High-fat Western diet alters silica-induced airway epithelium ion exchange but not airway smooth muscle reactivity

Animals and Diet

Six-wk old male Fischer (CDF) rats (F344/DuCrl) obtained from Charles River Laboratories, Inc. (Wilmington, MA) were divided into two dietary groups and fed either a commercially available "Western" diet (high-fat Western diet, HFWD; 45% fat Kcal, sucrose 22.2% by weight) or a standard rat chow (standard diet, STD; fat 6.2% by weight, sucrose-free) for 16 wk, prior to the commencement of silica inhalation exposures, and continued for the duration of the study. After 16 wk of diet consumption, animals were exposed by whole-body inhalation to silica for 6 h/d, 5 d/wk, 39 d or filtered air (control) and handled identically. Animals were euthanized at 0, 4, and 8 wk post-exposure to silica for use in endpoint measures of airway epithelial ion transport and smooth muscle reactivity ex vivo.

All studies were conducted in facilities accredited by AAALAC International, were approved by the Institutional Animal Care and Use Committee (protocol 18-011) and were in compliance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals and the NIH Guide for the Care and Use of Laboratory Animals. All animals were free of viral pathogens, parasites, mycoplasm, *Heliobacter* and cilia-associated respiratory bacillus. Animals were acclimated for one week upon arrival and housed in ventilated micro-isolator units supplied with HEPA-filtered laminar flow air (Lab Products OneCage; Seaford, DE), with Teklad Sanichip and Teklad Diamond Dry cellulose bedding (or Shepherd Specialty Paper's Alpha-Dri cellulose; Shepherd Specialty Papers; Watertown, TN). They were provided filtered tap water and autoclaved Teklad Global 18% protein rodent diet (Harlan Teklad; Madison, WI) *ad libitum*. Rats were housed in pairs under controlled light cycle (12 h light/12 h dark) and temperature (22 – 25 °C) conditions.

Silica-Inhalation Exposure

Crystalline silica (Min-U-Sil 5®; Berkeley Springs, WV; "SIL") was aerosolized using an automated exposure system (1) that delivered airborne particles with median aerodynamic diameter of 1.6 μm and geometric standard deviation of 1.6. Target silica concentration (15±1 mg/m_3) was monitored and controlled within the exposure chamber in real time and control animals were exposed to filtered air and handled identically. Animals from both STD and HFWD-consuming groups were exposed to 15 mg/m^3 x 6 h/d x 39 d to crystalline silica, or filtered air (control groups), and endpoints were measured at 0, 4 and 8 wk post-exposure to silica.

Measurement of Airway Epithelial Ion Transport *ex vivo*

The Ussing chamber was employed to determine changes in ion transport or resistance across the tracheal epithelium. Animals were euthanized (300 to 500 mg/kg pentobarbital), tracheal tissue was removed and mounted in a Ussing chamber system (Physiologic Instruments, Inc; Reno, 112 NV) containing modified Krebs-Henseleit solution gassed with 5% CO_2 / 95% O_2 at 37 °C. The tissue was stabilized under open circuit conditions for measurement of transepithelial voltage (V_t, mV) followed by application of a 0-mV voltage clamp and delivery of 1 mV pulses (5 sec duration, 55 sec interval). Short-circuit current (I_{SC}) was recorded (BioPac Systems) from which transepithelial resistance (R_t) was calculated using Ohm's law. To determine changes in ion transport the following ion channel inhibitors were used: Na^+ channel inhibitor amiloride (3.5×10^{-5} M, apical bath), Cl^- channel inhibitor 5-nitro-2-(3-phenylpropyl-amino) benzoic acid (NPPB; 10^{-4} M, apical bath), and Na^+,K^+-pump inhibitor ouabain (10^{-4} M, basolateral bath). For all responses measures, three data points were taken, and the average value was used. % I_{sc} and % R_t values for each tissue were calculated using the following formula:

% response from baseline = ((average agent response value – baseline value) / baseline value) X 100

Measurement of Airway Smooth Muscle Reactivity *ex vivo*

A 25 mm segment of rat trachea was removed and mounted on a tracheal perfusion holder. The holder contains indwelling cannulas with side holes, which are attached to the positive and negative sides of a differential pressure transducer; this allows measurement of basal tone as well as the change in pressure within the trachea to be determined by the inlet minus outlet pressure difference in response to addition of various agents. The mounted isolated trachea was bathed and perfused in modified Krebs-Henseleit solution (MKHS; 113 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, and 5.7 mM glucose) saturated with 95% O_2 / 5% CO_2, with a pH 7.4 at 37 °C, equilibrated for 1 h and washed at 15 min intervals. Methacholine (MCh) concentration-response curves were obtained by addition of stepwise increases of MCh concentrations added to the extraluminal (EL) bath, followed by a 90 min wash period at 15 min intervals, and then intraluminal (IL) additions of MCh. MCh, a muscarinic receptor agonist, was diluted in saline. MCh concentration-response curves were derived from the increase in inlet minus outlet pressures (cm H20; raw data) and normalized as a percentage of the maximal contractile response. Both EL and IL MCh concentration-

responses were measured; EL and IL % maximum responses, as well as EC50 values, were calculated for each individual preparation using the following calculation:

% maximal response = ((response value – baseline value) / maximal response value) X 100

1. McKinney W, Chen B, Schwegler-Berry D, Frazer DG. 2013. Computer-automated silica aerosol generator and animal inhalation exposure system. Inhalation toxicology. 25(7):363-372.