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Ozone induces glucose intolerance and systemic metabolic effects in young and aged brown Norway rats ☆

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ABSTRACT

Air pollutants have been associated with increased diabetes in humans. We hypothesized that ozone would impair glucose homeostasis by altering insulin signaling and/or endoplasmic reticular (ER) stress in young and aged rats. One, 4, 12, and 24 month old Brown Norway (BN) rats were exposed to air or ozone, 0.25 or 1.0 ppm, 6 h/day for 2 days (acute) or 2 d/week for 13 weeks (subchronic). Additionally, 4 month old rats were exposed to air or 1.0 ppm ozone, 6 h/day for 1 or 2 days (time-course). Glucose tolerance tests (GTT) were performed immediately after exposure. Serum and tissue biomarkers were analyzed 18 h after final ozone for acute and subchronic studies, and immediately after each day of exposure in the time-course study. Age-related glucose intolerance and increases in metabolic biomarkers were apparent at baseline. Acute ozone caused hyperglycemia and glucose intolerance in rats of all ages. Ozone-induced glucose intolerance was reduced in rats exposed for 13 weeks. Acute, but not subchronic ozone increased α_2-macroglobulin, adiponectin and osteopontin. Time-course analysis indicated glucose intolerance at days 1 and 2 (2 > 1), and a recovery 18 h post ozone. Leptin increased day 1 and epinephrine at all times after ozone. Ozone tended to decrease phosphorylated insulin receptor substrate-1 in liver and adipose tissues. ER stress appeared to be the consequence of ozone induced acute metabolic impairment since transcriptional markers of ER stress increased only after 2 days of ozone. In conclusion, acute ozone exposure induces marked systemic metabolic impairments in BN rats of all ages, likely through sympathetic stimulation.

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Introduction

Epidemiological studies have demonstrated a positive association between long-term exposures to ambient air pollutants, namely particulate matter (PM) and ozone (O_3), and incidence of cardiovascular diseases. Atherosclerosis as measured by increasing carotid intimal medial thickness has been associated with ambient PM (Adar et al., 2013; Brook and Rajagopalan, 2010; Gan et al., 2011; Künzli, 2013). The role of life-stage as a determinant of an individual's susceptibility to the cardiovascular health effects of air pollution is poorly defined. Because more than 100 million Americans live in areas of the US that do not meet EPA air quality standards for O_3 (U.S. Environmental Protection Agency, 2012) and the age distribution within the US is rapidly shifting upwards, age-related susceptibility has important public health relevance. Advancing age heralds an increased likelihood of the appearance of cardiovascular risk factors, systemic inflammation, endothelial dysfunction, insulin resistance, metabolic syndrome, diabetes, the progression of atherosclerotic vascular disease and subsequent clinical events. Animal studies and new epidemiological studies are now beginning to provide some mechanistic insights.

Recently air pollution, especially PM, has been linked to increased incidence of metabolic syndrome (Brook et al., 2008; Chen and Schwartz, 2008; Liu et al., 2013; Sun et al., 2013). Long-term exposure to vehicular traffic, as estimated by proximity of residence to highways, is positively associated with increased insulin resistance in children (Thiering et al., 2013). Yet, none of these studies provide any information about the effects of ozone and the role of age as a determinant of cellular metabolic response in relation to PM or O_3. To address this gap in knowledge, we sought to characterize the metabolic response to O_3 exposure in Brown Norway (BN) rats and the influence of age. In contrast to Sprague–Dawley, Wistar and Fischer 344 rats that develop spontaneous or diet related diseases and obesity (Christian et al., 1998; Newby et al., 1990), the BN rat represents a model of normative human aging without development of such complications. To simulate healthy human aging, we used

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BN rats (Lipman et al., 1996) of different ages to examine metabolic effects of acute and subchronic O_3 exposure.

There are two potential mechanisms by which exposure to air pollutants, such as ozone, can cause systemic metabolic effects. 1) Lung injury from pollutant exposure might cause systemic release of mediators, such as cytokines, oxidatively modified proteins and lipids, or vasoactive substances which can provoke metabolic response in distant organs (liver, muscle and adipose tissues). 2) Inhalation of pollutants (ozone) can stimulate C-fiber mediated neuronal response that through stress pathway produce metabolic effects in distant organs. Cardiovascular effects of air pollutants have been postulated to occur through autonomic stimulation and systemic mediators (Gackiere et al., 2011). Yet, systemic mediators or neurohumoral factors responsible for metabolic alterations have not been identified. It has been shown that chronic ambient PM exposure induces metabolic impairments and liver endoplasmic reticular stress (Laing et al., 2010; Özcan et al., 2004; Sun et al., 2009), however it is not known if O_3 exposure also produces such effects in the liver, and if so, could produce ER stress. Moreover, it is not known whether air pollution-induced metabolic effects could lead to, or exacerbate, insulin resistance, obesity, and type2 diabetes in susceptible individuals. The primary goals of this study were to examine 1) if O_3, a prototypic air pollutant, might induce metabolic alterations in glucose homeostasis in BN rats, 2) whether these alterations are associated with increased circulating cytokines or neurohormonal mediators of the stress response, and 3) whether there is greater susceptibility of very young versus very old rats relative to young adult animals, commonly used in air pollution and other toxicology research. We hypothesized that acute ozone exposure will cause impaired glucose homeostasis and liver endoplasmic reticular stress associated with increases in stress hormones and that these effects will be exacerbated in old rats due to their compromised metabolic processes. We also hypothesized that subchronic ozone exposure would result in exacerbated impairment in glucose homeostasis, which could potentially result in impaired insulin sensitivity. We examined acute and subchronic effects of O_3 with a time-course evaluation for systemic metabolic alterations and liver ER stress in healthy aging BN rats.

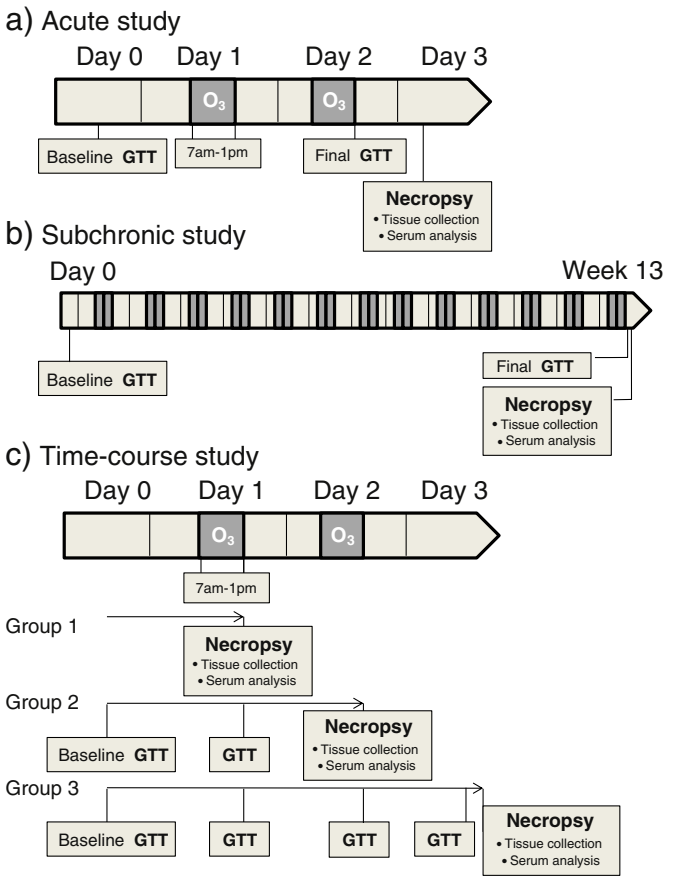

Fig. 1. Schematics of the study design for acute, subchronic and time-course experiments. Timing of glucose tolerance test (GTT), O_3 exposures, and necropsy/serum and tissue collection are shown for each sub-study. Post-exposure GTTs were done immediately following exposure whereas necropsy and tissue and serum collection were done either 18 h after exposure (acute, subchronic, and group 3 time-course study) or immediately following exposure (group 1 and 2 time-course study).

Materials and methods

Animals. Male Brown Norway rats were purchased from (Charles River Laboratories, Kingston, NY, or Portage, MI). Except for 1 and 3 month old rats, retired breeders were purchased at 8–10 month age and allowed to age at our NHEERL, EPA facility. All rats were maintained in AAALAC approved facilities, housed individually in polycarbonate cages (25 cm × 15 cm × 50 cm) containing laboratory-grade pine shavings (Granville Mills, Creedmore, NC). Colony rooms were maintained at constant temperature (22 °C), humidity (50% RH) and a 12 h light, 12 h dark illumination-cycle. Access to food (Rodent Chow 5001: Ralston Purina Laboratories, St. Louis, MO) and tap water was available ad libitum. The Institutional Animal Care and Use Committee (U.S. EPA NHEERL) approved the animal research protocol.

Ozone generation and animal exposures. A silent arc discharge generator (OREC, Phoenix, AZ) generated O_3 from oxygen. Mass flow controllers regulated the entry of O_3 into the Rochester style "Hinners" chambers. Photometric O_3 analyzers (API Model 400) monitored the O_3 concentrations in the chambers. Three different types of exposure studies were conducted and are described as the "acute", "subchronic" and "time-course" studies (Fig. 1). Exposure chamber conditions and actual ozone concentrations achieved are shown in Supplementary Material, Table 1. For the acute study, 1, 4, 12 and 24 month old BN rats (n = 8–12/age group) were exposed for 6 h/day on 2 consecutive days to either FA (0 ppm) or O_3 (0.25 ppm and 1.0 ppm) (Fig. 1a). For the subchronic study, 1, 9 and 21 month old BN rats (n = 8–12/age group) were exposed to FA or O_3 (0.25 or 1.0 ppm) for 6 h/day × 2 days/week over a 13 week period (referred to here by their ages at the end of the 13 week period: 4, 12, and 24 months) (Fig. 1b). For the time-course study, 4 month old BN rats (n = 6) were exposed to FA or 1.0 ppm O_3 6 h/day for 1 day or for two consecutive days. One group of rats was allowed to recover for 18 h after 2 days of O_3 exposure (Fig. 1c).

Glucose tolerance testing (GTT). For animals undergoing subchronic FA or O_3 exposure, GTT was performed 2–3 days prior to the first O_3 exposure (baseline), and immediately after 2 consecutive days of exposure during week 1 and week 13, to obtain data for acute and subchronic exposures, respectively. For the time-course study, rats designated for the 18 h recovery group underwent GTT. In these rats, GTT was performed 2–3 days prior to O_3 exposure, immediately after the 1st day of 6 h O_3 exposure, immediately after the 2nd day of O_3 exposure, and after an 18 h recovery period prior to necropsy. Rats were fasted for 8 h prior to glucose tolerance tests. In instances where GTT followed immediately after O_3 exposure, rats were fasted during exposure periods. For the 18 h recovery period, food was removed 8–10 h prior to GTT and necropsy (overnight starting from 10 pm). Prior to glucose injections, baseline glucose levels were measured by pricking the distal surface of rats' tails with a sterile needle, to obtain ~1 μl of blood. A Bayer Contour glucose meter was used to determine blood glucose levels, using test strips, which require 0.6 μL whole blood. After the 1st measurement, rats were given an intraperitoneal injection of glucose solution with a dose of 2 g/kg/10 ml (20% D-glucose; 10 ml/kg). Measurement with the glucose meter was repeated every 30 min over the course of 2 h.