Taylor & Francis
Taylor & Francis Group

Journal of Toxicology and Environmental Health, Part A
Current Issues

ISSN: 1528-7394 (Print) 1087-2620 (Online) Journal homepage: http://www.tandfonline.com/loi/uteh20

# Oxidative stress response to air particle pollution in a rat nutritional growth retardation model

Melisa L. Kurtz, Francisco Astort, Christian Lezon, Sebastián A. Ferraro, Guillermo A. Maglione, Nadia S. Orona, Silvia M. Friedman, Patricia M. Boyer & Deborah R. Tasat

**To cite this article:** Melisa L. Kurtz, Francisco Astort, Christian Lezon, Sebastián A. Ferraro, Guillermo A. Maglione, Nadia S. Orona, Silvia M. Friedman, Patricia M. Boyer & Deborah R. Tasat (2018): Oxidative stress response to air particle pollution in a rat nutritional growth retardation model, Journal of Toxicology and Environmental Health, Part A

**To link to this article:** https://doi.org/10.1080/15287394.2018.1519747

Published online: 21 Sep 2018.

JOURNAL OF TOXICOLOGY AND ENVIRONMENTAL HEALTH, PART A
https://doi.org/10.1080/15287394.2018.1519747



# Oxidative stress response to air particle pollution in a rat nutritional growth retardation model

Melisa L. Kurtz[a,b], Francisco Astort[a,b], Christian Lezon[c], Sebastián A. Ferraro[a,b], Guillermo A. Maglione[a,d], Nadia S. Orona[a,b], Silvia M. Friedman[e], Patricia M. Boyer[c], and Deborah R. Tasat[a,d]

[a]Center for The Studies in Health and Environment, School of Science and Technology, National University of San Martín, San Martín, Buenos Aires, Argentina; [b]Committee for Scientific Research, Buenos Aires, Argentina; [c]Department of Physiology, School of Dentistry, University of Buenos Aires, Buenos Aires, Argentina; [d]Department of Histology and Embryology, School of Dentistry, University of Buenos Aires, Buenos Aires, Argentina; [e]Department of General and Oral Biochemistry, School of Dentistry, University of Buenos Aires, Buenos Aires, Argentina

**ABSTRACT**

Air pollution consisting of gases and particulate matter-(PM) represents a health problem in cities worldwide. However, air pollution does not impact equally all individuals, as children appear to be more vulnerable subpopulations. Air pollution and malnutrition are two distinct factors that have been associated with oxidative damage. Therefore, the interaction between environmental exposure and nutritional status in populations at risk needs to be explored. The aim of this study was to examine oxidative metabolism in lung, heart and liver in malnourished young rats exposed to residual oil fly ash (ROFA). A Nutritional Growth Retardation (NGR) model was developed in weanling male rats placed on a 20% restricted balanced diet for 4 weeks. Then, NGR and control rats were intranasally instilled with either ROFA (1mg/kg BW) or phosphate buffered saline (PBS). Twenty-four hr post-exposure lung, heart and liver were excised, and serum collected. ROFA induced lung and liver inflammation in control and NGR animals as evidenced by lung polymorphonuclear neutrophil (PMN) recruitment and alveolar space reduction accompanied by liver lymphocyte and binucleated hepatocyte level increase. In lung and liver, antioxidant defense mechanisms reduced lipoperoxidation. In contrast, only in NGR animals did ROFA exposure alter heart oxidative metabolism leading to lipid peroxidation. Although histological and biochemical tissue alterations were detected, no marked changes in serum liver and heart systemic biomarkers were observed. In conclusion, NGR animals responded differently to PM exposure than controls suggesting that nutritional status plays a key role in responsiveness to ambient air contaminants.

**ARTICLE HISTORY**
Received 6 July 2018
Revised 31 August 2018
Accepted 1 September 2018

**KEYWORDS**
Particulate matter; oxidative metabolism; lung; heart; chronic undernutrition

## Introduction

Air pollution consisting of gases and particulate matter (PM) represents a major global health problem in urban areas. In 2012, the World Health Organization (WHO) estimated that intramural (indoor) and extramural (outdoor) air pollution was attributed to result in 6.5 million premature deaths annually with 90% mortality in low or median-income countries (WHO 2014). However, air pollution does not impact each population in the same manner. Particulate matter (PM) predominantly affects subpopulations considered to be more vulnerable such as young children, individuals older than 65 years and those with previous cardiorespiratory failures (Salvi 2007; Simoni et al. 2015).

It is important to emphasize that children and adolescents are not only more vulnerable to the adverse effects of the air environmental pollutants (Deng et al. 2018) but, in addition nutritional deficiencies exert a confounding further negative impact on individual, social and economic development in these subpopulations (Caulfield et al. 2004; Miller and Rayalam 2017). On a global scale, growth retardation as a result of chronic undernourishment affects 165 million children younger than 5 years, and is characterized by a deficit in longitudinal growth evidenced as low height for age (UNICEF, 2013). This delay in growth is predominantly attributed to inadequate food intake as a result of insufficient access to foods in quantity and quality (Kaplan and Toshima 1992). This type of malnutrition was considered as a deficit of the

**CONTACT** Melisa L. Kurtz mkurtz@unsam.edu.ar
Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/uteh.

anthropometric weight/age indices and height/age with a weight/height as well as biochemical indicators of malnutrition within normal limits (Lifshitz et al. 2012; Sandberg et al. 1991).

Ambient air PM adversely affects the respiratory system, its main route for entering the organism (Castaneda et al. 2017; Stiegel et al. 2017). The adverse effects observed depend, among other characteristics, upon the size and composition of the particles. Residual oil fly ash (ROFA), widely used as a surrogate of ambient pollution, is a type of PM that results from oil combustion which contains metallic traces such as vanadium, aluminum, silica and iron (Dreher et al. 1997; Figueroa, Rodriguez-Sierra, and Jimenez-Velez 2006; Huang and Ghio 2006). Vanadium is not only found in ROFA chemical composition, but constitutes a major pollutant found in the air of highly contaminated urban localities and this metal accumulates in lungs, bone tissue and liver (Roshchin, Ordzhonikidze, and Shalganova 1980). Previously, investigators showed that acute exposure to ROFA alters pulmonary morpho-physiology (Magnani et al. 2011; Orona et al. 2016). It is worthwhile noting that ambient air PM act not only on the respiratory system but also at the systemic level on various organs including heart, liver and brain (Brook 2008; Chen and Yang 2018; Orona et al. 2016; Tsai, Tsai, and Yang 2018).

Air PM induces in the lung the release of oxidant and proinflammatory mediators such as reactive oxygen species (ROS), reactive nitrogen species (RNS), tumor necrosis factor (TNF-α) and interleukins (IL-1, IL-6, and e IL-10) (Castaneda et al. 2017; Chen, Chiu, and Yang 2016; Gonzalez-Flecha 2004; Hiraiwa and van Eeden 2013). Therefore, the distant effects of air PM may be indirect, as a result of the release of mediators resulting from their interaction with pulmonary cells, and/or direct as a result of particle access to the blood circulation after crossing the alveolar-capillary barrier (Brook 2008; Kido et al. 2011; Stiegel et al. 2017). Emerging evidence in human and animal studies demonstrated that inhaled particles travel from the lungs into the bloodstream, potentially raising the risk of heart attack and strokes (Chen and Yang 2018; Miller et al. 2017; Tsai, Tsai, and Yang 2018). The World Health Organization (WHO, 2014) estimated that in 2012, approximately 72% of premature deaths related to outdoor air pollution were due to ischemic heart disease and strokes. These epidemiological findings provided evidence of an association between air pollution and cardiovascular diseases.

The liver, the major organ responsible for metabolism, biotransformation and detoxification of drugs and xenobiotics, responds to air PM exposure by increasing generation of reactive oxygen species (ROS) that may result in oxidative stress which leads to hepatotoxicity (Simeonova et al. 2014). Several investigators demonstrated that air PM exposure predisposes individuals to initiation of liver dysfunction, metabolic disease and cancer (Brocato et al. 2014; Kim et al. 2014). It was thus postulated that individuals suffering from chronic malnutrition may be more vulnerable to air PM-mediated effects as micronutrients essential for growth and functions are altered by air contaminant exposure (Miller and Rayalam 2017). Although there are no apparent data regarding effects of air pollution on malnourished subpopulations, it is postulated that the use of experimental animal models might be useful in predicting whether malnutrition is a risk factor capable of making these rodents susceptible to consequences of exposure to air contaminants. Therefore, the aim of this study was to determine in a murine model of nutritional chronic growth retardation the impact of acute ROFA exposure.

## Materials and methods

### Animals

Weanling male Wistar rats weighing 60 ± 5 g were provided by the Animal Resources Lab of the Department of Biochemistry, School of Dentistry, University of Buenos Aires, Argentina. Animals were fed a standard diet (Purina chow) of the following composition (g/100 g): protein, 22.7; lipids, 7.09; fiber, 6; Ca, 1.3; P, 0.8; ashes, 6.5; water,7.96; dextrin up to 100g, and were kept under standard lab conditions (light-dark 12:12 hr, 21°C ± 1°C and 50–60% humidity). The guidelines for the care and use of animals were approved by the University of Buenos Aires Ethics Committee, according to the Principles of Laboratory Animal Care (NIH 2 publication number 85–23, revised 1985, 3 http://grants1.nih.gov/grants/olaw/references/phspol.htm).

### Nutritional animal model

Rats (21–23 days old, n = 60) were randomly assigned to 2 groups: control (Control, n = 30) and nutritional growth retardation (NGR, n = 30). During the 4-week study, control rats were fed *ad libitum* with a standard diet, and NGR rats were fed 80% of the amount of food consumed by controls. The food amount was corrected by body weight (food intake in grams per 100 g body weight per day). Body weight and length were recorded throughout the whole experiment.

### Particulate matter collection and characterization

Residual Oil Fly Ash (ROFA) was collected from Boston Edison Co., Mystic Power Plant, Mystic, CT, USA, and kindly provided by Dr. J. Godleski (Harvard School of Public Health, MA, USA). ROFA was previously morphologically and chemically characterized by SEM and EDX (Ferraro et al. 2011; Ghio et al. 2002). ROFA is morphologically heterogeneous with metal traces (iron, vanadium and nickel) in its composition.

### ROFA animal exposure- experimental protocol

After 4 weeks of diet treatment, animals were intranasally instilled either with 1mg/kg BW ROFA or phosphate buffer saline (PBS) solution-vehicle, thus yielding 4 groups (n = 15 per group): Control, ROFA, NGR, NGR+ ROFA. Euthanasia was performed 24hr after intranasal instillation with an overdose of ketamine/xylazine. Lungs, hearts and livers were excised for histological and biochemical analyses. Blood was obtained by cardiac puncture and serum was obtained by centrifugation at 800xg for 10 min. Samples were kept at −80°C until use (Figure 1). The selection of ROFA dose was based upon previous studies (Ferraro et al. 2011; Magnani et al. 2013; Marchini et al. 2016; Orona et al. 2016). Intranasal instillation is an effective and noninvasive technique commonly used in toxicity studies (Leong et al. 1998; Southam et al. 2002).

### Bronchoalveolar lavage fluid (BALF), lung total cell number (TCN) and differential cell count (DCC)

Bronchoalveolar lavage fluid (BALF) was obtained as previously described by Tasat and de Rey (1987). Briefly, the thoracic cavity was partly dissected, the trachea was cannulated with an 18-gauge needle, and infused 12 times with 1 ml cold PBS. To avoid inaccuracies, BALF cells were incubated strictly under the same cell density condition, and employed 350,000 cell/ml. BALF was immediately centrifuged at 800xg for 10 min at 4°C and total cell number (TCN) determined using a hemocytometer. Differential cell count (DCC) was counted by light microscopy after cell fixation with methanol and staining with hematoxylin-eosin (Ferraro, Curutchet, and Tasat 2012; Orona et al. 2016).

### Superoxide anion generation

Superoxide anion ($O_2^-$), a predominant reactive oxygen species (ROS) generated during the respiratory burst, was determined in BALF cells using the 2,2-bis (4-nitrophenyl)-5,5-diphenyl-3,3-(3,3-dimethoxy-4,4-diphenylene (NBT, Sigma–Aldrich) reduction test (Hedley and Currie 1978). BALF cells were treated with NBT in the presence or absence of 12-O-tetradecanoylphorbol-13-acetate (TPA), a known inducer of $O_2^-$ production. All samples were incubated with NBT for 45 min at 37°C. $O_2^-$intracellular production was measured by the amount of a blue formazan precipitate in the cells after NBT reduction. Cells were scored by light microscopy as described elsewhere (Molinari et al. 2000).

### Blood samples and serum biomarkers

Biochemical analysis was carried out for all the blood samples collected from control and ROFA exposed rats.

***Aminotransaminases activities.*** Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were quantified by standardized methods (Roche Diagnostics, Mannheim, Germany) using a COBAS C501 autoanalyzer (Roche Diagnostics, Mannheim, Germany).

***Paraoxonase 1 (PON 1) and arylesterase (ARE) activities.*** The enzyme PON 1 was determined employing two different substrates: paraoxon (Sigma Chemical Co.; PON 1 activity) and phenylacetate (Sigma Chemical Co.; ARE activity). Both activities were measured in serum samples following the method of Furlong et al. (1989). PON 1 activity was expressed as nmol/ml/min. ARE activity was expressed as μmol/ml/min. (Furlong et al. 1989). All measurements were carried out within the same assay.

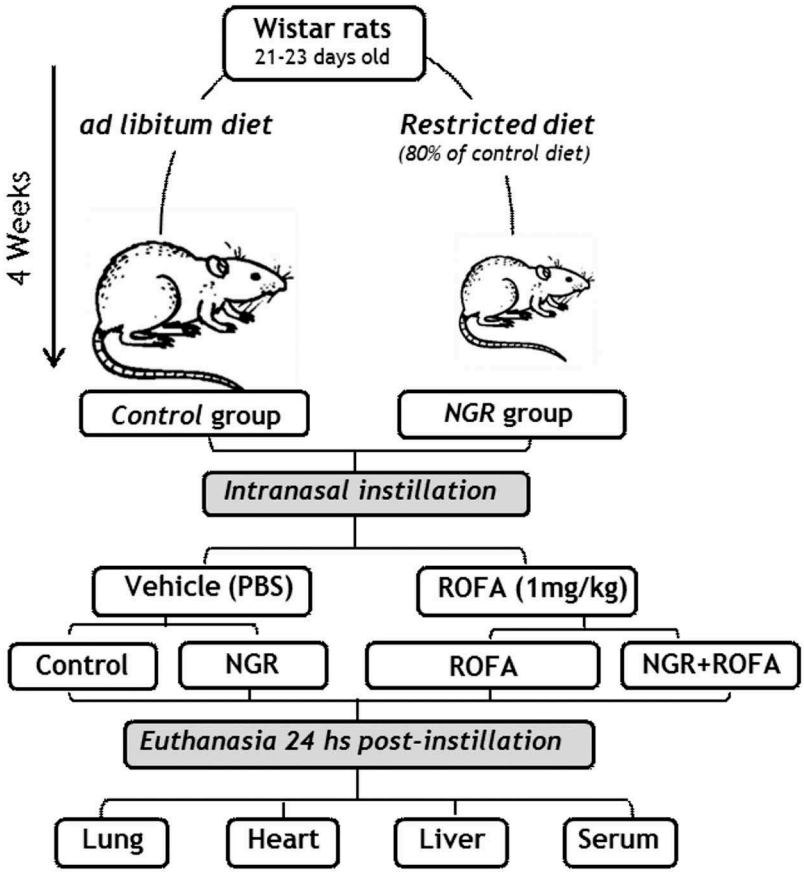


**Figure 1.** Chronic nutritional animal model and experimental design.

Wistar rats were randomized in two groups regarding food intake: 1) *ad libitum* food intake (Control) or 2) 80% of the amount of food consumed by control (NGR). After 4 weeks, animals were intranasally instilled either with ROFA (1mg/kg BW) or PBS creating 4 groups: Control, ROFA, NGR, NGR+ ROFA. Twenty four hr post-exposure, lung, heart and liver were excised from all animals for histological and biochemical analyses, and serum was collected in order to determine cardiac and hepatic risk biomarkers.

### *Lung, heart and liver histology*

Lung, heart and liver from control and NGR animals exposed to ROFA or vehicle were excised, fixed in 10% buffered formalin (pH = 7.4) and processed for histology. Briefly, tissues were dehydrated and embedded in paraffin. Sections of 5–7 μm-thickness were cut with a Reichert-Jung micrometer (Nossloch, Germany) and mounted on slides. The tissue sections were deparaffinized, hydrated and stained for light microscopy with Mayer's hematoxylin and alcoholic eosin (H&E) to perform routine histological analysis. Lung microphotographs were employed to perform morphometric analysis using an image processing software (Image-Pro Plus, Version 4.5.0.29, Media Cybernetics. Inc.). The alveolar space area relative to the total alveolar area of the lung tissue was determined. Results were expressed as % lumen area. Samples were qualitatively screened by means of a Leica microscope (model DM500) at a magnification of 100x and 400x; images were taken using a Leica ICC50 camera.

Changes in hepatocyte polyploidy are considered a marker of liver injuries. Percentage (%) of binucleated cells was evaluated in tissue sections by light microscopy according to Cano-Gutierrez et al. (2012).

### *Lung, heart and liver homogenates*

Lungs, hearts and livers from all animal groups were homogenized in PBS (pH 7.4) at 4°C (1:5 w/v). The suspension was centrifuged at 6000xg for 10 min at 4°C to remove cell debris. The pellet was discarded and supernatant was used as "homogenate" (Llesuy et al. 1994).

***Superoxide dismutase (SOD) and catalase (CAT) activities.*** Tissue supernatant was used for antioxidant activity determinations. The activity of

catalase (CAT) was assessed following Maehly and Chance (1954). Rates were calculated as the change in optical density of the assay mixture/min/mg protein.

The activity of SOD was assayed by the method of Misra and Fridovich (1972). One unit of SOD was defined as enzyme amount producing 50% inhibition epinephrine autoxidation. Enzyme activity was reported as units/mg protein in the homogenate.

***Determination of lipid peroxidation.*** Lipid peroxidation was estimated by measuring the concentrations of thiobarbituric acid reactive substances (TBARS). Briefly, homogenate was added with trichloroacetic acid (40% w/v) and centrifuged at 1000xg for 10 min. The supernatant was added with an equal volume of thiobarbituric acid (46 mM) (Sigma-Aldrich Co.), and the solution was heated at 95°C for 15 min. Then, samples were cooled and quantified spectrophotometrically at 535 nm. Malondialdehyde (MDA, Sigma-Aldrich Co.), subjected to the same conditions as the tissue homogenates, was used as a standard.

***Protein determination.*** Protein was measured by the method of Bradford (1976) using bovine serum albumin as the standard.

## Statistical analysis

Data are presented as the mean ± SEM. Groups were compared by two-way ANOVA in conjunction with Bonferroni's post-test. Statistical significance was set at $p < 0.05$.

## Results

In agreement with previous studies (Friedman et al. 2001; Tasat et al. 2014), a 4-week food restriction diet resulted in a significant loss of body weight in NGR rats compared to controls (Control: 276.6 ± 4.3 g vs. NGR: 153.2 ± 0.4 g). Similarly, a significant decrease in body length was observed between these two groups (Control: 21.6 ± 0.3 cm vs. NGR: 18.4 ± 0.4 cm). Final body length of NGR group was correlated to reduced body weight.

### Lung morphological and biochemical evaluation

***Lung histopathology.*** Histopathological analysis showed no marked differences between control and NGR animals (Figure 2A and 2C). ROFA instillation induced a marked recruitment of inflammatory cells polymorphonuclear (PMN) and lymphocytes into the parenchyma and alveolar spaces compared to control (Figure 2A and 2B). Similarly, NGR animals instilled with ROFA exhibited a rise in number of inflammatory cells and greater number of lymph nodes (Figure 2C and 2D).

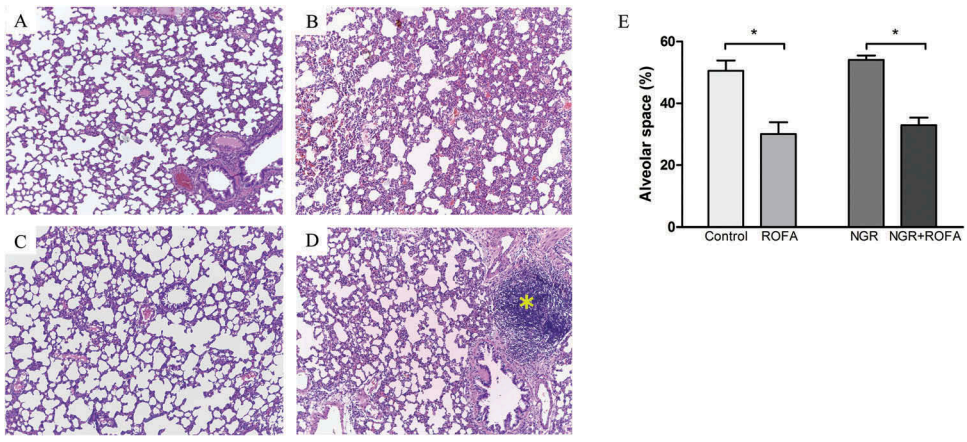


**Figure 2.** Lung histology and histomorphometry.

Representative microphotographs showing the lower respiratory tract from exposed and non-exposed Control and NGR animals. A: Control, B: ROFA, C: NGR, D: NGR+ ROFA. ROFA induced inflammatory cells recruitment (Figure 2B and D). The asterisk (*) shows the presence of lymphocytes in NGR + ROFA group. Hematoxylin-eosin stain, orig. mag. 100X. E: Histomorphometic analysis of alveolar space shows the comparative percentage for all experimental animal groups. Results are expressed as mean ± SEM; n = 5–7 animals per group. Two-way ANOVA in conjunction with Bonferroni's test was performed, * $p < 0.05$.

Lung histomorphometry of the lower respiratory tract from all experimental groups is illustrated in Figure 2E. Morphometric evaluation of the alveolar space revealed that ROFA induced a significant reduction in airspace % due to cell infiltration irrespective of the nutritional state of the animal (Control: 50.5 ± 3.4 vs. ROFA: 30.1 ± 3.8%.; NGR: 54.1 ± 1.4 vs. NGR+ ROFA: 32.9 ± 2.5%).

***Bronchoalveolar lung fluid (BALF) analysis.*** As shown in Figure 3A, total cell number (TCN) was significantly augmented after ROFA exposure. The contribution of different cell populations in the BALF was assessed by quantifying the % alveolar macrophages (AM), PMN and lymphocytes (L). The differential cell count (DCC) analysis showed that ROFA exposure induced in control as well as in NGR animals a significant rise in PMN % (Figure 3B).

***BALF superoxide anion generation.*** Superoxide anion generation in BALF cells from all 4 experimental groups using the NBT test is presented in Figure 4. BALF total cell number from ROFA exposed animals was always higher than BALF from non-exposed animals. ROFA exposure induced a significant elevation in % reactive BALF cells compared with non-exposed animals. The rise in reactive cells was higher for ROFA than NGR+ ROFA. It is noteworthy that the NGR group elicited a baseline reaction to NBT higher than that observed in control.

***Lung oxidative metabolism.*** As shown in Figure 5A, lungs from NGR rats exhibited increased CAT activity compared to control. ROFA acute exposure did not markedly alter CAT activity in ROFA or NGR+ ROFA rats. Figure 5B illustrates that SOD activity was significantly decreased in ROFA instilled groups with respect to control or NGR+ ROFA. NGR+ ROFA animals displayed lower SOD activity compared to ROFA. Regarding lipid peroxidation, no marked alterations were seen among groups (Figure 5C).

## Heart morphological and biochemical evaluation

***Heart histopathology.*** Hearts from control and NGR animals stained with H&E showed normal myocardium. In ROFA exposed groups, no apparent signs of inflammation in parenchyma or in epicardium were detected (data not shown).

### Heart oxidative metabolism

Analysis of CAT and SOD activities in heart homogenates showed no marked changes in all experimental groups (Figure 6A and 6B). With respect to TBARS concentration, a significant increase was found when NGR+ ROFA rats were compared to NGR indicating that exposure induced an oxidative damage (Figure 6C).

## Liver morphological and biochemical evaluation

### Liver histopathology

Rat liver tissue was evaluated for the presence of histological alterations. Controls presented normal hepatic histology with no apparent signs of inflammatory response or necrotic cells in any of the three zones of the hepatic lobule (Figure 7A). In contrast, ROFA exposure induced histopathological changes in

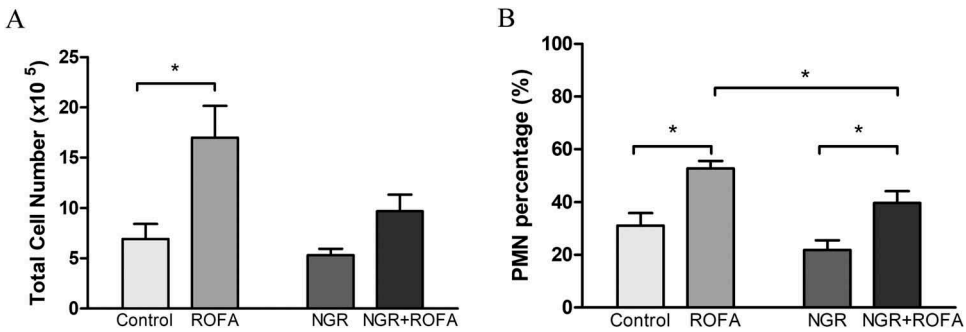


**Figure 3.** Total cell number and polymorphonuclear cells percentage (PMNs) in bronchoalveolar lavage fluid.
Bronchoalveolar lavage Total Cell Number (A) and PMNs percentage (B) from exposed and non-exposed Control and NGR animals. Results are expressed as mean ± SEM, n = 5–7 animals per group. Two-way ANOVA in conjunction with Bonferroni's test was performed, * $p < 0.05$.

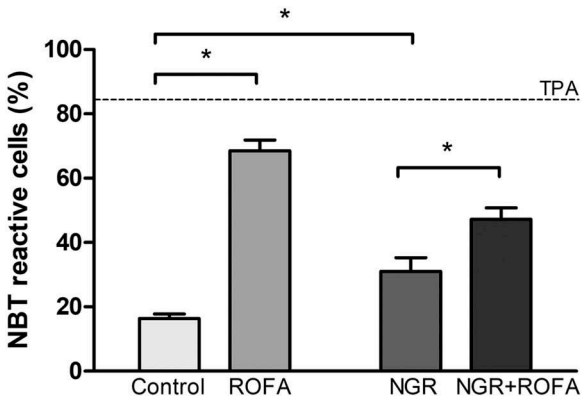


**Figure 4.** Superoxide anion generation in BALF cells.

Superoxide anion generation was evaluated as percentage of reactive dark-blue cells, measured by de NBT test in BALF cells. Results are expressed as mean ± SEM, n = 5–7 animals per group. Two-way ANOVA in conjunction with Bonferroni's test was performed, * $p < 0.05$.

control and NGR animals. As a marker of hepatotoxicity, the number of binucleated cells in the liver parenchyma was determined. The number of binucleated hepatocytes in the NGR group depicted a significant 6.3 fold elevation in number of binucleated cells compared to controls (Figure 7 - Table 1). As seen in Figure 7 and in Table 1, ROFA increased binucleation rate in liver parenchyma in in fed and NGR animals in comparison with non-exposed. Further, the liver from ROFA exposed animals exhibited inflammatory infiltration. It is noteworthy that Figure 7B clearly shows inflammatory foci in the liver parenchyma.

### *Liver oxidative metabolism*

CAT and SOD antioxidant enzymes data are presented in Figure 8 (A and B). Non-exposed NGR rats displayed a reduction in CAT activity compared to control (Figure 8A). In contrast, liver homogenates from NGR and control rats showed no marked changes in SOD activity (Figure 8B). ROFA acute exposure induced a significant reduction in CAT activity both in fed and NGR experimental animal groups (Figure 8A). In addition, ROFA instillation produced a significant decrease on SOD antioxidant enzyme activity in NGR exposed rats. No marked changes in lipid peroxidation were found in NGR or ROFA animals with respect to Control (Figure 8C).

### *Systemic response after acute ROFA exposure*

Systemic biomarkers of possible heart or liver damage were evaluated by measuring ALT and AST in serum.

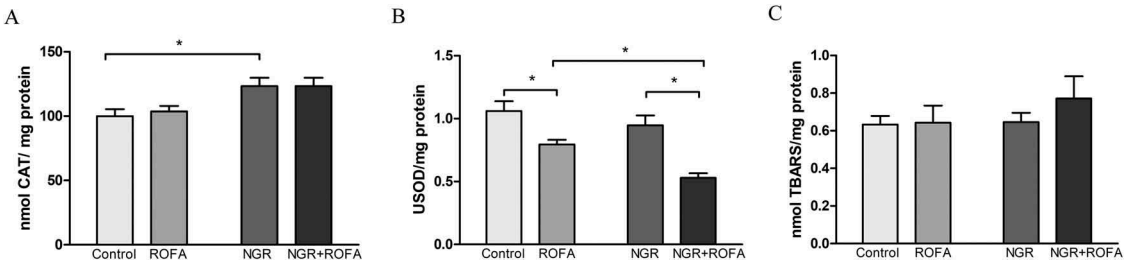


**Figure 5.** Lung oxidative metabolism.

Catalase (A) and Superoxide Dismutase (B) activities and lipoperoxidation (C) determined in lung homogenates. Results are expressed as mean ± SEM, n = 5–7 animals per group. Two-way ANOVA in conjunction with Bonferroni's test was performed, *$p < 0.05$.

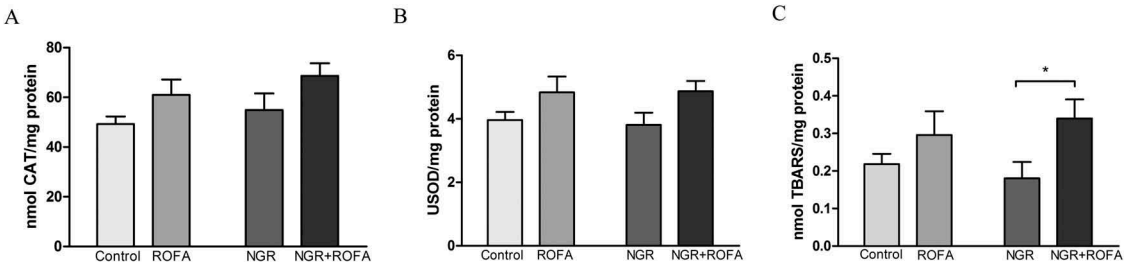


**Figure 6.** Heart oxidative metabolism.

Catalase (A) and Superoxide Dismutase (B) activities and lipoperoxidation (C) determined in heart homogenates. Results are expressed as mean ± SEM, n = 5–7 animals per group. Two-way ANOVA in conjunction with Bonferroni's test was performed, * $p < 0.05$.

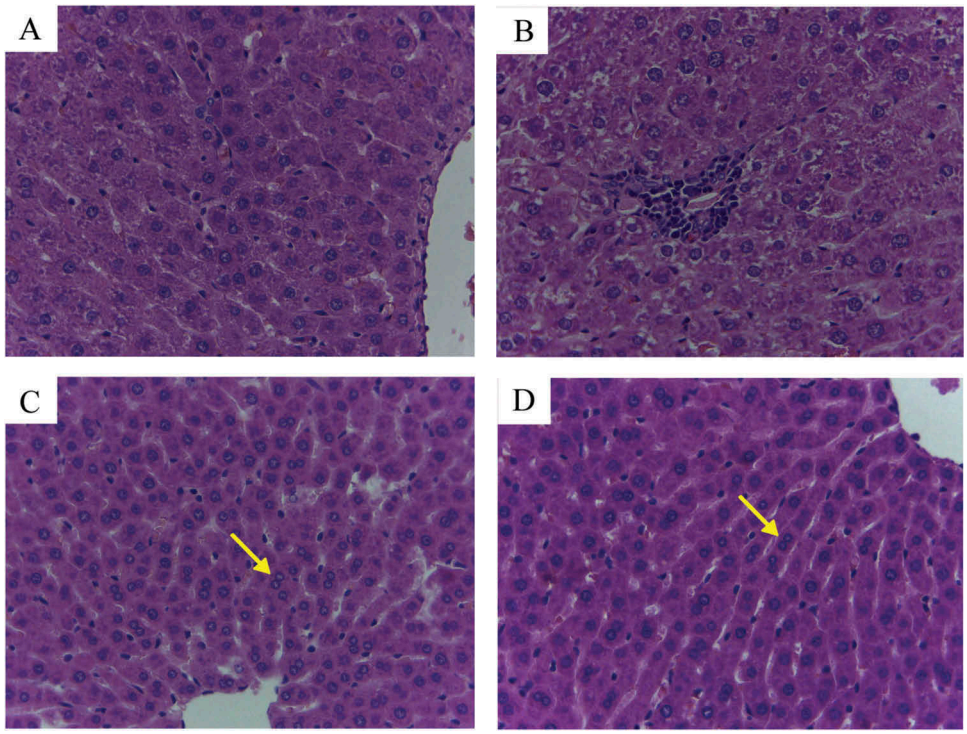


**Figure 7.** Liver Histology.

Representative microphotographs showing the liver from all animal groups. Control (A), ROFA (B), NGR (C), NGR+ ROFA (D). Hematoxylin-eosin stain, orig. mag. 100X. The arrows indicate binucleated cells.

**Table 1.** Binucleated hepatocytes percentage.

| Control | ROFA | NGR | NGR+ ROFA |
|---|---|---|---|
| 1.33 ± 0.23 | 3.42 ± 0.89* | 8.38 ± 1.27* | 14.15 ± 1.03# † |

Results are expressed as mean ± SEM, n = 5–7 animals per group. Two-way ANOVA in conjunction with Bonferroni's test was performed, * $p < 0.05$ vs. Control, # $p < 0.05$ vs. NGR, † $p < 0.05$ vs. ROFA.

In regard to these transaminases, no significant differences were found between control and NGR animals. Further, exposure to ROFA did not produce changes in fed or NGR rats (Figure 8A and 8B). PON-1 activity assayed for both substrates showed a reduction in non-exposed NGR rats with respect to controls. Similar to transaminases, ROFA exposure did not markedly modify PON-1 activity (Figure 9)

## Discussion

Air pollution does not affect the entire population equally (Pope 2000; Salvi 2007). Evidence of the interrelationship between air pollution and certain nutritional status such as obesity and micronutrients deficiency, has been documented (Miller et al. 2017). Oxidative stress has been linked not only to air pollution (Nel, Diaz-Sanchez, and Li 2001; Riva et al. 2011; Tao, Gonzalez-Flecha, and Kobzik 2003), but might be involved in malnutrition pathophysiology as a result of an imbalance in the production of reactive oxygen species (ROS) and/or altered antioxidant protection mechanisms

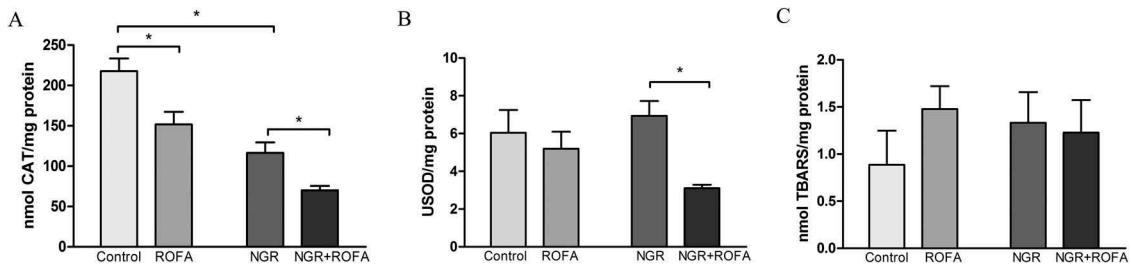


**Figure 8.** Liver oxidative metabolism.

Catalase (A) and Superoxide Dismutase (B) activities and lipoperoxidation (C) determined in liver homogenates. Results are expressed as mean ± SEM, n = 5–7 animals per group. Two-way ANOVA in conjunction with Bonferroni's test was performed, *$p < 0.05$.

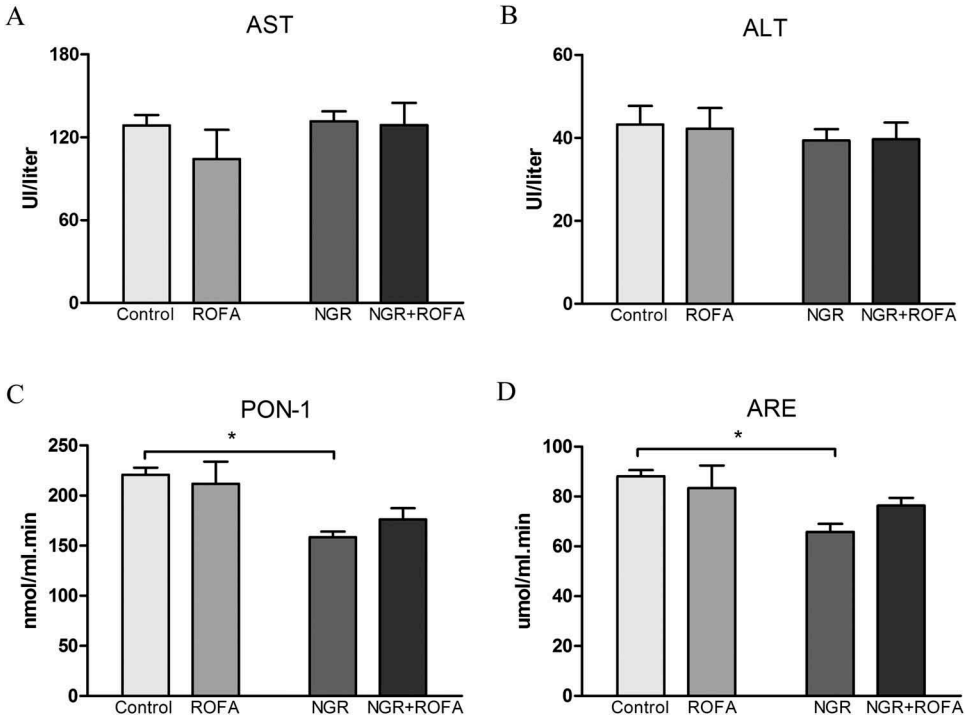


**Figure 9.** Systemic biomarkers.

AST and ALT (9A and 9B), PON-1 and ARE activity (9C and 9D) evaluated in serum. Results are expressed as mean ± SEM, n = 5–7 animals per group. Two-way ANOVA in conjunction with Bonferroni's test was performed, *$p < 0.05$.

(Gavia-Garcia et al. 2015). Several studies demonstrated that severe malnutrition was correlated with increased lipid peroxidation in children (Boşnak et al., 2010; Khare et al. 2014) as well as in rat brain, liver, kidneys, lungs, and heart (Jimoh, Odutuga, and Oladiji 2005). However, no apparent studies reported either an association of air pollution and world nutrient deficiency or the possible role of oxidative stress as the mechanism responsible for the negative impact of air contaminants on vulnerable malnourished subpopulation.

Histology was used to identify pulmonary, hepatic and cardiac alterations in response to acute air particle intranasal instillation. Following ROFA exposure, the predominant effect observed in the lung was cell infiltration which correlated well with marked reduction in alveolar space. ROFA in fed and NGR animals increased in BALF TCN and % inflammatory cells. It is well documented that metals enhance reactive oxygen species (ROS) generation and subsequently, the rise in ROS leads to an imbalance in cell oxidative metabolism producing tissue damage (Ghio, Carraway, and Madden 2012; Roberts et al. 2003). It is worthwhile noting that ROFA, which contains metallic traces in its chemical composition, mainly vanadium (Ferraro et al. 2011), induced a significant rise in superoxide anion generation. It is also worth noting that basal NGR reactive cell % was higher than the basal reactivity of BALF in control rats. Despite the fact that ROFA particles in both control and NGR BALF cells stimulated superoxide anion, no apparent tissue damage was observed as evidenced by lipoperoxidation evaluation. This response may be due to the mobilization of SOD, one of the main antioxidant enzymes tested (Gavia-Garcia et al. 2015).

In the liver, the predominant physiological mechanism leading to polyploidy is the impairment of cytokinesis (Margall-Ducos et al. 2007). Alternatively, hepatocytes may modulate its ploidy in response to a variety of stimuli and/or toxic injury related to ROS generation (Abdelhalim and Jarrar 2012; Gentric and Desdouets 2014; Tormos et al. 2015). In fact, NGR liver histology exhibited a significant elevation in the number of binucleated hepatocytes as compared to control. A reduction in nutrients intake may be viewed as a stressor which, subsequently might initiate an increase in liver polyploidy. However, further

studies need to be carried out in order to elucidate this finding. In this context, ROFA exposure modified hepatocyte ploidy by further elevating the number of binuclated cells in fed and NGR rats. This liver response might be attributed to the hepatotoxic effect of the metallic traces (vanadium) adsorbed to ROFA-PM (Cano-Gutierrez et al. 2012). In addition, ROFA treatment triggered inflammatory cells infiltration which was predominantly observed surrounding the peri-vascular zone.

As ROFA increased ROS generation in lung and liver binucleated cells it was of interest to evaluate oxidative metabolism in liver homogenates. As expected, ROFA administration induced antioxidant mobilization in fed and NGR rats. Similar to lung, no marked changes regarding lipoperoxidation were observed in liver homogenates. Therefore, our results suggest that NGR exposed to ROFA might constitute an adaptive response.

In heart homogenates, ROFA instillation failed to initiate changes in the levels of any of the two antioxidant enzymes. Damiani et al (2012) found that ROFA triggered an increase in ROS production accompanied by absence of SOD and CAT alterations which consequently resulted in oxidative damage at the membrane level as evidenced by high levels of lipoperoxidation. In contrast in our study ROFA did not stimulate cardiac antioxidant mechanisms. Our findings show that with respect to oxidative metabolism, distinctive tissue responsiveness to PM exposure was found. The lung and liver were capable of neutralizing the elevation in ROS induced by ROFA, thus avoiding the oxidative damage but this was not the case for cardiac tissue.

Antioxidant enzymes are also present in plasma circulation both free and associated with lipoproteins. Among the latter, PON seems to be of particular relevance because it confers most of the antioxidant capacity to its carrier (HDL fraction). In this study, PON reduction was noted only in NGR rats. In agreement with other investigators, undernourishment might be attributed to result in PON1 enzyme reduction (Ece et al. 2007).

Heart and liver function are usually estimated through transaminases levels in sera (Chopra and Griffin 1985; Reichling and Kaplan 1988; Shen et al. 2015). No marked alterations were found in AST or ALT blood enzyme levels. Although acute ROFA exposure produced liver and lung tissue response, biomarkers in serum were not markedly affected.

Taking our results into consideration, the response to air particle pollution in NGR animals is in agreement with metabolic adaptation to mild chronic food restriction. These adaptive responses to suboptimal nutrition ensure substrate flux to tissues that require constant energy needed for body growth. Therefore, although the response to some parameters evaluated in our study show similarities between NGR and C groups, it is worth noting that ROFA exposure induced differing biological responses in lung, liver and heart.

## Conclusions

In summary our findings demonstrated in an integrative manner, assessing histological, biochemical and ROS-mediated modifications in lung, liver and heart and that NGR animals respond differently than controls to ROFA exposure. Further, this experimental protocol was carried out in conditions mimicking chronic undernutrition undergone by children living in highly polluted environments. More studies need to be done in order to consider the possible tissue damage induced by air pollution and its mechanism of action in vulnerable malnourished subpopulations.

## Acknowledgments

This investigation was supported by the ANPCyT-National Agency for the Promotion of Science and Technology, Argentina under Grant PICT 2012-0328 and University of Buenos Aires under Grant UBACyT20020130100100BA.

## Funding

This work was supported by the Fondo para la Investigación Científica y Tecnológica [PICT 2012-0328];Universidad de Buenos Aires [UBACyT20020130100100BA]. The authors specially thank Ms Mariela Lacave for her technical expertise on the histological study.

## References


Abdelhalim, M. A., and B. M. Jarrar. 2012. Histological alterations in the liver of rats induced by different gold nanoparticle sizes, doses and exposure duration. *Journal* Nanobiotechnology 10:1–9. doi:10.1186/1477-3155-10-1.

Boşnak, M., K. Selvi., S. Yel, Y. Koçyiğit, V. Şen, and A. Ece. 2010. Oxidative stress in Marasmic children: Relationships with leptin. *Abbrev Electronic Journal General Medica* 7:1–8.

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72:248-254. doi: 10.1016/0003-2697(76)90527-3.

Brocato, J., H. Sun, M. Shamy, T. Kluz, M. A. Alghamdi, M. I. Khoder, L. C. Chen, and M. Costa. 2014. Particulate matter from Saudi Arabia induces genes involved in inflammation, metabolic syndrome and atherosclerosis. *Journal Toxicogical Environment Health A* 77:751–66. doi:10.1080/15287394.2014.892446.

Brook, R. D. 2008. Cardiovascular effects of air pollution. *Clinical Sciences (Lond)* 115:175–87. doi:10.1042/CS20070444.

Cano-Gutierrez, G., S. Acevedo-Nava, A. Santamaria, M. Altamirano-Lozano, M. C. Cano-Rodriguez, and T. I. Fortoul. 2012. Hepatic megalocytosis due to vanadium inhalation: Participation of oxidative stress. *Toxicogical Industrial Health* 28:353–60. doi:10.1177/0748233711412424.

Castaneda, A. R., K. J. Bein, S. Smiley-Jewell, and K. E. Pinkerton. 2017. Fine particulate matter (PM2.5) enhances allergic sensitization in BALB/c mice. *Journal Toxicogical Environment Health A* 80:197–207. doi:10.1080/15287394.2016.1222920.

Caulfield, L. E., M. de Onis, M. Blossner, and R. E. Black. 2004. Undernutrition as an underlying cause of child deaths associated with diarrhea, pneumonia, malaria, and measles. *The American Journal of Clinical Nutrition* 80:193–98. doi:10.1093/ajcn/80.1.193.

Chen, C. C., and C. Y. Yang. 2018. Association between gaseous air pollution and hospital admissions for hypertension in Taipei, Taiwan. *Journal Toxicogical Environment Health A* 81:53–59. doi:10.1080/15287394.2017.1395573.

Chen, C. C., H. F. Chiu, and C. Y. Yang. 2016. Air pollution exposure and daily clinical visits for allergic rhinitis in a subtropical city: Taipei, Taiwan. *Journal Toxicogical Environment Health A* 79:494–501. doi:10.1080/15287394.2016.1182002.

Chopra, S., and P. H. Griffin. 1985. Laboratory tests and diagnostic procedures in evaluation of liver disease. *The American Journal of Medicine* 79:221–30.

Damiani, R. M., M. O. Piva, R. Petry, and H. Saldiva. 2012. Is cardiac tissue more susceptible than lung to oxidative effects induced by chronic nasotropic instillation of residual oil fly ash (rofa)?. 22:533–539. doi: 10.3109/15376516.2012.692109.

Deng, Q., C. Ou, J. Chen, and Y. Xiang. 2018. Particle deposition in tracheobronchial airways of an infant, child and adult. *The Science of the Total Environment* 612:339–46. doi:10.1016/j.scitotenv.2017.08.240.

Dreher, K. L., R. H. Jaskot, J. R. Lehmann, J. H. Richards, J. K. McGee, A. J. Ghio, and D. L. Costa. 1997. Soluble transition metals mediate residual oil fly ash induced acute lung injury. *Journal Toxicogical Environment Health* 50:285–305.

Ece, A., F. Gurkan, F. Celik, M. Bosnak, S. Yel, H. Balik, and O. Erel. 2007. Paraoxonase, total antioxidant activity and peroxide levels in marasmic children: Relationships with leptin. *Clinical Biochemistry* 40:634–39. doi:10.1016/j.clinbiochem.2007.03.001.

Ferraro, S. A., G. Curutchet, and D. R. Tasat. 2012. Bioaccessible heavy metals-sediment particles from Reconquista River induce lung inflammation in mice. *Environmental Toxicology and Chemistry / SETAC* 31:2059–68. doi:10.1002/etc.1911.

Ferraro, S. A., J. S. Yakisich, F. T. Gallo, and D. R. Tasat. 2011. Simvastatin pretreatment prevents ambient particle-induced lung injury in mice. *Inhal Toxicogical* 23:889–96. doi:10.3109/08958378.2011.623195.

Figueroa, D. A., C. J. Rodriguez-Sierra, and B. D. Jimenez-Velez. 2006. Concentrations of Ni and V, other heavy metals, arsenic, elemental and organic carbon in atmospheric fine particles (PM2.5) from Puerto Rico. *Toxicogical Industrial Health* 22:87–99. doi:10.1191/0748233706th247oa.

Friedman, S. M., C. A. Gamba, P. M. Boyer, M. B. Guglielmotti, M. I. Vacas, P. N. Rodriguez, C. Guerrero, and F. Lifshitz. 2001. Growth deceleration and bone metabolism in nutritional dwarfing rats. *International Journal Food Sciences Nutritional* 52:225–33.

Furlong, C. E., R. J. Richter, S. L. Seidel, L. G. Costa, and A. G. Motulsky. 1989. Spectrophotometric assays for the enzymatic hydrolysis of the active metabolites of chlorpyrifos and parathion by plasma paraoxonase/arylesterase. *Analytical Biochemistry* 180:242–47.

Gavia-Garcia, G., H. Gonzalez-Martinez, A. Miliar-Garcia, E. Bonilla-Gonzalez, L. Rosas-Trejo Mde, M. Konigsberg, O. Najera-Medina, A. Luna-Lopez, and M. C. Gonzalez-Torres. 2015. Oxidative damage and antioxidant defense in thymus of malnourished lactating rats. *Nutrition (Burbank, Los Angeles County, Calif.)* 31:1408–15. doi:10.1016/j.nut.2015.05.014.

Gentric, G., and C. Desdouets. 2014. Polyploidization in liver tissue. *The American Journal of Pathology* 184:322–31. doi:10.1016/j.ajpath.2013.06.035.

Ghio, A. J., M. S. Carraway, and M. C. Madden. 2012. Composition of air pollution particles and oxidative stress in cells, tissues, and living systems. *Journal Toxicogical Environment Health B* 15:1–21. doi:10.1080/10937404.2012.632359.

Ghio, A. J., R. Silbajoris, J. L. Carson, and J. M. Samet. 2002. Biologic effects of oil fly ash. *Environmental Health Perspectives* 1:89–94. doi:10.1289/ehp.02110s1189.

Gonzalez-Flecha, B. 2004. Oxidant mechanisms in response to ambient air particles. *Molecular Aspects Medica* 25:169–82. doi:10.1016/j.mam.2004.02.017.

Hedley, D. W., and G. A. Currie. 1978. Monocytes and macrophages in malignant melanoma. III. Reduction of nitroblue tetrazolium by peripheral blood monocytes. *British Journal Cancer* 37:747–52.

Hiraiwa, K., and S. F. van Eeden. 2013. Contribution of lung macrophages to the inflammatory responses induced by exposure to air pollutants. *Mediators of Inflammation* 2013:619523. doi:10.1155/2013/619523.

Huang, Y. C., and A. J. Ghio. 2006. Vascular effects of ambient pollutant particles and metals. *Current Vascular Pharmacology* 4:199–203.

Jimoh, F. O., A. A. Odutuga, and A. T. Oladiji. 2005. Status of lipid peroxidation and antioxidant enzymes in the tissues of rats fed low-protein diet. *Pakistan Journal Nutritional* 4:431–34. doi:10.3923/pjn.2005.431.434.

Kaplan, R. M., and M. T. Toshima. 1992. Does a reduced fat diet cause retardation in child growth?. *Prevention Medica* 21:33–52.

Khare, M., C. Mohanty, B. K. Das, A. Jyoti, B. Mukhopadhyay, and S. P. Mishra. 2014. Free radicals and antioxidant status in protein energy malnutrition. *International Journal Pediatric* 2014:254396.

Kido, T., E. Tamagawa, N. Bai, K. Suda, H. H. Yang, Y. Li, G. Chiang, K. Yatera, H. Mukae, D. D. Sin, and S. F. Van Eeden. 2011. Particulate matter induces translocation of IL-6 from the lung to the systemic circulation. *American Journal Respiratory Cellular Molecular Biologic* 44:197–204. doi:10.1165/rcmb.2009-0427OC.

Kim, J. W., S. Park, C. W. Lim, K. Lee, and B. Kim. 2014. The role of air pollutants in initiating liver disease. *Toxicogical Researcher* 30:65–70. doi:10.5487/TR.2014.30.2.065.

Leong, B. K., J. K. Coombs, C. P. Sabaitis, D. A. Rop, and C. S. Aaron. 1998. Quantitative morphometric analysis of pulmonary deposition of aerosol particles inhaled via intratracheal nebulization, intratracheal instillation or nose-only inhalation in rats. *Journal Applications Toxicogical* 18:149–60.

Lifshitz, F., P. M. Pintos, C. E. Lezon, E. V. Macri, S. M. Friedman, and P. M. Boyer. 2012. Dyslipidemia is not associated with cardiovascular disease risk in an animal model of mild chronic suboptimal nutrition. *Nutritional Researcher* 32:52–58. doi:10.1016/j.nutres.2011.11.002.

Llesuy, S., P. Evelson, B. Gonzalez-Flecha, J. Peralta, M. C. Carreras, J. J. Poderoso, and A. Boveris. 1994. Oxidative stress in muscle and liver of rats with septic syndrome. *Free Radical Biology & Medicine* 16:445–51.

Maehly, A. C., and B. Chance. 1954. The assay of catalases and peroxidases. *Methods Of Biochemical Analysis* 1:357–424.

Magnani, N. D., T. Marchini, D. R. Tasat, S. Alvarez, and P. A. Evelson. 2011. Lung oxidative metabolism after exposure to ambient particles. *Biochemical and Biophysical Research Communications* 412:667–72. doi:10.1016/j.bbrc.2011.08.021.

Magnani, N. D., T. Marchini, V. Vanasco, D. R. Tasat, S. Alvarez, and P. Evelson. 2013. Reactive oxygen species produced by NADPH oxidase and mitochondrial dysfunction in lung after an acute exposure to residual oil fly ashes. *Toxicology and Applied Pharmacology* 270:31–38. doi:10.1016/j.taap.2013.04.002.

Marchini, T., D. Wolf, N. A. Michel, M. Mauler, B. Dufner, N. Hoppe, J. Beckert, M. Jackel, N. Magnani, D. Duerschmied, D. Tasat, S. Alvarez, J. Reinohl, C. von Zur Muhlen, M. Idzko, C. Bode, I. Hilgendorf, P. Evelson, and A. Zirlik. 2016. Acute exposure to air pollution particulate matter aggravates experimental myocardial infarction in mice by potentiating cytokine secretion from lung macrophages. *Basic Researcher Cardiology* 111:44. doi:10.1007/s00395-016-0562-5.

Margall-Ducos, G., S. Celton-Morizur, D. Couton, O. Bregerie, and C. Desdouets. 2007. Liver tetraploidization is controlled by a new process of incomplete cytokinesis. *Journal of Cell Science* 120:3633–39. doi:10.1242/jcs.016907.

Miller, C. N., and S. Rayalam. 2017. The role of micronutrients in the response to ambient air pollutants: Potential mechanisms and suggestions for research design. *Journal Toxicogical Environment Health B* 20:38–53. doi:10.1080/10937404.2016.1261746.

Miller, M. R., J. B. Raftis, J. P. Langrish, S. G. McLean, P. Samutrtai, S. P. Connell, S. Wilson, A. T. Vesey, P. H. B. Fokkens, A. J. F. Boere, P. Krystek, C. J. Campbell, P. W. F. Hadoke, K. Donaldson, F. R. Cassee, D. E. Newby, R. Duffin, and N. L. Mills. 2017. Inhaled nanoparticles accumulate at sites of vascular disease. *ACS Nano* 11:4542–52. doi:10.1021/acsnano.6b08551.

Misra, H. P., and I. Fridovich. 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *The Journal of Biological Chemistry* 247:3170–75.

Molinari, B. L., D. R. Tasat, M. L. Fernandez, H. A. Duran, J. Curiale, A. Stoliar, and R. L. Cabrini. 2000. Automated image analysis for monitoring oxidative burst in macrophages. *Analytical and Quantitative Cytology and Histology / the International Academy of Cytology [And] American Society of Cytology* 22:423–27.

Nel, A. E., D. Diaz-Sanchez, and N. Li. 2001. The role of particulate pollutants in pulmonary inflammation and asthma: Evidence for the involvement of organic chemicals and oxidative stress. *Current Opinion Pulmonary Medica* 7:20–26.

Orona, N. S., S. A. Ferraro, F. Astort, C. Morales, F. Brites, L. Boero, G. Tiscornia, G. A. Maglione, P. H. Saldiva, S. Yakisich, and D. R. Tasat. 2016. Acute exposure to Buenos Aires air particles (UAP-BA) induces local and systemic inflammatory response in middle-aged mice: A time course study. *Environmental Pollution (Barking, Essex : 1987)* 208:261–70. doi:10.1016/j.envpol.2015.07.020.

Pope, C. A., 3rd. 2000. Epidemiology of fine particulate air pollution and human health: Biologic mechanisms and who's at risk? *Environmental Health Perspectives* 4:713–23. doi:10.2307/3454408.

Reichling, J. J., and M. M. Kaplan. 1988. Clinical use of serum enzymes in liver disease. *Digestive Diseases and Sciences* 33:1601–14.

Riva, D. R., C. B. Magalhaes, A. A. Lopes, T. Lancas, T. Mauad, O. Malm, S. S. Valenca, P. H. Saldiva, D. S. Faffe, and W. A. Zin. 2011. Low dose of fine particulate matter (PM2.5) can induce acute oxidative stress, inflammation and pulmonary impairment in healthy mice. *Inhal Toxicogical* 23:257–67. doi:10.3109/08958378.2011.566290.

Roberts, E. S., J. H. Richards, R. Jaskot, and K. L. Dreher. 2003. Oxidative stress mediates air pollution particle-induced acute lung injury and molecular pathology. *Inhal Toxicogical* 15:1327–46. doi:10.1080/08958370390241795.

Roshchin, A. V., E. K. Ordzhonikidze, and I. V. Shalganova. 1980. Vanadium–Toxicity, metabolism, carrier state. *Journal Hygiene Epidemiology Microbiologic Immunology* 24:377–83.

Salvi, S. 2007. Health effects of ambient air pollution in children. *Paediatrica Respiratory Reviews* 8:275–80. doi:10.1016/j.prrv.2007.08.008.

Sandberg, D. E., M. M. Smith, V. Fornari, M. Goldstein, and F. Lifshitz. 1991. Nutritional dwarfing: Is it a consequence of disturbed psychosocial functioning? *Pediatrics* 88:926–33.

Shen, J., J. Zhang, J. Wen, Q. Ming, J. Zhang, and Y. Xu. 2015. Correlation of serum alanine aminotransferase and aspartate aminotransferase with coronary heart disease. *International Journal Clinical Experiments Medica* 8:4399–404.

Simeonova, R., M. Kondeva-Burdina, V. Vitcheva, and M. Mitcheva. 2014. Some in vitro/in vivo chemically-induced experimental models of liver oxidative stress in rats. *Biomedical Researcher International* 2014:706302.

Simoni, M., S. Baldacci, S. Maio, S. Cerrai, G. Sarno, and G. Viegi. 2015. Adverse effects of outdoor pollution in the elderly. *Journal Thoracic Diseases* 7:34–45. doi:10.3978/j.issn.2072-1439.2014.12.10.

Southam, D. S., M. Dolovich, P. M. O'Byrne, and M. D. Inman. 2002. Distribution of intranasal instillations in mice: Effects of volume, time, body position, and anesthesia. *American Journal Physiological Lung Cellular Molecular Physiological* 282:L833–L839. doi:10.1152/ajplung.00173.2001.

Stiegel, M. A., J. D. Pleil, J. R. Sobus, T. Stevens, and M. C. Madden. 2017. Linking physiological parameters to perturbations in the human exposome: Environmental exposures modify blood pressure and lung function via inflammatory cytokine pathway. *Journal Toxicogical Environment Health A* 80:485–501. doi:10.1080/15287394.2017.1330578.

Tao, F., B. Gonzalez-Flecha, and L. Kobzik. 2003. Reactive oxygen species in pulmonary inflammation by ambient particulates. *Free Radical Biology & Medicine* 35:327–40.

Tasat, D. R., and B. M. de Rey. 1987. Cytotoxic effect of uranium dioxide on rat alveolar macrophages. *Environmental Research* 44:71–81.

Tasat, D. R., C. E. Lezon, F. Astort, P. M. Pintos, E. V. Macri, S. M. Friedman, and P. M. Boyer. 2014. mRNA of cytokines in bone marrow and bone biomarkers in response to propranolol in a nutritional growth retardation model. *Pharmacology Reports* 66:867–73. doi:10.1016/j.pharep.2014.05.005.

Tormos, A. M., R. Talens-Visconti, A. Bonora-Centelles, S. Perez, and J. Sastre. 2015. Oxidative stress triggers cytokinesis failure in hepatocytes upon isolation. *Free Radical Research* 49:927–34. doi:10.3109/10715762.2015.1016019.

Tsai, S. S., C. Y. Tsai, and C. Y. Yang. 2018. Fine particulate air pollution associated with increased risk of hospital admissions for hypertension in a tropical city, Kaohsiung, Taiwan. *Journal Toxicogical Environment Health A* 81:567–75. doi:10.1080/15287394.2018.1460788.

UNICEF-*United Nations Children's Fund*. 2013. Improving child nutrition: The achievable imperative for global progress. Accessed June 20, 2018. https://www.unicef.org/nutrition/index_68661.html

WHO- World Health Organization. 2014. "7 million premature deaths annually linked to air pollution" Accessed June 25, 2018. http://www.who.int/mediacentre/news/releases/2014/air-pollution/en/