

Original Paper

# Pancreatic Effects of Diesel Exhaust Particles in Mice with Type 1 Diabetes Mellitus

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## Key Words

Particulate air pollution • Diesel exhaust particles • Pancreas • Oxidative stress • Streptozotocin • Type I diabetes • Mice

## Abstract

**Background/Aims:** Epidemiologically, diabetics are more prone to the adverse health effects of particulate air pollution than healthy individuals. We recently demonstrated an increased cardiovascular and respiratory susceptibility to diesel exhaust particles (DEP) in mice with type 1 diabetes. However, the pancreatic effects of DEP in healthy and diabetic mice are unknown.

**Methods:** Presently, we evaluated the pancreatic impact of DEP in healthy mice, and mice with streptozotocin-induced type 1 diabetes. Four weeks following induction of diabetes, mice were intratracheally instilled (i.t.) with either DEP (0.4 mg/kg) or saline, and several histological and biochemical endpoints were measured 24 h thereafter. **Results:** Neither the histology nor the stain for apoptosis in the pancreatic islets and exocrine glands were affected by DEP. In diabetic mice exposed to saline, the islet cells showed cellular vacuolation and apoptotic islet cells ( $71.6 \pm 2.6\%$ ). In diabetic mice exposed to DEP, a more marked decrease in the size and number of islet cells with cellular vacuolation along with a significant increase of apoptotic islet cells ( $79.1 \pm 1.7\%$ ,  $P < 0.05$ ) were observed. In diabetic mice, DEP increased significantly pancreatic amylase activity and markers of oxidative stress including 8-isoprostane, superoxide dismutase and reduced glutathione compared with either diabetic mice exposed to saline or non-diabetic mice exposed to DEP. Staining for inducible nitric oxide synthase (iNOS) in healthy mice exposed to either saline or DEP showed no staining in either pancreatic islets cells or acini. In saline-treated diabetic mice, a mild cytoplasmic staining for iNOS in some pancreatic islet cells was observed. Notably, in diabetic mice exposed to DEP, a marked cytoplasmic staining for iNOS in most pancreatic islet cells and some acinar cells was seen. **Conclusion:** We conclude that DEP caused detrimental effects on the pancreas of diabetic mice, and that oxidative stress is responsible, at least partially, for the observed effects.

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## Introduction

Exposure to particulate air pollution has been linked with increased pulmonary and extrapulmonary mortality and morbidity [1]. The World Health Organization estimated that the mass of fine particles  $<2.5 \mu\text{m}$  in aerodynamic diameter ( $\text{PM}_{2.5}$ ) contributed to  $\approx 800,000$  premature deaths per year, ranking  $\text{PM}_{2.5}$  as the 13th leading cause of worldwide mortality [2].

Diesel exhaust particles (DEP), the main component of  $\text{PM}_{2.5}$  and ultrafine (nano) particles (diameter  $\leq 0.1 \mu\text{m}$ ) in urban areas, are a significant contributor to inhaled particulate matter pollution [3]. These ultrafine particles have high alveolar deposition and ability to translocate to the bloodstream [3]. Acute exposure to DEP in healthy human volunteers caused pulmonary and extrapulmonary effects [4].

Particulate air pollution influences vulnerability to adverse events and may be detrimental to high-risk groups such as diabetic patients [5-8]. It has been reported that among diabetics, the risk of hospital admissions for heart diseases is 2-fold greater than that for non-diabetic people [9]. To verify and explain these observations, several experimental studies have been performed to test whether and to what extent the effects of particulate air pollution are aggravated in animal models of type 1 and type 2 diabetes [10-14].

We have recently demonstrated an increase in respiratory, systemic and coagulation susceptibility in mice with type 1 diabetes acutely exposed to DEP [12, 13]. However, the possible effects of DEP on the pancreas in healthy mice and those with type 1 diabetes have, as far as we are aware, not been investigated so far.

The hypothesis tested here is whether and to what extent can DEP exert injurious effects on the pancreas in type 1 diabetic mice. Consequently, the goal of this study is to evaluate the pancreatic effects of DEP in healthy mice, and mice with streptozotocin (STZ)-induced type 1 diabetes by measuring several histological and biochemical endpoints 24 h post-DEP exposure.

## Material and Methods

### *Diesel exhaust particles (DEP)*

The DEP (SRM 2975) were obtained from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA), and were suspended in sterile saline (NaCl 0.9 %) containing Tween 80 (0.01 %). To minimize aggregation, particle suspensions were always sonicated (Clifton Ultrasonic Bath, Clifton, New Jersey, USA) for 15 min and vortexed before their dilution and prior to intratracheal (i.t.) administration. Control animals received saline containing Tween 80 (0.01 %).

These particles were previously analysed by transmission electron microscopy, and shown to have a substantial amount of ultrafine (nano) sized particle aggregates, and larger particle aggregates [15, 16].

### *Animals and treatments*

This project was reviewed and approved by the Institutional Review Board of the United Arab Emirates University, College of Medicine and Health Sciences, and experiments were performed in accordance with protocols approved by the Institutional Animal Care and Research Advisory Committee.

Male TO mice (HsdOla:TO, Harlan, UK) were housed in light (12-h light:12-h dark cycle) and temperature-controlled ( $22 \pm 1^\circ\text{C}$ ) rooms. They had free access to commercial laboratory chow and were provided tap water *ad libitum*.

Type 1 diabetes mellitus was induced in male TO mice (6 to 8 weeks old) by intraperitoneal (i.p.) injection of 200 mg/kg body weight of STZ (Sigma Chemical, St. Louis, MO) [12, 13, 17, 18]. Tail vein blood glucose samples were measured before and during 4 weeks after injection to ensure induction of diabetes. The nondiabetic (control) mice were injected i.p. with the vehicle (0.1 mol/l citrate buffer, pH 4.5). Four-weeks post-STZ injection, mice were anesthetized with sodium pentobarbital (60 mg/kg, i.p.), and placed supine with extended neck on an angled board. A Becton Dickinson 24 Gauge cannula was inserted via the mouth into the trachea. Either the DEP suspensions (0.4 mg/kg) or saline-only were instilled intratracheally

(i.t.) (50  $\mu$ l) via a sterile syringe and followed by an air bolus of 50  $\mu$ l to diabetic and non-diabetic mice.

#### *Measurement of pancreatic enzymes and markers of oxidative stress*

After the exposure to either DEP or saline, animals were sacrificed by an overdose of sodium pentobarbital, and their pancreases were quickly collected and rinsed with ice-cold PBS (pH 7.4) before homogenization in 0.1M phosphate buffer, pH 7.4, containing 0.15M KCl, 0.1mM EDTA, 1mM DTT and 0.1mM phenylmethylsulfonylfluoride at 4°C. The homogenates were centrifuged for 10 min at 3000  $\times g$  to remove cellular debris and supernatants were used for further analysis. Protein content was measured by Bradford's method, as described before [19, 20].

The activities of amylase and lipase were measured using colorimetric assays (Roche Diagnostics, Indianapolis, USA).

The concentrations of 8-isoprostane were determined using an ELISA Kit (Cayman Chemicals, Michigan, USA). The concentration of reduced glutathione (GSH) and the activity of superoxide dismutase (SOD) were determined using colorimetric assay kits (Cayman Chemicals, Michigan, USA).

#### *Histology*

Light microscopy : pancreas was excised, washed with ice-cold saline, blotted with filter paper and weighed. Each pancreas was cassetted and fixed directly in 10% neutral formalin for 24 hours, which was followed by dehydration in increasing concentrations of ethanol, clearing with xylene and embedding with paraffin. Five- $\mu$ m sections were prepared from paraffin blocks and stained with haematoxylin and eosin. The stained sections were evaluated blindly using light microscopy by the histopathologist who participated in this project.

Staining for apoptosis : this was performed using a signal stain cleaved caspase-3 Immunohistochemical detection kit (Cell Signaling Technology, Boston, USA). This kit was used to detect the activation of caspase-3 using the avidin- biotin immunoperoxidase method to detect intracellular cleaved caspase-3 protein. Staining was performed on 5  $\mu$ m paraffin sections from the pancreas by a standard technique using rabbit anti-cleaved caspase 3 (clone Asp175, 1:50) [21]. Known positive control sections for apoptosis were used. For the negative control, primary antibody was replaced with normal rabbit serum.

Staining for inducible nitric oxide synthase (iNOS): five-micrometer sections were prepared and mounted on aminopropyltriethoxysilane (APES) coated slides. After dewaxing with xylene and rehydrating with graded alcohol, slides were placed in a 0.01M citrate buffer solution (pH=6.0), and pretreatment procedures to unmask the antigens were performed in a microwave oven for 10 minutes. After that, sections were treated with peroxidase block for 20 minutes followed by protein block for 10 minutes. Then, the sections were stained using the streptavidin-biotin immunohistochemical method for induced nitric oxide synthase (iNOS).

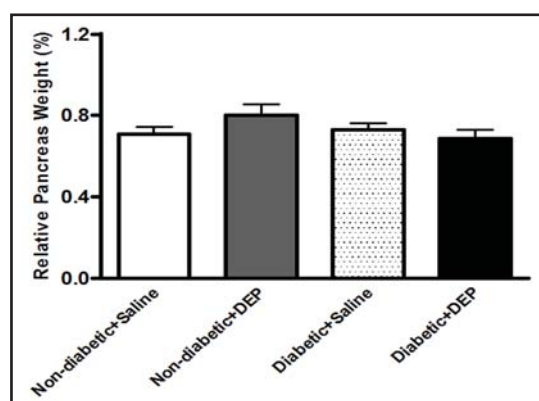
Anti- iNOS (Thermo Scientific, USA, 1:200 rabbit polyclonal) was applied on sections overnight at 4°C, followed by washing the sections in phosphate buffer saline (PBS) for 15 minutes in three changes, then EnVision-FLEX-HRP (DAKO Cytomation, Denmark) was added for 20 minutes at room temperature, which was followed by washing in PBS for 15 minutes and addition of 3,3' diaminobenzidine chromogen for 5 minutes ; this was followed by washing in PBS and counter staining with haematoxylin. Then the sections were dehydrated, cleared and mounted in DPX. An appropriate positive control was used. For negative control, the primary antibody was replaced by phosphate buffer and carried for the whole procedure.

Interpretation of pancreatic sections : apoptotic cells were counted in pancreatic islets in all sections in each group using Image J software (<http://rsbweb.nih.gov/ij/>). Apoptotic cells are defined by the presence of cytoplasmic brown granular staining. The percentage of apoptotic cells in relation to the total number of cells in each pancreatic islet was determined for the different groups. Positive cells for iNOS were defined by cytoplasmic brown granular staining.

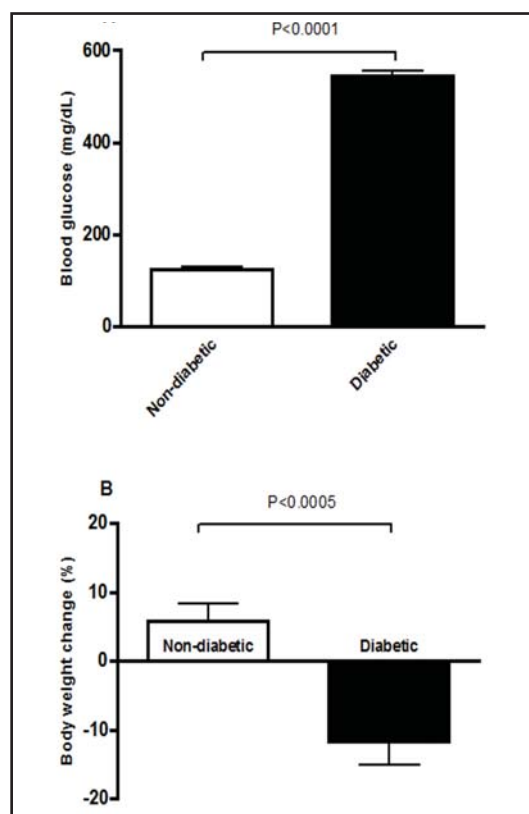
#### *Statistics*

Data were expressed as means  $\pm$  SEM, and were analyzed with GraphPad Prism Version 4.01 for Windows software (Graphpad Software Inc., San Diego, USA). For blood glucose concentrations and body weight change, comparisons were performed using unpaired Student's t-test. For all the other measured parameters, comparisons between groups were performed by analysis of variance (ANOVA), followed by Newman-Keuls multiple-range tests. *P* values <0.05 are considered significant.

**Fig. 1.** Characteristics of study animals. Body weight (A) and blood glucose concentrations (B). Data are mean  $\pm$  SEM (n=24).



**Fig. 2.** Relative pancreas weight, 24 h after intratracheal instillation of either saline or diesel exhaust particles (DEP, 0.4 mg/kg) in either non-diabetic or diabetic mice. Data are mean  $\pm$  SEM (n=6).



## Results

### *General characteristics of the diabetic and non-diabetic mice*

The mean blood glucose concentration was significantly ( $P<0.0001$ ) increased in diabetic mice compared with that of nondiabetic mice (Fig. 1A).

The mean body weight of diabetic mice was significantly ( $P<0.0005$ ) decreased compared with nondiabetic mice (Fig. 1B).

### *Effect of DEP on relative pancreatic weight in diabetic and non-diabetic mice*

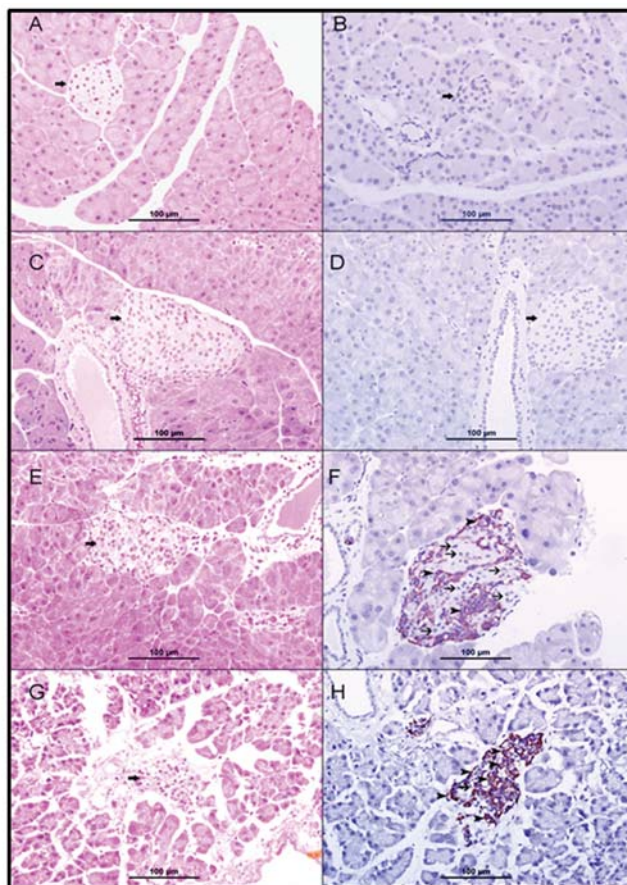
Figure 2 shows that the pancreatic weight relative to body weight was not significantly affected by DEP in either diabetic or non-diabetic mice.

### *Effect of DEP on pancreatic histology and apoptosis in diabetic and non-diabetic mice*

Figure 3 shows representative micrographs of pancreas from the four studied groups. Pancreas obtained from non-diabetic mice exposed to saline showed normal architecture and histology of pancreatic islets and exocrine glands (Fig. 3A). In the last group, the stain for apoptosis showed no evidence of apoptotic cells in the examined sections (Fig. 3B). In the non-diabetic group exposed to DEP, the pancreas showed normal architecture and histology of pancreatic islets and exocrine glands (figure 3C). In the last group, the stain for apoptosis showed no evidence of apoptotic cells in the examined sections (Fig. 3D). The pancreas obtained from diabetic mice treated with saline displayed normal architecture and histology of pancreatic exocrine glands. The islet cells show cellular vacuolation and irregularity in morphology (Fig. 3E). In the last group, the stain for apoptosis showed apoptotic islet cells in the examined sections (Fig. 3F). The pancreas of diabetic mice exposed to DEP presented normal architecture and histology of pancreatic exocrine glands. The islet cells displayed marked decrease in size and number with cellular vacuolation and irregularity in



**Fig. 3.** Cleaved caspase 3 in pancreatic tissue 24 h after intratracheal instillation of either saline or diesel exhaust particles (0.4 mg/kg) in either non-diabetic or diabetic mice. A: the saline-treated non-diabetic group shows normal architecture and histology of pancreatic islets (thick arrow) and exocrine glands, H&E. B: in the saline-treated non-diabetic group, the stain for apoptosis shows no evidence of apoptotic cells in the examined sections (thick arrow) using the streptavidin-biotin immunoperoxidase method. C: the DEP-treated non-diabetic group shows normal architecture and histology of pancreatic islets (thick arrow) and exocrine glands, H&E. D: in the DEP-treated non-diabetic group, the stain for apoptosis shows no evidence of apoptotic cells in the examined sections (thick arrow), streptavidin-biotin immunoperoxidase method. E: the saline-treated diabetic group shows normal architecture and histology of pancreatic exocrine glands. The islet cells (thick arrow) show cellular vacuolation and irregularity in morphology, H&E. F: in the saline-treated diabetic group, the stain for apoptosis shows apoptotic islet cells in the examined sections (arrow head) and normal cells (thin arrow) using the streptavidin-biotin immunoperoxidase method. G: the DEP-treated diabetic group shows normal architecture and histology of pancreatic exocrine glands. The islet cells (thick arrow) analysis shows a marked decrease in their numbers with cellular vacuolation and irregularity in morphology, H&E. H: in the DEP-treated diabetic group, the stain for apoptosis shows many apoptotic islet cells (arrow head) and few normal cells (thin arrow) using the streptavidin-biotin immunoperoxidase method.

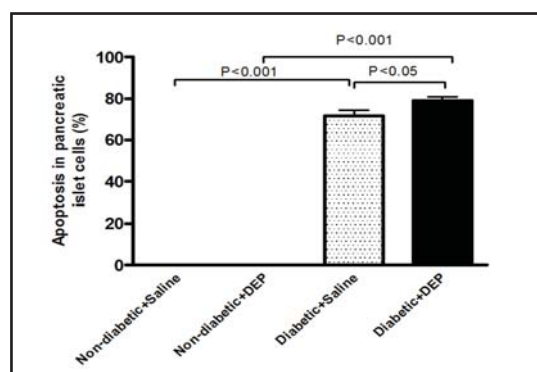


morphology (Fig. 3G). In the last group, the stain for apoptosis showed many apoptotic islet cells in the examined sections involving almost all the islet (Fig. 3H).

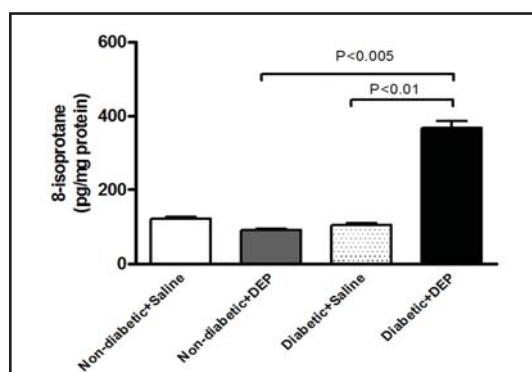
Figure 4 shows no evidence for apoptotic cells in non-diabetic mice exposed to either saline or DEP. In saline-treated diabetic mice, about 72% of islet cells were apoptotic when examining 800 islet cells, and 28% of them did not show any staining for apoptosis. In diabetic mice exposed to DEP, the number of apoptotic islet cells increased significantly ( $P < 0.05$ ) compared with the diabetic+saline group. When examining 800 islet cells, about 79% were apoptotic and 21% showed no staining for apoptosis.

#### *Effect of DEP on the activity of pancreatic amylase and lipase in diabetic and non-diabetic mice*

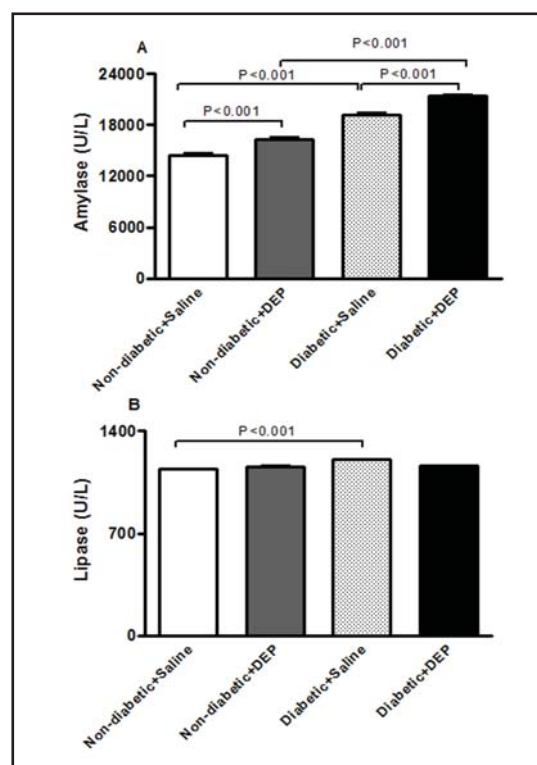
In non-diabetic mice, DEP exposure induced a significant increase in the activity of pancreatic amylase compared with the saline-treated group. Diabetic mice exposed to saline exhibited higher amylase activity compared with non-diabetic mice exposed to saline. Pulmonary administration of DEP caused a significant increase in pancreatic amylase activity in diabetic mice compared with either diabetic mice exposed to saline or non-diabetic mice exposed to DEP (Fig. 5A).



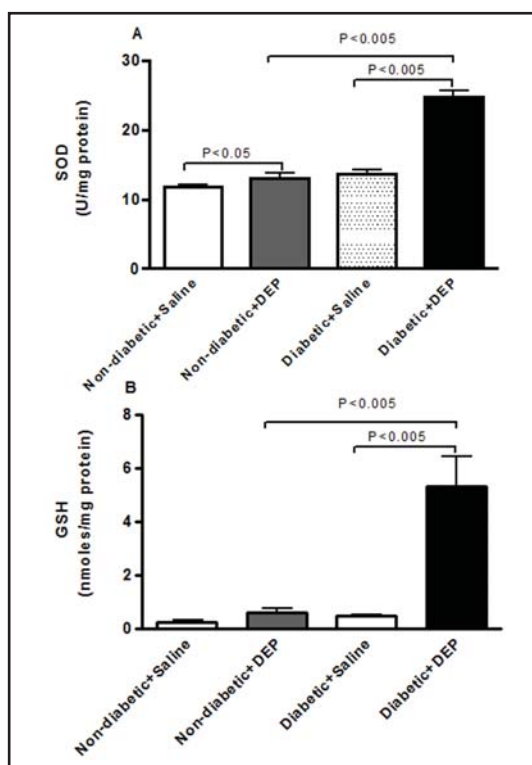
**Fig. 4.** Percentage apoptosis in pancreatic islet cells, 24 h after intratracheal instillation of either saline or diesel exhaust particles (DEP, 0.4 mg/kg) in either non-diabetic or diabetic mice. Data are mean  $\pm$  SEM (n=6).



**Fig. 6.** 8-Isoprostane concentration in pancreatic tissue, 24 h after intratracheal instillation of either saline or diesel exhaust particles (DEP, 0.4 mg/kg) in either non-diabetic or diabetic mice. Data are mean  $\pm$  SEM (n=6).



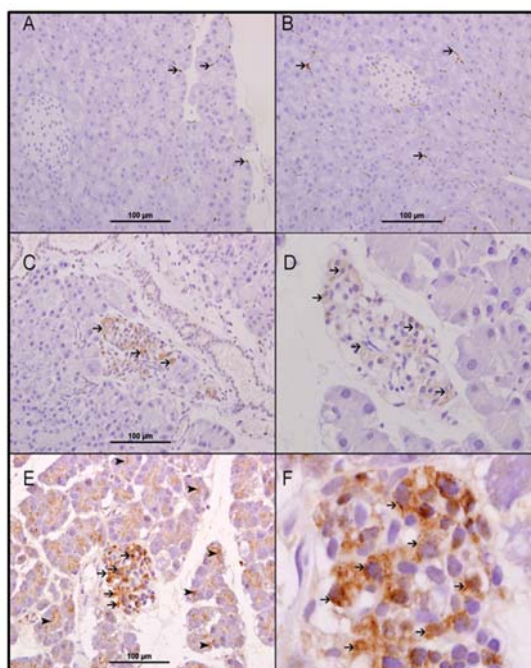
**Fig. 5.** Amylase (A) and lipase (B) activity in pancreatic tissue 24 h after intratracheal instillation of either saline or diesel exhaust particles (DEP, 0.4 mg/kg) in either non-diabetic or diabetic mice. Data are mean  $\pm$  SEM (n=6). Error bars in figure 5B are very small that is why they do not appear.



**Fig. 7.** Superoxide dismutase activity (SOD, A) and reduced glutathione concentration (GSH, B) in pancreatic tissue, 24 h after intratracheal instillation of either saline or diesel exhaust particles (DEP, 0.4 mg/kg) in either non-diabetic or diabetic mice. Data are mean  $\pm$  SEM (n=6).

The activity of pancreatic lipase was significantly increased in diabetic mice exposed to saline compared with non-diabetic exposed to saline. However, DEP exposure did not affect the activity of pancreatic lipase in either diabetic or non-diabetic mice (Fig. 5B).

**Fig. 8.** Expression of inducible nitric oxide (iNOS) in pancreatic tissue, 24 h after intratracheal instillation of either saline or diesel exhaust particles (0.4 mg/kg) in either non-diabetic or diabetic mice. A: the saline-treated non-diabetic group shows no staining for iNOS in pancreatic islets cells and acini. There is mild cytoplasmic staining for iNOS in endothelial cells (arrow) using the streptavidin-biotin immunoperoxidase method. B: the DEP-treated non-diabetic group shows no staining for iNOS in pancreatic islets cells and acini. There is mild cytoplasmic staining for iNOS in endothelial cells (arrow) using the streptavidin-biotin immunoperoxidase method. C and D: the saline-treated diabetic group shows staining for iNOS in some pancreatic islet cells (arrows) using the streptavidin-biotin immunoperoxidase method. E and F: the DEP-treated diabetic group shows staining for iNOS in most pancreatic islet cells (thin arrows), and pancreatic acini (arrow head) using the streptavidin-biotin immunoperoxidase method.



#### *Effect of DEP on pancreatic markers of oxidative stress in diabetic and non-diabetic mice*

In non-diabetic mice, DEP exposure did not affect the concentration of 8-isoprostane compared with the saline-treated group. In diabetic mice, DEP caused a significant increase in the concentrations of 8-isoprostane compared with either diabetic mice exposed to saline or non-diabetic mice exposed to DEP (Fig. 6).

Figure 7A illustrates that, in the non-diabetic group, DEP exposure induced a slight but significant increase in SOD activity compared with the saline-treated group. Interestingly, in diabetic mice, DEP caused a significant increase in SOD activity compared with either the diabetic mice exposed to saline or non-diabetic mice exposed to DEP (Fig. 7A).

Similarly, in diabetic mice, DEP caused a significant increase in the concentrations of reduced GSH compared to either diabetic mice exposed to saline or non-diabetic mice exposed to DEP (Fig. 7B).

#### *Effect of DEP on pancreatic staining for iNOS in diabetic and non-diabetic mice*

Staining for inducible nitric oxide synthase (iNOS) in non-diabetic mice exposed to either saline (Fig. 8A) or DEP (Fig. 8B) showed only mild cytoplasmic staining for iNOS in endothelial cells, and no staining in either pancreatic islets cells or acini.

In saline-treated diabetic mice, a mild cytoplasmic staining for iNOS in some pancreatic islet cells was observed (Fig. 8C-D).

In diabetic mice exposed to DEP, a marked cytoplasmic staining for iNOS in most pancreatic islet cells and some acinar cells was seen (Fig. 8 E-F).

## Discussion

In the present study, we showed an aggravating effect on the pancreas of diabetic mice exposed to DEP compared with non-diabetic ones. We observed a marked decrease in the size and number of islet cells with cellular vacuolation along with a significant increase of apoptotic islet cells in diabetic mice exposed to DEP compared with those exposed to saline. Moreover, DEP caused a significant increase in pancreatic amylase activity and markers of oxidative stress including 8-isoprostane, superoxide dismutase and reduced glutathione in



diabetic mice compared with either diabetic mice exposed to saline or non-diabetic mice exposed to DEP. A more marked cytoplasmic staining for iNOS in most pancreatic islet cells and some acinar cells was seen in diabetic mice exposed to DEP compared with the saline-treated diabetic group.

It is well-established that the effects of particulate air pollution are not only limited to the lung but they can affect distant organs including heart, brain and kidney [2, 3, 22]. There are three primary hypotheses which are being investigated to explain the extrapulmonary effect of nanoparticles [3]. The first one relates to the effect of particles on their ability to impact the autonomic nervous system. Inhaled particles may affect the extrapulmonary sites through inflammatory mediators produced in the lungs and released into the circulation [2, 3, 22]. Moreover, several studies have showed that nanoparticles, owing to their small size, could avoid normal phagocytic defenses in the respiratory system and gain access to the systemic circulation and, therefore reach different extrapulmonary sites [23-25]. While several studies have investigated the effects of pollutant particles on distant organs such as heart, brain and kidney [1, 3, 25, 26], so far, no study has, as far as we are aware, investigated the effect of particles on the pancreas.

We have recently assessed the respiratory and cardiovascular effects of DEP in the mouse model of type 1 diabetes, and found an aggravation of the respiratory and cardiovascular effects. Here, we continued to use the same animal model of diabetes, the dose of DEP (0.4 mg/kg) and time point (24 h post-DEP exposure) [12, 13], and assessed the pancreatic impact of these particles by assessing histological and biochemical endpoints.

Our data show that in saline-treated diabetic mice, islet cells showed cellular vacuolation and irregularity in morphology and the presence of apoptotic islet cells in the examined sections. These effects were aggravated following the exposure to DEP where islet cells displayed a marked decrease in size and number with cellular vacuolation and a significant increase in the number of apoptotic islet cells. We have recently demonstrated the presence of many apoptotic cells in the examined lung sections obtained from diabetic mice exposed to DEP [12].

In this work a significant increase in pancreatic amylase activity in non-diabetic mice exposed to DEP compared with saline ones was found. Interestingly, we also found a significant increase in pancreatic amylase activity in diabetic mice exposed to DEP compared with either diabetic mice exposed to saline or non-diabetic mice exposed to DEP. This finding suggests that DEP interact either directly [23, 24] or indirectly through the induction of systemic inflammation [13], with pancreatic acinar cells and cause the increase of pancreatic secretion of amylase. We have recently demonstrated that DEP cause systemic inflammation and oxidative stress in diabetic mice [13]. However, the lack of effect of DEP on pancreatic lipase activity cannot be readily explained. Additional experiments are required to clarify this point.

To assess the mechanism underlying the pancreatic effects of DEP in diabetic mice, we have measured the pancreatic concentrations of 8-isoprostane and reduced GSH and the activity of SOD. Oxidative damage to lipids (lipid peroxidation) leads to the production of isoprostanes. Isoprostanes, of which 8-isoprostane is the best-characterized isomer, are produced independently of the cyclooxygenase enzyme by the peroxidation of arachidonic acid, catalyzed by free radicals. They are considered to be a reliable index of *in vivo* oxidative stress because they are structurally stable and are produced *in vivo* [27, 28]. Our data show that, in diabetic mice, DEP caused a significant increase in the concentrations of 8-isoprostane compared with either diabetic mice exposed to saline or non-diabetic mice exposed to DEP. We also found that the antioxidant activity of SOD and the concentrations of reduced GSH in pancreas were significantly decreased in diabetic mice exposed to DEP compared with saline-treated diabetic and DEP-treated non diabetic mice. This was possibly due to a development of oxidative stress that is accompanied by an adaptive response that counterbalances the potentially damaging activity of oxygen free radicals by antioxidant defence mechanisms. We and others have reported an increase of pulmonary antioxidants following short-term exposure to cigarette smoking [19, 29].



Elevated plasma markers of oxidative stress have been reported after the exposure to either DEP in mice [13] or to PM<sub>2.5</sub> in rats [14] with type 1 diabetes.

Nitric oxide (NO), a highly reactive free radical, is produced from the amino acid L-arginine by the action of a family of isoenzymes, the nitric oxide synthases (NOS). Two broad groups can be identified: constitutive (cNOS) and iNOS [30]. Induction of iNOS produces excessive NO accompanied by increased production of reactive oxygen species, including peroxynitrite and superoxide, which are detrimental to various organs including the pancreas [31]. Our data show, in saline-exposed diabetic mice, the presence of only mild cytoplasmic staining for iNOS in some pancreatic islet cells. Importantly, in diabetic mice, following exposure to DEP, a marked cytoplasmic staining for iNOS in most pancreatic islet cells and some acinar cells was seen. This finding, along with the increase of markers of oxidative stress (8-isoprostane, reduced GSH and SOD) that we found in pancreatic homogenate, highlights the importance of oxidative stress in DEP-induced injurious pancreatic effects. iNOS overexpression was shown to be responsible for DEP-induced lung inflammation [32], while iNOS knockout mice had a significant reduction of cytokines in the lung after exposure to ambient particles [33]. iNOS expression is up-regulated by NF- $\kappa$ B, which is sensitive to inflammation and oxidative stress stimulation [34]. Additional experimental studies are necessary to investigate further the mechanisms that may be underlying our observations.

In conclusion, our data showed, probably for the first time, evidence for an aggravating pancreatic effect of DEP in diabetic mice. Our findings expand the list of extrapulmonary organs that can be adversely affected by particulate air pollution to include the pancreas in a mouse model of type 1 diabetes. Epidemiological and clinical studies are needed to assess the effect of particulate air pollution on the pancreas in healthy individuals and those with pre-existing chronic diseases such as diabetes.

### Grants

This work was supported by funds of the NRF-UAEU grant, and the College of Medicine and Health Sciences grant.

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