

# **Effects of Silica Inhalation on Metabolic Obesity in a Western Diet-Induced F344 Rat Model; silica inhibits adipose function and diet-induced inflammation\_Dataset**

## **Detailed Materials and Methods**

### **Animals and Diet**

Six-wk old male F344 Fischer rats (Charles River, NC) were divided into one of two diet groups and fed either a commercially available “Western” diet (HFWD)[45% fat Kcal , sucrose 22.2% by weight] or a standard rat chow (STD)(fat 6.2% by weight] for 16 wk, prior inhalation exposure. After 16 wk, animals were exposed to silica or air for 6 h per day, 5 days per wk, for 39 days or filtered air (control). Animals were used for terminal metabolic measures at 0, 4, and 8 wk post-exposure. Animals designated for the 8-wk post-exposure studies were used for repeated measure laser doppler and fasting glucose studies. This study was approved by the Institutional Animal Care and Use Committee and conducted in facilities fully accredited by the AAALAC International.

### **Silica Exposure**

Silica (Min-U-Sil 5; US Silica, WV) was aerosolized using an automated exposure system which delivered airborne silica particles with median aerodynamic diameter of 1.6  $\mu\text{m}$  and geometric standard deviation of 1.6. Target silica concentration ( $15 \pm 1 \text{ mg/m}^3$ ) was monitored and controlled within the exposure chamber in real time (McKinney et al. 2013. Computer-automated silica aerosol generator and animal inhalation exposure system. *Inhal Tox.* 25(7):363-372).

### **Bronchoalveolar lavage (BAL) and Lung Histology**

Animals were euthanized by an overdose of sodium pentobarbital (200 – 300 mg/kg, Vortech, Dearborn, Michigan) administered by intraperitoneal injection. With the right lung clamped, the left lung used for collection of BAL fluid. In situ, an opening was made exposing the trachea; a small incision was made, and a catheter was inserted and secured with suture. The right lung was lavaged 5 times with 5 ml of ice-cold PBS containing 0.6 mM EDTA. The first lavage was kept separate from the 4 subsequent washes, and all were stored on ice until further use; BAL samples were then centrifuged at 600 x g for 10 minutes. The supernatant from the first lavage was removed, aliquoted, and used for LDH analysis; all remaining supernatant aliquots were stored at -80 °C until further use in cytokine analysis.

The cell pellet from the first lavage was resuspended in 1 ml of Hanks' balanced salt solution (Sigma-Aldrich, St. Louis, MO) and combined with the cell pellet from the subsequent lavage sample. First, the total number of BALF cells was determined by electronic cell counter/sizer (Coulter Multisizer II, Coulter Electronics, Hialeah, FL, USA). The remaining BAL cells were spun onto a glass microscope slide using a Cytospin 3 Cyto centrifuge (Thermo Shandon, Pittsburgh, PA). Slides were air-dried and then stained with Hema 3 (Biochemical Sciences, Swedesboro, NJ). Three hundred cells were counted by hemocytometer evaluated under a by light microscopy by a board-certified veterinary pathologist (AFH) for the differential cell analysis.

### **BAL Proinflammatory Cytokine Analysis**

BAL cytokines were measured using the MSD V-PLEX Proinflammatory Panel 2 (rat) kit and MESO QuickPlex SQ 120 (Meso Scale Diagnostics, Rockville, MD) following the manufacturer's protocol.

### **Lung Histology**

Following lavage of the right lung, the right lung was tied off and removed, the left lung lobe, which had not been lavaged, was inflated with 3.5 ml of 10% neutral buffered formalin (NBF) and collected for

histological examination. The left lung was paraffin-embedded and 5  $\mu\text{m}$  sections were prepared for hematoxylin and eosin (H&E), Masson's trichrome, and picosirius red staining in order to examine sections for indicators of pulmonary injury, inflammation, and fibrosis by a board-certified veterinary pathologist (REP). Unblinded semiquantitative histopathologic assessment was used to determine distribution and severity of silica-induced lesions in the lung at 0, 4 and 8 wk post-exposure to silica of both diet groups by a board-certified veterinary pathologist.