Nitrogen Dioxide Air Pollution near Ambient Levels Is an Atherogenic Risk Primarily in Obese Subjects: A Brief Communication

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Ambient exposure to nitrogen dioxide, a critical air pollutant in developed countries, is positively associated with cardiovascular mortality and morbidity. Although its cardiovascular effects are predominantly shown in patients with high risk of atherogenesis, no studies have elucidated whether daily exposure to nitrogen dioxide air pollution enhances atherogenic metabolisms, primarily in obese subjects who are susceptible to atherogenesis and subsequent cardiovascular diseases. We used male Otsuka Long-Evans Tokushima Fatty (OLETF) rats as obese subjects and Long-Evans Tokushima (LETO) rats as nonobese controls. The animals were continuously exposed to nitrogen dioxide at a concentration of 0, 0.16, 0.8, or 4.0 ppm from 8 weeks of age through 32 weeks. At 40 weeks of age, levels of body weight, triglyceride, and total cholesterol were significantly greater in the OLETF rats than in the LETO rats. A ratio of high-density lipoprotein (HDL) to total cholesterol was significantly smaller in the former than in the latter. In the LETO rats, nitrogen dioxide exposure significantly decreased only the levels of HDL as compared with clean air exposure. In the OLETF rats, however, nitrogen dioxide exposure at a concentration of 0.16 ppm significantly elevated triglyceride concentration and decreased the ratio of HDL to total cholesterol as well as the levels of HDL. Nitrogen dioxide air pollution near ambient levels is an atherogenic risk primarily in obese subjects. Exp Biol Med 229:361–364, 2004

Key words: atherosclerosis; obesity; cholesterol; lipids; nitrogen dioxide

Obesity, which enhances the risk of death from cardiovascular diseases, is increasing in the population of developed countries. The relative risk associated with an increment of body mass index has been reported to be 1.10 for mortality from cardiovascular diseases (1). Furthermore, abdominal obesity is frequently associated with highly atherogenic metabolic complications such as hypertension, dyslipidemia, and glucose intolerance (2). On the other hand, air pollution has also been reported to increase the risk of death from cardiovascular diseases. Recent studies have linked air pollution to tens of thousands of premature cardiovascular deaths per year (3). Among a variety of air pollutants, an interquartile range increase in nitrogen dioxide is associated with an increase of 6.1% of cardiovascular mortality (4, 5). Furthermore, concentration of nitrogen dioxide is associated with daily hospital emergency transports for ischemic heart diseases such as angina pectoris and myocardial infarction (6), as well as for subsequent cardiac insufficiency (7) and arrhythmia (8). Intriguing aspects of these epidemiologic data are that the cardiovascular effects of nitrogen dioxide are predominantly observed in patients with diabetes mellitus (9) and those with cardiovascular diseases aged 65 years or older who have high risks of atherogenesis (10).

However, no studies have elucidated the effect of nitrogen dioxide air pollution on atherogenic lipid metabolisms primarily in obese subjects who are susceptible to atherogenesis and subsequent cardiovascular diseases. The

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Table 1. Body Weights and the Levels of Triglyceride, High-Density Lipoproteins (HDL), Total Cholesterol, and Sugar in the Blood of Obese (Otsuka Long-Evans Tokushima Fatty [OLETF]) Rats and Normal Control (Long-Evans Tokushima [LETO]) Rats[1]

Strains	Number	Exposure (ppm)	Body weight (g)	Triglyceride (mg/dl)	HDL (mg/dl)	Total cholesterol (mg/dl)	HDL/total cholesterol	Sugar (mg/dl)
Normal control subjects								
LETO	10	0 (clean air)	497 ± 25.9	24.5 ± 15.8	48.4 ± 4.3	111.0 ± 7.4	0.438 ± 0.025	227 ± 88
LETO	14	0.16	504 ± 50.2	36.1 ± 22.8	42.9 ± 4.7*	100.6 ± 12.5	0.427 ± 0.021	232 ± 107
LETO	13	0.8	487 ± 24.0	15.4 ± 6.10	43.7 ± 2.4*	101.9 ± 7.4	0.430 ± 0.018	245 ± 84
LETO	14	4.0	492 ± 21.2	19.9 ± 10.6	43.6 ± 3.8*	100.9 ± 9.3	0.434 ± 0.032	248 ± 104
Obese subjects								
OLETF	9	0 (clean air)	624 ± 51.2**	156 ± 130**	62.2 ± 7.4**	154.5 ± 17.4**	0.403 ± 0.023**	405 ± 112***
OLETF	13	0.16	631 ± 33.1**	264 ± 210*,**	52.8 ± 10.2**,****	148.4 ± 23.7**	0.356 ± 0.045**,****	304 ± 100
OLETF	12	0.8	614 ± 37.4**	154 ± 97**	56.7 ± 5.6*,**	143.0 ± 17.7**	0.398 ± 0.022**	327 ± 78
OLETF	10	4.0	616 ± 29.6**	121 ± 64***	57.9 ± 7.8**	146.8 ± 20.1**	0.395 ± 0.019**	364 ± 112***

[1] Data are expressed as mean ± SD. Data were analyzed using ANOVA.
* $P < 0.05$ versus clean air exposure in the identical strain.
** $P < 0.01$ versus LETO rats that were exposed to nitrogen dioxide at the identical concentration.
*** $P < 0.05$ versus LETO rats that were exposed to nitrogen dioxide at the identical concentration.
**** $P < 0.01$ versus clean air exposure in the identical strain.

present study was undertaken to determine whether daily exposure to nitrogen dioxide air pollution near ambient levels is an atherogenic risk primarily in obese subjects.

Materials and Methods

Four-week-old male Otsuka Long-Evans Tokushima Fatty (OLETF) rats and Long-Evans Tokushima (LETO) rats serving as normal controls were kindly supplied from the Otsuka Tokushima Research Institute (Otsuka Pharmaceutical, Tokushima, Japan). The OLETF rat strain was established by Kawano *et al.* (11) as an inbred strain of spontaneous mutants that show obesity, hypertriglycemia, hypertension, and insulin resistance. The rats were housed in an animal facility that was maintained at 24°–26°C with 55%–75% humidity and a 14:10-hr light:dark cycle; they were fed a commercial diet (Japan Clea Co., Tokyo, Japan) and water *ad libitum*.

The animals were continuously exposed to nitrogen dioxide at a concentration of 0, 0.16, 0.8, or 4.0 ppm from 8 weeks of age for 32 weeks in our exposure chambers. The volume of chambers was 1.39 m^3. Filtered clean air flowed through one chamber, and in the other chambers for nitrogen dioxide exposure, filtered clean air was mixed with 5000 ppm NO_2/N_2 (>99.9%; Sumitomo Seika Chemicals, Tokyo, Japan) from a bomb before it entered the chambers. The chambers were operated under dynamic conditions at 25°C ± 1°C, 55% ± 5% humidity, −5 mm H_2O relative to atmospheric pressure with an air flow of 110 m^3/hr. The concentrations of nitrogen dioxide were controlled by continuous monitoring with an NOx analyzer (Monitor Labs model 8440, San Diego, CA) that operates on a chemiluminescence principle. NO was undetectable in each chamber.

At 40 weeks of age, body weight of the animals was measured. Routine laboratory examinations including triglyceride, high-density lipoproteins (HDL), total cholesterol, and sugar on peripheral blood were conducted after exsanguination under deep anesthesia with diethyl ether. The lungs were fixed in 10% neutral phosphate-buffered formalin, and sections were stained with hematoxylin and eosin as previously reported (12).

Data were reported as mean ± SD. Differences among groups were determined using analysis of variance (ANOVA; Stat-View version 4.0; Abacus Concepts, Inc., Berkeley, CA). If differences among groups were significant ($P < 0.05$), Fisher's protected least significant difference test was used to distinguish between pairs of groups.

Results

The levels of body weight, triglyceride, and total cholesterol were significantly greater in the OLETF rats than in the LETO rats under exposure to nitrogen dioxide at each concentration ($P < 0.01$, but $P < 0.05$ for triglyceride at 4.0 ppm of nitrogen dioxide; Table 1). A ratio of HDL to total cholesterol was significantly smaller in the OLETF rats than in the LETO rats under exposure to nitrogen dioxide at each concentration ($P < 0.01$). The levels of blood sugar were greater in the OLETF rats than in the LETO rats under exposure to nitrogen dioxide at each concentration ($P < 0.05$ at 0 and 4.0 ppm of nitrogen dioxide).

In the LETO rats, nitrogen dioxide exposure at each concentration significantly decreased only the levels of HDL compared with clean air exposure ($P < 0.05$; Table 1). In the OLETF rats, however, nitrogen dioxide exposure at a concentration of 0.16 ppm significantly elevated triglyceride concentration ($P < 0.05$), and significantly decreased the ratio of HDL to total cholesterol ($P < 0.01$) as well as the levels of HDL ($P < 0.01$) in comparison with

clean air exposure. As compared with clean air exposure, nitrogen dioxide exposure at a concentration of 0.8 ppm also showed a significant decrease in the levels of HDL ($P < 0.05$). However, nitrogen dioxide exposure at a concentration of 4.0 ppm did not show any significant changes in the OLETF rats. The levels of blood sugar were not affected by nitrogen dioxide exposure in both strains. Histological studies revealed that nitrogen dioxide exposure dose-dependently increased infiltration of lymphocytes and hyperplasia of the bronchial epitheliums. However, there were no significant differences between the OLETF rats and the LETO rats.

Discussion

The present study has demonstrated that daily exposure to nitrogen dioxide air pollution near ambient levels (0.16 ppm) enhances atherogenic lipid metabolisms primarily in the OLETF rats, but less in the LETO rats.

The OLETF rats have been reported to show obesity, hypertriglycemia, hypertension, insulin resistance, a late onset of hyperglycemia, hyperplastic foci of pancreatic islets, and renal nodular lesions with a chronic disease course, which is a good animal model of human obesity usually accompanied by Type 2 diabetes mellitus (11). Also in the present study, the OLETF rats developed obesity and atherogenic lipid metabolisms, whereas the control LETO rats did not. Thus, the OLETF rats and the LETO rats are useful to determine the differential effects of air pollution on the atherogenic lipid metabolisms in obese and nonobese subjects.

In the present study, nitrogen dioxide exposure at a concentration of 0.16 ppm enhanced atherogenic lipid metabolisms including triglyceride, HDL, and a ratio of HDL to total cholesterol in the OLETF rats, whereas it enhanced only HDL levels in the LETO rats. These results clearly indicate that obese subjects who have a high risk of atherogenesis and cardiovascular diseases are susceptible further to the atherogenic cardiovascular effects of nitrogen dioxide air pollution at a relatively low concentration.

Although the National Ambient Air Quality Standards of nitrogen dioxide is 0.053 ppm per annual arithmetic mean, continuous monitoring of in-vehicle (13) or commuter exposures (14) to nitrogen dioxide have ranged from 0.03 to 0.17 ppm in the United States. In addition, levels as high as 0.5–0.6 ppm of nitrogen dioxide have been demonstrated in the vicinity of an operating gas stove (15). In the present study, it is striking that the atherogenic effects of nitrogen dioxide are shown primarily at a concentration of 0.16 ppm, which is near ambient levels, but less at higher toxic concentrations. Comparable results have been demonstrated by a previous human study: nitrogen dioxide exposure significantly increased airway resistance at a relatively low concentration (1.5 ppm), but not at higher concentrations up to 8.0 ppm (16). It has been reported that several of the dose-response curves for health effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin are U-shaped, which appears to result because the integrated toxicologic response depends on multiple underlying processes, each of which has a different relationship with respect to the pollutant (17).

Although nitrogen dioxide is *in vivo* counteracted by an arsenal of antioxidants (18), the levels of lipid peroxides were not significantly different among the experimental groups (data not shown).

In conclusion, nitrogen dioxide air pollution near ambient levels is an atherogenic risk primarily in obese subjects. Future epidemiologic and experimental studies should focus on the atherogenic cardiovascular effects of air pollution primarily in obese subjects.

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