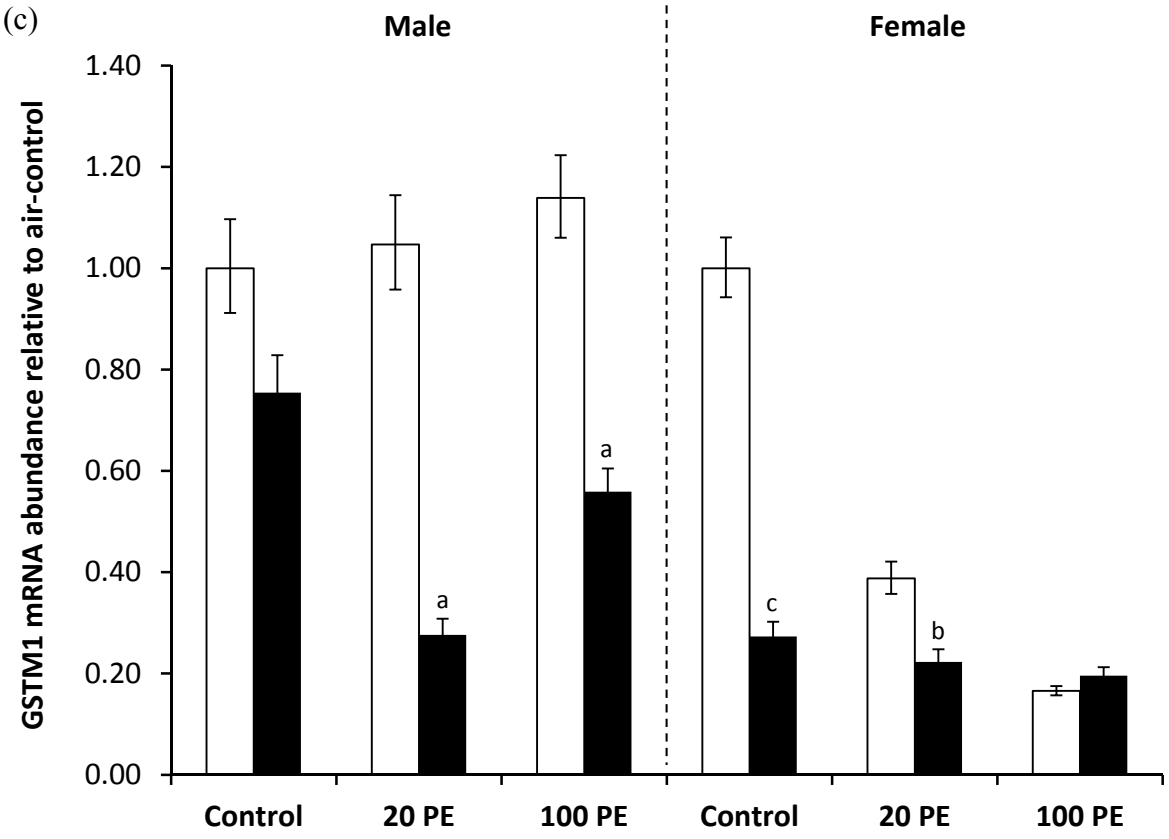
(a)


Male
Female
ET1 mRNA abundance relative to air-control
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
a
Control
20 PE
100 PE
Control
20 PE
100 PE

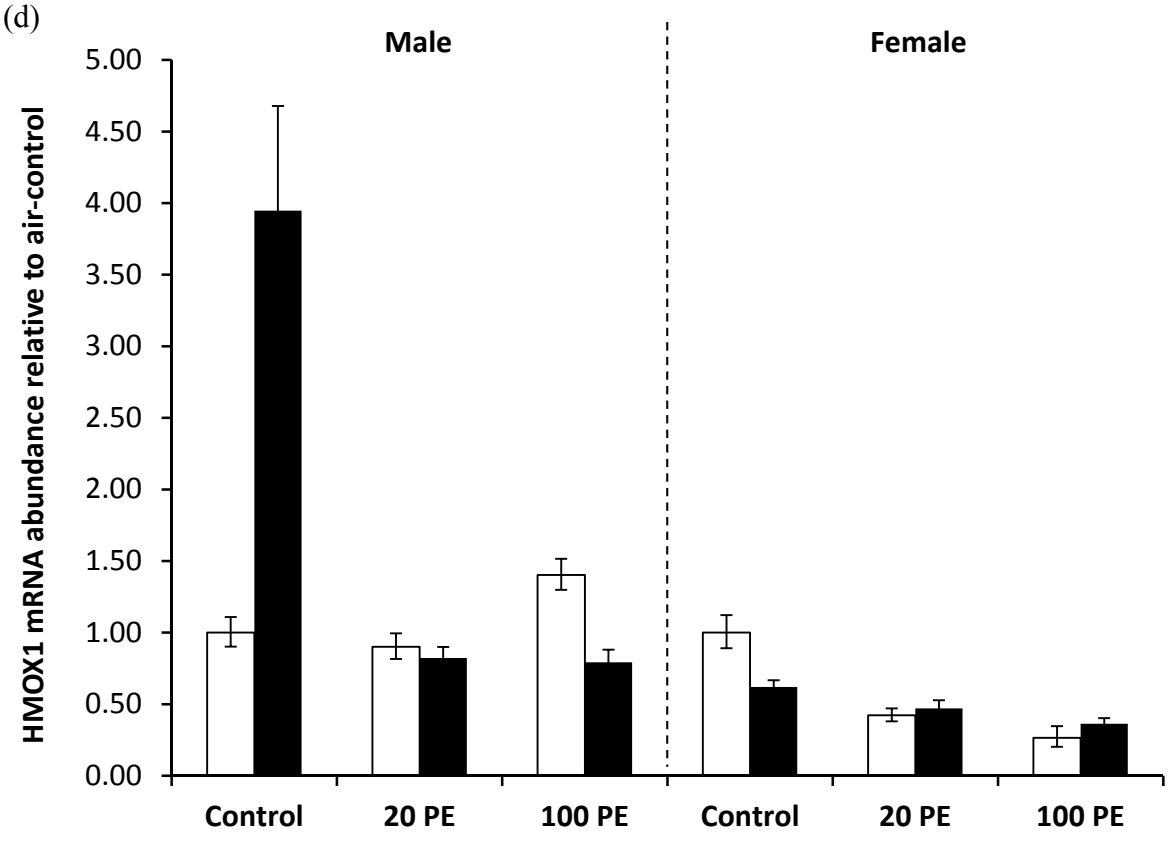
(b)


Male
Female
GPX1 mRNA abundance relative to air-control
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
b
b
b,d
Control
20 PE
100 PE
Control
20 PE
100 PE

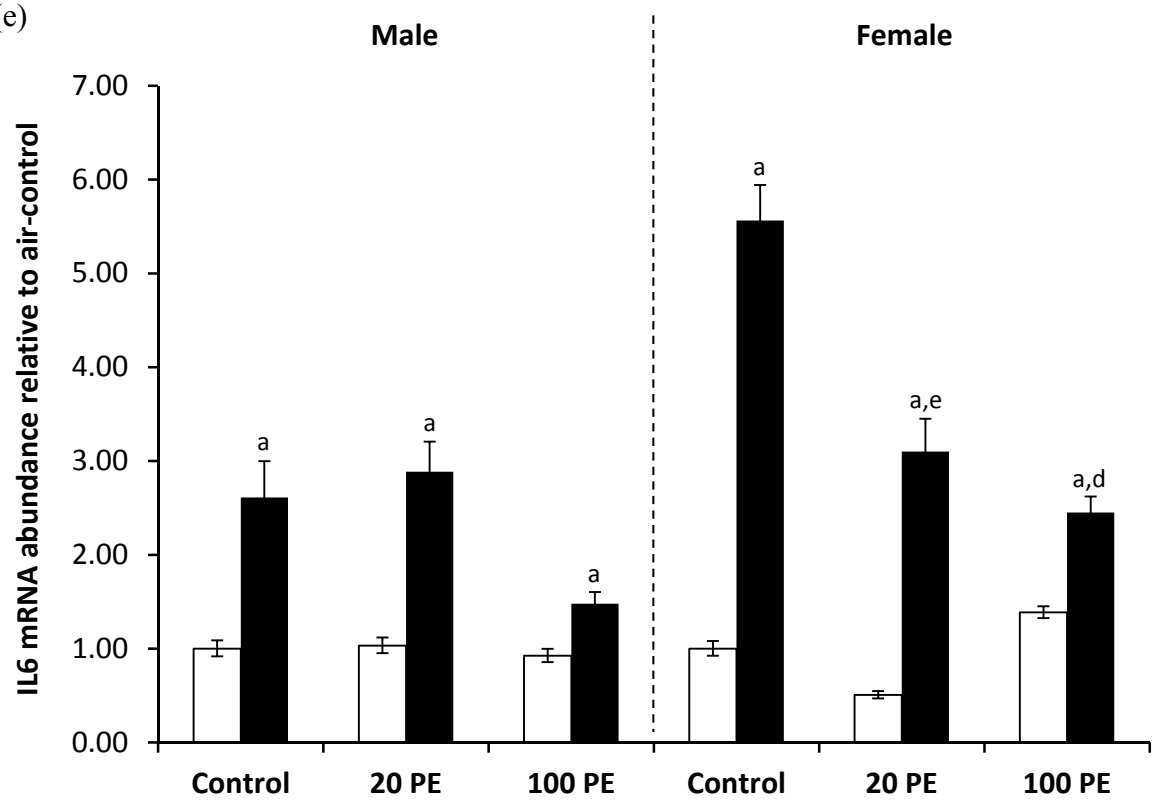
(c)


Male
Female
GSTM1 mRNA abundance relative to air-control
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
a
a
c
b
Control
20 PE
100 PE
Control
20 PE
100 PE

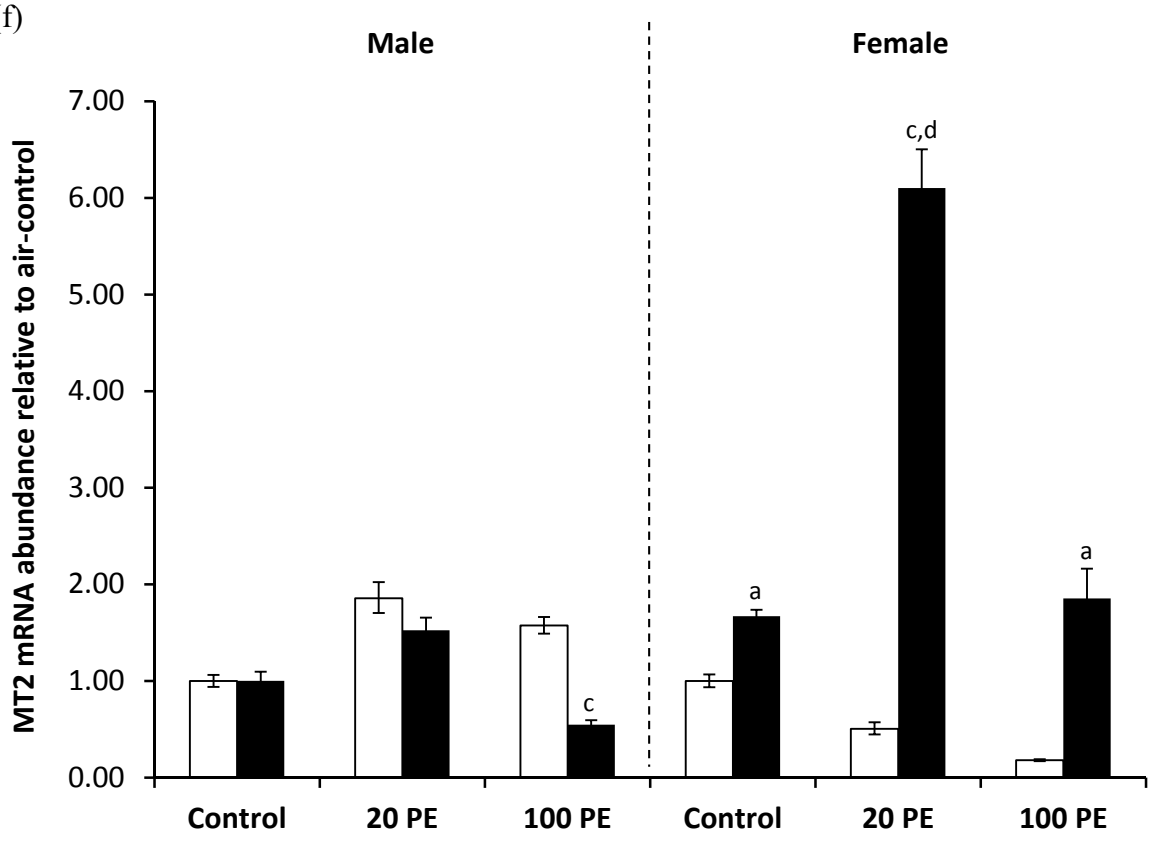
(d)


Male
Female
HMOX1 mRNA abundance relative to air-control
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Control
20 PE
100 PE
Control
20 PE
100 PE

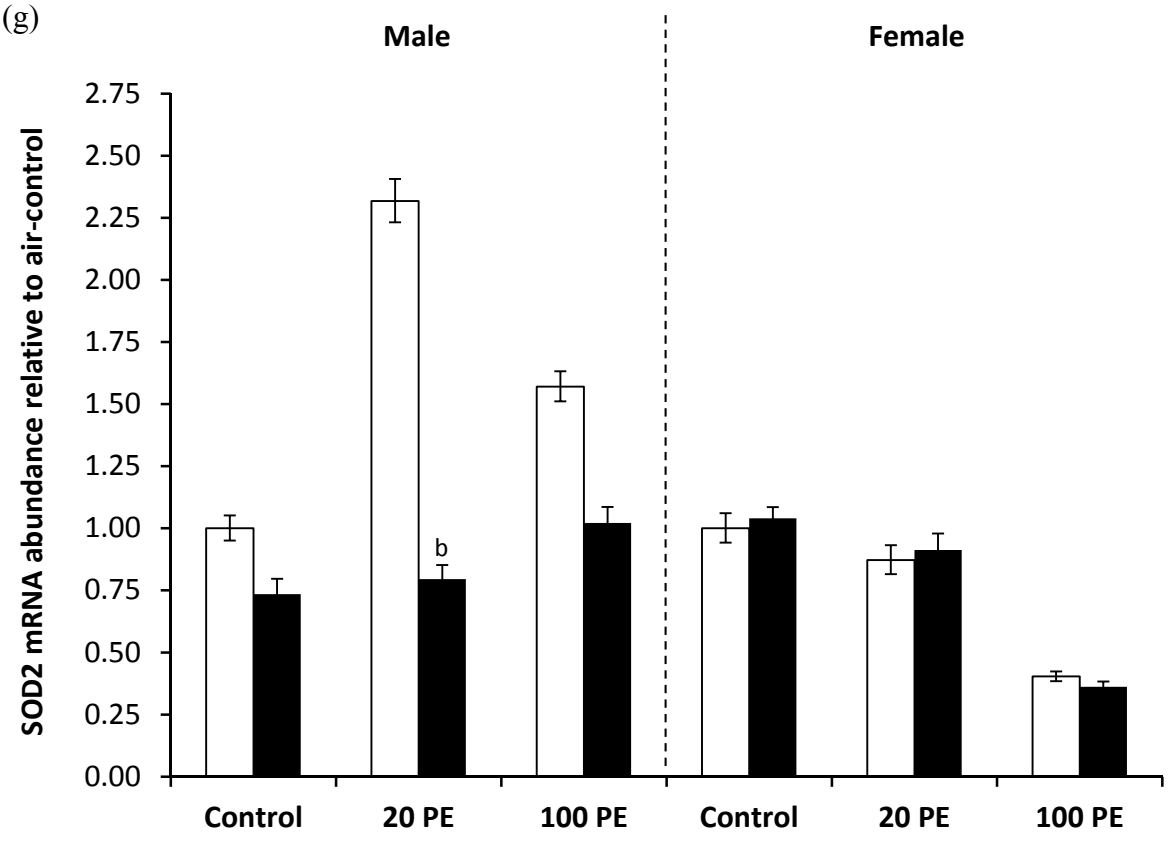
(e)


Male
Female
IL6 mRNA abundance relative to air-control
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
a
a
a
a
a,e
a,d
Control
20 PE
100 PE
Control
20 PE
100 PE

(f)


Male
Female
MT2 mRNA abundance relative to air-control
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
c
a
c,d
a
Control
20 PE
100 PE
Control
20 PE
100 PE

(g)



[a] $p < 0.05$ versus respective air group within dietary treatment

[b] $p \approx 0.05$ versus respective air group within dietary treatment, trending

[c] $p = 0.05$ versus respective air group within dietary treatment

[d] $p < 0.05$ versus respective ozone control group

[e] $p \approx 0.05$ versus respective ozone control group, trending

FIGURE 9. The mRNA abundance of antioxidative and inflammatory mediators, including endothelin 1 (*ET1*) **(a)**, glutathione peroxidase 1 (*GPX1*) **(b)**, glutathione-*S*-transferase Mu 1 (*GSTM1*) **(c)**, heme oxygenase (decycling) 1 (*HMOX1*) **(d)**, interleukin 6 (*IL-6*) **(e)**, metallothionein 2 (*MT2*) **(f)**, and superoxide dismutase 2 (*SOD2*) **(g)** in the lung tissues of male and female mice are presented as mean ± SE (n = 4–8) and were determined by the ΔC_t method with the level of β-actin as internal control. White bars: air; black bars: ozone.

VII. DISCUSSION

The present study was the first to examine the dose-responsive effects of a polyphenol-rich diet on ozone-mediated lung inflammation/injury by evaluating the relevant immunocytology profile and biomarkers, but most importantly, the influence on gene expression as related to the endogenous antioxidant defenses under conditions of oxidative stress. Ozone-induced pulmonary injury is characterized by epithelial disruption, impaired pulmonary function, and inflammation, all of these effects have been extensively examined in animal models [5, 19, 23, 30, 35]. These responses are a consequence of the reactive nature of ozone in conjunction with the depletion of endogenous antioxidant defenses leading to oxidative injury [46-48]. Much of the ozone-mediated toxicity can be indirectly evaluated based on the release of cytokine/chemokine in association with inflammatory cell recruitment/activation along with increased protein in the alveolar epithelium. Furthermore, since the gene transcription of endogenous antioxidants in epithelial cells and macrophages is tightly balanced under the Nrf2 and NF-κB transcription network, the changes in the transcriptional activity of antioxidant and pro-inflammatory genes are thus excellent indicators of the epithelial redox status in response to ozone.

Not only PE supplemented treatment conferred protection in counteracting the metabolic abnormalities, its preventive capacity extended to ameliorating both pulmonary inflammation and tissue injury mediated by ozone as evidenced by a significant reduction in neutrophil migration ($p < 0.05$; Table 8) and improved epithelial integrity ($p < 0.001$; Fig. 7) in both 20 and 100 PE-treated male and female mice. These findings are consistent with decreased hyperpermeability of the alveolar/capillary barrier, which is associated with acute pulmonary injury [16]. In addition, both PE treatments were not associated with increased concentrations of pulmonary alveolar macrophages upon ozone challenge (Table 8), which is supportive of the hypothesis that PE supplementation inhibited ozone-mediated influx of macrophages into the alveolar space, thereby preventing an inflammatory cascade that can trigger neutrophil accumulation (pulmonary inflammation) and vascular permeability (pulmonary injury). A diminished involvement/infiltration of secondary macrophages and neutrophil characterize an attenuated production of pro-inflammatory cytokines and neutrophil chemoattractants as observed in the BALF concentrations of the inflammatory biomarkers (Table 9 and Fig. 8A and B). The lack of

significant differences between air- and ozone-exposed PE-supplemental groups does not necessarily imply a lack of responsiveness to the PE treatment; on the contrary, it shows that PE was effective in normalizing the levels of pro-inflammatory cytokines and chemokines to the levels similar to that in the air-exposed group. These data were consistent with another *in vivo* study where they monitored ozone-induced changes in the lung function and airway inflammation in healthy non-smoking male and female adults (age 18–35) on a diet low in ascorbate for 3 wk [130]. After 1 wk of dietary antioxidant restriction, each subject was exposed to filtered air while intermittently resting and exercising for 2 hr. Cohorts supplemented with 250 mg of ascorbate, 50 IU of α-tocopherol, and 12 oz of vegetable cocktail daily for 2 wk were found to have significantly increased plasma levels of antioxidants and improved pulmonary function, but most importantly, the inflammatory response as represented by the percent neutrophils and the concentration of IL-6 recovered in the BALF of ozone-exposed subjects was not different from the placebo group. The anti-inflammatory effects of CGA are well documented. CGA was found to inhibit staphylococcal exotoxin-stimulated human peripheral blood mononuclear cells in a concentration-dependent manner [176]. Near complete suppression of cytokines (IL-1β, IL-6) and chemokines (MCP-1, MIP-1α, MIP-1β) was observed at cell culture concentrations of 200 μg/mL ($p < 0.05$) but not at 20 μg/mL.

As PE supplementation was associated with significant protective effects against acute pulmonary injury in both sexes, the gene expression of several antioxidant and pro-inflammatory genes were also studied to further understand their adaptive activity in response to acute ozone challenge after a long-term dietary treatment with polyphenols. Findings from this study are in support of the concept that PE supplementation altered the transcriptional activity of these genes, which could imply a reduction in hyperoxic toxicity induced by ozone exposure, thereby improving the overall inflammatory milieu in the bronchial epithelium. For instance, ET1 is a potent vasoconstrictor peptide that has been detected in both animal models and humans after exposure to ambient fine particulate matter and ozone since the lungs are the primary source of circulating ET-1 [2, 161]. An increase in the plasma ET-1 profile within a few hours is indicative of ambient ozone or other pollutant exposure [2]. Both 20 PE males and 100 PE females showed decreased lung *ET-1* expression in response to ozone, which is indicative of PE pre-treatment providing protection from hyperoxic insult (Fig. 9A). Additionally, females under 100 PE

supplemented condition, regardless of exposure parameter, demonstrated a reduced *ET-1* abundance levels relative to those in the control group. This indicates that PE supplementation extended its health benefits (in a dose- and sex-dependent manner) beyond the presence of an acute oxidant insult, thus could provide protection against detrimental cardiac outcomes associated with chronic pollutant exposure, including elevated blood pressure, decreased heart rate, increased myocardial infarction, and disruption of systemic vascular function [2, 177, 178]. GPX1 is one of the earliest enzymatic antioxidants found in ELF to restore the redox status that is altered with oxidative stress by catalyzing the reduction of hydrogen peroxide and lipid peroxides [179, 180]. *GPX1* overexpression is especially crucial to inhibit the pro-inflammatory cascade as it is associated with the downregulation of hydrogen peroxide-induced NF-κB activation [181, 182]. This is in accordance with the respiratory *GPX1* mRNA abundance showing excessive activity in male mice without the PE pre-treatment upon ozone exposure (Fig. 9B). Previous findings have shown that *GPX1* overexpression is a protective response following environmental toxic challenges [181, 182]. In contrast, PE treatment groups showed significant reduction in the *GPX1* activity levels especially among the higher dosage (0.72-fold vs. 5.77-fold, $p < 0.05$ versus respective ozone-exposed control group) which could be due to the decreased pulmonary toxicity associated with PE supplementation. Similar observations were noted with the *GSTM1* mRNA abundance levels in males, whereby the interaction of ozone exposure with either of the PE treatments had the effect of downregulating *GSTM1* expression activity significantly ($p < 0.05$ versus respective air group within the same dietary treatment) (Fig. 9C). The female counterpart, however, showed neither such expression pattern nor interaction as ozone-exposed controls, 20 PE, and 100 PE treatment groups shared similar mRNA abundance levels (Fig. 9C). A possible explanation for the above observations is that PE supplementation may have effectively improved the redox status in the epithelial lining of the lungs such that enzymatic *GPX1* and *GSTM1* upregulation at the transcription level was dispersible. Support for this latter assumption was particularly evident among the females where the *GPX1* expression levels did not differ between either the untreated or the treated PE groups, implying that the PE-supplemented diet succeeded in restoring homeostatic balance to the level comparable to that of air-exposed group without PE treatment (Fig. 9B). Consistent with the *GPX1* expression profile, the *HMOX1* overexpression by type II pneumocytes and alveolar macrophages is another cellular antioxidative defense to counteract acute lung injury induced by heme, hypoxia, hyperoxia, nitric oxide, endotoxin, and

pro-inflammatory cytokines (e.g., IL-6, TNF-α) [183-186]. The excessive upregulation of *HMOX1* as seen in the ozone-exposed male mice was suppressed by both 20 and 100 PE treatments to a level below 1.00-fold (Fig. 9D). The female mice exhibited similar expression pattern for *HMOX1* under both PE conditions (Fig. 9D). A marked increase of IL-6 mRNA abundance in ELF is a common observation in the mouse model of acute lung injury upon exposure to environmental toxicants, including ozone [187, 188]. IL-6 is a well-known pleiotropic cytokine which has been reported to exhibit both pro- and anti-inflammatory properties [189]. Even though an overexpression of *IL6* levels was noted in male and female mice after PE pre-treatment with respect to its air control within the same diet parameter, 100 PE significantly supressed *IL6* upregulation versus respective ozone control group (no statistical significance was reached in males, but $p < 0.05$ in females; Fig. 9E). These findings highlight that PE-mediated suppression of IL-6 release by alveolar epithelial cells and macrophages uncouples the lymphocytic infiltration and tissue injury that are responsible for perpetuating an oxidative state in the alveolar space. Typically, an overexpression of IL-6 confers to protection against hyperoxic condition as seen in the transgenic mouse model with remarkably diminished pro-inflammatory cell influx, airway reactivity, and lung lipid peroxidation [190, 191]. By improving the redox status in the epithelial lining of the lungs, the higher polyphenol dosage conferred similar protection with markedly diminished acute phase response in the lungs, as evidenced by a significant reduction in inflammatory cell influx, alveolar-capillary protein permeability, and cytokine/chemokine release. In this case, sex factor did not play a significant role in the *IL6* expression activity, as the effect was entirely dictated by PE in a dose-dependent manner (Fig. 9E). It is important to mention that mRNA expression and protein levels do not have to correspond, as was the case here for IL6 protein concentrations to be elevated upon ozone exposure in both sexes (Table 9). Numerous factors can affect the mRNA stability, including alternative splicing and/or post-translational modifications, all of which can account for the discrepancy observed in the expression levels between protein and mRNA.

Metallothionein proteins play an important role in the homeostasis and detoxification of heavy metals (e.g., zinc, cadmium and copper) as well as protecting cells and tissues against the oxidative effects posed by pro-inflammatory cytokines, including TNF-α, IL-6, and IFN-γ [192-194]. Depending on the pathophysiological conditions, metallothionein also possesses the dual

pro- and anti-inflammatory properties similar to IL-6 [195, 196]. Unlike IL-6, the polyphenol pre-treatment had a divergent influence on the expression activity of *MT2* in male and female mice upon ozone challenge (Fig. 9F). In males, 100 PE attenuated *MT2* mRNA abundance ($p = 0.05$ versus respective air group within dietary treatment), whereas both dosages significantly enhanced its expression with 20 PE showing a 12-fold increase relative to air group within the same dietary treatment ($p = 0.05$). An overexpression of endogenous metallothionein, in the context of ozone exposure, typically corresponds to maintaining pulmonary endothelial and epithelial cellular integrity, in conjunction to regulating the formation of oxidative stress-related molecules such as inducible nitric oxide synthase and nitrotyrosine [192]. Yet, this was not observed in the 100 PE supplemented male group, which rather shared a similar suppressed expression pattern with *GPX1* and *GSTM1* (Fig. 9B–C). As stated earlier, the PE pre-treatment (in a sex- and dose-dependent manner) had effectively minimized transient oxidant damage by indirectly maintaining the redox equilibrium such that the upregulation of stress-activated NF-κB signaling pathway in epithelial cells and macrophages was no longer obligatory. It is not entirely clear why the induction of *SOD2* was selectively observed in the female group (Fig. 9F). In line with the other findings, regardless of dosage, the polyphenol-rich potato extract suppressed the transcription activation of *SOD2* well below the respective air group within each dietary treatment (Fig. 9G). The 100 PE-treated female mice demonstrated a tendency for decreased transcription activation of SOD2 even without the presence of an oxidant as the *SOD2* abundance levels were reduced to more than half of what the control group exhibited (0.40-fold vs. 1.00-fold). SOD2 is essential for the mitochondrial antioxidant capacity, whereby its impairment can increase susceptibility to the development of cardiovascular disease caused by oxidative stressors, including chronic systemic cholesterol levels due to unhealthy lifestyles or environmental tobacco smoke [197, 198]. The decreased SOD2 activity observed in this study did not compromise the proper cellular processes necessary to resolve an inflammatory response, as supported by the improved pulmonary cellular, inflammatory and total protein indices, because the PE pre-treatment prevented homeostatic imbalance from occurring upon the transient hyperoxic insult [199, 200].

When oxidative stress occurs, cells respond to counteract the oxidative constituents through an *adaptive* upregulation of antioxidants to minimize the impact/extent of further oxidant injury [45, 174]. These adaptive oxidative responses evolved around the activation or silencing of

genes which in turn are modulated by a complex cross-talk between signaling pathway mediators (e.g., transcription factors, kinases, phosphatases, cytokines, antioxidant response elements). Many of these biochemical transductions are redox sensitive. Therefore, the gene expression results from this study strongly suggest that the proposed mechanistic properties associated with polyphenols are likely not of a direct effect on the signaling pathway mediators (Table 2), but rather through altering the redox status in the epithelial milieu. Genetic variations are common among the enzymatic antioxidants studied. For instance, the significance of GSTM1 polymorphism in the human population has been identified to correlate with inter-individual susceptibility to environmental toxicants, the extent of inflammation pathogenesis, and variability in the response to oxidative stress and antioxidant supplementation [201, 202]. Such inter-individual variations in the studied mice could explain partly the variability of gene expression observed in the data. Inbred mice are ideal for investigating multifaceted gene-environment interactions because they share several chromosomal regions of conserved synteny with humans [203]. However, their genetic background contributes significantly to the differential susceptibility to ozone exposure based on the examination of lung inflammation [204], hyperpermeability [205], acute lung injury [206], and alveolar macrophage activity [207]. According to phenotypic analysis of several recombinant inbred strains, C57BL/6J represents a good mouse model to study ozone exposure because it is O_3-sensitive, whereas C3H/HeJ is O_3-resistant. It is also documented that O_3-sensitivity is a recessive multigenic trait [204, 206].

In recent years, there is an increasing amount of interest in the possible health impacts associated with low dose chemical exposures and human health, especially chemicals that can alter functions of the endocrine system [208]. In this context, low dose is referred to as the environmentally relevant levels. A non-monotonic dose response (NMDR) is sometimes observed in low doses where the biological effects of the chemical do not follow a linear trajectory within the tested range, but rather contain one or two points of inflection along the slope of the response curve [209]. In this study, it is not biologically plausible for the mice to exhibit a NMDR towards the polyphenol treatment since, as reported by the World Health Organization, NMDRs are more frequently identified in *in vitro* studies, high-dose range studies, or short-term studies. In addition, the question of reproducibility often comes up for the evaluation of the same chemical. This lack of reproducibility may be due to the variations in sample size, experimental design issues,

inappropriate statistical analysis, or the lack of a true NMDR [208, 209]. When NMDRs are present and reproducible in *in vivo* studies, they are typically associated with apical adverse effects after a high dose exposure [209].

Similar to genetic polymorphisms, sex underlies the inter-individual differences in terms of susceptibility to environmental toxicants [140]. Several other factors have been identified to account for the inter-individual variability in the acute responses to ozone. Age, sex, race and antioxidant intake are the notable ones [210, 211]. Another possible cause for the discrepancy seen in male and female might be related to the influence of sex hormones on pulmonary inflammatory processes as evidenced by the compelling data from the current study. The concept of sex hormones in influencing the lung response to injurious stimuli such as ozone, however, has not been previously investigated. In a mouse monocytic cell line (RAW 264.7), 17β-estradiol (E2) administration inhibited LPS-induced DNA binding and transcriptional activity of the p65 subunit of NF-κB by preventing its nuclear translocation [212]. Similarly, in rat vascular smooth muscle cells, E2 inhibited both constitutive and IL-1β-stimulated NF-κB activation [213]. Those receiving E2 treatment had a higher recovery rate with significant reduced infarct volume, neuronal apoptosis, and inflammatory responses. Moreover, estrogen treatment restored cellular immunity in ethanol/burn-injured male mice as indicated by combination of improved splenocyte-proliferative responses, reduced macrophage-mediated IL-6 production, and increased survival after bacterial challenge [214]. E2 pro-inflammatory effects on cytokine secretion appear to only occur at periovulatory (proestrus) to pregnancy levels, which are evident for IL-6, IL-8, and TNF. Collectively, the evidence presented indicates that sex factor might confound the interaction between oxidants and dietary factors. The present data underscore the importance of how sex can influence not just the immune responses towards ozone-induced toxicity, but can also dictate the underlying mechanisms responsible for male and female to differ in their responsiveness towards an exogenous dietary treatment. The efficacy thus appears not to be merely dependent on the dose as sex also appears to play an integral role in dictating the anti-inflammatory properties of PE supplementation.

The current diet-exposure model is reflective of the lifestyle observed in an urban setting with considerable levels of air pollution. The present study showed that PE supplementation in

male and female C57BL/6 mice fed a high-fat diet for 4 wk prior to acute exposure to ozone was associated with protection against diet-induced metabolic impairments in addition to ozone-mediated lung injury and inflammation. PE supplementation was associated with attenuated weight gain in a dose-dependent manner, with the 100 PE diet demonstrating a notably higher efficacy than 20 PE in lowering the final body weight in males by 30% (Fig. 1B). The female mice exhibited similar but even greater results in body weight attenuation with both supplemented dosages (Fig. 2B). This observation was corroborated by an overall improved adiposity in both sexes as the 100 PE-treated group demonstrating a significant reduction ($p < 0.01$) in the total fat pads relative to the untreated control group. Furthermore, both 20 and 100 PE treatments restored fasting blood glucose homeostasis ($p = 0.001$) and improved insulin sensitivity ($p = 0.05$), albeit in a non-consisting responsive manner between the sexes as indicated by both metabolic endpoints (Fig. 3–5). It appears that perhaps male and female differ in their dietary adaptation to a high-fat diet that may inadvertently predispose to their divergent response patterns to PE or vice-versa. Interestingly, the concept of sex-specific predisposition to obesity was investigated in another study using 6-wk-old C57BL/6 male and female mice receiving one of three diet regimens: 30% calorie-restricted, low-fat (5% fat), or high-fat (35% fat) [215]. Regardless of the dietary composition, male mice had a greater propensity of gaining body weight than female mice, and that ovariectomy eliminated the protection against weight gain in female mice; in fact, ovariectomized female mice appear to mimic the male mice in their susceptibility to weight gain and percent body fat levels [215]. In other words, sex hormones account for the metabolic differences between males and females. This latter study thus provides an explanation as to why the female mice in this study appeared to demonstrate less pronounced body weight gain and fat deposition, along with enhanced glucose tolerance in the 100 PE treatment. It is possible that the sex hormones of the female mice could have protected them from the effects of diet-induced obesity, which may have further augmented the anti-obesity metabolic effects associated with the CGA and FA-rice potato extract (refer to Table 4). Regardless, the results presented here are comparable to the beneficial effects of resveratrol on the overall metabolic health and well-being of mice on a high-calorie diet [216]. There have been numerous reports strongly supporting a link between low-grade inflammation associated with obesity and respiratory complications [217]. Clinical investigations showed a strong correlation between transient elevations in pro-inflammatory cytokine levels (e.g., TNF-α, IL-6) due to obesity and the risk of developing adverse

lung complications in patients with sepsis [218, 219]. Serum elevations of TNF-α and IL-6 mimic the symptomatic response related to acute lung injury, including depletion of antioxidant stores, upregulation of adhesion molecules, and enhanced susceptibility to injury [220]. Additionally, the insulin-sensitizing and anti-inflammatory adipokine known as adiponectin is proposed to be inhibited by the same IL-6 and TNF-α in obese individuals [221]. This is relevant to the respiratory system as adiponectin has been shown to play a key role in regulating immune responses and promoting an anti-inflammatory phenotype in the lung [222-224]. Isolated alveolar macrophages and lung endothelium from adiponectin-deficient mice demonstrated a propensity to upregulate pro-inflammatory genes compared to the wild-type. This suggests that conditions leading to hypoadiponectinemia, such as obesity, may lead to increase lung susceptibility to pro-inflammatory challenges [217]. Further studies are needed to explore the possible relationship between the protective effects of PE supplementation on weight gain as a mechanism of its protective action on ozone-induced pulmonary injury.

Due to experimental constraints, factors that are useful in the monitoring of the body weight gain (e.g., daily food intake, physical activity, energy expenditure, and respiratory quotient) were not measured during the study. It is possible that PE supplementation may have an unintended effect of controlling the daily caloric intake of mice for the duration of the study which might have contributed to their protective effect against ozone as supported by an *in vivo* study, where Kari and colleagues subjected male F344 rats to either an *ad libitum* or a 25% restricted calorie diet for 20 d before exposure to 2.0 O_3 ppm [225]. They reported that calorie-restricted rats exhibited a markedly diminished inflammatory response to O_3 inhalation, including reduced levels of IL-6, PMN infiltration, and protein. They attributed the mechanism responsible for this effect to an increase in ascorbate and glutathione levels in the ELF, thereby providing an antioxidant milieu which helps to minimize the toxic effects of O_3 to reach biological targets [225]. On the other hand, Kubow *et al*. reported that PRPE (based on various compositional combinations of CGA and FA per kg of 60% HFD for 10 wk) conferred body weight gain protection and reduced adiposity in diet-induced obesity of C56BL/J male and female mice despite notably higher concurrent food intake and activity levels [111].

In conclusion, the work presented here is the first to report the synergistic effects of using two potato-derived polyphenols in the protection against pulmonary inflammation and injury mediated by ozone at the acceptable environmental level of 0.8 ppm. Moreover, the findings here support the hypothesis that supplementation of dietary PE can greatly improve weight gain, adiposity, glucose intolerance, and fasting plasma insulin associated with long-term consumption of WD.

VIII. CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Unhealthy eating habits and environmental pollutants are recognized as two major adverse health risk factors in urban areas, especially in relation to cardiopulmonary morbidity and mortality. Both inconvenient by-products of urbanization are no doubt highly correlated. Recent epidemiological studies have reported that among the specific population groups with chronic obesity-related conditions (i.e., diabetes mellitus, hypertension, and myocardial infarction), they are greatly susceptible to the adverse effects of ozone, especially among women, comparable to those with pre-existing chronic pulmonary diseases [226, 227]. Ozone is a harmful oxidant produced by the photochemical reaction between nitrogen oxides and volatile organic compounds [51].

The results in previous disease models studied have concluded that the therapeutic properties of polyphenols are primarily of anti-inflammatory, antioxidant and antimicrobial nature [228]. In particular, they counteract the various ROS-mediated activation of transcription factors, notably NF-κB and AP-1, by changing the nuclear histone acetylation and deacetylation patterns [73]. Even though they undergo a series of degradation which affect their bioaccessibility in the surrounding tissues, this study provides indisputable evidence that shows potato extracts are able to respond to oxidative stress and influence inflammation cascades accordingly. The current diet-exposure model was reflective of the lifestyle observed in any urban setting with considerable levels of air pollution, but it was only representative of one given time point. This is because the level and duration of ozone exposure (0.8 ppm for 4 h) in the study merely represents a transient, acute perturbation that can induce a pulmonary inflammatory response, but not a systemic one. Therefore, the extent of the ozone-induced toxicity was not adequate to significantly alter glucose tolerance, insulin homeostasis and visceral adiposity. In order for inhaled ozone to elicit a systemic low-grade inflammatory response and atherogenic symptoms mimicking the development of WD-related metabolic perturbation, the exposure frequency needs to be increased to a minimum of 4 hr a day and 4 days a week until the week prior euthanization as seen in most animal studies [157, 161, 225, 229-232]. For this reason, only the effect of PE supplementation was considered for the fasting blood glucose and insulin parameters. Due to experimental constraints, factors that are useful in the monitoring of the body weight gain (e.g., daily food intake, physical activity, energy

expenditure, and respiratory quotient) were not measured during the study. As such, it was not possible to properly assess how much of the protective effect against ozone was attributed to PE supplementation, reduced caloric intake, increased physical activity, or a combination of all. This is the major drawback of the present study. With the limited sample size in each dietary treatment group and short-term duration of ozone exposure, the longitudinal effect of ambient ozone exposure on the cardiopulmonary health outcomes of interest was not comprehensively explored. As a consequence, the mechanisms underlying the complex interaction between WD, PE supplementation, ozone exposure, and sex factors remain incomplete. Therefore, this demonstrates the need for additional chronic, dose-response WD × ozone exposures to further determine whether the biological outcomes observed with PE treatment was sustainable, and how WD alters ozone susceptibility or vice-versa. Nevertheless, the findings in the current study do provide new insights into a potential biological basis for the use of hydroxycinnamic acid derivatives (in addition to the use of vitamin C, α-tocopherol, or resveratrol as seen in most literature) pertaining to the protection against air pollution together with a WD regimen. The fact that sex factor confounds the co-occurrence of environmental factors in some human diseases further underscores the difficulty in establishing the individual roles of each causative factor, let alone determining the efficacy of an antioxidant treatment. Hence, the concept of sex differences and how it might influence concurrent ozone exposure and polyphenol metabolism should be prioritized as an underlying factor in the extent of oxidative stress when outcome variables are being measured in future studies. Similarly, understanding the metabolic differences between the sexes can allow appropriate preventive modality to be dispensed for chronic diseases associated with metabolic disorders (e.g., type II diabetes and cardiovascular diseases).

Oxidative stress and the generation of reactive radical species belong to an intricate intracellular redox-signalling network that is essential for life. Under normal physiological conditions, most ROS are by-products of energy metabolism and are obligatory components for maintaining cellular functions in the body (e.g., oxidative modification of DNA [233] or removal of damaged cells and pathogens in phagosomes [234]). In that case, a continual occurrence of cellular oxidation to a certain degree is not considered deleterious. Since the respiratory system comprises such a large epithelial surface area and is directly linked to the external environment, it has to encompass an armamentarium of tightly regulated antioxidant defenses to compensate for

the higher risk of oxidant exposure. This includes consequences of oxidative stress arising from metabolic abnormalities [235]. Prolonged metabolic imbalances engage a similar set of molecules and signalling pathways that are typically involved in mounting an inflammatory response [236, 237]. In fact, many of the metabolic and immune response pathways are highly integrated, evolutionarily conserved throughout species, and the proper function of each is dependent on the other [236]. This implies that it is unlikely for exogenous antioxidants to replace the role of such a highly conserved, highly efficient endogenous antioxidant defense system against any environmental oxidant that may disrupt normal physiological functioning of the lung. Following such logic, perhaps what contributes to the biological actions of dietary antioxidants might not be related to their ability to exert direct antioxidant activity under pathological conditions, it is *more* plausible to infer their role as "pro-oxidants" (rather than compensatory) acting to aggravate/promote the transcriptional activity of various ARE-responsive and pro-inflammatory genes in epithelial cells and macrophages, thereby strengthening the overall antioxidant defense in the respiratory system upon a transient environmental insult (i.e., high-fat diet and/or ozone exposure) [238]. The biological effects ascribed to PE supplementation in the present study were likely due to PE pre-treatment exerting a pre-conditioning effect on the redox status in the epithelial lining of the lungs so that to minimize the impact associated with the stress-activated NF-κB signaling pathway. Hence, lung morphology integrity, cellular, and biochemical pro-inflammatory mediators remained relatively unaltered parallel to the air-exposed groups as reflected in the results.

With the biological significance of reactive oxygen species and the role of dietary antioxidants defined, it explains why most human intervention trials involving the administration of antioxidants failed to reveal any positive effects or reverse the adverse effects in oxidative stress-driven pathologies [239-247]. This does not mean phytochemicals or polyphenols alike are not essential for human health. It simply means that diet-derived antioxidants do not behave the same way as pharmacologics, because their mode of action is not to target an existing oxidative damage *per se*, but rather to challenge the endogenous adaptation systems in the body so that to bring forth more protection against possible disease development/susceptibility. In other words, having a well-balanced, consistent dietary regimen with plenty of cereal grains, fruits, and vegetables for a prolonged period of time (in the absence of an oxidative stress) confer the most

health benefits than the consumption of large doses of vitamins as seen in most of the aforementioned intervention studies [248]. Not only does this knowledge validate the data from the current study, it reinforces the need to further understand polyphenols in order to apply it in other disease context. As stated earlier, the true biological properties of polyphenols are not governed by their ability to respond to oxidative stress, inflammation, and endothelial function, *in vivo* bioavailability, biotransformation, and utilization must also be considered in order to fully understand their functionality. This adds on another layer of complexity to the topic of polyphenols [249, 250]. The interaction between the gut microbiota and polyphenolic metabolites is pivotal to delineate the metabolic (induced by WD) and immune response (induced by ozone exposure) pathways in perpetuating inflammation. By comparing the compositional/genomic shifts between affected and healthy individuals, in conjunction with measuring localized oxidative biomarkers, this could help identify metabolites with the most biological significance.

VIX. REFERENCES

1. Pryor W.A., Squadrito G.L., and Friedman M. **(1995)** The cascade mechanism to explain ozone toxicity: The role of lipid ozonation products. *Free Radical Biology and Medicine* 19(6): 935-941.

2. Brook R.D., Brook J.R., Urch B., Vincent R., Rajagopalan S., and Silverman F. **(2002)** Inhalation of fine particulate air pollution and ozone causes acute arterial vasoconstriction in healthy adults. *Circulation* 105(13): 1534-1536.

3. Gilmour M.I., Park P., Doerfler D., and Selgrade M.K. **(1993)** Factors that Influence the Suppression of Pulmonary Antibacterial Defenses in Mice Exposed to Ozone. *Experimental Lung Research* 19(3): 299-314.

4. Weinmann G.G., Bowes S.M., Gerbase M.W., Kimball A.W., and Frank R. **(1995)** Response to acute ozone exposure in healthy men. Results of a screening procedure. *Am J Respir Crit Care Med* 151(1): 33-40.

5. Savov J.D., Whitehead G.S., Wang J., Liao G., Usuka J., Peltz G.*, et al.* **(2004)** Ozone-induced acute pulmonary injury in inbred mouse strains. *American journal of respiratory cell and molecular biology* 31(1): 69-77.

6. Jorres R., Nowak D., and Magnussen H. **(1996)** The effect of ozone exposure on allergen responsiveness in subjects with asthma or rhinitis. *Am J Respir Crit Care Med* 153(1): 56-64.

7. Kerr H.D., Kulle T.J., McIlhany M.L., and Swidersky P. **(1975)** Effects of ozone on pulmonary function in normal subjects. An environmental-chamber study. *The American review of respiratory disease* 111(6): 763-773.

8. Devlin R.B., McDonnell W.F., Mann R., Becker S., House D.E., Schreinemachers D.*, et al.* **(1991)** Exposure of humans to ambient levels of ozone for 6.6 hours causes cellular and biochemical changes in the lung. *American journal of respiratory cell and molecular biology* 4(1): 72-81.

9. Kehrl H.R., Vincent L.M., Kowalsky R.J., Horstman D.H., O'Neil J.J., McCartney W.H.*, et al.* **(1987)** Ozone exposure increases respiratory epithelial permeability in humans. *The American review of respiratory disease* 135(5): 1124-1128.

10. Foster W.M., Brown R.H., Macri K., and Mitchell C.S. **(2000)** Bronchial reactivity of healthy subjects: 18-20 h postexposure to ozone. *J Appl Physiol* 89(5): 1804-1810.

11. Foster W.M., Costa D.L., and Langenback E.G. **(1987)** Ozone exposure alters tracheobronchial mucociliary function in humans. *J Appl Physiol* 63(3): 996-1002.

12. McDonnell W.F., Stewart P.W., and Smith M.V. **(2007)** The temporal dynamics of ozone-induced FEV1 changes in humans: an exposure-response model. *Inhal Toxicol* 19(6-7): 483-494.

13. Brown J.S., Bateson T.F., and McDonnell W.F. **(2008)** Effects of exposure to 0.06 ppm ozone on FEV1 in humans: a secondary analysis of existing data. *Environ Health Perspect* 116(8): 1023-1026.

14. Koren H.S., Devlin R.B., Graham D.E., Mann R., McGee M.P., Horstman D.H.*, et al.* **(1989)** Ozone-induced inflammation in the lower airways of human subjects. *The American review of respiratory disease* 139(2): 407-415.

15. Aris R.M., Christian D., Hearne P.Q., Kerr K., Finkbeiner W.E., and Balmes J.R. **(1993)** Ozone-induced airway inflammation in human subjects as determined by airway lavage and biopsy. *The American review of respiratory disease* 148(5): 1363-1372.

16. Mudway I.S. and Kelly F.J. **(2000)** Ozone and the lung: a sensitive issue. *Mol Aspects Med* 21(1-2): 1-48.

17. Kinney P.L., Nilsen D.M., Lippmann M., Brescia M., Gordon T., McGovern T.*, et al.* **(1996)** Biomarkers of lung inflammation in recreational joggers exposed to ozone. *Am J Respir Crit Care Med* 154(5): 1430-1435.

18. Krishna M.T., Madden J., Teran L.M., Biscione G.L., Lau L.C., Withers N.J.*, et al.* **(1998)** Effects of 0.2 ppm ozone on biomarkers of inflammation in bronchoalveolar lavage fluid and bronchial mucosa of healthy subjects. *The European respiratory journal : official journal of the European Society for Clinical Respiratory Physiology* 11(6): 1294-1300.

19. Ishii Y., Yang H., Sakamoto T., Nomura A., Hasegawa S., Hirata F.*, et al.* **(1997)** Rat alveolar macrophage cytokine production and regulation of neutrophil recruitment following acute ozone exposure. *Toxicology and applied pharmacology* 147(2): 214-223.

20. Pendino K.J., Laskin J.D., Shuler R.L., Punjabi C.J., and Laskin D.L. **(1993)** Enhanced production of nitric oxide by rat alveolar macrophages after inhalation of a pulmonary irritant is associated with increased expression of nitric oxide synthase. *J Immunol* 151(12): 7196-7205.

21. Pendino K.J., Gardner C.R., Quinones S., and Laskin D.L. **(1996)** Stimulation of nitric oxide production in rat lung lavage cells by anti-Mac-1beta antibody: effects of ozone inhalation. *American journal of respiratory cell and molecular biology* 14(4): 327-333.

22. Lee I.T. and Yang C.M. **(2013)** Inflammatory signalings involved in airway and pulmonary diseases. *Mediators of inflammation* 2013: 791231.

23. Cho H.Y., Zhang L.Y., and Kleeberger S.R. **(2001)** Ozone-induced lung inflammation and hyperreactivity are mediated via tumor necrosis factor-alpha receptors. *American journal of physiology. Lung cellular and molecular physiology* 280(3): L537-546.

24. Yang I.A., Holz O., Jorres R.A., Magnussen H., Barton S.J., Rodriguez S.*, et al.* **(2005)** Association of tumor necrosis factor-alpha polymorphisms and ozone-induced change in lung function. *Am J Respir Crit Care Med* 171(2): 171-176.

25. Maekawa Y., Ishikawa K., Yasuda O., Oguro R., Hanasaki H., Kida I.*, et al.* **(2009)** Klotho suppresses TNF-alpha-induced expression of adhesion molecules in the endothelium and attenuates NF-kappaB activation. *Endocrine* 35(3): 341-346.

26. Cho H.Y., Morgan D.L., Bauer A.K., and Kleeberger S.R. **(2007)** Signal transduction pathways of tumor necrosis factor--mediated lung injury induced by ozone in mice. *Am J Respir Crit Care Med* 175(8): 829-839.

27. Kelly F.J., Mudway I., Krishna M.T., and Holgate S.T. **(1995)** The free radical basis of air pollution: focus on ozone. *Respiratory medicine* 89(10): 647-656.

28. Comhair S.A. and Erzurum S.C. **(2002)** Antioxidant responses to oxidant-mediated lung diseases. *American journal of physiology. Lung cellular and molecular physiology* 283(2): L246-255.

29. Pryor W.A. **(1994)** Mechanisms of radical formation from reactions of ozone with target molecules in the lung. *Free radical biology & medicine* 17(5): 451-465.

30. Hatch G.E., Slade R., Harris L.P., McDonnell W.F., Devlin R.B., Koren H.S.*, et al.* **(1994)** Ozone dose and effect in humans and rats. A comparison using oxygen-18 labeling and bronchoalveolar lavage. *Am J Respir Crit Care Med* 150(3): 676-683.

31. Postlethwait E.M., Langford S.D., and Bidani A. **(1994)** Determinants of inhaled ozone absorption in isolated rat lungs. *Toxicology and applied pharmacology* 125(1): 77-89.

32. Postlethwait E.M., Cueto R., Velsor L.W., and Pryor W.A. **(1998)** O3-induced formation of bioactive lipids: estimated surface concentrations and lining layer effects. *The American journal of physiology* 274(6 Pt 1): L1006-1016.

33. Langford S.D., Bidani A., and Postlethwait E.M. **(1995)** Ozone-reactive absorption by pulmonary epithelial lining fluid constituents. *Toxicology and applied pharmacology* 132(1): 122-130.

34. Kafoury R.M., Pryor W.A., Squadrito G.L., Salgo M.G., Zou X., and Friedman M. **(1999)** Induction of inflammatory mediators in human airway epithelial cells by lipid ozonation products. *Am J Respir Crit Care Med* 160(6): 1934-1942.

35. Johnston R.A., Schwartzman I.N., Flynt L., and Shore S.A. **(2005)** Role of interleukin-6 in murine airway responses to ozone. *American journal of physiology. Lung cellular and molecular physiology* 288(2): L390-397.

36. Johnston R.A., Mizgerd J.P., and Shore S.A. **(2005)** CXCR2 is essential for maximal neutrophil recruitment and methacholine responsiveness after ozone exposure. *American journal of physiology. Lung cellular and molecular physiology* 288(1): L61-67.

37. Bates D.V., Bell G.M., Burnham C.D., Hazucha M., Mantha J., Pengelly L.D.*, et al.* **(1972)** Short-term effects of ozone on the lung. *J Appl Physiol* 32(2): 176-181.

38. Pryor W.A., Das B., and Church D.F. **(1991)** The ozonation of unsaturated fatty acids: aldehydes and hydrogen peroxide as products and possible mediators of ozone toxicity. *Chemical research in toxicology* 4(3): 341-348.

39. Boutten A., Goven D., Boczkowski J., and Bonay M. **(2010)** Oxidative stress targets in pulmonary emphysema: focus on the Nrf2 pathway. *Expert opinion on therapeutic targets* 14(3): 329-346.

40. Cho H.Y., Reddy S.P., and Kleeberger S.R. **(2006)** Nrf2 defends the lung from oxidative stress. *Antioxid Redox Signal* 8(1-2): 76-87.

41. Cho H.Y., Jedlicka A.E., Reddy S.P., Kensler T.W., Yamamoto M., Zhang L.Y.*, et al.* **(2002)** Role of NRF2 in protection against hyperoxic lung injury in mice. *American journal of respiratory cell and molecular biology* 26(2): 175-182.

42. Umemura T., Kuroiwa Y., Kitamura Y., Ishii Y., Kanki K., Kodama Y.*, et al.* **(2006)** A crucial role of Nrf2 in in vivo defense against oxidative damage by an environmental pollutant, pentachlorophenol. *Toxicological sciences : an official journal of the Society of Toxicology* 90(1): 111-119.

43. Chan K. and Kan Y.W. **(1999)** Nrf2 is essential for protection against acute pulmonary injury in mice. *Proceedings of the National Academy of Sciences of the United States of America* 96(22): 12731-12736.

44. Osburn W.O. and Kensler T.W. **(2008)** Nrf2 signaling: an adaptive response pathway for protection against environmental toxic insults. *Mutation research* 659(1-2): 31-39.

45. Cross C.E., van der Vliet A., O'Neill C.A., Louie S., and Halliwell B. **(1994)** Oxidants, antioxidants, and respiratory tract lining fluids. *Environ Health Perspect* 102 Suppl 10: 185-191.

46. Rahman I. and Massaro D. **(1992)** Endotoxin treatment protects rats against ozone-induced lung edema: with evidence for the role of manganese superoxide dismutase. *Toxicology and applied pharmacology* 113(1): 13-18.

47. Avissar N.E., Reed C.K., Cox C., Frampton M.W., and Finkelstein J.N. **(2000)** Ozone, but not nitrogen dioxide, exposure decreases glutathione peroxidases in epithelial lining fluid of human lung. *Am J Respir Crit Care Med* 162(4 Pt 1): 1342-1347.

48. Mudway I.S., Krishna M.T., Frew A.J., MacLeod D., Sandstrom T., Holgate S.T.*, et al.* **(1999)** Compromised concentrations of ascorbate in fluid lining the respiratory tract in human subjects after exposure to ozone. *Occupational and environmental medicine* 56(7): 473-481.

49. Rahman I., Clerch L.B., and Massaro D. **(1991)** Rat lung antioxidant enzyme induction by ozone. *The American journal of physiology* 260(6 Pt 1): L412-418.

50. Hayden M.S. and Ghosh S. **(2008)** Shared principles in NF-kappaB signaling. *Cell* 132(3): 344-362.

51. Tong N., Leung D., and Liu C.-H. **(2011)** A Review on Ozone Evolution and Its Relationship with Boundary Layer Characteristics in Urban Environments. *Water, Air, and Soil Pollution* 214(1-4): 13-36.

52. Alexandros G., Bertil F., Klea K., and Antonis A. **(2004)** Acute Effects of Ozone on Mortality from the "Air Pollution and Health: A European Approach" Project. *American Journal of Respiratory and Critical Care Medicine* 170(10): 1080-1087.

53. Jerrett M., Burnett R.T., Pope C.A., Ito K., Thurston G., Krewski D.*, et al.* **(2009)** Long-Term Ozone Exposure and Mortality. *The New England Journal of Medicine* 360(11): 1085-1095.

54. Gent J.F., Triche E.W., Holford T.R., Belanger K., Bracken M.B., Beckett W.S.*, et al.* **(2003)** Association of low-level ozone and fine particles with respiratory symptoms in children with asthma. *JAMA : the journal of the American Medical Association* 290(14): 1859-1867.

55. Schwartz J. **(1995)** Short term fluctuations in air pollution and hospital admissions of the elderly for respiratory disease. *Thorax* 50(5): 531-538.

56. U.S.-Environmental-Protection-Agency **(1997)** National Ambient Air Quality Standards for Ozone. *Fed. Regist.* 62(138).

57. Hubbell B.J., Hallberg A., McCubbin D.R., and Post E. **(2005)** Health-related benefits of attaining the 8-hr ozone standard. *Environ Health Perspect* 113(1): 73-82.

58. Hollingsworth J.W., Kleeberger S.R., and Foster W.M. **(2007)** Ozone and pulmonary innate immunity. *Proc Am Thorac Soc* 4(3): 240-246.

59. Alexeeff S.E., Litonjua A.A., Suh H., Sparrow D., Vokonas P.S., and Schwartz J. **(2007)** Ozone exposure and lung function: effect modified by obesity and airways hyperresponsiveness in the VA normative aging study. *Chest* 132(6): 1890-1897.

60. Bell M.L. and Dominici F. **(2008)** Effect modification by community characteristics on the short-term effects of ozone exposure and mortality in 98 US communities. *American journal of epidemiology* 167(8): 986-997.

61. Zanobetti A. and Schwartz J. **(2008)** Mortality displacement in the association of ozone with mortality: an analysis of 48 cities in the United States. *Am J Respir Crit Care Med* 177(2): 184-189.

62. Lisabeth L.D., Escobar J.D., Dvonch J.T., Sanchez B.N., Majersik J.J., Brown D.L., *et al.* **(2008)** Ambient air pollution and risk for ischemic stroke and transient ischemic attack. *Annals of neurology* 64(1): 53-59.

63. Meng Y.Y., Wilhelm M., Rull R.P., English P., and Ritz B. **(2007)** Traffic and outdoor air pollution levels near residences and poorly controlled asthma in adults. *Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology* 98(5): 455-463.

64. Chen C., Arjomandi M., Balmes J., Tager I., and Holland N. **(2007)** Effects of chronic and acute ozone exposure on lipid peroxidation and antioxidant capacity in healthy young adults. *Environ Health Perspect* 115(12): 1732-1737.

65. Rainham D.G.C., Smoyer-Tomic K.E., Sheridan S.C., and Burnett R.T. **(2005)** Synoptic weather patterns and modification of the association between air pollution and human mortality. *International Journal of Environmental Health Research* 15(5): 347-360.

66. Kolb S., Radon K., Valois M.F., Heguy L., and Goldberg M.S. **(2007)** The short-term influence of weather on daily mortality in congestive heart failure. *Archives of environmental & occupational health* 62(4): 169-176.

67. Ruidavets J.B., Cournot M., Cassadou S., Giroux M., Meybeck M., and Ferrieres J. **(2005)** Ozone air pollution is associated with acute myocardial infarction. *Circulation* 111(5): 563-569.

68. Goldberg M.S., Burnett R.T., Yale J.-F., Valois M.-F., and Brook J.R. **(2006)** Associations between ambient air pollution and daily mortality among persons with diabetes and cardiovascular disease. *Environmental Research* 100(2): 255-267.

69. Cornblatt B.S., Ye L., Dinkova-Kostova A.T., Erb M., Fahey J.W., Singh N.K., *et al.* **(2007)** Preclinical and clinical evaluation of sulforaphane for chemoprevention in the breast. *Carcinogenesis* 28(7): 1485-1490.

70. Gagandeep, Dhanalakshmi S., Mendiz E., Rao A.R., and Kale R.K. **(2003)** Chemopreventive effects of Cuminum cyminum in chemically induced forestomach and uterine cervix tumors in murine model systems. *Nutrition and cancer* 47(2): 171-180.

71. Kode A., Rajendrasozhan S., Caito S., Yang S.R., Megson I.L., and Rahman I. **(2008)** Resveratrol induces glutathione synthesis by activation of Nrf2 and protects against cigarette smoke-mediated oxidative stress in human lung epithelial cells. *American journal of physiology. Lung cellular and molecular physiology* 294(3): L478-488.

72. Niestroy J., Barbara A., Herbst K., Rode S., van Liempt M., and Roos P.H. **(2011)** Single and concerted effects of benzo[a]pyrene and flavonoids on the AhR and Nrf2-pathway in the human colon carcinoma cell line Caco-2. *Toxicology in vitro : an international journal published in association with BIBRA* 25(3): 671-683.

73. Rahman I., Biswas S.K., and Kirkham P.A. **(2006)** Regulation of inflammation and redox signaling by dietary polyphenols. *Biochem Pharmacol* 72(11): 1439-1452.

74. Gonzalez R., Ballester I., Lopez-Posadas R., Suarez M.D., Zarzuelo A., Martinez-Augustin O.*, et al.* **(2011)** Effects of flavonoids and other polyphenols on inflammation. *Critical reviews in food science and nutrition* 51(4): 331-362.

75. Hertog M.G.L., Feskens E.J.M., Kromhout D., Hollman P.C.H., and Katan M.B. **(1993)** Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *The Lancet* 342(8878): 1007-1011.

76. Virgili F. and Marino M. **(2008)** Regulation of cellular signals from nutritional molecules: a specific role for phytochemicals, beyond antioxidant activity. *Free radical biology & medicine* 45(9): 1205-1216.

77. Manach C., Mazur A., and Scalbert A. **(2005)** Polyphenols and prevention of cardiovascular diseases. *Current opinion in lipidology* 16(1): 77-84.

78. Nagao T., Meguro S., Hase T., Otsuka K., Komikado M., Tokimitsu I.*, et al.* **(2009)** A catechin-rich beverage improves obesity and blood glucose control in patients with type 2 diabetes. *Obesity (Silver Spring)* 17(2): 310-317.

79. Brasnyo P., Molnar G.A., Mohas M., Marko L., Laczy B., Cseh J.*, et al.* **(2011)** Resveratrol improves insulin sensitivity, reduces oxidative stress and activates the Akt pathway in type 2 diabetic patients. *The British journal of nutrition* 106(3): 383-389.

80. Arts I.C. and Hollman P.C. **(2005)** Polyphenols and disease risk in epidemiologic studies. *The American journal of clinical nutrition* 81(1 Suppl): 317S-325S.

81. Yang C.S., Landau, J.M., Huang, M.T., Newmark, H.L. **(2001)** Inhibition of carcinogenesis by dietary polyphenolic compounds. *Annual review of nutrition* 21: 381-406.

82. Lau F.C., Shukitt-Hale B., and Joseph J.A. **(2006)** Beneficial effects of berry fruit polyphenols on neuronal and behavioral aging. *JSFA Journal of the Science of Food and Agriculture* 86(14): 2251-2255.

83. Yang C.S., Sang S., Lambert J.D., Hou Z., Ju J., and Lu G. **(2006)** Possible mechanisms of the cancer-preventive activities of green tea. *Molecular nutrition & food research* 50(2): 170-175.

84. Riedl K.M. and Hagerman A.E. **(2001)** Tannin-protein complexes as radical scavengers and radical sinks. *J Agric Food Chem* 49(10): 4917-4923.

85. Higdon J.V. and Frei B. **(2003)** Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions. *Critical reviews in food science and nutrition* 43(1): 89-143.

86. Rechner A.R., Smith M.A., Kuhnle G., Gibson G.R., Debnam E.S., Srai S.K.S*., et al.* **(2004)** Colonic metabolism of dietary polyphenols: influence of structure on microbial fermentation products. *Free Radical Biology and Medicine* 36(2): 212-225.

87. Renouf M., Guy P.A., Marmet C., Fraering A.L., Longet K., Moulin J*., et al.* **(2010)** Measurement of caffeic and ferulic acid equivalents in plasma after coffee consumption: small intestine and colon are key sites for coffee metabolism. *Mol Nutr Food Res* 54(6): 760-766.

88. Rechner A.R., Kuhnle G., Bremner P., Hubbard G.P., Moore K.P., and Rice-Evans C.A. **(2002)** The metabolic fate of dietary polyphenols in humans. *Free Radic Biol Med* 33(2): 220-235.

89. Schaffer S. and Halliwell B. **(2012)** Do polyphenols enter the brain and does it matter? Some theoretical and practical considerations. *Genes Nutr* 7(2): 99-109.

90. Wheeler D.S., Catravas J.D., Odoms K., Denenberg A., Malhotra V., and Wong H.R. **(2004)** Epigallocatechin-3-gallate, a green tea-derived polyphenol, inhibits IL-1 beta-dependent proinflammatory signal transduction in cultured respiratory epithelial cells. *J Nutr* 134(5): 1039-1044.

91. Tanigawa S., Fujii M., and Hou D.X. **(2007)** Action of Nrf2 and Keap1 in ARE-mediated NQO1 expression by quercetin. *Free Radic Biol Med* 42(11): 1690-1703.

92. Canali R., Comitato R., Ambra R., and Virgili F. **(2010)** Red wine metabolites modulate NF-kappaB, activator protein-1 and cAMP response element-binding proteins in human endothelial cells. *Br J Nutr* 103(6): 807-814.

93. Moridani M.Y., Scobie H., Salehi P., and O'Brien P.J. **(2001)** Catechin metabolism: glutathione conjugate formation catalyzed by tyrosinase, peroxidase, and cytochrome p450. *Chem Res Toxicol* 14(7): 841-848.

94. Nishinaka T., Ichijo Y., Ito M., Kimura M., Katsuyama M., Iwata K*., et al.* **(2007)** Curcumin activates human glutathione S-transferase P1 expression through antioxidant response element. *Toxicol Lett* 170(3): 238-247.

95. Spencer J.P., Schroeter H., Kuhnle G., Srai S.K., Tyrrell R.M., Hahn U*., et al.* **(2001)** Epicatechin and its in vivo metabolite, 3'-O-methyl epicatechin, protect human fibroblasts from oxidative-stress-induced cell death involving caspase-3 activation. *Biochem J* 354(Pt 3): 493-500.

96. Kobuchi H., Roy S., Sen C.K., Nguyen H.G., and Packer L. **(1999)** Quercetin inhibits inducible ICAM-1 expression in human endothelial cells through the JNK pathway. *Am J Physiol* 277(3 Pt 1): C403-411.

97. Saeki K., Kobayashi N., Inazawa Y., Zhang H., Nishitoh H., Ichijo H*., et al.* **(2002)** Oxidation-triggered c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein (MAP) kinase pathways for apoptosis in human leukaemic cells stimulated by

epigallocatechin-3-gallate (EGCG): a distinct pathway from those of chemically induced and receptor-mediated apoptosis. *Biochem J* 368(Pt 3): 705-720.

98. Tarahovsky Y.S. **(2008)** Plant polyphenols in cell-cell interaction and communication. *Plant Signal Behav* 3(8): 609-611.

99. Kong A.N., Yu R., Chen C., Mandlekar S., and Primiano T. **(2000)** Signal transduction events elicited by natural products: role of MAPK and caspase pathways in homeostatic response and induction of apoptosis. *Arch Pharm Res* 23(1): 1-16.

100. Camire M.E., Kubow S., and Donnelly D.J. **(2009)** Potatoes and Human Health. *Critical reviews in food science and nutrition* 49(10): 823-840.

101. Nara K., Miyoshi T., Honma T., and Koga H. **(2006)** Antioxidative Activity of Bound-Form Phenolics in Potato Peel. *Bioscience, Biotechnology, and Biochemistry* 70(6): 1489-1491.

102. Kanatt S.R., Chander R., Radhakrishna P., and Sharma A. **(2005)** Potato Peel Extracta Natural Antioxidant for Retarding Lipid Peroxidation in Radiation Processed Lamb Meat. *J. Agric. Food Chem.* 53(5): 1499-1504.

103. Friedman M. **(1997)** Chemistry, Biochemistry, and Dietary Role of Potato Polyphenols. A Review. *J. Agric. Food Chem.* 45(5): 1523-1540.

104. Rodriguez de Sotillo D.V. and Hadley M. **(2002)** Chlorogenic acid modifies plasma and liver concentrations of: cholesterol, triacylglycerol, and minerals in (fa/fa) Zucker rats. *The Journal of Nutritional Biochemistry* 13(12): 717-726.

105. Johnston K.L., Clifford M.N., and Morgan L.M. **(2003)** Coffee acutely modifies gastrointestinal hormone secretion and glucose tolerance in humans: glycemic effects of chlorogenic acid and caffeine. *The American journal of clinical nutrition* 78(4): 728-733.

106. Tunnicliffe J.M., Eller L.K., Reimer R.A., Hittel D.S., and Shearer J. **(2011)** Chlorogenic acid differentially affects postprandial glucose and glucose-dependent insulinotropic polypeptide response in rats. *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme* 36(5): 650-659.

107. Jin Son M., C W.R., Hyun Nam S., and Young Kang M. **(2010)** Influence of oryzanol and ferulic Acid on the lipid metabolism and antioxidative status in high fat-fed mice. *Journal of clinical biochemistry and nutrition* 46(2): 150-156.

108. Srinivasan M., Sudheer A.R., and Menon V.P. **(2007)** Ferulic Acid: therapeutic potential through its antioxidant property. *Journal of clinical biochemistry and nutrition* 40(2): 92-100.

109. Belcaro G., Cesarone M.R., Ledda A., Cacchio M., Ruffini I., Ricci A*., et al.* **(2008)** 5-Year control and treatment of edema and increased capillary filtration in venous hypertension and diabetic microangiopathy using O-(beta-hydroxyethyl)-rutosides: a prospective comparative clinical registry. *Angiology* 59 Suppl 1: 14S-20S.

110. Kwon K.H., Murakami A., Tanaka T., and Ohigashi H. **(2005)** Dietary rutin, but not its aglycone quercetin, ameliorates dextran sulfate sodium-induced experimental colitis in mice: attenuation of pro-inflammatory gene expression. *Biochemical Pharmacology* 69(3): 395-406.

111. Kubow S., Hobson L., Iskandar M.M., Sabally K., Donnelly D.J., and Agellon L.B. **(2014)** An extract of Irish potatoes (Solanum tuberosum L.) decreases body weight gain and adiposity and improves glucose control in the mouse model of diet-induced obesity. *Molecular nutrition & food research*: In press.

112. Hanhineva K., Torronen R., Bondia-Pons I., Pekkinen J., Kolehmainen M., Mykkanen H.*, et al.* **(2010)** Impact of dietary polyphenols on carbohydrate metabolism. *International journal of molecular sciences* 11(4): 1365-1402.

113. Tabak C., Smit H.A., Heederik D., Ocke M.C., and Kromhout D. **(2001)** Diet and chronic obstructive pulmonary disease: independent beneficial effects of fruits, whole grains, and alcohol (the MORGEN study). *Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology* 31(5): 747-755.

114. Walda I.C., Tabak C., Smit H.A., Rasanen L., Fidanza F., Menotti A.*, et al.* **(2002)** Diet and 20-year chronic obstructive pulmonary disease mortality in middle-aged men from three European countries. *European journal of clinical nutrition* 56(7): 638-643.

115. Harik-Khan R.I., Muller D.C., and Wise R.A. **(2004)** Serum Vitamin Levels and the Risk of Asthma in Children. *American journal of epidemiology* 159(4): 351-357.

116. Rubin R.N., Navon L., and Cassano P.A. **(2004)** Relationship of Serum Antioxidants to Asthma Prevalence in Youth. *American Journal of Respiratory and Critical Care Medicine* 169(3): 393-398.

117. Dosil-Diaz O., Ruano-Ravina A., Gestal-Otero J.J., and Barros-Dios J.M. **(2008)** Consumption of fruit and vegetables and risk of lung cancer: a case-control study in Galicia, Spain. *Nutrition* 24(5): 407-413.

118. Satia J.A., Littman A., Slatore C.G., Galanko J.A., and White E. **(2009)** Long-term use of beta-carotene, retinol, lycopene, and lutein supplements and lung cancer risk: results from the VITamins And Lifestyle (VITAL) study. *American journal of epidemiology* 169(7): 815-828.

119. Feskanich D., Ziegler R.G., Michaud D.S., Giovannucci E.L., Speizer F.E., Willett W.C.*, et al.* **(2000)** Prospective study of fruit and vegetable consumption and risk of lung cancer among men and women. *Journal of the National Cancer Institute* 92(22): 1812-1823.

120. Miyake Y., Sasaki S., Yokoyama T., Chida K., Azuma A., Suda T.*, et al.* **(2004)** Vegetable, fruit, and cereal intake and risk of idiopathic pulmonary fibrosis in Japan. *Annals of nutrition & metabolism* 48(6): 390-397.

121. Baur J.A. and Sinclair D.A. **(2006)** Therapeutic potential of resveratrol: the in vivo evidence. *Nature reviews. Drug discovery* 5(6): 493-506.

122. Siedlinski M., Boer J.M., Smit H.A., Postma D.S., and Boezen H.M. **(2012)** Dietary factors and lung function in the general population: wine and resveratrol intake. *The European respiratory journal : official journal of the European Society for Clinical Respiratory Physiology* 39(2): 385-391.

123. Kolleck I., Sinha P., and Rustow B. **(2002)** Vitamin E as an antioxidant of the lung: mechanisms of vitamin E delivery to alveolar type II cells. *Am J Respir Crit Care Med* 166(12 Pt 2): S62-66.

124. Elsayed N.M. **(2001)** Antioxidant mobilization in response to oxidative stress: a dynamic environmental-nutritional interaction. *Nutrition* 17(10): 828-834.

125. Chatham M.D., Eppler J.H., Jr., Sauder L.R., Green D., and Kulle T.J. **(1987)** Evaluation of the effects of vitamin C on ozone-induced bronchoconstriction in normal subjects. *Annals of the New York Academy of Sciences* 498: 269-279.

126. Trenga C.A., Koenig J.Q., and Williams P.V. **(2001)** Dietary antioxidants and ozone-induced bronchial hyperresponsiveness in adults with asthma. *Archives of environmental health* 56(3): 242-249.

127. Sienra-Monge J.J., Ramirez-Aguilar M., Moreno-Macias H., Reyes-Ruiz N.I., Del Rio-Navarro B.E., Ruiz-Navarro M.X.*, et al.* **(2004)** Antioxidant supplementation and nasal inflammatory responses among young asthmatics exposed to high levels of ozone. *Clinical and experimental immunology* 138(2): 317-322.

128. Grievink L., Jansen S.M., van't Veer P., and Brunekreef B. **(1998)** Acute effects of ozone on pulmonary function of cyclists receiving antioxidant supplements. *Occupational and environmental medicine* 55(1): 13-17.

129. Grievink L., Zijlstra A.G., Ke X., and Brunekreef B. **(1999)** Double-blind intervention trial on modulation of ozone effects on pulmonary function by antioxidant supplements. *American journal of epidemiology* 149(4): 306-314.

130. Samet J.M., Hatch G.E., Horstman D., Steck-Scott S., Arab L., Bromberg P.A.*, et al.* **(2001)** Effect of Antioxidant Supplementation on Ozone-Induced Lung Injury in Human Subjects. *American Journal of Respiratory and Critical Care Medicine* 164(5): 819-825.

131. Steck-Scott S., Arab L., Craft N.E., and Samet J.M. **(2004)** Plasma and lung macrophage responsiveness to carotenoid supplementation and ozone exposure in humans. *European journal of clinical nutrition* 58(12): 1571-1579.

132. Institute-of-Medicine **(2001)** Exploring the biological contributions to human health: does sex matter? *Journal of women's health & gender-based medicine* 10(5): 433-439.

133. Caracta C.F. **(2003)** Gender differences in pulmonary disease. *The Mount Sinai journal of medicine, New York* 70(4): 215-224.

134. Melgert B.N., Ray A., Hylkema M.N., Timens W., and Postma D.S. **(2007)** Are there reasons why adult asthma is more common in females? *Current allergy and asthma reports* 7(2): 143-150.

135. Mannino D.M., Homa D.M., Akinbami L.J., Ford E.S., and Redd S.C. **(2002)** Chronic obstructive pulmonary disease surveillance--United States, 1971-2000. *MMWR Surveill Summ* 51(6): 1-16.

136. Chapman K.R. **(2004)** Chronic obstructive pulmonary disease: are women more susceptible than men? *Clinics in Chest Medicine* 25(2): 331-341.

137. Egleston B.L., Meireles S.I., Flieder D.B., and Clapper M.L. **(2009)** Population-Based Trends in Lung Cancer Incidence in Women. *Seminars in oncology* 36(6): 506-515.

138. Siegfried J.M., Hershberger P.A., and Stabile L.P. **(2009)** Estrogen Receptor Signaling in Lung Cancer. *Seminars in oncology* 36(6): 524-531.

139. Rich S., Dantzker D.R., Ayres S.M., Bergofsky E.H., Brundage B.H., Detre K.M.*, et al.* **(1987)** Primary pulmonary hypertension. A national prospective study. *Annals of internal medicine* 107(2): 216-223.

140. Straub R.H. **(2007)** The Complex Role of Estrogens in Inflammation. *Endocrine Reviews* 28(5): 521-574.

141. Lambert K.C., Curran E.M., Judy B.M., Milligan G.N., Lubahn D.B., and Estes D.M. **(2005)** Estrogen Receptor α (ERα) Deficiency in Macrophages Results in Increased Stimulation of CD4+ T Cells while 17β-Estradiol Acts through ERα to Increase IL-4 and GATA-3 Expression in CD4+ T Cells Independent of Antigen Presentation. *The Journal of Immunology* 175(9): 5716-5723.

142. Anand S.S. and Yusuf S. **(2011)** Stemming the global tsunami of cardiovascular disease. *The Lancet* 377(9765): 529-532.

143. Berrington de Gonzalez A., Hartge P., Cerhan J.R., Flint A.J., Hannan L., MacInnis R.J.*, et al.* **(2010)** Body-Mass Index and Mortality among 1.46 Million White Adults. *New England Journal of Medicine* 363(23): 2211-2219.

144. Finucane M.M., Stevens G.A., Cowan M.J., Danaei G., Lin J.K., Paciorek C.J.*, et al.* **(2011)** National, regional, and global trends in body-mass index since 1980: systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9·1 million participants. *The Lancet* 377(9765): 557-567.

145. Wilson P.W., D'Agostino R.B., Parise H., Sullivan L., and Meigs J.B. **(2005)** Metabolic syndrome as a precursor of cardiovascular disease and type 2 diabetes mellitus. *Circulation* 112(20): 3066-3072.

146. Davis B.B., Zeki A.A., Bratt J.M., Wang L., Filosto S., Walby W.F.*, et al.* **(2013)** Simvastatin inhibits smoke-induced airway epithelial injury: implications for COPD therapy. *European Respiratory Journal* 42(2): 350-361.

147. Lokhandwala T., West-Strum D., Banahan B.F., Bentley J.P., and Yang Y. **(2012)** Do statins improve outcomes in patients with asthma on inhaled corticosteroid therapy? A retrospective cohort analysis. *BMJ Open* 2(3).

148. Baldán Á., Gomes A.V., Ping P., and Edwards P.A. **(2008)** Loss of ABCG1 Results in Chronic Pulmonary Inflammation. *The Journal of Immunology* 180(5): 3560-3568.

149. Brook R.D., Franklin B., Cascio W., Hong Y., Howard G., Lipsett M.*, et al.* **(2004)** Air Pollution and Cardiovascular Disease: A Statement for Healthcare Professionals From the Expert Panel on Population and Prevention Science of the American Heart Association. *Circulation* 109(21): 2655-2671.

150. Bhatnagar A. **(2006)** Environmental Cardiology: Studying Mechanistic Links Between Pollution and Heart Disease. *Circulation Research* 99(7): 692-705.

151. Pope C.A., Burnett R.T., Thurston G.D., Thun M.J., Calle E.E., Krewski D.*, et al.* **(2004)** Cardiovascular Mortality and Long-Term Exposure to Particulate Air Pollution: Epidemiological Evidence of General Pathophysiological Pathways of Disease. *Circulation* 109(1): 71-77.

152. Miller K.A., Siscovick D.S., Sheppard L., Shepherd K., Sullivan J.H., Anderson G.L.*, et al.* **(2007)** Long-Term Exposure to Air Pollution and Incidence of Cardiovascular Events in Women. *New England Journal of Medicine* 356(5): 447-458.

153. Peters A., Liu E., Verrier R.L., Schwartz J., Gold D.R., Mittleman M.*, et al.* **(2000)** Air pollution and incidence of cardiac arrhythmia. *Epidemiology* 11(1): 11-17.

154. Zanobetti A. and Schwartz J. **(2005)** The effect of particulate air pollution on emergency admissions for myocardial infarction: a multicity case-crossover analysis. *Environ Health Perspect* 113(8): 978-982.

155. Dockery D.W., Pope C.A., 3rd, Xu X., Spengler J.D., Ware J.H., Fay M.E.*, et al.* **(1993)** An association between air pollution and mortality in six U.S. cities. *N Engl J Med* 329(24): 1753-1759.

156. Zanobetti A. and Schwartz J. **(2007)** Particulate air pollution, progression, and survival after myocardial infarction. *Environ Health Perspect* 115(5): 769-775.

157. Sun Q., Yue P., Deiuliis J.A., Lumeng C.N., Kampfrath T., Mikolaj M.B.*, et al.* **(2009)** Ambient Air Pollution Exaggerates Adipose Inflammation and Insulin Resistance in a Mouse Model of Diet-Induced Obesity. *Circulation* 119(4): 538-546.

158. Dubowsky S.D., Suh H., Schwartz J., Coull B.A., and Gold D.R. **(2006)** Diabetes, obesity, and hypertension may enhance associations between air pollution and markers of systemic inflammation. *Environ Health Perspect* 114(7): 992-998.

159. O'Neill M.S., Veves A., Zanobetti A., Sarnat J.A., Gold D.R., Economides P.A., *et al.* **(2005)** Diabetes enhances vulnerability to particulate air pollution-associated impairment in vascular reactivity and endothelial function. *Circulation* 111(22): 2913-2920.

160. Vunnam R. **(2010)** Antioxidant capacity and polyphenolic content of potato tubers are affected by cultivar and hormetic treatment. M.Sc. Thesis (McGill University, Macdonald Campus, Ste-Anne-de-Bellevue).

161. Thomson E., Kumarathasan P., Goegan P., Aubin R.A., and Vincent R. **(2005)** Differential regulation of the lung endothelin system by urban particulate matter and ozone. *Toxicological sciences : an official journal of the Society of Toxicology* 88(1): 103-113.

162. Kumarathasan P., Goegan P., and Vincent R. **(2001)** An Automated High-Performance Liquid Chromatography Fluorescence Method for the Analyses of Endothelins in Plasma Samples. *Analytical Biochemistry* 299(1): 37-44.

163. Smith P.K., Krohn R.I., Hermanson G.T., Mallia A.K., Gartner F.H., Provenzano M.D., *et al.* **(1985)** Measurement of protein using bicinchoninic acid. *Anal Biochem* 150(1): 76-85.

164. Zuker M. **(2003)** Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Research* 31(13): 3406-3415.

165. Thomson E. and Vincent R. **(2005)** Reagent volume and plate bias in real-time polymerase chain reaction. *Analytical Biochemistry* 337(2): 347-350.

166. Chen D., Pan X., Xiao P., Farwell M.A., and Zhang B. **(2011)** Evaluation and identification of reliable reference genes for pharmacogenomics, toxicogenomics, and small RNA expression analysis. *Journal of Cellular Physiology* 226(10): 2469-2477.

167. Vandesompele J., De Preter K., Pattyn F., Poppe B., Van Roy N., De Paepe A., *et al.* **(2002)** Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biology* 3(7): research0034.0031 - research0034.0011.

168. Pfaffl M., Tichopad A., Prgomet C., and Neuvians T. **(2004)** Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: BestKeeper – Excel-based tool using pair-wise correlations. *Biotechnology Letters* 26(6): 509-515.

169. Andersen C.L., Jensen J.L., and Orntoft T.F. **(2004)** Normalization of real-time quantitative reverse transcription-PCR data: a model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. *Cancer Res* 64(15): 5245-5250.

170. Silver N., Best S., Jiang J., and Thein S. **(2006)** Selection of housekeeping genes for gene expression studies in human reticulocytes using real-time PCR. *BMC Molecular Biology* 7(1): 33.

171. Thomson E.M., Vladisavljevic D., Mohottalage S., Kumarathasan P., and Vincent R. **(2013)** Mapping Acute Systemic Effects of Inhaled Particulate Matter and Ozone: Multiorgan Gene Expression and Glucocorticoid Activity. *Toxicological Sciences Toxicological Sciences* 135(1): 169-181.

172. Livak K.J. and Schmittgen T.D. **(2001)** Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. *Methods* 25(4): 402-408.

173. Valacchi G., Pagnin E., Corbacho A.M., Olano E., Davis P.A., Packer L.*, et al.* **(2004)** In vivo ozone exposure induces antioxidant/stress-related responses in murine lung and skin. *Free radical biology & medicine* 36(5): 673-681.

174. Janssen Y.M., Driscoll K.E., Timblin C.R., Hassenbein D., and Mossman B.T. **(1998)** Modulation of mitochondrial gene expression in pulmonary epithelial cells exposed to oxidants. *Environ Health Perspect* 5: 1191-1195.

175. Leikauf G.D., McDowell S.A., Bachurski C.J., Aronow B.J., Gammon K., Wesselkamper S.C.*, et al.* **(2001)** Functional genomics of oxidant-induced lung injury. *Adv Exp Med Biol* 500: 479-487.

176. Krakauer T. **(2002)** The Polyphenol Chlorogenic Acid Inhibits Staphylococcal Exotoxin-induced Inflammatory Cytokines and Chemokines. *Immunopharmacology and Immunotoxicology* 24(1): 113-119.

177. Vincent R., Kumarathasan P., Goegan P., Bjarnason S.G., Guenette J., Berube D.*, et al.* **(2001)** Inhalation toxicology of urban ambient particulate matter: acute cardiovascular effects in rats. *Res Rep Health Eff Inst* 104: 5-54.

178. Kang Y.J., Li Y., Zhou Z., Roberts A.M., Cai L., Myers S.R.*, et al.* **(2002)** Elevation of serum endothelins and cardiotoxicity induced by particulate matter (PM2.5) in rats with acute myocardial infarction. *Cardiovasc Toxicol* 2(4): 253-261.

179. Cheng W., Fu Y.X., Porres J.M., Ross D.A., and Lei X.G. **(1999)** Selenium-dependent cellular glutathione peroxidase protects mice against a pro-oxidant-induced oxidation of NADPH, NADH, lipids, and protein. *Faseb J* 13(11): 1467-1475.

180. Cheng W.H., Ho Y.S., Valentine B.A., Ross D.A., Combs G.F., Jr., and Lei X.G. **(1998)** Cellular glutathione peroxidase is the mediator of body selenium to protect against paraquat lethality in transgenic mice. *The Journal of nutrition* 128(7): 1070-1076.

181. Li Q. and Engelhardt J.F. **(2006)** Interleukin-1β Induction of NFκB Is Partially Regulated by H2O2-mediated Activation of NFκB-inducing Kinase. *Journal of Biological Chemistry* 281(3): 1495-1505.

182. Li Q., Sanlioglu S., Li S., Ritchie T., Oberley L., and Engelhardt J.F. **(2001)** GPx-1 gene delivery modulates NFkappaB activation following diverse environmental injuries through a specific subunit of the IKK complex. *Antioxid Redox Signal* 3(3): 415-432.

183. Christou H., Morita T., Hsieh C.M., Koike H., Arkonac B., Perrella M.A.*, et al.* **(2000)** Prevention of hypoxia-induced pulmonary hypertension by enhancement of endogenous heme oxygenase-1 in the rat. *Circ Res* 86(12): 1224-1229.

184. Yet S.F., Pellacani A., Patterson C., Tan L., Folta S.C., Foster L.*, et al.* **(1997)** Induction of heme oxygenase-1 expression in vascular smooth muscle cells. A link to endotoxic shock. *The Journal of biological chemistry* 272(7): 4295-4301.

185. Motterlini R., Foresti R., Intaglietta M., and Winslow R.M. **(1996)** NO-mediated activation of heme oxygenase: endogenous cytoprotection against oxidative stress to endothelium. *The American journal of physiology* 270(1 Pt 2): H107-114.

186. Lee P.J., Alam J., Sylvester S.L., Inamdar N., Otterbein L., and Choi A.M. **(1996)** Regulation of heme oxygenase-1 expression in vivo and in vitro in hyperoxic lung injury. *American journal of respiratory cell and molecular biology* 14(6): 556-568.

187. Shore S.A., Johnston R.A., Schwartzman I.N., Chism D., and Krishna Murthy G.G. **(2002)** *Ozone-induced airway hyperresponsiveness is reduced in immature mice* pp 1019-1028.

188. Johnston C.J., Stripp B.R., Reynolds S.D., Avissar N.E., Reed C.K., and Finkelstein J.N. **(1999)** Inflammatory and antioxidant gene expression in C57BL/6J mice after lethal and sublethal ozone exposures. *Exp Lung Res* 25(1): 81-97.

189. Tilg H., Dinarello C.A., and Mier J.W. **(1997)** IL-6 and APPs: anti-inflammatory and immunosuppressive mediators. *Immunol Today* 18(9): 428-432.

190. DiCosmo B.F., Geba G.P., Picarella D., Elias J.A., Rankin J.A., Stripp B.R.*, et al.* **(1994)** Airway epithelial cell expression of interleukin-6 in transgenic mice. Uncoupling of airway inflammation and bronchial hyperreactivity. *The Journal of clinical investigation* 94(5): 2028-2035.

191. Ward N.S., Waxman A.B., Homer R.J., Mantell L.L., Einarsson O., Du Y.*, et al.* **(2000)** Interleukin-6-induced protection in hyperoxic acute lung injury. *American journal of respiratory cell and molecular biology* 22(5): 535-542.

192. Inoue K., Takano H., Kaewamatawong T., Shimada A., Suzuki J., Yanagisawa R.*, et al.* **(2008)** Role of metallothionein in lung inflammation induced by ozone exposure in mice. *Free radical biology & medicine* 45(12): 1714-1722.

193. Waelput W., Broekaert D., Vandekerckhove J., Brouckaert P., Tavernier J., and Libert C. **(2001)** A mediator role for metallothionein in tumor necrosis factor-induced lethal shock. *J Exp Med* 194(11): 1617-1624.

194. De S.K., McMaster M.T., and Andrews G.K. **(1990)** Endotoxin induction of murine metallothionein gene expression. *The Journal of biological chemistry* 265(25): 15267-15274.

195. Takano H., Inoue K., Yanagisawa R., Sato M., Shimada A., Morita T.*, et al.* **(2004)** Protective role of metallothionein in acute lung injury induced by bacterial endotoxin. *Thorax* 59(12): 1057-1062.

196. Inoue K., Takano H., Shimada A., Wada E., Yanagisawa R., Sakurai M.*, et al.* **(2006)** Role of metallothionein in coagulatory disturbance and systemic inflammation induced by lipopolysaccharide in mice. *Faseb J* 20(3): 533-535.

197. Harrison C.M., Pompilius M., Pinkerton K.E., and Ballinger S.W. **(2011)** Mitochondrial oxidative stress significantly influences atherogenic risk and cytokine-induced oxidant production. *Environ Health Perspect* 119(5): 676-681.

198. Knight-Lozano C.A., Young C.G., Burow D.L., Hu Z.Y., Uyeminami D., Pinkerton K.E.*, et al.* **(2002)** Cigarette smoke exposure and hypercholesterolemia increase mitochondrial damage in cardiovascular tissues. *Circulation* 105(7): 849-854.

199. Suzuki K., Nakamura M., Hatanaka Y., Kayanoki Y., Tatsumi H., and Taniguchi N. **(1997)** Induction of apoptotic cell death in human endothelial cells treated with snake venom: implication of intracellular reactive oxygen species and protective effects of glutathione and superoxide dismutases. *J Biochem* 122(6): 1260-1264.

200. Strassburger M., Bloch W., Sulyok S., Schuller J., Keist A.F., Schmidt A.*, et al.* **(2005)** Heterozygous deficiency of manganese superoxide dismutase results in severe lipid peroxidation and spontaneous apoptosis in murine myocardium in vivo. *Free radical biology & medicine* 38(11): 1458-1470.

201. Tujague J., Bastaki M., Holland N., Balmes J.R., and Tager I.B. **(2006)** Antioxidant intake, GSTM1 polymorphism and pulmonary function in healthy young adults. *The European respiratory journal : official journal of the European Society for Clinical Respiratory Physiology* 27(2): 282-288.

202. Romieu I., Sienra-Monge J.J., Ramírez-Aguilar M., Moreno-Macías H., Reyes-Ruiz N.I., Estela del Río-Navarro B.*, et al.* **(2004)** Genetic polymorphism of GSTM1 and antioxidant supplementation influence lung function in relation to ozone exposure in asthmatic children in Mexico City. *Thorax* 59(1): 8-10.

203. Vancza E.M., Galdanes K., Gunnison A., Hatch G., and Gordon T. **(2009)** Age, Strain, and Gender as Factors for Increased Sensitivity of the Mouse Lung to Inhaled Ozone. *Toxicological Sciences* 107(2): 535-543.

204. Kleeberger S.R., Levitt R.C., Zhang L.Y., Longphre M., Harkema J., Jedlicka A.*, et al.* **(1997)** Linkage analysis of susceptibility to ozone-induced lung inflammation in inbred mice. *Nature genetics* 17(4): 475-478.

205. Kleeberger S.R., Reddy S.P.M., Zhang L.Y., Cho H.Y., and Jedlicka A.E. **(2001)** Toll-like receptor 4 mediates ozone-induced murine lung hyperpermeability via inducible nitric oxide synthase. *American journal of physiology.* 280(2): L326-L333.

206. Prows D.R., Shertzer H.G., Daly M.J., Sidman C.L., and Leikauf G.D. **(1997)** Genetic analysis of ozone-induced acute lung injury in sensitive and resistant strains of mice. *Nature genetics.* 17(4): 471.

207. Ohtsuka Y., Brunson K.J., Jedlicka A.E., Mitzner W., Clarke R.W., Zhang L.-Y*., et al.* **(2000)** Genetic Linkage Analysis of Susceptibility to Particle Exposure in Mice. *American journal of respiratory cell and molecular biology* 22(5): 574-581.

208. Vandenberg L.N., Colborn T., Hayes T.B., Heindel J.J., Jacobs J., D. R. , Lee D.-H*., et al.* **(2012)** Hormones and Endocrine-Disrupting Chemicals: Low-Dose Effects and Nonmonotonic Dose Responses. *Endocrine Reviews* 33(3): 378-455.

209. United-Nations-Environment-Programme-and-the-World-Health-Organization **(2013)** State of the Science of Endocrine Disrupting Chemicals. Report (Editor: Bergman A., Heindel J.J., Jobling S., Kidd K.A., and Zoeller R.T.).

210. Hazucha M.J., Folinsbee L.J., and Bromberg P.A. **(1985)** Distribution and reproducibility of spirometric response to ozone by gender and age. *J Appl Physiol* 95(5): 1917-1925.

211. McDonnell W.F., Muller K.E., Bromberg P.A., and Shy C.M. **(1993)** Predictors of individual differences in acute response to ozone exposure. *The American review of respiratory disease* 147(4): 818-825.

212. Ghisletti S., Meda C., Maggi A., and Vegeto E. **(2005)** 17β-Estradiol Inhibits Inflammatory Gene Expression by Controlling NF-κB Intracellular Localization. *Molecular and Cellular Biology* 25(8): 2957-2968.

213. Sharma R.V., Gurjar M.V., and Bhalla R.C. **(1985)** Selected contribution: estrogen receptor-alpha gene transfer inhibits proliferation and NF-kappaB activation in VSM cells from female rats. *J Appl Physiol* 91(5): 2400-2406.

214. Messingham K.A., Heinrich S.A., and Kovacs E.J. **(2001)** Estrogen restores cellular immunity in injured male mice via suppression of interleukin-6 production. *J Leukoc Biol* 70(6): 887-895.

215. Hong J., Stubbins R.E., Smith R.R., Harvey A.E., and Nunez N.P. **(2009)** Differential susceptibility to obesity between male, female and ovariectomized female mice. *Nutrition journal* 8: 11.

216. Baur J.A., Pearson K.J., Price N.L., Jamieson H.A., Lerin C., Kalra A*., et al.* **(2006)** Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* 444(7117): 337-342.

217. Konter J.B.E.S.R.S.O.A.M.o.I. and Inflammation in the L. **(2013)** Obesity: "Priming" the lung for injury. *Pulmonary Pharmacology & Therapeutics* 26(4): 427-429.

218. Bhatia M. and Moochhala S. **(2004)** Role of inflammatory mediators in the pathophysiology of acute respiratory distress syndrome. *The Journal of Pathology* 202(2): 145-156.

219. Thijs L.G. and Hack C.E. **(1995)** Time course of cytokine levels in sepsis. *Intensive Care Med* 21(2): S258-S263.

220. Simpson S.Q. and Casey L.C. **(1989)** Role of tumor necrosis factor in sepsis and acute lung injury. *Crit Care Clin* 5(1): 27-47.

221. Ouchi N., Kihara S., Funahashi T., Matsuzawa Y., and Walsh K. **(2003)** Obesity, adiponectin and vascular inflammatory disease. *Current opinion in lipidology* 14(6): 561-566.

222. Medoff B.D., Okamoto Y., Leyton P., Weng M., Sandall B.P., Raher M.J.*, et al.* **(2009)** Adiponectin deficiency increases allergic airway inflammation and pulmonary vascular remodeling. *American journal of respiratory cell and molecular biology* 41(4): 397-406.

223. Shore S.A., Terry R.D., Flynt L., Xu A., and Hug C. **(2006)** Adiponectin attenuates allergen-induced airway inflammation and hyperresponsiveness in mice. *J Allergy Clin Immunol* 118(2): 389-395.

224. Summer R., Fiack C.A., Ikeda Y., Sato K., Dwyer D., Ouchi N.*, et al.* **(2009)** Adiponectin deficiency: a model of pulmonary hypertension associated with pulmonary vascular disease. *American journal of physiology. Lung cellular and molecular physiology* 297(3): 26.

225. Kari F., Hatch G., Slade R., Crissman K., Simeonova P.P., and Luster M. **(1997)** Dietary restriction mitigates ozone-induced lung inflammation in rats: a role for endogenous antioxidants. *American journal of respiratory cell and molecular biology* 17(6): 740-747.

226. Stafoggia M., Forastiere F., Faustini A., Biggeri A., Bisanti L., Cadum E.*, et al.* **(2010)** Susceptibility factors to ozone-related mortality: a population-based case-crossover analysis. *Am J Respir Crit Care Med* 182(3): 376-384.

227. Medina-Ramon M. and Schwartz J. **(2008)** Who is more vulnerable to die from ozone air pollution? *Epidemiology* 19(5): 672-679.

228. Scalbert A., Manach C., Morand C., Remesy C., and Jimenez L. **(2005)** Dietary polyphenols and the prevention of diseases. *Critical reviews in food science and nutrition* 45(4): 287-306.

229. Liu C., Xu X., Bai Y., Wang T.Y., Rao X., Wang A.*, et al.* **(2014)** Air pollution-mediated susceptibility to inflammation and insulin resistance: influence of CCR2 pathways in mice. *Environ Health Perspect* 122(1): 17-26.

230. Wiegman C.H., Li F., Clarke C.J., Jazrawi E., Kirkham P., Barnes P.J.*, et al.* **(2014)** A comprehensive analysis of oxidative stress in the ozone-induced lung inflammation mouse model. *Clin Sci* 126(6): 425-440.

231. Chuang G.C., Yang Z., Westbrook D.G., Pompilius M., Ballinger C.A., White C.R.*, et al.* **(2009)** Pulmonary ozone exposure induces vascular dysfunction, mitochondrial damage, and atherogenesis. *American journal of physiology. Lung cellular and molecular physiology* 297(2): 24.

232. Tilton S.C., Waters K.M., Karin N.J., Webb-Robertson B.-J.M., Zangar R.C., Lee K.M.*, et al.* **(2013)** Diet-induced obesity reprograms the inflammatory response of the murine lung to inhaled endotoxin. *Toxicology and applied pharmacology* 267(2): 137-148.

233. Burrows C.J. **(2009)** Surviving an Oxygen Atmosphere: DNA Damage and Repair. *ACS Symp Ser Am Chem Soc* 20: 147-156.

234. Halliwell B. **(2006)** Phagocyte-derived reactive species: salvation or suicide? *Trends Biochem Sci* 31(9): 509-515.

235. Furukawa S., Fujita T., Shimabukuro M., Iwaki M., Yamada Y., Nakajima Y.*, et al.* **(2004)** Increased oxidative stress in obesity and its impact on metabolic syndrome. *The Journal of clinical investigation* 114(12): 1752-1761.

236. Hotamisligil G.S. **(2006)** Inflammation and metabolic disorders. *Nature* 444(7121): 860-867.

237. Wellen K.E. and Hotamisligil G.S. **(2005)** Inflammation, stress, and diabetes. *The Journal of clinical investigation* 115(5): 1111-1119.

238. Halliwell B. **(2008)** Are polyphenols antioxidants or pro-oxidants? What do we learn from cell culture and in vivo studies? *Archives of biochemistry and biophysics* 476(2): 107-112.

239. Bjelakovic G., Nikolova D., Simonetti R.G., and Gluud C. **(2008)** Systematic review: primary and secondary prevention of gastrointestinal cancers with antioxidant supplements. *Alimentary Pharmacology & Therapeutics* 28(6): 689-703.

240. Virtamo J., Pietinen P., Huttunen J.K., Korhonen P., Malila N., Virtanen M.J.*, et al.* **(2003)** Incidence of cancer and mortality following alpha-tocopherol and beta-carotene supplementation: a postintervention follow-up. *JAMA : the journal of the American Medical Association* 290(4): 476-485.

241. Goodman G.E., Thornquist M.D., Balmes J., Cullen M.R., Meyskens F.L., Jr., Omenn G.S.*, et al.* **(2004)** The Beta-Carotene and Retinol Efficacy Trial: incidence of lung cancer and cardiovascular disease mortality during 6-year follow-up after stopping beta-carotene and retinol supplements. *Journal of the National Cancer Institute* 96(23): 1743-1750.

242. Christen W.G., Gaziano J.M., and Hennekens C.H. **(2000)** Design of Physicians' Health Study II--a randomized trial of beta-carotene, vitamins E and C, and multivitamins, in prevention of cancer, cardiovascular disease, and eye disease, and review of results of completed trials. *Ann Epidemiol* 10(2): 125-134.

243. Bairati I., Meyer F., Gelinas M., Fortin A., Nabid A., Brochet F.*, et al.* **(2005)** Randomized trial of antioxidant vitamins to prevent acute adverse effects of radiation therapy in head and neck cancer patients. *J Clin Oncol* 23(24): 5805-5813.

244. Prieme H., Loft S., Nyyssonen K., Salonen J.T., and Poulsen H.E. **(1997)** No effect of supplementation with vitamin E, ascorbic acid, or coenzyme Q10 on oxidative DNA damage estimated by 8-oxo-7,8-dihydro-2'-deoxyguanosine excretion in smokers. *The American journal of clinical nutrition* 65(2): 503-507.

245. Kelly R.P., Poo Yeo K., Isaac H.B., Lee C.Y., Huang S.H., Teng L.*, et al.* **(2008)** Lack of effect of acute oral ingestion of vitamin C on oxidative stress, arterial stiffness or blood pressure in healthy subjects. *Free Radic Res* 42(5): 514-522.

246. Rytter E., Vessby B., Asgard R., Ersson C., Moussavian S., Sjodin A.*, et al.* **(2010)** Supplementation with a combination of antioxidants does not affect glycaemic control, oxidative stress or inflammation in type 2 diabetes subjects. *Free Radic Res* 44(12): 1445-1453.

247. Lee C.-Y.J., Isaac H.B., Huang S.H., Long L.H., Wang H., Gruber J.*, et al.* **(2009)** Limited antioxidant effect after consumption of a single dose of tomato sauce by young males, despite a rise in plasma lycopene. *Free Radical Research* 43(6): 622-628.

248. Halliwell B. **(2011)** Free radicals and antioxidants - quo vadis? *Trends Pharmacol Sci* 32(3): 125-130.

249. Laparra J.M. and Sanz Y. **(2010)** Interactions of gut microbiota with functional food components and nutraceuticals. *Pharmacological research : the official journal of the Italian Pharmacological Society* 61(3): 219-225.

250. Selma M.V., Espin J.C., and Tomas-Barberan F.A. **(2009)** Interaction between phenolics and gut microbiota: role in human health. *J Agric Food Chem* 57(15): 6485-6501.