Supplementary Online Content

Moheimani RS, Bhetraratana M, Yin F, et al. Increased cardiac sympathetic activity and oxidative stress in habitual electronic cigarette users: implications for cardiovascular risk. *JAMA Cardiol*. Published online February 1, 2017. doi:10.1001/jamacardio.2016.5303

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This supplementary material has been provided by the authors to give readers additional information about their work.

eMETHODS-SUPPLEMENT

Paraoxonase-1 (PON-1) enzymatic activity:

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 considering a path length of 0.58 cm.

eTABLE 1-SUPPLEMENT
Baseline Characteristics (Includes 5 e-cigarette users with detectable nicotine)

	E-Cigarette User (n=21)	Non-User Control (n=18)	*p* value
Age (years)	28.7 ± 1.1	26.6 ± 1.5	0.25
Sex (M/F)	15/6	7/11	0.05
BMI (kg/m^2)	25.3 ± 0.7	23.0 ± 0.9	0.04
Ethnicity			
African American	1	2	
Asian	3	3	
Hispanic	2	2	
White (Non-Hispanic)	15	11	
Former smoker	15	2	
Pack-years	2.3 ± 0.5	0.6 ± 0.4	
Interval since quitting (years)	2.3 ± 0.6	13 ± 7	
E-cigarette use			
Minutes/day	247 ± 284 (5-960)		
Duration (years)	1.8 ± 0.6 (1-3)		
SBP (mmHg)	116.4 ± 2.2	109.0 ± 2.6	0.07
DBP (mmHg)	74.6 ± 2.3	70.0 ± 2.0	0.11
MAP (mmHg)	88.5 ± 2.0	83.0 ± 2.0	0.07
HR (bpm)	65.9 ± 2.3	63.0 ± 2.0	0.35

BMI = body mass index, bpm = beats per minute, DBP = diastolic blood pressure, HR = heart rate, MAP = mean arterial pressure, SBP = systolic blood pressure

eTable 2-SUPPLEMENT

E-Cigarette Use

User	Device*	Liquid/day (ml)	Flavor	Nicotine(mg)[T]	
1	Pen		Strawberry cheesecake, Unicorn	6	Unknown
2	Mod		Milk	3	15+
3	Cigalike, Pen	Unknown	Blu, Cherry	2.4%, 3	
4	Pen		Fruit	6	2
5	Cigalike	Unknown	Mint	18	
6	Mod		Fruity	6	3
7	Pen		Menthol	12	3
8	Pen		Savory Flavors	3	3
9	Pen		Custards and Creams	8	Unknown
10	Pen		Gogurt	3	Unknown
11	Pen		Chase the Vapor	8	Unknown
12	Cigalike	Unknown	Menthol	12	
13	Unknown		Apple, Fruit	1.2%	5
14	Unknown		Vaping Rabbit, Milkman	3	2
15	Mod		Vapor Chef, Honeydew	3	4-7
16	Mod		Fruity, Menthol	3	6
**17	Pen		Vanilla	6	3-5
**18	Pen		Vapor nuvola, Guava banana	12	Unknown
**19	Mod		Enigma, Blueberry, Strawberry	3	22
**20	Pen		Vapism, White Girl	6	15
**21	Cigalike		Menthol Ice	2.4%	8

*E-cigarette devises are generally divided into 1st generation "Cigalike" devices, which come with a chamber preloaded with e-liquid; 2nd generation, "Pen" devices, in which the

user can mix and refill customized flavors; and 3rd generation, "Mods" in which the voltage of the heating element can be modified, providing a larger quantifiy of aerosol.

Ŧ Units of nicotine content is mgs in the eLiquid used in the Pen and Mods, % in the Cigalike devices

**17-21 were excluded from analysis since they had detectable nicotine levels consistent with recent e-cigarette use.

eTable 3-SUPPLEMENT

Heart Rate Variability (Includes 5 e-cigarette users with detectable nicotine)

(normalized units)

	E-Cigarette User (n=21)	Non-User Control (n=18)	p value
HF (nu)	48.2 ± 3.2	57.8 ± 3.5	<0.05
LF (nu)	51.0 ± 3.4	39.9 ± 3.6	0.03
LF/HF ratio	1.25 ± 0.16	0.85 ± 0.17	0.09

HF = high frequency, LF = low frequency, nu=normalized units; Mean values are displayed since these data followed a parametric distribution.

eTable 4-SUPPLEMENT

Heart Rate Variability (Includes 5 e-cigarette users with detectable nicotine)

(absolute units)

	E-Cigarette User (n=21)	Non-User Control (n=18)	p value
HF (μs^2)	615.0 ± 259.8	1376.5 ± 574.2	0.14
LF (μs^2)	483.0 ± 220.6	1316.0 ± 504.0	0.09
VLF (μs^2)	942.5 ± 409.0	987.1 ± 432.5	0.66
Total power (μs^2)	1970.0 ± 601.8	4502.0 ± 1279.8	0.03

HF = high frequency, LF = low frequency, VLF = very low frequency; Median values are displayed since these data followed a nonparametric distribution.

eTable 5-SUPPLEMENT

Blood tests (Includes 5 e-cigarette users with detectable nicotine)

	E-Cigarette User (n=12)	Non-User Control (n=18)	*p* value
LDL-Ox (units)	3741.7 ± 351.6	2413.3 ± 310.1	0.008
PON-1 (nmol p-nitrophenol/min/ml)	612.3 ± 109.3	892.8 ± 110.0	0.08
HOI (units)	0.49 ± 0.05	0.38 ± 0.04	0.09
Fibrinogen(mg/dL)	268.9 ± 9.7	251.4 ± 10.5	0.23

HOI = HDL anti-oxidant index, LDL-Ox = LDL-Oxidizability, PON-1 = Paraoxonase-1 activity; Mean values are displayed since these data followed a parametric distribution.

eFigure 1.
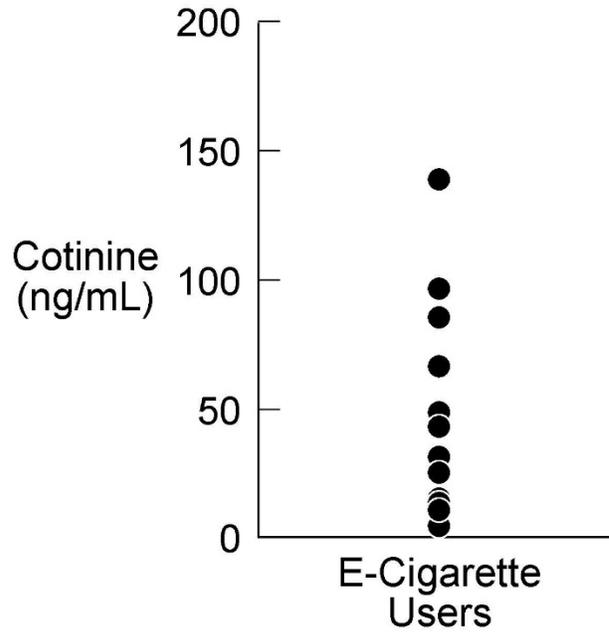
eFigure 1. Cotinine levels on the day of the study in e-cigarette users. Cotinine levels are well-distributed across the range of values from 3.8-139 ng/mL.