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The Metabolic Response to Ozone

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The respiratory effects of O_3 are well established. High ambient O_3 concentrations are associated with respiratory symptoms, declines in pulmonary function, asthma exacerbations, and even mortality. The metabolic effects of O_3 are less well appreciated. Here we review data indicating that O_3 exposure leads to glucose intolerance and hyperlipidemia, characteristics of the metabolic syndrome. We also review the role of stress hormones in these events. We describe how the metabolic effects of O_3, including effects within the lungs, are exacerbated in the setting of the metabolic derangements of obesity and we discuss epidemiological data indicating an association between ambient O_3 exposure and diabetes. We conclude by describing the role of the gut microbiome in the regulation of metabolism and by discussing data indicating a link between the gut microbiome and pulmonary responses to O_3.

Keywords: obesity, metabolome, microbiome, fatty acids, hyperglycemia

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INTRODUCTION

Ozone (O_3) is an air pollutant produced by exposure of automobile exhaust to sunlight. The respiratory effects of O_3 are well established. O_3 causes peroxidation of lipids in the nasal and airway lining liquid and epithelial cell membranes, leading to epithelial cell damage and subsequent sterile inflammation (1, 2). The inflammatory response to ozone includes production of inflammatory cytokines and chemokines as well as activation of innate lymphoid cells type 2 and subsequent release of type 2 cytokines (3–5). The details of these events vary with the concentration of O_3 and with the chronicity of exposure and the reader is referred to other reviews (1, 2) for an in depth description of these events. The net effect is that O_3 causes respiratory symptoms including cough, shortness of breath, and wheezing, as well as declines in pulmonary function. O_3 also increases the risk of pulmonary infections, of asthma exacerbations, and even of mortality, the latter mostly in patients with pre-existing cardiorespiratory conditions (6–10). What is less well-appreciated is that O_3 also has pronounced metabolic effects. Here we review data indicating that O_3 exposure impacts the function of the primary organs regulating metabolism leading to glucose intolerance and hyperlipidemia, characteristics of the metabolic syndrome. We describe how the effects of O_3, including effects on the lungs, are exacerbated in the setting of the metabolic derangements of obesity. We conclude by describing the next frontier. The gut microbiome is a key player in the regulation of metabolism. There is increasing evidence of a role for the microbiome in pulmonary responses to O_3. Whether the microbiome also contributes to the metabolic responses to O_3 remains to be established.

ACUTE EXPOSURE TO O_3 DECREASES THE METABOLIC RATE IN RODENTS

Almost four decades ago, Clemons and Garcia reported that acute O_3 exposure reduces plasma concentrations of thyroid hormones (11). Consistent with the role of thyroid hormones in setting

the metabolic rate, acute O_3 exposure also reduces core body temperature, heart rate, activity level, food consumption, and minute ventilation (12–16). These changes are proportional to the O_3 concentration administered and wane over time with repeated exposure. The reduction in minute ventilation that accompanies the reduction in metabolic rate would be expected to reduce the inhaled dose of O_3 and has consequently been viewed as protective against the toxic effects of O_3. Indeed, conditions that increase thyroid hormones, and thus increase the metabolic rate, including reductions in the ambient temperature and exogenous administration of thyroid hormones, and conditions that increase the metabolic rate, such as immaturity, increase the pulmonary inflammation and injury induced by acute O_3 exposure (14, 15, 17).

The torpor-like state described above is similar to what is observed in rodents during acute fasting (18), and acute O_3 exposure also has metabolic consequences similar to those observed during fasting: the adipose tissue initiates lipolysis mobilizing fatty acids that provide a source of energy, and the liver alters its handling of glucose. A metabolomic analysis of serum harvested from rats exposed to air or to O_3 (1 ppm) indicates that short and long-chain free fatty acids (FFAs) are uniformly elevated after O_3 exposure (19). Gene expression analysis on the livers from these rats indicated that O_3 exposure alters many genes involved in fatty acid metabolism and insulin signaling. Last et al. (20) also reported changes in genes related to lipid and fatty acid metabolism and to carbohydrate metabolism in livers from air vs. O_3 exposed mice. In addition, glucose tolerance tests performed on rats immediately after exposure to O_3 indicate hyperglycemia and impaired glucose clearance (19, 21, 22). Similarly, serum 1,5-anhydroglucitol, which is inversely related to long-term glucose control, is decreased in O_3-exposed rats (19). The effects of O_3 are concentration dependent: little effect is observed at 0.25 ppm, glucose intolerance is observed after 0.5 ppm, and both fasting hyperglycemia and glucose intolerance are observed after 1 ppm exposure (23). The latter concentration is higher than would be experienced by humans even in the most polluted of cities, but there are differences in O_3 dosimetry between rodents and humans (24). Importantly, glucose intolerance is also observed when rats are exposed to lower concentrations of O_3 for more extended periods of time (25). Serum insulin is also elevated after O_3 suggesting insulin resistance rather than impaired insulin release and experiments using euglycemic clamps verify insulin resistance (22). Acute O_3 exposure also causes reduced insulin sensitivity in liver and skeletal muscle but not adipose tissue harvested from O_3-exposed rats, as assessed by phosphorylation of AKT (21, 22).

There are sex differences in the metabolic response to acute O_3 exposure. Gordon et al. (26) reported that male rats developed the same fasting hyperglycemia and glucose intolerance after acute O_3 as described above, whereas females that were littermates of these males did not. In addition, although glucose tolerance tests performed after O_3 exposure indicated some glucose intolerance in females, the effect was much smaller than was observed in males. Interestingly, markers of O_3-induced pulmonary injury and inflammation were also lower in the female than male rats suggesting a link between the metabolic and inflammatory responses to O_3.

EVIDENCE OF METABOLIC EFFECTS OF O_3 IN HUMANS

The metabolic effects of acute O_3 exposure are not restricted to rodents. Miller et al. (27) performed a metabolomic analysis of serum collected 1 h after exposure of human subjects to filtered air or to O_3 (0.3 ppm) with a 15-min on-off exercise cycle. The results indicated O_3-induced increases in medium and long chain fatty acids and glycerol indicative of lipid mobilization from adipose tissue stores similar to what is observed in rats (19). Epidemiological studies also provide increasing evidence of an association between O_3 exposure and diabetes. For example, in a large study from Italy that included 376,157 individuals, the authors noted a positive association between average annual ambient O_3 concentrations and the risk of diabetes (28). A large (45,231 women) longitudinal analysis of African American women conducted over 16 years also indicated an association between ambient O_3 concentrations and the risk of incident diabetes (29). The association remained unaltered even after controlling for particulate air pollution, which is also associated with diabetes (30). No association between O_3 and hyperglycemia was observed in a study from the Framingham Heart cohort, even though associations were observed with $PM_{2.5}$ and NO_2, but the study was much smaller (5958 participants) and may not have been adequately powered to detect an association.

MECHANISTIC BASIS FOR THE METABOLIC EFFECTS OF O_3

Stress hormones likely account for the metabolic effects of O_3 (**Figure 1**). In rodents, serum corticosterone levels increase immediately following acute O_3 exposure (19, 31). A similar increase in cortisol is observed in human subjects after acute O_3 exposure (27). Serum concentrations of epinephrine are also increased following O_3 (19, 21, 31) and remain elevated even 18 h after cessation of exposure (21). These changes in stress hormones are thought to arise from O_3-induced stimulation of sensory afferents within the lungs and nose (32). These afferents have been shown to terminate in stress responsive regions of the brain (33). Importantly, the hyperglycemia and impaired glucose clearance observed after acute O_3 exposure are virtually abolished in rats in which either the adrenal medulla or the entire adrenal gland is surgically removed bilaterally (31). Acute O_3-induced increases in serum lipids are also ablated by removal of either the adrenal medulla or the whole adrenal gland. The data are consistent with the known effects of epinephrine and cortisol in promoting gluconeogenesis, insulin resistance, and lipolysis in the liver and adipose tissue during fasting.

It is has been reported that adrenalectomy and drugs that block either beta adrenergic receptors or glucocorticoid signaling attenuate the pulmonary inflammation and injury that occur with O_3 exposure (31, 34, 35). The genes whose expression in the lungs are impacted by O_3 are similar to

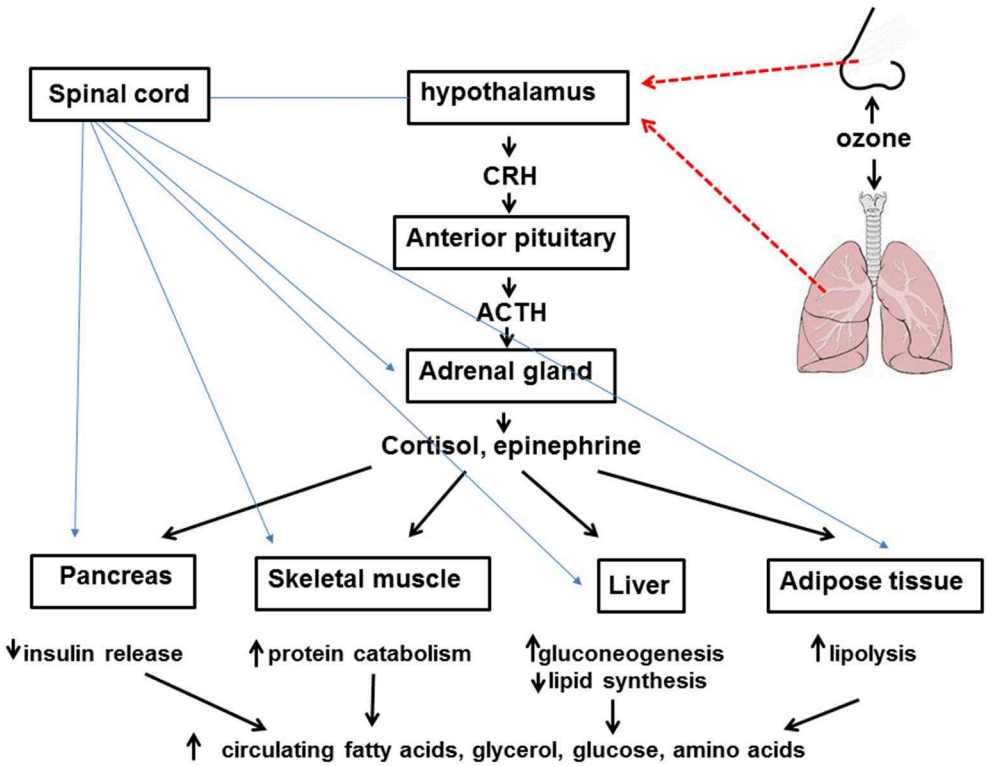

FIGURE 1 | Inhaled ozone stimulates sensory afferents in the nose and lungs (hatched red lines) ultimately leading to activation of the hypothalamus and activation of the sympathetic nervous system as well as release of corticotropin releasing hormone (CRH). CRH leads to release of adrenocorticotropic hormone (ACTH) from the anterior pituitary which acts on the adrenal cortex to cause release of cortisol. Activation of the sympathetic nervous system (blue lines) leads to release of epinephrine from the adrenal medulla. Cortisol, epinephrine, and the sympathetic nervous system lead to attenuated insulin release from the pancreas, increased protein catabolism within skeletal muscle, reduced lipid synthesis and increased gluconeogenesis in the liver, and lipolysis within adipose tissue. The net effect of these events is increased circulating levels of fatty acids, glycerol, glucose, and amino acids.

the genes whose expression changes with glucocorticoids or with agents that, like epinephrine, induce cAMP activation (34). Furthermore, adrenalectomy substantially reduces O_3-induced changes in gene expression within the lungs (34). O_3 exposure causes activation of NF-κB in the lungs and subsequent induction of a variety of inflammatory cytokines and chemokines that contribute to neutrophil recruitment (2, 36) and these events are inhibited by exogenous administration of dexamethasone (37, 38). Therefore, it is unlikely that the effects of adrenalectomy on O_3-induced changes in gene expression (34) are the result of loss of the effects of stress hormones on activation of inflammatory genes by O_3. Instead, the observed reductions in O_3-induced neutrophil recruitment that occur after adrenalectomy or after inhibition of endogenous stress hormones (31, 34, 35) may be the result of inhibition of the effects of stress hormones on metabolic pathways. Inhibition of O_3-induced NF-κB by corticosteroids occurs at higher concentrations of these steroids than are typically released endogenously following O_3. In this context, it is interesting to note that fatty acids that are released in a stress-hormone dependent manner following O_3 (31) should have the capacity activate neutrophils via the fatty acid receptor, FFAR1 (39).

Other events may also contribute to the metabolic effects of O_3. O_3 causes an inflammatory response in the lung characterized by release of acute phase cytokine and cytokines and increases in BAL neutrophils and macrophages (5). Lung specific overexpression of a constitutively active inhibitor of κB kinase (IKK2) not only causes a similar inflammatory response in the lungs, but also induces insulin resistance, perhaps by inducing systemic and adipose tissue inflammation (40), which are thought to mediate the insulin resistance associated with obesity (41). However, in mice, inhalation of another air pollutant, $PM_{2.5}$, also causes inflammation with adipose tissue and liver, and leads to insulin resistance but the insulin resistance is not attenuated when the hepatic and adipose tissue inflammation are ameliorated by genetic deficiency in CCR2, the receptor for the macrophage chemotactic cytokines, CCL2 (42).

EFFECTS OF O_3 IN ANIMALS WITH METABOLIC SYNDROME

Given the effects of acute O_3 exposure on lipid and carbohydrate metabolism, it is interesting to consider differences in the response to O_3 under circumstances in which lipid and

carbohydrate metabolism are already compromised: metabolic syndrome and obesity. In Goto-Kakizaki rats, a model of non-obese type 2 diabetes, exposure to approximately 0.4 ppm O_3 for 4 h results in decreased LDL cholesterol and neither hyperglycemia nor glucose intolerance (43). Unfortunately, no normal rats were included in the study and the concentration used was lower than the 0.5–1.0 ppm concentrations that evoked hyperlipidemia, hyperglycemia, and glucose intolerance in normal rats (19, 21–23), so it is difficult to determine whether the differences are the result of the diabetic state or the nature of the exposure. In contrast, chronic O_3 exposure does appear to affect glucose metabolism in insulin-resistant, diabetes-prone KK mice (44). When these mice are repeatedly exposed to O_3 (0.5 ppm, 4 h a day for 13 days), the mice develop even greater insulin resistance. There is marked fasting hyperglycemia even in air-exposed KK mice and O_3 does not cause any further increases in baseline glucose but does decrease fasting insulin, suggesting impaired insulin release. Injection of insulin gradually reduces blood glucose in air-exposed KK mice, but after O_3 exposure, no such reduction is observed. Increased insulin resistance is also observed in rats and mice with diet-induced obesity as well as in normal weight mice after chronic pulmonary exposure to another air pollutant, $PM_{2.5}$ (45–47). Adipose tissue inflammation and systemic inflammation are typically observed in obese mice, and repeated exposure to O_3 exacerbates this inflammation (44): the number of activated macrophages within adipose tissue and the number of circulating inflammatory monocytes are both elevated in O_3- vs. air-exposed KK mice. Expression of adipose tissue inflammatory genes linked to insulin resistance, including TNFα, is also elevated in O_3 vs. air exposed KK mice. Pulmonary exposure to another type of air pollution, silicon dioxide nanoparticles, also augments mRNA expression of pro-inflammatory genes within adipose tissue (48). The effects of repeated O_3 exposure on adipose tissue inflammation and insulin release but not insulin sensitivity were also observed in another model of obese type 2 diabetes, KKAy mice (49). The mechanistic basis for the effects of exposure to air pollution on adipose tissue gene expression, including inflammatory gene expression are not well-understood, but it is conceivable that changes in the gut microbiome may contribute (see below).

We have established that the pulmonary inflammation and injury induced by acute O_3 exposure are also increased in obese mice. This effect of obesity was observed in *ob/ob* and *db/db* mice which are obese because of a genetically deficiency in leptin or the leptin receptor, in mice obese because of a genetic deficiency in carboxypeptidase E (Cpe), an enzyme involved in processing neuropeptides related to eating behavior, and in mice diet-induced obesity (5, 50–54). These mice are also diabetic to varying degrees. There are marked effects of obesity on the serum and urinary metabolomes of humans, rats, and mice including changes in carbohydrate, lipid, and branched chain amino acid (BCAA) metabolism (55–57). Lungs of naive obese mice also exhibit metabolic changes, including changes in lipid, phospholipid, and cholesterol metabolism (58). As described above, O_3 has substantive metabolic effects that may be linked to effects of O_3 on the lung. To determine whether O_3 also affects metabolic processes within the lungs and whether these effects of O_3 were modified by obesity, we performed a metabolomic analysis of lung tissue from *db/db* and wildtype (WT) female mice exposed acutely to air or O_3 (54). Our results indicated substantial differences in the lung metabolomes of air-exposed *db/db* and WT mice including increases in lipids and lung carbohydrates. It is possible that increases in these substances in the lungs are due to corresponding increases in the blood (57) and subsequent diffusion into the lung extracellular fluid. Acute O_3 exposure also affected the lung metabolome and there were differential effects of O_3 in *db/db* and WT mice. For example, we observed effects of O_3 on the substrates used for energy production in the lungs and these effects differed in *db/db* and WT mice. In WT mice, O_3 exposure reduced BCAA metabolites consistent with increased reliance upon BCAA catabolism for energy, but no such effect was observed in *db/db* mice. Instead, in *db/db* mice, O_3 resulted in decreased long chain acylcarnitines consistent with increased reliance upon β-oxidation for energy after O_3 exposure. Changes in lung lipids are also observed in monkeys after chronic exposure to lower concentrations of O_3 (59, 60).

As discussed above, O_3-induced increases in stress hormones appear to mediate the hyperglycemia and hyperlipidemia that occur with acute O_3 exposure. Corticosteroids also promote β-oxidation (61) and attenuate BCAA catabolism (62), similar to the effects of O_3 in *db/db* mice. In our metabolomic analysis, lung corticosterone was greater in O_3- than air-exposed mice, presumably as a result of increases in serum corticosterone, but the effect of O_3 on corticosterone was only significant in *db/db* mice (54). Thus, greater O_3-induced increases in corticosterone in *db/db* than WT mice might account for the different effects of O_3 on lung β-oxidation and BCAA metabolism observed in *db/db* vs. WT mice.

O_3 AND THE MICROBIOME: THE NEXT FRONTIER

Data from animal models indicate that the gut microbiome contributes to variety of metabolic conditions including insulin resistance and also affects metabolic processes within the liver (63–68). For example, treatment with oral antibiotics attenuates both the glucose intolerance and the adipose tissue inflammation observed in obese mice (65). Germ free mice consuming a Western style diet are protected against the development of obesity and have changes in skeletal muscle and liver that promote fatty acid metabolism (64). One way that gut microbiota regulate metabolism is via the production of metabolites that can impact their host. For example, gut microbiota modify bile acids which signal in the intestines and liver to regulate lipid metabolism (68). Hence, it is possible that the gut microbiome also contributes to the changes in metabolism as well as to the changes in hepatic gene transcription observed following acute O_3 exposure.

Data from our lab also indicate a role for the microbiome in the metabolic changes observed in the lungs after O_3 exposure. Among the lung metabolites identified in the metabolomic

profiling experiment described above were several that require bacteria for their generation in mammals (54). Notably, each of these bacterial-mammalian co-metabolites was affected by obesity, by O_3 exposure, or by the combination of obesity and O_3 exposure. It is perhaps not surprising that obesity affects metabolites of bacterial origin. The community structure of the gut microbiome is altered by obesity both in rodents and in humans [see (69) for review] and there are differences in the metabolomic profile of tissues and blood harvested from germ free vs. conventionally housed mice and from antibiotic-treated vs. control mice (70–72). Thus, gut bacteria-derived metabolites can enter the blood and most are small enough to diffuse from the blood into the lungs. It is more surprising that O_3 also affects these metabolites. One potential explanation is the effects of O_3 on the liver (19, 20), since generation of many of the bacterial-mammalian co-metabolites identified in the lungs requires a metabolic step that occurs in the liver. However, it is also conceivable that O_3 alters either the gut or the lung microbiome. O_3 also affects the nose (3), and O_3-induced changes to the nasal microbiome could also contribute to responses to O_3 by altering metabolites that stimulate nasal afferents and contribute to activation of the HPA axis (**Figure 1**).

Our data indicate that bacteria also contribute to pulmonary responses to acute O_3 exposure (73). O_3-induced airway hyperresponsiveness and O_3-induced neutrophil recruitment are reduced in male C57BL/6 mice treated with antibiotics, as well as in germ free mice. Since these changes are observed both with antibiotics that can cross the intestines and enter the blood and with antibiotics that cannot, the data suggest that the origin of the bacteria involved in these events is the gut and not the lungs. Gut bacteria generate short chain fatty acids (SCFAs) from dietary fiber and our data suggest a role for SCFAs in the effects of the microbiome on responses to O_3. We observed reductions in serum SCFAs only in mice treated with those antibiotics that attenuated responses to O_3. Furthermore, exogenous administration of SCFAs via the drinking water and diets high in fermentable fiber that increased serum SCFAs also augmented responses to O_3 (73). Together, our data support a role for the gut microbiome in pulmonary responses to O_3. Whether the gut microbiome also contributes to the metabolic changes observed after O_3 exposure and whether O_3 itself has the capacity to alter the gut microbiome remains to be established.

SUMMARY

In rodents, acute O_3 exposure causes endocrine and metabolic changes similar to those observed during fasting: stress hormones are released and act on the liver, adipose tissue, and skeletal muscle countering the effects of insulin and promoting lipolysis, thus providing a ready source of energy. However, O_3 also lowers the metabolic rate, reducing the need for energy. The net effects of these changes are hyperglycemia and hyperlipidemia, characteristics of the metabolic syndrome. Similar, albeit attenuated effects are observed in rodents after repeated exposures at lower concentrations of O_3, an exposure paradigm that perhaps better reflects human exposures to ambient O_3. Humans do not experience the torpor-like state that characterizes rodents exposed to O_3, but hyperlipidemia is also observed after acute exposure of human subjects to O_3 and there is an increasing body of epidemiological data indicating an association between O_3 exposure and diabetes. Indeed, in certain types of obese diabetic rodents, O_3 exacerbates their already compromised insulin sensitivity and also induces adipose tissue and systemic inflammation, other characteristics of the metabolic syndrome. O_3 also differentially affects both energy metabolism and inflammation within the lungs of obese diabetic vs. normal lean mice. Better understanding of the mechanistic basis for the effects of O_3 on the liver and adipose tissue is needed to protect populations already at risk of metabolic disease.

There is increasing evidence that the gut microbiome contributes to energy regulation. It remains to be established whether the gut microbiome also contributes to the derangements in energy regulation that occur after O_3 exposure, but there is evidence of a link between the gut microbiome and pulmonary responses to O_3. Better understanding of this link could result in strategies to prevent or mitigate the deleterious effects of O_3 not only on the lungs, but also on metabolic health.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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