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Activation of the "Splenocardiac Axis" by Electronic and Tobacco Cigarettes in Otherwise Healthy Young Adults

Running head: Tobacco & E-Cigarettes Activate the Splenocardiac Axis

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ABSTRACT

The "Splenocardiac Axis" describes an inflammatory signaling network underlying acute cardiac ischemia, characterized by sympathetic nerve stimulation of hematopoietic tissues, such as the bone marrow and spleen, which then release pro-inflammatory monocytes that populate atherosclerotic plaques, thereby promoting ischemic heart disease. Electronic (e) cigarettes, like tobacco cigarettes, trigger sympathetic nerve activation, but virtually nothing is known about their influence on hematopoietic and vascular tissues and cardiovascular risks. The objective of this study was to determine if the Splenocardiac Axis is activated in young adults who habitually use either tobacco or e-cigarettes. In otherwise healthy humans who habitually use tobacco cigarettes or e-cigarettes (not both), we used ^{18}F-flurorodeoxyglucose positron emission tomography/computer tomography (FDG-PET/CT) to test the hypothesis that tobacco or e-cigarettes increased metabolic activity of the hematopoietic and vascular tissues. FDG uptake in the spleen increased from non-user controls (1.62±0.07), to the e-cigarette users (1.73±0.04), and was highest in tobacco cigarette smokers (1.82±0.09; monotone p=0.05). Similarly, FDG uptake in the aorta increased from the non-user controls (1.87±0.07) to the e-cigarette users (1.98±0.07), and was highest in tobacco cigarette smokers (2.10±0.07; monotone p=0.04). FDG uptake in skeletal muscle, which served as a control tissue, was not different between the groups. In conclusion, these findings are consistent with activation of the Splenocardiac Axis by emissions from

tobacco cigarettes and e-cigarettes. This activation suggests a mechanism by which tobacco cigarettes, and potentially e-cigarettes, may lead to increased risk of future cardiovascular events.

Key words: electronic cigarettes, tobacco cigarettes, arterial inflammation, hematopoietic activation, FDG-PET/CT

New & Noteworthy

In tobacco or electronic-cigarette smokers, FDG-PET/CT was used to measure metabolic activity in hematopoietic and vascular tissues. FDG uptake in the spleen and aorta increased from controls, to e-cigarette users, to tobacco cigarette smokers, possibly indicative of graded activation of the Splenocardiac Axis, an inflammatory signaling network underlying cardiac ischemia.

INTRODUCTION

Using an integrative biological approach to inflammation, the existence of a signaling network, termed the "Splenocardiac Axis," linking the brain, autonomic nervous system, bone marrow, and spleen to the development of atherosclerosis and acute myocardial infarction, has been proposed (Figure 1) (18). Recent studies in rodent models support the concept that during acute stress, increased central sympathetic outflow activates bone marrow progenitor cells and leukocytes via β-3 receptor stimulation(6, 17). The leukocyte progenitor cells migrate from the bone marrow to the spleen, where they multiply in response to stem cell factors. Augmented numbers of pro-inflammatory monocytes then leave the spleen to enter the circulation, reaching the arterial wall where increased monocyte recruitment promotes and accelerates atherosclerosis. Not simply a transient process, the pro-inflammatory changes detected in the blood vessel wall have been found to persist for months (6, 14, 17, 18).

This Splenocardiac Axis may explain the observation in humans that heightened sympathetic tone, for example, that accompanies acute or chronic mental stress, pain, or even an acute myocardial infarction, is a risk factor for future acute ischemic cardiovascular events (11, 22) (28). Emami and colleagues used ^{18}F-flurorodeoxyglucose positron emission tomography/computer tomography (FDG-PET/CT) to demonstrate augmented inflammatory activity in the spleen and arterial wall in patients following an acute coronary syndrome compared to control subjects (7). Furthermore,

increased splenic metabolic activity in patients was an independent predictor of adverse cardiovascular events during follow up (7).

Tobacco cigarettes, the most common preventable risk factor for premature cardiovascular death in the United States, produce a relative hyper-adrenergic state(19). Nicotine, one of the 7000 constituents present in tobacco cigarette smoke, acts on receptors in the brain, autonomic ganglia, and sympathetic nerve terminals to increase adrenergic tone and norepinephrine release(12, 19). Furthermore, previous reports have confirmed that tobacco cigarette smoking is associated with a leukocytosis(15). We reasoned that in habitual tobacco cigarette smokers who are regularly exposed to nicotine, activation of the Splenocardiac Axis and arterial inflammation may be present and is detectable and quantifiable by FDG-PET/CT, explaining in part the increased risk for acute coronary syndromes and sudden death conferred by smoking.

Importantly, a new tobacco product, the electronic (e) cigarette, is gaining skyrocketing popularity, especially among young people, but the cardiovascular risk associated with e-cigarettes remains unknown. Although e-cigarettes deliver much lower levels of toxicants including carcinogens, compared to tobacco cigarettes(10), they typically deliver nicotine. Recent evidence from our laboratory supports the concept that habitual e-cigarette users who do not smoke tobacco cigarettes also have increased sympathetic activation(20). Accordingly, we hypothesized that in habitual e-cigarette users, who do not smoke tobacco cigarettes, increased hematopoietic and

vascular metabolic activity may be intermediate between tobacco cigarettes smokers and non-smokers, identifying a mechanism by which e-cigarettes may too increase future cardiovascular risk.

MATERIALS AND METHODS

<u>Study Population</u>

Otherwise healthy habitual tobacco cigarette smokers or habitual e-cigarette users (not dual users) between the ages of 21-45 years, who had used tobacco cigarettes or e-cigarettes, respectively, most days for a minimum of 1 year, in whom plasma cotinine levels were elevated, were eligible for the study if they met the study criteria: 1) no known health problems, 2) non-obese ($\leq$30 kg/m^2 BMI), 3) not taking prescription medications except oral contraceptive pills, 4) alcoholic intake $\leq$ 2 drinks per day and no illicit drug use, and 5) not exposed to secondhand smoke, or using licensed nicotine replacement therapies. Healthy volunteers meeting these inclusion criteria, who were not e-cigarette users or tobacco cigarettes smokers, were eligible to be enrolled as non-user controls. Participants who were former tobacco cigarette smokers were eligible for the study if they had quit smoking > 1 year prior to the study. Participants were enrolled with the goal to balance age and sex among the groups. The experimental protocol was approved by the Institutional Review Board at the University of California, Los Angeles and written, informed consent was obtained from each participant. The study is registered at ClinicalTrials.gov (NCT02734888).

FDG-PET/CT Imaging

FDG-PET/CT imaging was performed according to previously reported standards and guidelines to optimize FDG uptake in the hematopoietic tissues and arterial wall(3, 4, 7, 8, 16, 25, 26). Briefly, following an overnight fast, and confirmation of fasting blood glucose <200 mg/dL, 0.14 mCi/kg of FDG was injected intravenously. The subject rested without unnecessary motion for 90 minutes and then images of the neck, chest and abdomen were obtained. High count five-minute scans per bed position were obtained, compared to shorter acquisition of 2 minutes, or less, typically done for oncology imaging, producing higher count rate and decreased image noise, resulting in better image quality for more reliable and reproducible quantitative assessment.

Image Analysis.

All scans were read by a single investigator (P.G.) blinded to participant identification or study group affiliation. As previously reported, inflammation of the aorta, spleen, vertebral bone marrow, and adjacent erector spinae skeletal muscle (control tissue) were measured by placing a region of interest over axial sections(3, 7, 8, 26). For the aorta, measurements were made every 5 mm, starting 1 cm above the aortic valve annulus, continuing to the bottom of the aortic arch. The maximum standardized uptake value (SUVmax) was recorded for each region of interest(4, 16). Parenthetically, the sample size calculations (below) were based on the previously reported(26) variable "most diseased segment–tissue to background ratio

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(MDS-TBR)." The TBR was calculated as a time- and dose-corrected tissue radioactivity ratio of the SUVmax of the arterial wall compared to background superior vena cava venous SUV mean activity, and MDS was the 1.5 cm segment with the highest TBR and each 1.5 cm segment of either side. Since the time that these sample size calculations were performed, it has been argued that the SUVmax of the aorta is the preferred method of analysis (4, 16). Both analyses are reported, herein. For the spleen, bone marrow, and skeletal muscle control, the SUVmax in the axial plane was measured. Analysis of FDG in the carotid arteries was neither planned nor attempted since identification and measurement of the carotid vessel wall metabolic activity, in the absence of intravenous contrast administration, is poor and prone to error, such as partial volume artifact.

Blood tests. Venipuncture was performed by trained Nuclear Medicine staff on the day of the study. Blood was tested for glucose, and sent to the UCLA Clinical Laboratory for measurement of 1) cotinine ($t_{1/2}$ 20 hours), 2) carboxyhemoglobin (COHb, marker for tobacco cigarette, but not e-cigarette use), 3) inflammatory markers, including C-reactive protein (CRP) and fibrinogen. Blood samples were also centrifuged to separate into plasma samples, which were frozen at $-80\,°C$ in a cryopreservative solution(2) for later analysis for the following anti-oxidant parameters: 1) paraoxonase-1 activity, (PON-1 activity), a protective ester hydrolase enzyme associated with HDL in blood that prevents the formation of oxidized LDL(29) (described below) 2) LDL Oxidizability (LDL Ox), indicative of susceptibility of apoB-

containing lipoproteins to oxidation as previously reported(30), 3) HDL anti-oxidant/anti-inflammatory capacity, expressed as a HDL anti-oxidant index (HOI), which assesses the ability of HDL to inhibit LDL oxidation.

<u>Paraoxonase-1 (PON-1) enzymatic activity</u>

The enzymatic activity of human plasma PON-1 was determined by its capacity to hydrolyze paraoxon substrate to p-nitrophenol. Assays were performed in duplicate in clear, flat-bottom, 96-well plates (Corning® Costar®), and measurements were conducted using the BioTek Synergy Mx microplate reader and Gen5 software. From each plasma sample, 5 µL was incubated with paraoxon (Chem Service Inc., catalog # N-12816-100MG) in the assay buffer (0.1 M Tris-HCl buffer at pH 8.5, with 2 M NaCl and 2 mM CaCl2) at room temperature. The kinetics of p-nitrophenol formation were immediately measured every 15 seconds at 405 nm for a total of four minutes in the BioTek microplate reader. The absorbance readings (OD/min) were converted into nanomoles p-nitrophenol/min/ml with the use of the molar extinction coefficient for p-nitrophenol, determined to be 16,734 M-1cm-1 at a pH of 9.18, and a path length of 0.58 cm.

<u>Sample size calculation</u>. In a retrospective analysis of FDG-PET/CT scans performed for clinical purposes, aortic inflammation was estimated by MDS-TBR(26). In tobacco cigarette smokers (n=8), MDS-TBR was 1.34 with SD of 0.16, and non-smokers (n=9), MDS-TBR was 1.17 with a SD of 0.12. Thus, we computed that a sample size of 8 per group allowed confirmation a difference of 22% and 10 per group allowed confirmation of a difference of

8% between groups, assuming 80% power using a 2-sided alpha = 0.05. Our analysis included 9 per group.

Statistical analysis. Means were compared across controls, e-cigarette users, and tobacco users using an analysis of variance model with ordered trend F tests. Under this model, F tests for ordered, monotone dose trend (control, e-cigarette users, tobacco cigarette smokers) were computed where the null hypothesis of no change was tested against the alternative of monotone change. The monotone test was used since the response should be ordered, where the group having the intermediate exposure or level should also have the intermediate response, at least on average. The mean and its standard error (SEM) are reported. For comparing binary data such as gender, p values were computed using Fisher's exact test. Associations of continuous variables with cotinine were assessed using the Spearman (r_s) correlation. Differences or associations were considered statistically significant when $p \leq 0.05$.

RESULTS

Study population (Figure 1)

A total of 31 participants meeting the above criteria were initially enrolled in this study, including 10 habitual tobacco cigarette users, 11 habitual e-cigarette users, and 10 healthy control subjects. Nine in each group were included in the final analysis (see Figure 2).

Baseline Characteristics (Table)

The non-user control, habitual electronic-cigarette user, and tobacco cigarette smoker groups were intentionally well-matched by age and sex. Cotinine levels, an estimate of nicotine burden, were not different between the tobacco cigarette and e-cigarette groups.

Hematopoietic tissue metabolic activity is increased in tobacco and e-cigarette users (Figures 3 & 4) Representative cross-sectional PET images from a non-user, e-cigarette user, and tobacco cigarette smoker are displayed in Figure 3. FDG uptake in the spleen as measured by SUVmax increased from non-user controls (1.62±0.07) to the e-cigarette users (1.73±0.04), and was highest in tobacco cigarette smokers (1.82±0.09; monotone p=0.05; Figure 4A). FDG uptake in the bone marrow as measured by SUVmax was lowest in the controls (1.88±0.06) and was higher in both the e-cigarette users (2.17±0.12) and the tobacco cigarette smokers (2.14±0.15), but the monotone trend did not reach statistical significance (p=0.12; Figure 4B). FDG uptake as measured by SUVmax in skeletal muscle, which served as a control tissue, was not different between the groups (Figure 4C).

Aortic wall metabolic activity is increased in tobacco and e-cigarette users. Aortic wall metabolic activity as measured by SUVmax, was increased from non-user controls (1.87±0.07) to the e-cigarette users (1.98±0.07), and was highest in tobacco cigarette smokers (2.10±0.07; monotone p=0.04; Figure 4D). When measured by MDS-TBR, aortic wall metabolic activity was not different among the 3 groups (non-user controls (1.87±0.05, e-cigarette

users (1.81±0.09), tobacco cigarette smokers (1.91±0.09; monotone p=0.75).

Relationship of hematopoietic tissue metabolic activity with cigarette burden.

Plasma cotinine, an estimate of tobacco cigarette and e-cigarette burden, was weakly correlated with bone marrow activity (r_s 0.39, p=0.05), but other correlations were not significant (data not shown).

Markers of inflammation and oxidative stress (Table, Figure 4)

Although markers of inflammation and oxidative stress did not differ among the groups (Table), PON-1 activity, a protective anti-oxidant enzyme, tended to exhibit higher activity levels in the non-user control group (971.6 ± 169 nmol p-nitrophenol/min/ml), intermediate in the e-cigarette users (682.6±169 nmol p-nitrophenol/min/ml), and lowest in the tobacco cigarette users (618.0±125.7 nmol p-nitrophenol/min/ml, monotone p=0.10; Figure 5).

DISCUSSION

FDG-PET/CT is a sensitive, non-invasive imaging modality used in many clinical situations, including sarcoidosis, human immunodeficiency virus disease, and fever of unknown origin, to detect the presence of active inflammation[8]. FDG is taken up by glucose transporters into metabolically active cells such as activated immune cells, including activated macrophages[25]. Blood vessel wall inflammation, characterized by infiltration of activated macrophages, plays a critical role in the initiation and progression of atherosclerosis. Additionally, in preclinical studies, increased

sympathetic activity has been shown to activate hematopoietic stems cells in bone marrow, which then replicate as pro-inflammatory monocytes in the spleen, leaving to infiltrate the blood vessel wall, initiating atherosclerosis(14, 18). In clinical studies of atherosclerosis using FDG-PET/CT, increased metabolic activity in hematopoietic tissues, including the bone marrow and spleen, is consistent with activation of the inflammatory Splenocardiac Axis, and has even been shown to confer increased cardiac risk(7, 18, 25). E-cigarettes and tobacco cigarettes increase sympathetic activity(19, 20), and thus are capable of initiating the Splenocardiac Axis. The major new finding in this study is that FDG uptake is increased in both the spleen and the aorta in a striking linear dose-response relationship from non-smoking healthy controls to habitual e-cigarette users to tobacco cigarette smokers. These findings of increased metabolic activity in both spleen and blood vessel wall support the hypothesis of activation of the Splenocardiac Axis in smokers.

Although increased metabolic activity is detectable in the aorta in smokers, the "most diseased segment" (MDS-TBR) variable was not increased in smokers versus non-smokers. Perhaps this is not surprising, since the MDS-TBR analytic approach was developed in patients with known atherosclerosis, which is not likely to be present in our young, otherwise healthy smokers(26). Rather, in the present study in smokers, we have detected increased metabolic activity in the wall of the aorta, consistent with

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increased vessel wall inflammation, concerning for the future development of atherosclerosis and cardiac ischemia(25).

Tobacco cigarette smoking is a risk factor for myocardial ischemia and sudden death, a risk that dissipates shortly - within months to a few years – after quitting(23, 27). As supported by this study, activation of the Splenocardiac Axis, which leads to increased numbers of proinflammatory monocytes that infiltrate arterial atheroma, thereby contributing to plaque instability, may underlie this increased risk. It is plausible that the reversal of sympathetic activation that accompanies smoking cessation is also accompanied by a de-activation of the Splenocardiac Axis, explaining the marked fall in cardiac risk following smoking cessation(13). Investigations into hematopoietic tissue and blood vessel metabolic activity in former smokers would be of interest.

From these studies, one cannot be certain which component in tobacco cigarette smoke is the causal agent, but the fact that FDG uptake in hematopoietic tissues was also found to be increased in habitual e-cigarette users is strongly suggestive of a prominent role for nicotine. Although the carcinogenic toxins present in the aerosol generated by e-cigarettes are orders of magnitude lower than those present in tobacco smoke(10), nicotine levels achieved by each exposure are similar(9). In our study, plasma cotinine levels, a metabolite of nicotine with a half-life of 20 hours and an estimate of nicotine burden, were not different between the tobacco cigarette smokers and e-cigarette users. Nicotine has a strong

sympathomimetic effect, increasing peripheral sympathetic activity, and catecholamine release from the post-ganglionic nerve terminals and adrenal gland.

Although we did not find biomarker evidence for increased inflammation in our study, Emami and colleagues reported up-regulated gene expression of proinflammatory leukocytes and elevated CRP levels in their study of the Splenocardiac Axis(7). We did find a trend for decreased PON-1 activity, a protective anti-oxidant enzyme that is associated with HDL, in tobacco cigarette and e-cigarette users compared to controls. This inverse relationship is consistent with our prior report in young women, in whom we found a correlation between decreased PON-1 activity and increased number of tobacco cigarettes smoked(21). Decreased PON-1 activity has been found to be an independent predictor of premature coronary artery disease in patients younger than 45 years(24).

Limitations

Human studies rely on self-reporting for many of the behaviors that occur outside the laboratory, and thus are vulnerable to inaccuracies(5). To circumvent this, in this study we required biochemical verification of e-cigarette use, and absence of tobacco cigarette use(1, 5). Tobacco cigarette exposure is typically quantified by the number of tobacco cigarettes smoked per day, but there is not an equivalent measurement unit for e-cigarettes. Attempts to quantify e-cigarette exposure seemed less reliable, since most participants had difficulty quantifying time per day using their e-cigarette, or

milliliters of liquid used per day. The plasma cotinine level measured on the day of the study seemed the most objective means to assess e-cigarette burden, but remains a rough estimate. Furthermore, some of the e-cigarette users were former tobacco cigarette users (quit > 1 year). We cannot exclude that certain inflammatory changes were residual from prior tobacco cigarette smoking, persisting for > 1 year.

Finally, and importantly, this is a small study. Although the number of FDG-PET/CT scans performed was not large, it exceeded the number required by our sample size calculations. Furthermore, the FDG-PET/CT scans were prospectively performed according to a rigorous research protocol designed to maximize count rate and decrease image noise, and were analyzed by a single, expert reader blinded to group affiliation, all ensuring accuracy and consistency. Finally, the finding of a statistically significant, graded increase in FDG activity – lowest in the non-user, intermediate in the e-cigarette user, and greatest in the tobacco cigarette smoker, in not only one, but two tissues (spleen and aorta) of the three tissues analyzed makes random variation less likely as the explanation. Nonetheless, as all new research findings warrant, this research warrants replication by other investigators.

CONCLUSIONS

In summary, in this cross-sectional study of 3 groups, evidence is presented demonstrating activation of the Splenocardiac Axis in a graded manner from

non-user healthy control subjects to habitual e-cigarette users to tobacco cigarette smokers. This activation suggests a mechanism by which tobacco cigarettes, and potentially e-cigarettes, may lead to increased risk of future cardiovascular events.

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DISCLOSURES

None

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FIGURE CAPTIONS

Figure 1. Splenocardiac Axis. The Splenocardiac Axis is an inflammatory signalling network, initiated by increased sympathetic nerve activity(SNA), that underlies the development of atherosclerosis and acute myocardial ischemia. Nicotine, from tobacco and electronic cigarettes, increases SNA directly, and through activation of oxidative stress. Increased SNA mobilizes bone marrow progenitor cells, which migrate to the spleen, where they multiply. Pro-inflammatory monocytes enter the circulation, reaching the arterial wall, where increased monocytes, oxidative stress, and prothrmombotic factors, promote atherosclrosis. (Figure adapted, with permission, from reference 18).

Figure 2. Patient enrollment. Thirty-one participants were enrolled in the study. One was excluded from the healthy non-user control group when her CRP returned threefold normal values, and it was learned that she was a frequent platelet donor. Two participants were excluded from the e-cigarette group: one had elevated plasma COHb consistent with tobacco cigarette smoking(1), and one without detectable plasma cotinine level, indicative of insufficient e-cigarette exposure. One participant was excluded from the tobacco cigarette group due to undetectable plasma cotinine level, indicative of insufficient tobacco cigarette exposure. COHb = carboxyhemoglobin, CRP = C-reactive protein, EC = electronic cigarette, TC = tobacco cigarette

Figure 3. Panels A-C. Representative cross-sectional PET scans from a non-user (Panel A), e-cigarette user (Panel B) and tobacco cigarette smoker (Panel C). The white arrow in each panel identifies the spleen.

Figure 4. Activation of the Splenocardiac Axis in E-Cigarette and Tobacco Cigarette Users. Panel 4A. FDG uptake in the spleen increased from non-user controls (1.62±0.07), to the e-cigarette users (1.73±0.04), and was highest in tobacco cigarette smokers (1.82±0.09; monotone p=0.05). The individual between group comparisons were: tobacco cigarette smokers versus the non-user controls, p=0.056, e-cigarette users and controls, p=0.29, e-cigarette users and tobacco cigarette smokers, p=0.35. Panel 4B. FDG uptake in the bone marrow was lowest in the controls (1.88±0.06) and was higher in both the e-cigarette users (2.17±0.12) and the tobacco cigarette smokers (2.14±0.15), but the monotone p did not reach statistical significance (p=0.12). The individual between group comparisons were: e-cigarette users versus non-user controls, p=0.09, tobacco cigarette smokers versus the non-user controls, p=0.12, e-cigarette users versus non-user controls, p=0.85. Panel 4C. As expected, FDG uptake in skeletal muscle, which served as a control tissue, was not different between the groups. Panel 4D. FDG uptake in the aorta increased from non-user controls (1.87±0.07) to the e-cigarette users (1.98±0.07), and was highest in tobacco cigarette smokers (2.10±0.07; monotone p=0.04). The individual between group comparisons were: tobacco cigarette smokers versus the non-user controls,

p=0.04, e-cigarette users and controls, p=0.27, e-cigarette users and

tobacco cigarette smokers, p=0.32. FDG= ^{18}F-flurorodeoxyglucose, SUVmax

= maximum standardized uptake value

Figure 5. Oxidative stress. PON-1 activity, a protective anti-oxidant enzyme,

tended to be higher in the non-user control group (971.6 ± 169 nmol p-

nitrophenol/min/ml), intermediate in the e-cigarette users (682.6±169 nmol

p-nitrophenol/min/ml), and lowest in the tobacco cigarette users

(618.0±125.7 nmol p-nitrophenol/min/ml, monotone p=0.10). The individual

between group comparisons were: e-cigarette users versus non-user

controls, p=0.16, tobacco cigarette smokers versus the non-user controls,

p=0.17, tobacco cigarette smokers versus e-cigarette users, p=0.82. PON-1

= Paraoxonase-1

TABLE

Study Population Characteristics

	Non-User Control (n=9)	E-Cigarette User (n=9)	T-Cigarette Smoker (n=9)	p value
Age (years)	28 ± 1.6	29 ± 1.5	27.1 ± 1.6	0.80
Sex (M/F)	6/3	7/2	7/2	0.82
Ethnicity				
African American	0	1	0	
Asian	2	1	1	
Hispanic	1	1	1	
White(Non-Hispanic)	6	6	7	
Cotinine (ng/mL)	0	120.4 ± 31.6	192.0 ± 55.8	0.18
Present E-Cigarette use				
Minutes/day	85 ± 30 (20-300)	NA	NA	
Duration (years)	2.1 ± 1.0 (1-4)	NA	NA	
Present T-Cigarette use				
Pack-Years	NA	NA	7.3 ± 3.2 (0.7-30)	
SBP (mmHg)	112.7 ± 4.5	112.6 ± 4.4	120.3 ± 5.8	0.47
DBP (mmHg)	70.4 ± 2.9	71.3 ± 3.0	73.6 ± 3.9	0.77
MAP (mmHg)	84.4 ± 3.3	84.9 ± 3.3	87.7 ± 4.3	0.85
HR (bpm)	66.5 ± 4.2	61.1 ± 2.6	64.5 ± 3.4	0.55
Glucose (mg/dL)	85.9 ± 1.9	87.8 ± 2.9	88.7 ± 3.0	0.75
Fibrinogen (mg/dL)	239.7 ± 16.3	262.8 ± 10.7	247.6 ± 11.9	0.67
LDL-Ox (units)	2725 ± 334	2365 ± 131	2801 ± 370	0.91
HOI (units)	1.36 ± 0.17	0.99 ± 0.25	1.24 ± 0.17	0.62

CRP (mg/dL)   0.31 ± 0.01   0.30 ± 0.00   0.41 ± 0.10
     0.32

bpm = beats per minute, CRP = C-reactive protein, DBP = diastolic blood pressure, HOI = HDL anti-oxidant index, HR= heart rate, LDL-Ox = LDL oxidizability, MAP = mean arterial pressure, SBP = systolic blood pressure, T-Cigarette=tobacco cigarette
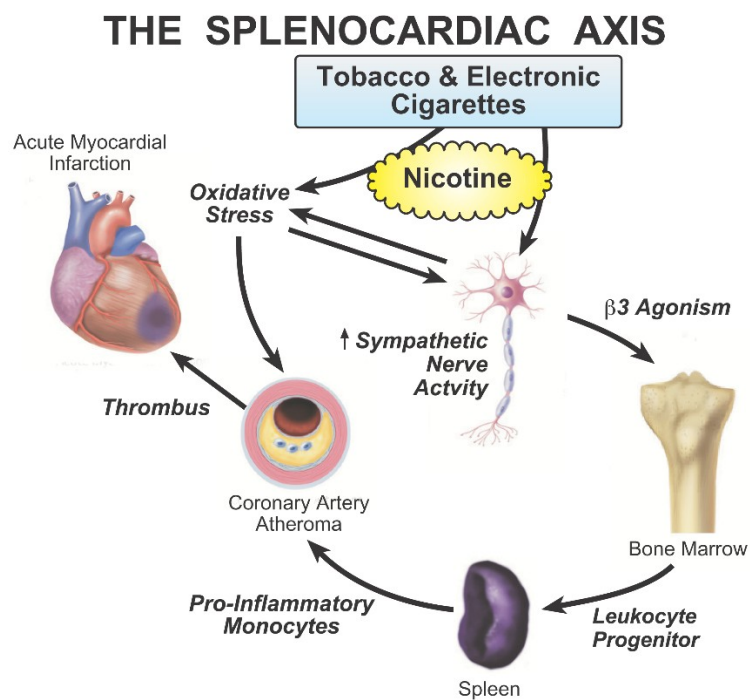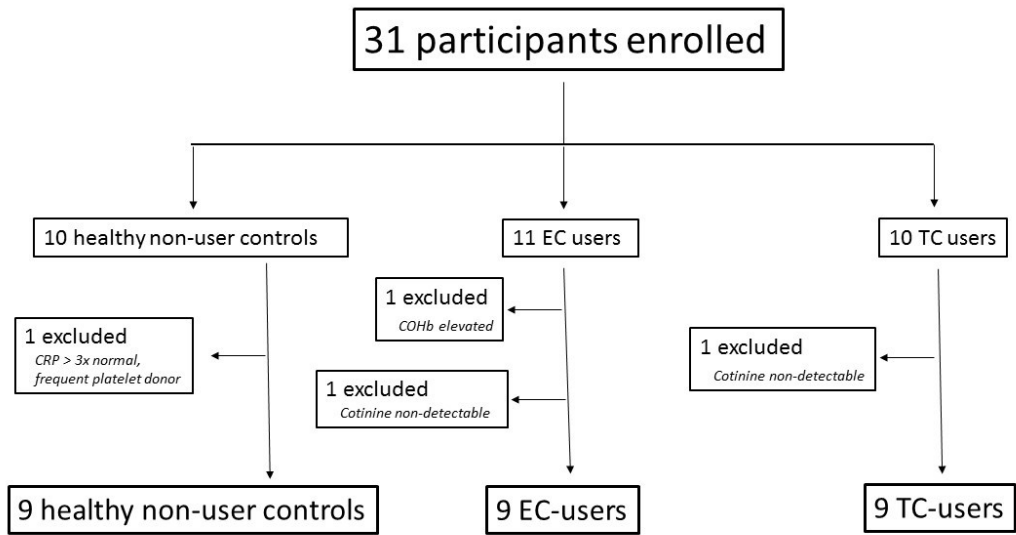
Figure 1 The Splenocardiac Axis

THE SPLENOCARDIAC AXIS

Figure 2 Participant Enrollment

Figure 3. Representative cross-sectional PET scans
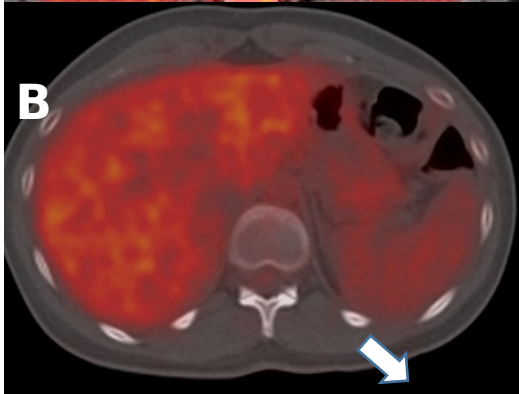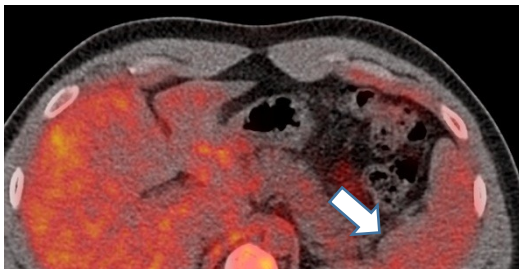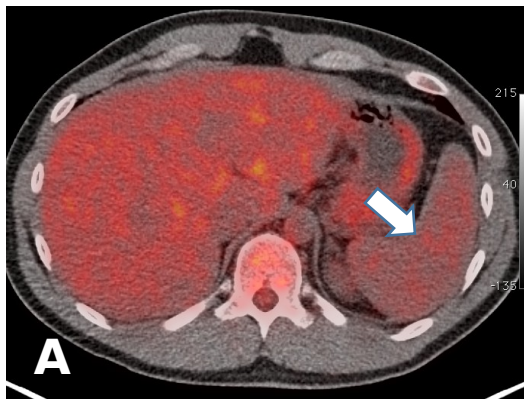
Figure 4. Activation of the Splenocardaic Axis in E-Cigarette and Tobacco Cigarette Users.
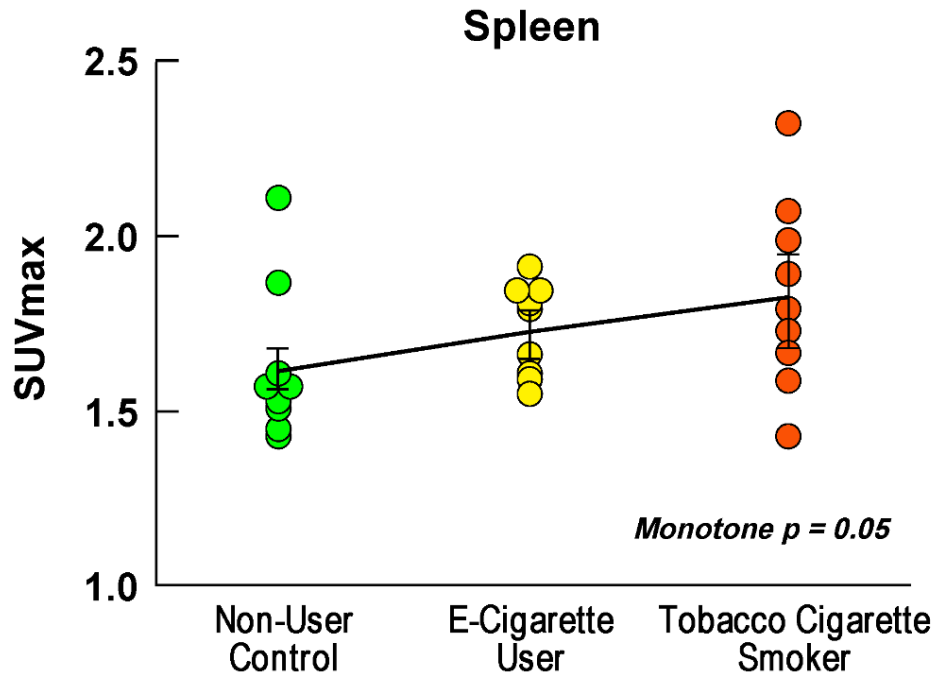
Figure 4A Metabolic Activity in the Spleen

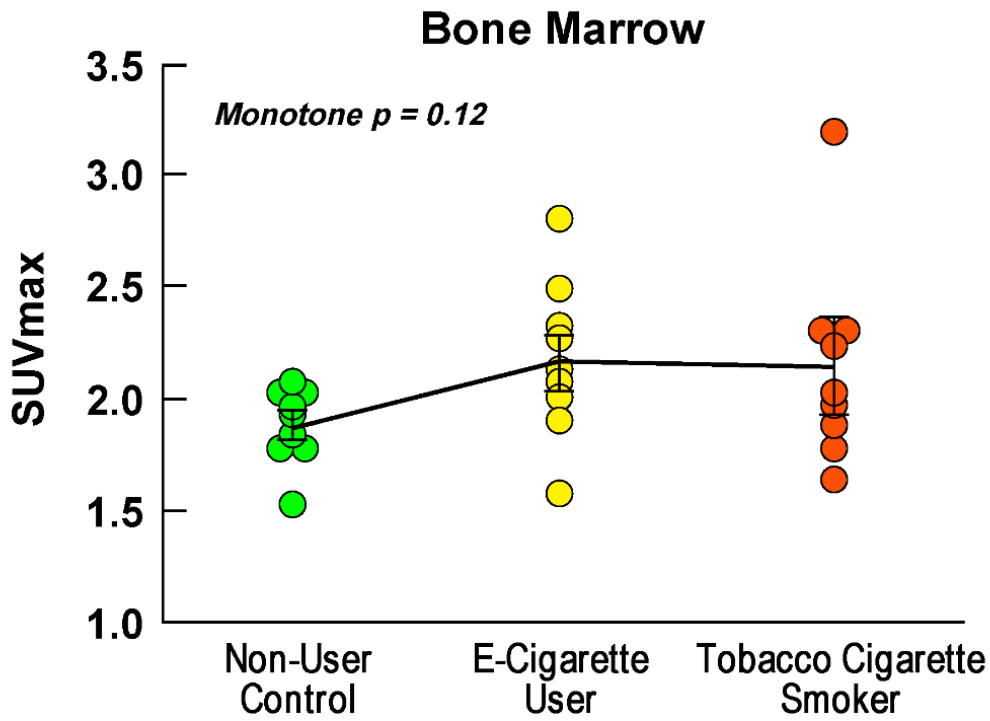**Spleen**

Figure 4B Metabolic Activity in the Bone Marrow

Bone Marrow

Figure 4C Metabolic Activity in the Skeletal Muscle

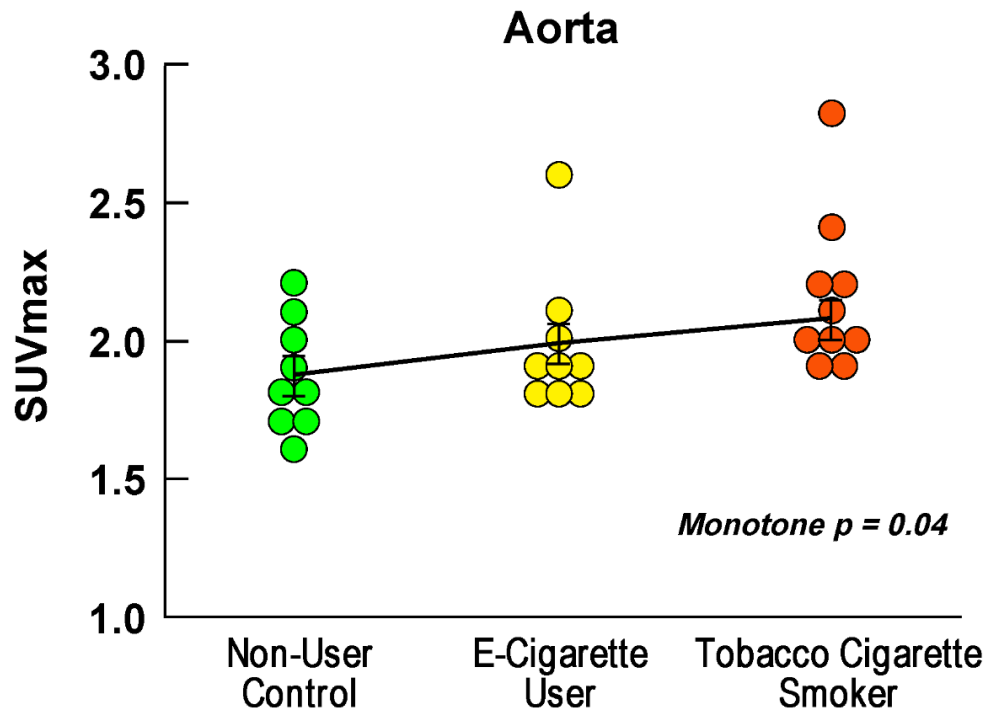**Skeletal Muscle Control**
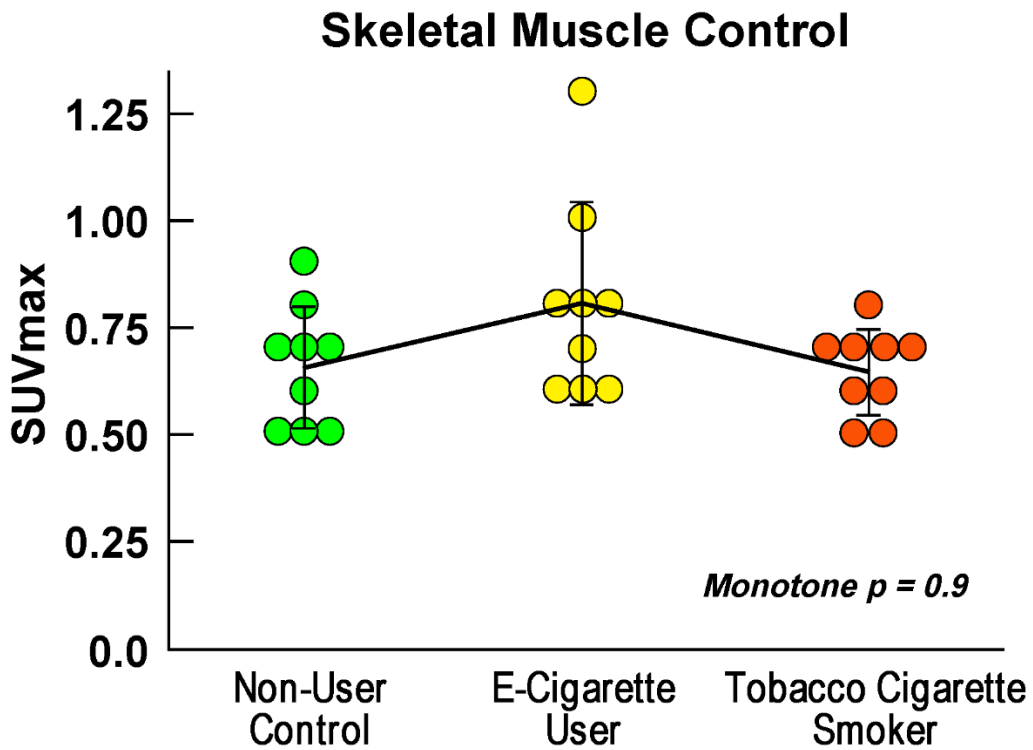
Figure 4D Metabolic Activity in the Aorta

Aorta

Monotone p = 0.04

Figure 5 Oxidative stress

Pon-1 Activity

Monotone p = 0.10