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

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 (Fig. 1). This study was approved by the Institutional Animal Care and Use Committee and conducted in facilities fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care 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 μm and geometric standard deviation of 1.6. Target silica concentration (15 ± 1 mg/m3) was monitored and controlled within the exposure chamber in real time (McKinney et at. 2013).

Anthropometric Measures

Animals were euthanized by pentobarbital overdose (200-300mg/kg). Weight, length, and abdominal girth were measured; body mass index (BMI) was calculated. Blood collected in EDTA-treated collection tubes, cells were measured using ProCyte Dx Hematology Analyzer (IDEXX Laboratories, Inc., Norcross, GA). Blood used for serum samples were collected in BD Vacutainer™ Serum Separation Tubes, left at RT for 1.5 hour and centrifuged 25,000g for 20 min. Fresh serum was used for measurements of high-density lipoprotein (HDL) (ab65390, Abcam) and blood chemistry (Catalyst Dx Chemistry Analyzer, IDEXX Laboratories, Inc.). Serum samples were stored at -80˚ C until used for ELISA and cytokine analysis. ELISAs were conducted following the manufacturers protocol: leptin (#MOB00B, R&D Systems, Minneapolis, MN) and adiponectin (#Acrp30, R&D Systems). Insulin was measured using Ultra-Sensitive Rat Insulin Elisa Kit (#90060, Crystal Chem, Elk Grove Village, IL). Serum cytokines were measured using the MSD V-PLEX Proinflammatory Panel 2 (rat) kit and MESO QuickPlex SQ 120 (Meso Scale Diagnostics, Rockville, MD).

Repeated Measures

Repeated measures (fasting glucose, weight, laser-Doppler flowmetry (LDF)) were made at pre-exposure, 0, 4, 8 wk post-exposure to silica. Animals were fasted 12-15 hours prior to fasting glucose measure. LDF measures were made in unfasted animals, placed in Broome-style restrainers, and recorded over a 15-minute interval. Mean blood flow was calculated and analyzed. Time-series analyses were performed on the raw LSDF data. Peaks at 0.88- 1 Hz and 0.2 – 0.4 Hz were identified and the area under the curves for these peaks were compared between groups.