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Impact of electronic cigarette vaping on the cardiovascular function in young and old rats

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Background While the acute exposure to electronic cigarette (E-cig) vapor has been associated with an increase in blood pressure, the chronic effect of E-cig vapor on blood pressure compared to standard cigarette smoke has not been extensively studied. We determined the effect of E-cig exposure on blood pressure and other measures of cardiac function in both young and old rats.
Methods Young Sprague Dawley rats (6 weeks old, both sexes) were randomly exposed to air ($n=34$), E-cig with nicotine (E-cig Nic+; $n=30$), E-cig without nicotine (E-cig Nic-; $n=28$) or standard cigarette smoke ($n=27$). Old Fischer 344 rats (25 months old, both sexes) were randomized into 2 groups: (1) 26 rats in the purified air (negative control) group and (2) 17 rats in the electronic cigarette vapor plus nicotine group (E-cig Nic+). After 12 weeks of exposure, hemodynamics were determined by Millar catheter, echocardiography, and thermodilution catheter, a few days after their last exposure.
Results In young rats, cigarette smoke was associated with higher systolic, diastolic and mean blood pressures and peak LV systolic pressure, compared to air or E-cig Nic + or E-cig Nic- groups. Neither fractional shortening nor cardiac output differed among the groups. Absolute value for dp/dt min, a measure of diastolic LV function, was lowest in the E-cig Nic- group. Tau, a measure of LV relaxation was worse in this group as well. In old rats, E-cig vaping did not change heart rate, blood pressure, and cardiac function. However, E-cig Nic + exposure was associated with a greater heart weight/BW and LV weight/BW compared to air exposure in old rats.
Conclusions Chronic exposure to E-cig vaping did not cause an increase in blood pressure or heart rate, nor did it change cardiac function compared to air in young rats after 12 weeks of exposure, while standard cigarette smoking was associated with an increase in blood pressure. E-cig vaping was associated with a greater heart weight/BW and LV weight/BW compared to air exposure in old rats, suggested that older animals might be more vulnerable to E-cig stimulus than younger ones.

Keywords Electronic cigarettes, Cardiovascular function

Whether older individuals are more susceptibility to the effects of tobacco cigarette smoke remains controversial. Some animal studies show that there are age-dependent differences in response to cigarette smoke exposure[1,2]. Moriyama et al.[1] subjected 9-week-old and 69-week-old C57BL/6J mice to cigarette smoke and found that the older animals had a greater neutrophil influx in the lungs, higher levels of the neutrophil chemo-attractants MIP-2 and keratinocyte-derived chemokine in bronchoalveolar lavage fluid, and greater nuclear translocation of NF-κB in bronchiolar epithelium. These results demonstrated that aging increases susceptibility to cigarette smoke-induced pulmonary inflammation in a mouse model. However, other animal studies did not observe this type of age-dependent differences. For example, Zhou et al.[3] exposed 3 months and 12 months old C57Bl/6 female mice to daily cigarette smoke for 6 months (controls exposed to air). There were no differences in cigarette smoke induced emphysema, the severity of small airway remodeling, and the numbers of tissue macrophages and neutrophils and levels of 8-hydroxyguanosine between young and old mice. The authors concluded that aging did not enhance cigarette smoke-induced chronic obstructive lung disease in this model.

Electronic cigarettes (E-cig) vaping is marketed as a less harmful alternative to tobacco cigarette smoking and is gaining rapid popularity among all age groups in United States[4]. However, experimental and clinical studies support the concept that different constituents of E-cigs such as nicotine, propylene glycol and glycerol (and their heat degradation or other reaction products), various flavoring additives, sweeteners, and metals released

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from the heating elements carry potential risk for cardiovascular diseases[5]. There are no studies examining the age-dependent difference in E-cig vaping induced cardiovascular dysfunction. We therefore investigated whether older animals were more vulnerable to E-cig stimulus than younger ones.

Methods

All animal study protocols were reviewed and approved by the Institutional Animal Care and Use Committees at the Huntington Medical Research Institutes, and the University of California, Irvine. Both institutes are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. The experiments were performed in accordance with the "Guide for the Care and Use of Laboratory Animals" (NIH publication No. 85 − 23, National Academy Press, Washington DC, revised 2011). This study is reported in accordance with ARRIVE guidelines.

Young Sprague Dawley (SD) rats (6 weeks old, both sexes) and aged Fischer 344 (F344) rats (25 months old, both sexes) were used in this study. Sprague-Dawley rats were purchased from Charles River Laboratories (Hollister, CA), and Fischer 344 rats were obtained from the National Institute of Aging (Bethesda, MD). The young SD rats were randomly exposed to 4 groups: (1) 34 rats in the purified air (negative control) group; (2) 30 rats in the electronic cigarette vapor plus nicotine group (E-cig Nic+); (3) 28 rats in the electronic cigarette vapor without nicotine group (E-cig Nic-); and (4) 27 rats in the combustion cigarettes (1R6F) group. The aged F344 rats were randomized into 2 groups: (1) 26 rats in the purified air (negative control) group and (2) 17 rats in the electronic cigarette vapor plus nicotine group (E-cig Nic+).

All rats were exposed to inhalation exposure in a nose-only exposure system (In-Tox Products, Clinton MS) using the methods described previously[6] at Dr. Kleinman' lab (the Air Pollution Health Effects Laboratory located at the University of California, Irvine). Briefly, the rats were acclimated to the exposure system. Prior to the 12-week exposure period, the rats were exposed daily to purified air in the nose-only system for increasing durations (0.5, 1.0, 2.0, and 4.0 h). They did not exhibit common signs of stress, such as changes in food or water consumption, aggressive behavior, abnormal grooming activity, or red tears; however, no assessments of stress levels, including cortisol or corticosterone measurements, were conducted. For the designed 12-week exposure experiments, rats were restrained in individual tubes that were plugged into an exposure manifold with just the snout exposed to the exposure atmosphere, which served to minimize dermal exposure. The puff duration was 2 s per 30 s (2 puffs/minute) at a flow rate of 1.67 L per minute (equating to a 35mL puff volume as described in the International Organization for Standardization (ISO) smoking conditions). All of the exposures, including E-cig with or without nicotine, cigarette, or air, took place for 5 h/day, 4 days/week for a total of 12 weeks.

E-cig aerosols were generated from commercially available mod-style vape units with stainless steel atomizers. In the E-cig Nic- group, rats were exposed to the E-cig liquid that had a 50/50 (vol/vol) propylene glycol/vegetable glycerin (PG/VG) matrix to which were added tobacco flavor but did not contain nicotine (www.VaporFi.Com). In the E-cig Nic + group, 15 mg/mL of pure nicotine (L-Nicotine, Acros Organics, Lot: A0382410) was added to the above E-cig liquid to ensure consistent control of nicotine concentrations over the exposure period. The 15 mg/mL of nicotine was chosen to match that used in the original NIDA Standard Research E- cigarette (SREC) produced at the time of the experiment. In the cigarette group, rats were exposed to the standard nicotine-containing combustion cigarette smoke generated from 1R6F Standard Research Cigarettes (Center for Tobacco Reference Products, University of Kentucky).

Measurement of LV end-diastolic and end-systolic diameters at the midpapillary muscle level, LV wall thickness, and LV fractional shortening (LVFS) were obtained by echocardiogram. Arterial blood pressure, LV end-systolic pressure (LV Pes), LV end-diastolic pressure (LV Ped), the maximal slope of LV systolic pressure increment (positive dP/dt max), and diastolic pressure decrement (negative dP/dt min) were recorded by a 2 F high-fidelity catheter-tipped micromanometer. Cardiac output was measured using a thermodilution catheter. Femoral artery flow-mediated vasodilation was assessed by ultrasound imaging.

After 12 weeks of exposure, rats were transferred to Dr. Kloner's lab at Huntington Medical Research Institutes (Pasadena, CA) for cardiovascular function assessment. After 2 to 4 days of acclimation, the rats were anesthetized with an intraperitoneal injection of ketamine (90 mg/kg) and xylazine (10 mg/kg), intubated and mechanically ventilated. The chest and neck area were shaved and cleaned; echocardiography was performed using a 15-MHz transducer (Sonos 5500 ultrasound system, Philips Medical System, Andover, MA). LV end-diastolic and end-systolic internal diameters at the mid- papillary muscle level, systolic and diastolic LV wall thickness, as well as LV fractional shortening were measured and calculated. Then a catheter (PE-50) was inserted through the right jugular vein and its tip placed into the right atrium. A thermocouple catheter was introduced into the right carotid artery, and the tip of the probe was advanced into the aortic arch immediately distal to the aortic valve for cardiac output measurement using a thermodilution technique previously described[7]. In brief, 0.2 ml 0 °C saline solution was injected into the right atrium through the catheter within the jugular vein and the thermodilution curve was recorded. Three measurements of cardiac output were calculated using the PowerLab system (LabChart 8.0, PowerLab, ADInstruments) and the results were averaged in each rat.

For recording of the arterial blood pressure (BP) and heart rate (HR), a 2 F high-fidelity catheter-tipped micromanometer (model SPR-869, Millar, Inc.) was inserted into the right carotid artery and advanced into the ascending aorta. Then the micromanometer was further advanced into the left ventricular (LV) cavity to record LV end-systolic pressure (LVESP), LV end-diastolic pressure (LVEDP), the maximal slope of LV systolic pressure increment (dP/dt max), diastolic pressure decrement (dP/dt min), and Tau (a measure of diastolic function).

The technique of endothelium-dependent flow-mediated vasodilation was used to assess femoral artery endothelial function using the methods described previously[8]. Briefly, a blood pressure cuff was placed at the inguinal level and was inflated to 200 mmHg to occlude the femoral artery for 5 min to induce ischemia, followed by rapid deflation to induce a brief high-flow reperfusion state through the femoral artery (reactive hyperemia). Prior to the artery occlusion and after artery reperfusion, the diameter of the femoral artery was measured using

	Air	E-cig Nic+	E-cig Nic-	Cigarette	*p* value
	n = 34	*n* = 29	*n* = 28	*n* = 27	
LV diastolic ID (mm)	6.52 ± 0.12	6.44 ± 0.12	6.03 ± 0.12	6.58 ± 0.18	0.027[#]
LV systolic ID (mm)	3.23 ± 0.12	3.34 ± 0.17	2.87 ± 0.11	3.31 ± 0.18	0.114
LVFS (%)	50.8 ± 1.3	48.70 ± 2.1	52.7 ± 1.4	50.5 ± 1.8	0.266
Systolic LV wall thickness (mm)	3.00 ± 0.09	2.94 ± 0.10	3.00 ± 0.09	3.01 ± 0.10	0.957
Diastolic LV wall thickness (mm)	1.99 ± 0.08	1.88 ± 0.07	2.01 ± 0.09	2.02 ± 0.11	0.814
Cardiac output (ml/min)	47.5 ± 2.7	46.5 ± 2.5	45.0 ± 3.3	45.4 ± 3.0	0.795

Table 1. Cardiac function of the young rats. LV: left ventricular; ID: internal diameter; LVFS: left ventricular fractional shortening. #: Cigarette vs. Nic-.

	Air	E-cig Nic+	E-cig Nic-	Cigarette	*P* value
	n = 34	*n* = 30	*n* = 28	*n* = 27	
Systolic Pressure (mmHg)	92 ± 2	94 ± 3	89 ± 3	102 ± 3	0.02[#]
Diastolic Pressure (mmHg)	68 ± 2	69 ± 2	68 ± 3	79 ± 3	0.003[$]
Mean Pressure (mmHg)	79 ± 2	81 ± 3	78 ± 3	90 ± 3	0.006[&]
Pulse Pressure (mmHg)	24 ± 1	25 ± 1	21 ± 1	22 ± 1	0.013[*]
Heart Rate (BPM)	231 ± 5	220 ± 6	226 ± 9	229 ± 6	0.736
Pes (mmHg)	91 ± 2	92 ± 3	90 ± 3	104 ± 4	0.04[£]
Ped (mmHg)	5 ± 0.4	3 ± 1	5 ± 1	5 ± 0.4	0.01 €
+dP/dt max (mmHg/s)	5977 ± 149	5936 ± 188	5825 ± 202	6312 ± 231	0.344
-dP/dt min (mmHg/s)	-5324 ± 159	-5238 ± 215	-4729 ± 225	-6149 ± 294	0.001[₱]
Tau (ms)	12.5 ± 0.7	12.3 ± 1.1	14.2 ± 0.8	13.1 ± 0.5	0.014[₾]

Table 2. Hemodynamic parameters of young rats. Pes: LV end-systolic pressure; Ped: LV end-diastolic pressure; +dp/dt; positive change in left ventricular pressure over change in time; -dp/dt: negative change in left ventricular pressure over change in time; #: Cigarette vs. Nic-; $: Cigarette vs. air, Nic-, and Nic+; &: Cigarette vs. air and Nic-; *: Nic + vs. Nic-; £: Cigarette vs. Nic-; €: Nic- vs. Nic+; ₱: Cigarette vs. Nic-; ₾: Nic- vs. Nic+.

a M-mode display with high resolution ultrasound (15-MHz transducer and Sonos 5500 ultrasound system, Philips Medical System, Andover, MA), and blood flow rate (ml/min) was measured by ultrasound perivascular flow probe technology (TS410, Transonic Systems Inc., NY, USA).

Finally, the animals were euthanized at the end of the experiment under deep anesthesia by intraperitoneal injection of ketamine/xylazine followed by intravenous injection of KCL (149 mg/ml, 0.5 ml/250 gm body weight) to stop the hearts in a relatively diastolic or relaxed state. Ether was not used for euthanasia in this study. After the body weight was obtained, the heart was excised and weighed. The left and right ventricles were weighed separately. The left ventricle was fixed with formalin at pressure equal to 13 cm water column, and the postmortem LV volumes were measured by filling the cavity with water and weighing, repeated three times and averaged.

Statistical analysis

All data are reported as mean ± SEM. In the young rats, values among 4 groups were compared with one-way analysis of variance (ANOVA). Statistically significant differences were established at $p < 0.05$. If an ANOVA three-way comparisons were significant, than 2 way comparisons were performed using Holm-Sidak method. Student's *t*-test was used for comparison between the 2 groups in aged rat study. The significance threshold was set at $p < 0.05$. Additionally, we conducted ANOVAs for all outcomes, where we included exposure groups for air versus E cig plus nicotine for young plus old animals.

Results

Data from young rat study

Cardiac function data derived from the echocardiograms of the young rats is summarized in Table 1. There were no significant differences among the 4 groups in LV end-systolic diameter, LV fractional shortening (LVFS), systolic and diastolic LV wall thickness, and cardiac output. However, there was a significantly decreased LV diastolic ID in the E-cig Nic- group compared to the cigarette group.

The hemodynamics data of young rats are demonstrated in Table 2. Cigarettes exposure significantly increased blood pressure (Fig. 1A) and left ventricular end-systolic pressure, and LV dp/dt min (mmHg/s; more negative). The absolute value of LV dp/dt minimum was numerically lower and Tau numerically higher in the

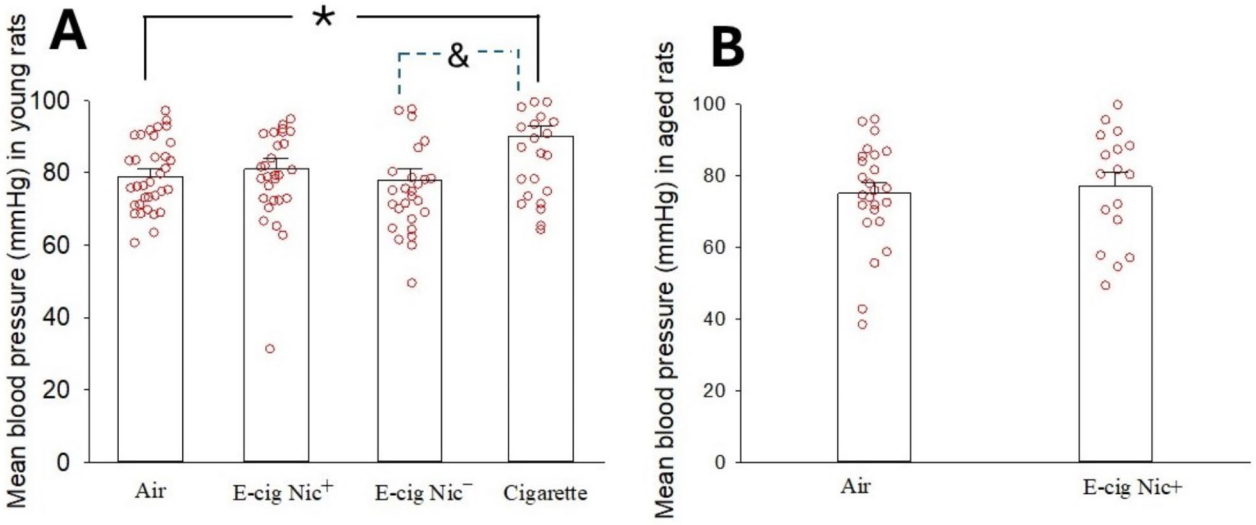

Fig. 1. Cigarette smoke increased blood pressure, while E-cig Nic + exposure did not alter blood pressure. **A**: In young rats, exposure to cigarette smoke was associated with higher mean blood pressure (90 ± 3, $n = 27$) compared to air exposure (79 ± 2, $n = 34$), while no significant effects were observed in the E-cig Nic + group (81 ± 3, $n = 30$). Statistical significance: *: Cigarette vs. air, $p = 0.0019$; **&**: Cigarette vs. Nic- (78 ± 3, $n = 28$), $p = 0.009$. **B**: In older rats, mean blood pressure was comparable between the air (75 ± 3, $n = 26$) and E-cig Nic + vaping groups (77 ± 4, $n = 177$; $p = 0.682$).

	Air	E-cig Nic+	E-cig Nic-	Cigarette	*p* value
	***n* = 34**	***n* = 30**	***n* = 28**	***n* = 27**	
Artery ID (mm) before occlusion	0.63 ± 0.03	0.57 ± 0.03	0.56 ± 0.02	0.62 ± 0.04	0.264
Flow velocity (CM/S) before occlusion	61.91 ± 3.63	64.61 ± 4.12	51.48 ± 2.98	53.36 ± 4.67	0.021
Artery ID (mm) after reperfusion	0.63 ± 0.02	0.58 ± 0.03	0.57 ± 0.03	0.87 ± 0.26	0.337
Flow velocity (CM/S) after reperfusion	61.28 ± 3.94	51.29 ± 4.25	47.92 ± 2.18	54.39 ± 4.65	0.078
	n = 24	*n* = 16	*n* = 24	*n* = 27	
Flow rate before occlusion (ml/min)	0.57 ± 0.08	0.52 ± 0.06	0.62 ± 0.06	0.54 ± 0.04	0.685
Peak of flow rate after artery reperfusion (ml/min)	2.00 ± 0.22	2.07 ± 0.17	2.22 ± 0.18	2.16 ± 0.20	0.783

Table 3. Femoral artery internal diameter (ID), blood flow velocity (CM/S), blood flow rate (ml/min) of young rats.

E-cigarette, nicotine negative group, which may suggest an abnormality in diastolic function in the E-cigarette, nicotine negative group.

Femoral artery endothelial function assessed by flow-mediated vasodilation was comparable after release of a 5-minute common femoral artery occlusion among the 4 groups. Table 3 lists the femoral artery internal diameter and flow velocity, and blood flow rate (ml/min).

Table 4 summarizes the postmortem parameters of young rats, including postmortem LV volume, body weight, heart weight, tibia length, lung wet and dry weight. These results, including heart weight/body weight ratio (Fig. 2A). are comparable among the 4 groups, except there were a decreased LV weight/body weight and right ventricle wall thickness, and smaller lung wet/dry weight ratio in the E-cig Nic- exposed rats.

Data for the old rat study

The data of cardiac function of old rats was shown in Table 5. There were no differences in LV end-diastolic internal diameter, LV end-systolic internal diameter, LV fractional shortening, LV diastolic and systolic wall thickness, and cardiac output between the 2 groups.

Hemodynamic data of old rats were summarized in Table 6 and showed that E-cig exposure did not affect arterial blood pressure (Fig. 1B), the heart rate, LV end-diastolic pressure (Ped), LV end-systolic pressure (Pes), dP/dt max, dP/dt min, Tau.

Femoral artery endothelial function assessed by flow-mediated vasodilation demonstrated that there was no significant difference in the diameter of the femoral artery and flow velocity (CM/S) in the femoral artery, and

	Air	E-cig Nic+	E-cig Nic-	Cigarette	*p* value
	n = 29	*n* = 26	*n* = 24	*n* = 23	
Postmortem LV volume (ml)	0.529 ± 0.030	0.443 ± 0.028	0.451 ± 0.027	0.470 ± 0.024	0.129
BW (grams)	353 ± 22	312 ± 19	335 ± 22	351 ± 22	0.448
Tibia length (mm)	41 ± 0.7	40 ± 0.5	41 ± 0.6	42 ± 0.5	0.271
Heart weight (grams)	0.9685 ± 0.0493	0.9008 ± 0.0435	0.8788 ± 0.0460	1.0122 ± 0.0623	0.255
LV weight (grams)	0.7862 ± 0.0368	0.7418 ± 0.0366	0.6794 ± 0.0328	0.7802 ± 0.0421	0.171
Right ventricle weight (grams)	0.1631 ± 0.0094	0.1486 ± 0.0084	0.1418 ± 0.0103	0.1734 ± 0.0097	0.1
Heart weight/BW (grams/grams)	0.0028 ± 0.0001	0.0030 ± 0.0001	0.0027 ± 0.0001	0.0029 ± 0.0001	0.072
LV weight/BW (grams/grams)	0.0023 ± 0.0001	0.0024 ± 0.0001	0.0021 ± 0.0000	0.0023 ± 0.0000	$<0.001^{\&}$
Heart weight/Tibia (grams/mm)	0.0234 ± 0.0009	0.0222 ± 0.0009	0.0212 ± 0.0009	0.0239 ± 0.0012	0.238
LV weight/tibia (grams/mm)	0.0190 ± 0.0007	0.0183 ± 0.0008	0.0165 ± 0.0006	0.0185 ± 0.0008	0.052
LV wall thickness (mm)	1.6034 ± 0.0737	1.5212 ± 0.0728	1.4854 ± 0.0732	1.7309 ± 0.0482	0.132
Right ventricle wall thickness (mm)	0.7303 ± 0.0424	0.7415 ± 0.0532	0.5808 ± 0.0204	0.7496 ± 0.0353	$0.008^{\#}$
Lung wet weight (grams)	1.2312 ± 0.0675	1.2760 ± 0.0488	1.1663 ± 0.1198	1.3857 ± 0.0658	0.151
Lung Dry weight (grams)	0.1954 ± 0.0071	0.1820 ± 0.0116	0.2126 ± 0.0107	0.2069 ± 0.0087	0.051
Lung wet/dry weight ratio (grams/grams)	6.47 ± 0.33	7.26 ± 0.22	5.71 ± 0.48	6.75 ± 0.24	0.017^{*}

Table 4. Postmortem parameters of young rats. LV: left ventricular; BW: Body weight. &: E-cig Nic + vs. E-cig Nic-; Air vs. E-cig Nic-; #: Cigarette vs. E-cig Nic-; *: E-cig Nic + vs. E-cig Nic-.

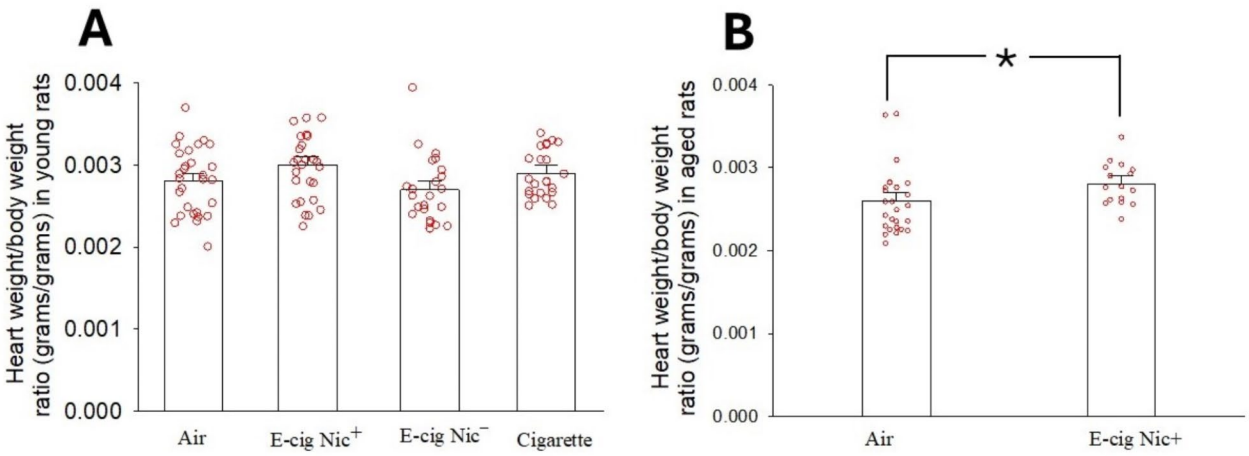

Fig. 2. E-cig Nic + vaping (0.0028 ± 0.0001, $n = 17$) was associated with a significantly greater heart weight/body weight (Panel **B**) compared to air exposure (0.0026 ± 0.0001, $n = 26$; *: $p = 0.037$) in older rats, although this effect was not statistically significant in young rats (Panel **A**. Air: 0.0028 ± 0.0001, $n = 29$; E-cig Nic+: 0.0030 ± 0.0001, $n = 26$; E-cig Nic-: 0.0027 ± 0.0001, $n = 24$; Cigarette: 0.0029 ± 0.0001, $n = 23$; $p = 0.072$).

	Air	E-cig Nic+	*p* value
	n = 26	*n* = 17	
LV diastolic ID (mm)	6.15 ± 0.14	6.26 ± 0.17	0.618
LV systolic ID (mm)	2.69 ± 0.15	2.78 ± 0.21	0.728
LVFS (%)	56.80 ± 1.84	55.92 ± 2.96	0.803
Systolic LV wall thickness (mm)	3.64 ± 0.09	3.38 ± 0.11	0.072
Diastolic LV wall thickness (mm)	2.31 ± 0.06	2.12 ± 0.09	0.077
Cardiac output (ml/min)	31.9 ± 2.3	29.6 ± 2.1	0.461

Table 5. Cardiac function of old rats assessed by echocardiography. LV: left ventricular; ID: internal diameter; LVFS: left ventricular fractional shortening.

	Air	E-cig Nic+	*p* value
	n = 26	*n* = 17	
Systolic Pressure (mmHg)	88 ± 3	91 ± 5	0.609
Diastolic Pressure (mmHg)	64 ± 3	66 ± 3	0.616
Mean Pressure (mmHg)	75 ± 3	77 ± 4	0.682
Pulse Pressure (mmHg)	24 ± 1	25 ± 2	0.735
Heart Rate (BPM)	202 ± 6	191 ± 6	0.219
Pes (mmHg)	87 ± 3	92 ± 4	0.311
Ped (mmHg)	4 ± 2	14 ± 5	0.091
+dP/dt max (mmHg/s)	5118 ± 258	4604 ± 404	0.292
-dP/dt min (mmHg/s)	-3567 ± 212	-3096 ± 273	0.183
Tau (ms)	20 ± 2	38 ± 11	0.124

Table 6. Hemodynamic parameters and cardiac output of old rats.

the femoral artery blood rate (ml/min) between the E-cig Nic + and air exposure group at before and after release of a 5-minute common femoral artery occlusion (Table 7).

Table 8 summarized the postmortem parameters of old rats, including postmortem LV volume, body weight, heart weight, tibia length, lung wet and dry weight. There were significantly higher heart weight/body weight (Fig. 2B) and LV weight/ body weight in the E-cig Nic + group compared to the air control group. Heart weight and LV weights were numerically greater in the E-cig Nic + group compared to air. Other postmortem parameters were comparable between the 2 groups.

	Air	E-cig Nic+	*p* value
	n = 26	*n* = 16	
Artery ID (mm) before occlusion	0.83 ± 0.03	0.77 ± 0.02	0.094
Flow velocity (CM/S) before occlusion	25.63 ± 1.73	21.59 ± 1.59	0.093
Artery ID (mm) after reperfusion	0.84 ± 0.03	0.77 ± 0.03	0.142
Flow velocity (CM/S) after reperfusion	25.33 ± 1.60	22.56 ± 1.53	0.232
	n = 26	*n* = 17	
Flow rate before occlusion (ml/min)	0.32 ± 0.04	0.30 ± 0.06	0.821
Peak of flow rate after artery reperfusion (ml/min)	2.04 ± 0.23	2.52 ± 0.30	0.212

Table 7. Femoral artery internal diameter (ID) and blood flow velocity (CM/S), and blood flow rate (ml/min) of old rats.

	Air	E-cig Nic+	*P* value
	n = 26	*n* = 17	
Postmortem LV volume (ml)	0.3955 ± 0.0173	0.4122 ± 0.0270	0.608
BW (grams)	336 ± 14	315 ± 16	0.327
Tibia length (mm)	42 ± 0.7	41 ± 0.8	0.376
Heart weight (grams)	0.8555 ± 0.0314	0.8699 ± 0.0499	0.808
Left ventricle weight (grams)	0.7039 ± 0.0240	0.7207 ± 0.0445	0.741
Right Ventricle Weight (grams)	0.1496 ± 0.0071	0.1427 ± 0.0109	0.600
Heart weight/BW (grams/grams)	0.0026 ± 0.0001	0.0028 ± 0.0001	0.037
LV weight/BW (grams/grams)	0.0021 ± 0.0001	0.0023 ± 0.0001	0.019
Heart weight/Tibia (grams/mm)	0.0201 ± 0.0005	0.0211 ± 0.0009	0.339
LW weight/tibia (grams/mm)	0.0165 ± 0.0004	0.0175 ± 0.0008	0.294
Left ventricle wall thickness (mm)	1.9558 ± 0.0629	1.7841 ± 0.0577	0.051
Right ventricle wall thickness (mm)	0.6696 ± 0.0215	0.7094 ± 0.0495	0.469
Lung wet weight (grams)	1.9162 ± 0.1177	2.0261 ± 0.2854	0.733
Lung Dry weight (grams)	0.2723 ± 0.0163	0.3512 ± 0.0768	0.343
Lung wet/dry weight ratio (grams/grams)	7.1307 ± 0.2056	6.8733 ± 0.3757	0.563

Table 8. Postmortem parameters of old rats.

Discussion

Key findings in young rats were that chronic E-cig vaping did not cause an increase in blood pressure or heart rate, although standard cigarette smoking, as a positive control, was associated with an increase in blood pressure at 12 weeks (Fig. 1A). In addition, E-cig did not alter arterial endothelial function. E-cig Nic- was associated with worse diastolic function. Standard cigarette smoking may be associated with a greater sympathetic surge causing elevated blood pressure compared to E-cig.

Key findings in old rats were that chronic E-cig vaping did not cause a significantly changes in arterial endothelial cell function, blood pressure (Fig. 1B), heart rate, and cardiac function. E-cig Nic + exposure is associated with a greater heart weight/BW (Fig. 2B) and LV weight/BW compared to air exposure in old rats.

Arterial flow-mediated dilation is a validated method to measure the ability of arteries to vasodilate in response to an increase in blood flow, which is used to assess the endothelial function. Given that endothelial dysfunction can be seen early in atherogenesis[9], flow-mediated dilation is a clinical prognostic indicator of cardiovascular health[10]. In human studies, impairment of flow-mediated dilation was reported after acute and chronic exposures to secondhand smoke and active smoking[11,12]. Carnevale et al.[13] performed a crossover, single-blind study in 40 healthy people, and showed that smoking both E-cig and traditional cigarettes led to unfavorable effects on flow-mediated dilation after single use, although E-cig seemed to have a lesser impact. Rao et al.[14] exposed rats to aerosol from E-cig for 10 cycles of 2 s inhalation over 5 min, and endothelial function was assessed by flow-mediated dilation pre- and post-exposure in right femoral artery. E-cig vaping impaired endothelial function in rats, comparable to impairment by cigarette smoke. An inherent problem of the published studies regarding impact of E-cig on endothelial function is their focus on short-term effects[15]. Our present study demonstrated that 12 weeks of E-cig vaping did not affect the femoral artery flow-mediated dilation.

One concern of E-cig vaping is that the nicotine in the E-cig aerosol stimulates the sympathetic nervous system, producing an increase in heart rate and blood pressure[16]. Numerous E-cig vaping studies reported that the use of E-cig with and without nicotine results in short-term elevations of both heart rate and blood pressure [for review, read references [17] and [18]. However, there are some studies that reported that daily use did

not increase the heart rate and blood pressure. Oncken et al.[19] recruited 20 nontreatment-seeking smokers who were willing to try E-cig (18 mg/mL nicotine e-Juice) for 2 weeks, found no changes in resting heart rate and resting blood pressure. In a prospective 3.5-year observational study, Polosa et al.[20] recruited nine daily E-cig users (29.7 ± 6.1 years old) who had never smoked in the test group and twelve age and sex-matched never-smoking non-E-cig users in the control group. In the E-cig group, systolic BP, diastolic BP, and heart rate were not significantly changed from baseline to any follow-up study visits during the 3.5-year follow-up period; and no significant difference was found between E-cig users and control subjects. The authors claimed that their results may provide some preliminary evidence that long-term use of E-cig is unlikely to raise significant health concerns in relatively young users. In our present study, tobacco cigarette smoking significantly increased blood pressure in young rats after 12 weeks exposure, while E-cig vaping did not, suggesting that E-cig vaping was less impactful than traditional tobacco smoking. E-cig vaping also did not change blood pressure in old rats after 12 weeks of exposure compared to air exposure.

There are limited long-term exposure studies that have been conducted to investigate the effects of E-cig vaping on cardiac mass and function, and these studies are generally performed in young animals. Shi et al.[21] subjected C57BL/6 mice (both sexes, 2–3 months old) to E-cig vaping (contains 24 mg/ml nicotine) for 2 weeks; and reported that E-cig vaping did not show any significant effects on cardiac contractility and geometry. Olfert et al.[22] randomly assigned 10-week-old female mice C57BL/6 to daily exposure to E-cig vapor with 18 mg/ml nicotine, standard (3R4F reference) cigarette smoke, or filtered air for 32 weeks. Upon completion of the 32 weeks exposure, whole heart mass was not statistically different, and cardiac function assessed by echocardiography revealed that heart rate, stroke volume, and cardiac output were similar among the 3 groups. In contrast to Olfert's study, El-Mahdy et al.[23] performed longitudinal studies to evaluate alterations in cardiovascular function associated with E-cig vaping (with 0, 6, or 24 mg/mL nicotine) exposure durations of up to 60 weeks in 20-week-old C57/BL6 male mice. E-cig vaping induced cardiac hypertrophy with elevated heart weight at 32 weeks of exposure, and these abnormalities further increased out to 60 weeks of exposure. The authors reported that severity of cardiac hypertrophy increases with E-cig exposure duration and vape nicotine content, whereas cardiac contractile function was not significantly altered. However, in our present study, 6-week-old and 25-month-old rats were exposed to E-cig vapor with 15 mg/ml nicotine for 12 weeks. E-cig with nicotine significantly increased heart weight/body and LV weight/body in old rats, but not in young rats. E-cig vaping did not affect cardiac function in either young or old rats. Our data suggested that the hearts of older rats were more vulnerable to E-cig stimulus than younger ones. The data also suggest that there may be non-hemodynamic mechanisms involved in the alteration of heart weight related to E-cig plus nicotine in older rats. In young rats, the potential time-course effects of varying exposure of E-cig vaping with different nicotine concentrations needs to be determined in future studies.

The limitations of our present study include the issue that different strains, young Sprague Dawley rats and aged Fischer 344 rats, were used. After completing the exposure studies with young Sprague Dawley rats, we discovered that 25-month-old Sprague Dawley rats were not commercially available. Due to time constraints imposed by our grant and the limited availability of aged rats from the National Institute of Aging, we opted to use 25-month-old Fischer 344 rats for this study. The influence of strains on cardiovascular responses to E-cig exposure must be considered when interpreting the effects of E-cig Nic + in this study. We cannot exclude the potential impact of strain on the cardiovascular analyses presented. In future studies, we will use the same strains and groups for comparison, including E-cig Nic- and standard cigarette groups in older rats. Another limitation is that our goal was to examine differences in young versus old and not by sex, where there has been controversy in the literature[24–27]; so future studies should examine the effects of e-cig between sexes.

Conclusions

In summary, E-cig vaping did not increase blood pressure or heart rate, nor did it alter cardiac function compared to air exposure in young rats after 12 weeks. In contrast, standard cigarette smoking was associated with increased blood pressure. However, we cannot rule out the possibility that longer exposure may affect cardiac function and structure, even in younger rats. Additionally, E-cig with nicotine did increase the heart weight-to-body weight ratio in older rats, suggesting that the effects of E-cigarettes may not be benign. Our findings indicate that older animals may be more vulnerable to E-cig exposure than younger ones.

We cannot conclude from this study that E-cig vaping is a safe alternative to traditional cigarette smoking, as many clinical studies have shown that E-cig use can cause more harm than good and may increase the risk of long-term cardiovascular morbidity[28]. Moreover, other studies from our group have demonstrated acute pulmonary inflammation in rodent models exposed to E-cig vaping[29]. We have also observed harmful effects on the cardiovascular system when E-cigs are administered to rats with pre-existing conditions, such as post-myocardial infarction[27]. In addition, advanced techniques for assessing cardiovascular health, such as the intrinsic frequency method for analyzing arterial waveforms, may detect abnormalities caused by E-cig exposure that are not evident with standard hemodynamic or echocardiographic analyses[30]. Therefore, the potential long-term effects of E-cigarettes on the cardiovascular system require further investigation in both young and old animals.

Data availability

The data presented in this study are available on request from the corresponding author Wangde Dai via email wangde.dai@hmri.org.

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References

1. Moriyama, C. et al. Aging enhances susceptibility to cigarette smoke-induced inflammation through bronchiolar chemokines. *Am. J. Respir Cell. Mol. Biol.* **42** (3), 304–311 (2010).
2. Gould, N. S. et al. Aging adversely affects the cigarette smoke-induced glutathione adaptive response in the lung. *Am. J. Respir Crit. Care Med.* **182** (9), 1114–1122 (2010).
3. Zhou, S., Wright, J. L., Liu, J., Sin, D. D. & Churg, A. Aging does not enhance experimental cigarette smoke-induced COPD in the mouse. *PLoS One*. **8** (8), e71410 (2013).
4. Bandi, P. et al. Changes in E-Cigarette Use among U.S. adults, 2019–2021. *Am. J. Prev. Med.* **65** (2), 322–326 (2023).
5. Rose, J. J. et al. Cardiopulmonary impact of electronic cigarettes and Vaping products: A Scientific Statement from the American Heart Association. *Circulation* **148** (8), 703–728 (2023).
6. Dai, W. et al. Effects of electronic cigarette exposure on myocardial infarction and No-Reflow, and cardiac function in a rat model. *J. Cardiovasc. Pharmacol. Ther.* **28**, 10742484231155992. https://doi.org/10.1177/10742484231155992 (2023 Jan-Dec).
7. Osborn, J. W. Jr, Barber, B. J., Quillen, E. W. Jr, Abram, R. J. & Cowley, A. W. Jr. Chronic measurement of cardiac output in unanesthetized rats using miniature thermocouples. *Am. J. Physiol.* **251** (6 Pt 2), H1365–H1372 (1986).
8. Machin, D. R. et al. Ultrasound Assessment of Flow-mediated dilation of the Brachial and superficial femoral arteries in rats. *J. Vis. Exp.* ;(117):54762. (2016).
9. Cheng, K. S., Baker, C. R., Hamilton, G., Hoeks, A. P. & Seifalian, A. M. Arterial elastic properties and cardiovascular risk/event. *Eur. J. Vasc Endovasc Surg.* **24** (5), 383–397 (2002).
10. Flammer, A. J. et al. The assessment of endothelial function: from research into clinical practice. *Circulation* **126** (6), 753–767 (2012).
11. Celermajer, D. S. et al. Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. *Circulation* **88** (5 Pt 1), 2149–2155 (1993).
12. Celermajer, D. S. et al. Passive smoking and impaired endothelium-dependent arterial dilatation in healthy young adults. *N Engl. J. Med.* **334** (3), 150–154 (1996).
13. Carnevale, R. et al. Acute Impact of Tobacco vs Electronic cigarette smoking on oxidative stress and vascular function. *Chest* **150** (3), 606–612 (2016).
14. Rao, P., Liu, J. & Springer, M. L. JUUL and Combusted cigarettes comparably impair endothelial function. *Tob. Regul. Sci.* **6** (1), 30–37 (2020).
15. Daiber, A., Kuntic, M., Oelze, M., Hahad, O. & Münzel, T. E-cigarette effects on vascular function in animals and humans. *Pflugers Arch.* **475** (7), 783–796 (2023).
16. Middlekauff, H. R., Park, J. & Moheimani, R. S. Adverse effects of cigarette and noncigarette smoke exposure on the autonomic nervous system: mechanisms and implications for cardiovascular risk. *J. Am. Coll. Cardiol.* **64** (16), 1740–1750 (2014).
17. Benowitz, N. L. & Fraiman, J. B. Cardiovascular effects of electronic cigarettes. *Nat. Rev. Cardiol.* **14** (8), 447–456 (2017).
18. Martinez-Morata, I., Sanchez, T. R., Shimbo, D. & Navas-Acien, A. Electronic cigarette use and blood pressure endpoints: a systematic review. *Curr. Hypertens. Rep.* **23** (1), 2. https://doi.org/10.1007/s11906-020-01119-0 (2020).
19. Oncken, C. A., Litt, M. D., McLaughlin, L. D. & Burki, N. A. Nicotine concentrations with electronic cigarette use: effects of sex and flavor. *Nicotine Tob. Res.* **17** (4), 473–478 (2015).
20. Polosa, R. et al. Health impact of E-cigarettes: a prospective 3.5-year study of regular daily users who have never smoked. *Sci. Rep.* **7** (1), 13825 (2017).
21. Shi, H. et al. The effect of electronic-cigarette vaping on cardiac function and angiogenesis in mice. *Sci. Rep.* **9** (1), 4085 (2019).
22. OlfertIM et al. Chronic exposure to electronic cigarettes results in impaired cardiovascular function in mice. *J. Appl. Physiol. (1985).* **124** (3), 573–582 (2018).
23. El-Mahdy, M. A. et al. Long-term electronic cigarette exposure induces cardiovascular dysfunction similar to tobacco cigarettes: role of nicotine and exposure duration. *Am. J. Physiol. Heart Circ. Physiol.* **320** (5), H2112–H2129 (2021).
24. Alam, F. & Silveyra, P. Sex differences in E-Cigarette Use and Related Health effects. *Int. J. Environ. Res. Public. Health*. **20** (22), 7079 (2023).
25. Hering, D. et al. Heightened acute circulatory responses to smoking in women. *Blood Press.* **17** (3), 141–146 (2008).
26. Kotlyar, M., Thuras, P., Hatsukami, D. K. & al'Absi, M. Sex differences in physiological response to the combination of stress and smoking. *Int. J. Psychophysiol.* **118**, 27–31 (2017).
27. Dai, W. et al. Effects of electronic cigarette vaping on Cardiac and vascular function, and post-myocardial infarction remodeling in rats. *Cardiovasc. Toxicol.* **24** (2), 199–208 (2024).
28. Rahman, A., Alqaisi, S., Alzakhari, R. & Saith, S. Characterization and summarization of the impact of electronic cigarettes on the Cardiovascular System: a systematic review and Meta-analysis. *Cureus* **15** (5), e39528 (2023).
29. Kleinman, M. T. et al. E-cigarette or Vaping Product Use-Associated Lung Injury Produced in an animal model from electronic cigarette vapor exposure without tetrahydrocannabinol or vitamin E oil. *J. Am. Heart Assoc.* **9** (18), e017368 (2020).
30. Alavi, R. et al. Adverse Cardiovascular effects of Nicotine delivered by Chronic Electronic cigarettes or Standard cigarettes captured by Cardiovascular intrinsic frequencies. *J. Am. Heart Assoc.* **13** (18), e035462 (2024).

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Author contributions

The authors made substantial contributions to the conception or design of the work (R.A.K., M.T.K.) or to the acquisition, analysis, or interpretation of data for the work (W.D., J.S., J.C., M.T.K., D.A.H., R.J.A., S.R., I.H., A.T., R.A.K.); participated in critically revising the manuscript (W.D., J.S., J.C., M.T.K., D.A.H., R.J.A., S.R., I.H., A.T., R.A.K); approved the final version to be published (W.D., J.S., J.C., M.T.K., D.A.H., R.J.A., S.R., I.H., A.T., R.A.K); and agreed to be accountable for all aspects of the work (W.D., J.S., J.C., M.T.K., D.A.H., R.J.A., S.R., I.H., A.T., R.A.K).

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Declarations

Competing interests

The authors declare no competing interests.

Conflict of interest
The authors claim to have no conflicts of interest.

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