MRI and Biological Markers of Acute E-Cigarette Exposure in Smokers and Vapers

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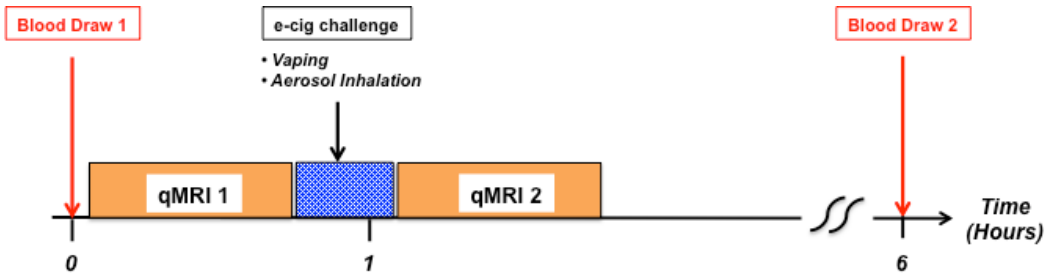
To *determine the acute effects of e-cig aerosol inhalation we will quantify surrogate markers of EDF at the micro- and macro-vascular level before and after a nicotine-free e-cig aerosol in healthy subjects without history of cardiovascular disease.*

In order to isolate and assess the acute effects of inhaled aerosol on surrogate MRI markers of EDF (endothelial dysfunction), nicotine-naïve (i.e. non-smokers) will undergo an e-cig aerosol inhalation challenge using a commercially available, disposable, non-flavored nicotine-free e-cig (ECO series, epuffer.com).

All MR procedures will be performed on a 3T Siemens TIM Trio imager (Siemens Medical Solutions) using standard RF coils (8-channel extremity coil for cuff paradigm studies at the location of the femoro-popliteal artery and vein, and a combination of two body matrix and 12-channel head coils for PWV (pulse wave velocity) of the aorta and neurovascular reactivity in the superior sagittal sinus (SSS) in conjunction with the custom-designed pulse sequences. Subjects will be asked to fast for eight hours, have abstained from taking vasoactive medications (including over-the-counter medications) as well as vigorous physical activity. Each visit will comprise two blood draws and two qMRI (quantitative magnetic resonance imaging) examinations, one before and one after an e-cig challenge to assess the changes in the functional and mechanical metrics described in the previous section. The e-cig challenge will consist of 16 two-second-long puffs (equivalent to smoking one conventional cigarette). Smoking will be directly supervised to prevent superficial smoking or hyperventilation by the study subjects. The challenge will last approximately 20 minutes. A second blood draw will be taken six hours after the e-cig challenge. From the blood samples collected, inflammatory biomarkers will be assessed.

Blood will be drawn from the antecubital vein of the subject's left or right arm. At least 2 mL of plasma will be isolated and kept frozen after centrifuging blood for 15 min.

The qMRI protocol will consist of three parts (cuff paradigm, *PWV* and breath-hold index). The patient set-up for *PWV* and breath-hold index (BHI) will be combined, i.e. the patient will enter the scanner head-first, supine, with head and body matrix coils in place. Vascular reactivity in the femoral artery and vein will be quantified first. After the cuff-induced hyperemia portion of the protocol the patient will be prepared for the second part designed to measure aortic *PWV* and BHI.



Data and Statistical Analysis

The fractional changes in functional and mechanical qMRI parameters between pre- and post-nicotine challenge will be determined. For each parameter paired two-tailed t-tests will be performed with $p<0.05$ indicating statistical significance. The data will allow us to determine which of the qMRI metrics (peripheral vascular reactivity, central arterial stiffness or neurovascular reactivity), are most sensitive to e-cig aerosol inhalation. We will also further conduct multivariate regression analysis on the within-subject changes controlling for other subject-specific covariates.

To quantify the changes in the levels of inflammatory biomarkers in serum collected from study participants before and after e-cigarette aerosol exposure and compare these metrics with qMRI-derived biomarkers.

Blood drawn from the antecubital vein by a phlebotomist from study participants in will be collected into tubes containing clot activator. Serum will be separated within 30 minutes by centrifugation and stored at −70°C prior to analysis. Inflammatory biomarkers will be detected via enzyme-linked immunosorbent assay (ELISA) techniques from blood draws #1 and 2. MCP-1 and MIP-1 concentrations will be measured using commercially available ELISA kits (R&D Systems, Abingdon, UK); sICAM, sRAGE, IFN-γ and TNF-α with RayBiotech kits

(RayBiotech, Inc., Norcross, GA 30092); HMGB1 will be assayed by ELISA kits supplied by IBL International (IBL GmbH).

Analyses

Descriptive statistics will initially be performed to determine the characteristics of the various distributions and to identify outliers by displaying the data in the form of box and whisker plots and by computing means and standard deviations. Acute changes from baseline (blood draws pre- and post e-cig aerosol challenge) will be evaluated by paired t-tests for each of the variables listed above. To compare the qMRI metrics with inflammatory indices, Spearman correlations will be performed. We expect moderate inter-modality correlations ($R^2 \sim 0.5$) for individual qMRI measures. We then use linear regression to create a classification model using the eight qMRI indices jointly. We expect $R^2 > 80\%$, substantially greater than each individual parameter alone, indicating that inhaled e-cig aerosol promotes *systemic EDF*.