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Effects of Electronic vs. Tobacco Cigarettes on Cardiovascular Health in Male and Female Mice

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### Authors

Lao, Candice

Jordan, Maria C

Friedman, Theodore C

et al.

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Peer reviewed

RESEARCH ARTICLE

Integrative Cardiovascular Physiology and Pathophysiology

## Conventional tobacco and electronic cigarettes differentially affect cardiovascular health in male and female mice

Candice J. Lao,<sup>1,2</sup> Maria C. Jordan,<sup>1</sup> Juan Carlos Rivera,<sup>2</sup> Yin Tintut,<sup>1,3,4</sup> Karla Luna,<sup>1</sup> William Ramirez-Trillo,<sup>2</sup> Trent Yamamoto,<sup>1</sup> Bilal Pandit,<sup>1</sup> Cherie Huang,<sup>1</sup> Angela Li,<sup>1</sup> Sophia Lewis Wang,<sup>1</sup> Thalia Nguyen,<sup>1</sup> Valentina Castro,<sup>1</sup> Ayushi Kothari,<sup>1</sup> Victoria C. Chen,<sup>1</sup> Xuesi M. Shao,<sup>2,5</sup> Theodore C. Friedman,<sup>2,3</sup> and Kenneth P. Roos<sup>1</sup>

<sup>1</sup>Department of Physiology, David Geffen School of Medicine at UCLA, Los Angeles, California, United States; <sup>2</sup>Department of Internal Medicine, Charles R. Drew University of Medicine and Science, Los Angeles, California, United States; <sup>3</sup>Department of Medicine, David Geffen School of Medicine at UCLA, Los Angeles, California, United States; <sup>4</sup>Department of Orthopedic Surgery, David Geffen School of Medicine at UCLA, Los Angeles, California, United States; and <sup>5</sup>Department of Neurobiology, David Geffen School of Medicine at UCLA, Los Angeles, California, United States

### Abstract

Although promoted as a safer alternative, the cardiovascular effects of electronic cigarettes (E-cigs) compared with tobacco cigarettes (T-cigs) are largely unknown. Thus, we evaluated aerosol exposures and the potential additive effects of a Western diet (WD) on cardiovascular health in multiple cohorts of hyperlipidemic (*ApoE*<sup>−/−</sup>) male and female mice. The mice were fed either a normal diet (ND) or WD and exposed daily during their dark cycle to either air (control), E-cig, or T-cig aerosol for 12 wk. Results showed that plasma nicotine levels in the E-cig and T-cig exposed mice were similar to those of human users. In both sexes, body weight was increased by WD, whereas caloric intake was increased by T-cig on both diets in both sexes and by E-cig on ND only in male mice. T-cig-exposed males and females on WD had significantly greater atherosclerotic lesions than their ND counterparts, whereas the E-cig effect was only in males. In addition, on WD, T-cig-exposed females had greater lesions than T-cig-exposed males and E-cig-exposed females. Left ventricular (LV) functional indices were significantly decreased to a similar extent by E-cigs and T-cigs on both diets in both sexes. Structurally, T-cigs increased LV mass on both diets and sexes, whereas E-cigs increased LV mass only on WD in both sexes. Overall, the findings demonstrated that both electronic and tobacco cigarettes impose differential adverse effects on caloric consumption, atherosclerosis, and cardiovascular health in diet- and sex-dependent manners, suggesting that E-cig is not a safer alternative to T-cig.

**NEW & NOTEWORTHY** Effects of electronic cigarettes (E-cigs) compared with tobacco cigarettes (T-cigs) in the Western population are largely undetermined. Here, we assessed chronic, intermittent exposures to either E-cigs or T-cigs on cardiovascular parameters in hyperlipidemic mice with similar nicotine levels. Our findings suggest that both E-cigs and T-cigs imposed adverse effects on cardiac function, heart chamber size, and atherosclerotic lesions in diet and sex-dependent manners, suggesting that electronic cigarettes are not a safer alternative to conventional cigarettes.

*atherosclerosis; electronic cigarettes; sex differences; tobacco cigarettes; ventricular dysfunction*

### INTRODUCTION

Nicotine in tobacco-based products has been associated with cardiovascular disease and increased mortality for decades (1–3). However, the introduction of electronic nicotine delivery systems (ENDS), or electronic cigarettes (E-cigs), added a new modality for nicotine exposure. E-cigs became widely available in 2009 and were marketed as a tool for smoking cessation and as a safer alternative to conventional tobacco cigarettes (T-cig) (4). E-cigs rapidly gained in popularity,

especially among young people, exceeding T-cigs by 2018 (5–7). Adolescents in the United States are especially enticed by the more attractive newer generation pod-mod device designs, the higher effective nicotine concentrations, and flavors (8–10). Their daily use among high school students peaked at 27.5% in 2019, although it subsequently decreased post-pandemic to 10.0% in 2023 and 8.0% in 2024 (6, 11). However, E-cig use continues to rise among all users worldwide to ~80 million in 2023 (8). Many adults who use E-cigs to get off T-cigs end up using both and become dual users (12). Unlike the overwhelming



Correspondence: K. P. Roos (kroos@mednet.ucla.edu).

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evidence that T-cigs are detrimental to health, less is known about the long-term effects of E-cigs on the health of cardiovascular and other organ systems in mice and humans (13). A recent meta-analysis reported similar cardiovascular odds ratios between E-cigs and T-cigs (14).

E-cigs deliver nicotine with a battery that heats a wire to aerosolize a liquid typically containing propylene glycol, glycerin, nicotine, and optionally flavors or other substances (7, 8, 13, 15, 16). The active ingredient in T-cigs and E-cigs is nicotine which is a highly addictive alkaloid chemical known to cause a wide variety of detrimental cardiovascular changes, including higher blood pressure, heart rates, arrhythmias, and atherosclerosis (7, 8, 13, 17).

Previously, we investigated the effects of chronic, intermittent whole body exposures to E-cig aerosol containing 2.4% nicotine in male *Apoe*<sup>-/-</sup> mice fed a Western Diet and in male wild-type mice on a high-fat diet (13, 18–20). After 12 wk of daily exposure, these mice exhibited impaired cardiac function, increased oxidative stress, myocardial ultrastructural abnormalities, and had greater atherosclerotic lesions compared with control mice exposed to E-cigs containing 0% nicotine or saline aerosols. Acute nicotine aerosol exposure also disrupted hemodynamics and induced cardiac arrhythmias in pregnant rats (21). We also recently reported altered cardiac rhythms and increased particulate matter in acute E-cig aerosol-exposed mice (17).

However, it has not been clearly established whether chronic exposure to E-cigs is a safer alternative than T-cigs. In addition, their effects on female mice compared with male mice, as well as potential additive effects with a high-fat Western diet (WD), are largely understudied. Thus, in this study, we used 12 cohorts of hyperlipidemic mice to compare the effects of diet, sex, and exposure delivery of T-cigs and E-cigs on caloric intake and cardiovascular health, including cardiac structure, function, and atherosclerosis in both male and female mice. The overriding hypothesis being evaluated is that E-cig is not a safer alternative to T-cig for maintaining cardiovascular health in both male and female mice.

## MATERIALS AND METHODS

### Mice and Diet

All animal studies followed the UCLA Animal Research Committee (IACUC) guidelines under approved protocols. We used an established mouse model (*Apoe*<sup>-/-</sup>) that is susceptible to atherosclerosis and cardiovascular dysfunction (22). One hundred twelve (112) male and female *Apoe*<sup>-/-</sup> mice (9–10 wk old), purchased from the Jackson Laboratory (RRID:IMSR\_JAX:002052; Bar Harbor, ME), were used for all the control and exposure studies reported here. Data from all of the mice are included in the results except one female E-cig cohort mouse, which unexpectedly died at week 10 in its home cage during the non-exposure period. The mice were randomly grouped into 12 cohorts, housing 4 per cage in a temperature-controlled study area room on a shifted 12 h-light-dark (LD) light cycle (Lights ON @ 2100; OFF @ 0900). For each sex, there were six cohorts: 1) ND, 2) ND + E-cig, 3) ND + T-cig, 4) WD, 5) WD + E-cig, and 6) WD + T-cig. The control and E-cig cohorts had 8 mice/group, and T-cig cohorts had 12 mice/group. The mice were fed either a normal diet (ND;

Teklad 7013, Inotiv, Madison, WI) or a Western Diet (WD; D12079B, Research Diets, New Brunswick NJ; with 60% of calories from fat consisting of 26.2% of protein, 26.3% of carbohydrate, and 34.9% of fat). Fresh food was supplied daily for E-cig-exposed chambers, every other day for the T-cig-exposure cages, and twice weekly for the control nonexposed cages. Mouse weight and cumulative food consumption were measured weekly.

### Exposure Protocols

The chronic-intermittent exposure protocol was designed to mimic human smoking and vaping behavior (16). All exposures were performed in the dark phase since mice are nocturnal and active at that time. We shifted the circadian cycle of the mice for our convenience, so the lights went off at 0900 and went on at 2100. This allowed us to visually monitor the studies from both exposure systems during our daytime to ensure animal welfare and equipment functionality. All the mice were monitored during the exposures with a low-level red light, which does not alter the circadian cycle. We used a chronic intermittent protocol of dosing every half hour that resembles the nicotine pharmacokinetics of typical human smokers and vapers (16, 18). The mice were exposed to either E-cig or T-cig aerosol for 10 h/day, 7 days/wk for 12 wk using specialized exposure equipment (Fig. 1, A–C). At the end of each 10-h exposure period, the mice were returned to their home cages in the study area, the exposure systems sanitized, and E-cig batteries recharged.

#### Air exposure (n = 32, 4 cohorts).

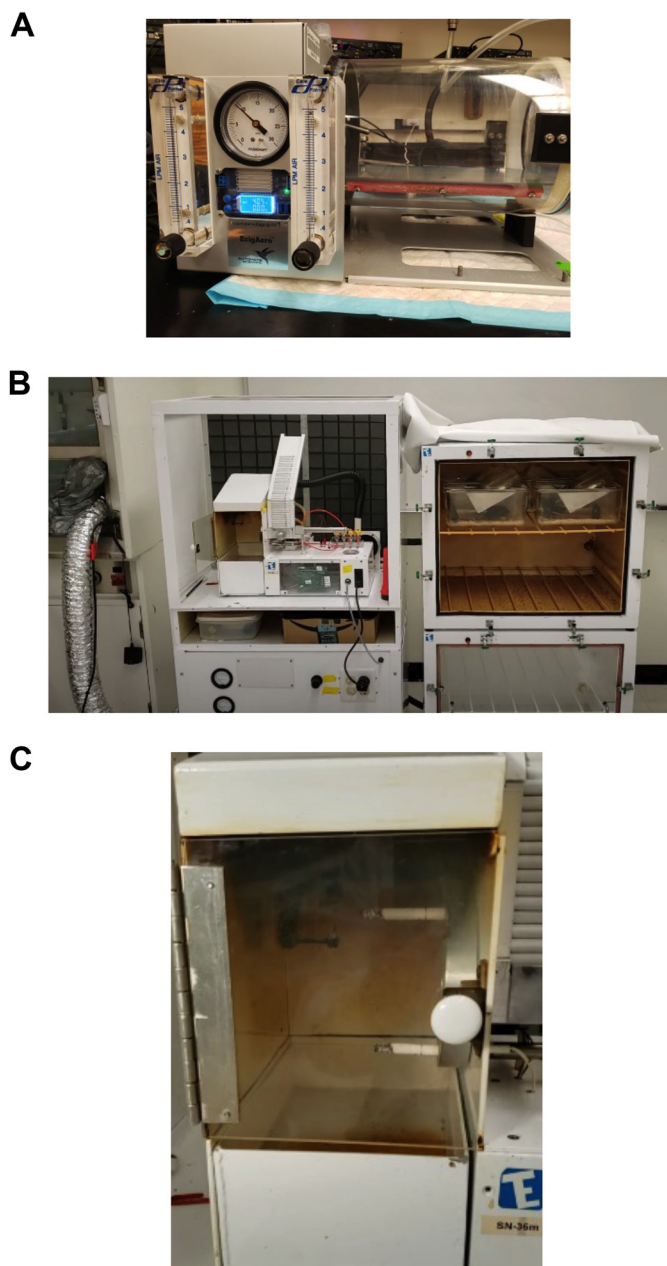
The control mice were exposed only to air and not to either E-cig or T-cig aerosols. The mice remained in their home cages in the study area.

#### E-cig exposure (n = 32, 4 cohorts).

Whole body E-cig exposures were performed in 8 ECigAero chambers (AutoMate Scientific, Berkeley, CA; US patent No. 11000065) (Fig. 1A) housing 4 mice each, as previously described (15, 18). Briefly, two Blu Plus (Classic Tobacco taste) E-cigs (Fontem US, LLC) were installed in each exposure chamber daily. Each Blu Plus E-cig contains 2.4% free-base nicotine in an approximate 33%/60% ratio of vegetable glycerin to propylene glycol liquid solution in water. Under computer control, they were alternately puffed for 4 s every 30 s, six puffs per exposure episode to provide the E-cig aerosol. These episodes were repeated every 30 min for the 10-h period. Between puffs and the 27-min nonexposure periods, fresh air flowed through the chamber at 4 L/min to prevent anoxia, to remove CO<sub>2</sub> and the E-cig aerosol residue (16).

#### T-cig exposure (n = 48, 4 cohorts).

Mice were exposed to T-cig in a Teague TE-10b smoking machine (Teague Enterprises, Woodland, CA). This automated system (Fig. 1B) can smoke multiple cigarettes at a time with programmable software-controlled protocols for prescribed periods of time. The T-cigs were loaded pneumatically from a cassette into a rotating wheel in the smoking chamber, where they were lit by a nichrome wire and puffed by a pump (Fig. 1C). The primary and side-stream smoke was sucked at a rate of 16 L/min into a large exposure chamber that holds up to 12 standard mouse cages at once.



**Figure 1.** Exposure systems. **A:** ECigAero chambers. **B:** Teague TE10b T-cig system with the cigarette cassette and adjacent smoking chamber on the *left*, and the mouse exposure chamber on the *right*. **C:** T-cig smoking chamber in use with the rotating wheel holding 2 lit 3R4F reference cigarettes. E-cig, electronic cigarette; T-cig, tobacco cigarette.

The air with smoke was exhausted into a chemical hood. Fresh room air continued to flow between puffs and nonexposure episodes. To start the exposures, the mice were transferred from their home cages into separate exposure cages with fresh food and water for the 10-h exposure period. As determined from the initial optimization of nicotine dosing, we used two 3R4F reference cigarettes (Center for Tobacco Reference Products, Lexington, KY) following the standardized US Federal Trade Commission (FTC) protocol of two-second puffs alternately from two individual research cigarettes (23). These filtered cigarettes were similar to commercially available

low-tar products. They contain 0.73 mg of nicotine and 9.4 mg of tar per cigarette. Each puff was repeated nine times, separated by 1 min, for a total 9-min duration from each cigarette. When completed, the cigarette was ejected pneumatically into a water bath and extinguished. There was a 21-min interval of room air flow that was repeated every 30 min for the 10-h exposure period, smoking 40 cigarettes (2 packs) daily. At the end of the daily exposure period, the mice were removed from the exposure chamber and returned to their home cages. The system was cleaned and sanitized for the next round of exposures.

### Nicotine Dose Verification

To optimize the Teague smoking system and to confirm that the exposure protocols from both systems provided doses of nicotine similar to those seen in moderate to heavy human smokers (10), plasma levels of nicotine were obtained from separate cohorts of 9-wk-old male C57BL/6 mice (RRID: IMSR\_JAX:000664; Jackson Laboratory, Bar Harbor, ME). Sixteen of these mice were used to determine the optimal number and timing of tobacco cigarettes and chamber flow rates for exposure with the Teague TE10b smoking machine (described in *T-cig exposure*). Additional mice were exposed to E-cigs ( $n = 5$ ) and/or T-cigs ( $n = 5$ ) for 10 days using the exposure protocols outlined above for dosing verification. Blood was collected within 20 min of their last exposure of the day by direct cardiac puncture of the left ventricle (16). Plasma samples were shipped to NMS Labs (Horsham, PA). Nicotine levels were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

### Echocardiography

All mice underwent ultrasound echocardiographic assessment twice—within days of the start of exposures and after 12 wk of exposure with a 15 MHz Siemens Acuson Sequoia Model C256 as previously described (18, 20, 24). Briefly, the mice were lightly anesthetized with isoflurane (1.5%) to immobilize them for imaging while retaining physiological heart rates ( $> 450$  beats/min). Two-dimensional, M-mode echocardiography and spectral Doppler images were obtained and later analyzed to obtain the standard measures of left ventricular (LV) dimensions and function. The parameters consisted of septal and posterior wall thickness (VST and PWT), LV dimensions at the end of diastole (EDD) and systole (ESD), aortic ejection time (Ao ET), heart rate (HR), LV ejection fraction (LVEF), LV fractional shortening (LVFS), velocity of circumferential fiber shortening (Vcf), LV mass, and indices of diastolic dysfunction (E, A, E/A). All echo images were acquired and analyzed by the same person (M.C.J.) to ensure consistency.

### Aortic Tissue Harvesting and en Face Staining

At the end of the 12-wk-exposure period, the mice were euthanized and perfused with 0.9% saline. The ascending and descending aortas were harvested, fixed in 7.5% sucrose solution, and stained with Sudan IV stain (RRID:SCR\_008988; Sigma-Aldrich, St. Louis, MO). The atherosclerotic lesions were imaged and quantified using Image Pro Plus software (version 7, RRID:SCR\_016879; Media Cybernetics, Rockville MD) (25). All aortas were processed and analyzed by the same person (C.J.L.) to ensure consistency.

## Statistical Analysis

Data were analyzed using three-way or four-way repeated measures ANOVA models with factors diet, sex, treatment (air, E-cig, and T-cig), and repeated measures on one factor (before and after the treatment). For the body weight and caloric intake data with the time factor of 12 wk (Fig. 2), we analyzed the slopes that reflect the body weight or caloric intake changes with time (as a continuous variable) using three-way repeated measure ANOVA models (factors: diet, treatment, time). The delta body weight change (Supplemental Fig. S1) and the atherosclerotic lesions (Fig. 3) were analyzed with three-way ANOVA (factors: diet, sex, and treatments). The echocardiographic data were analyzed using four-way repeated measure ANOVA models (factors: diet, sex, treatment, and time) (Fig. 4). Multiple pairwise comparisons were analyzed with Sidak method (26).  $P \leq 0.05$  was considered statistically significant. SAS 9.4 (Statistical Analysis System; RRID:SCR\_008567) was used to perform statistical analyses. Data are presented with mean  $\pm$  standard error (SE). More specific, detailed analyses are described in the legends of the Figures and Tables.

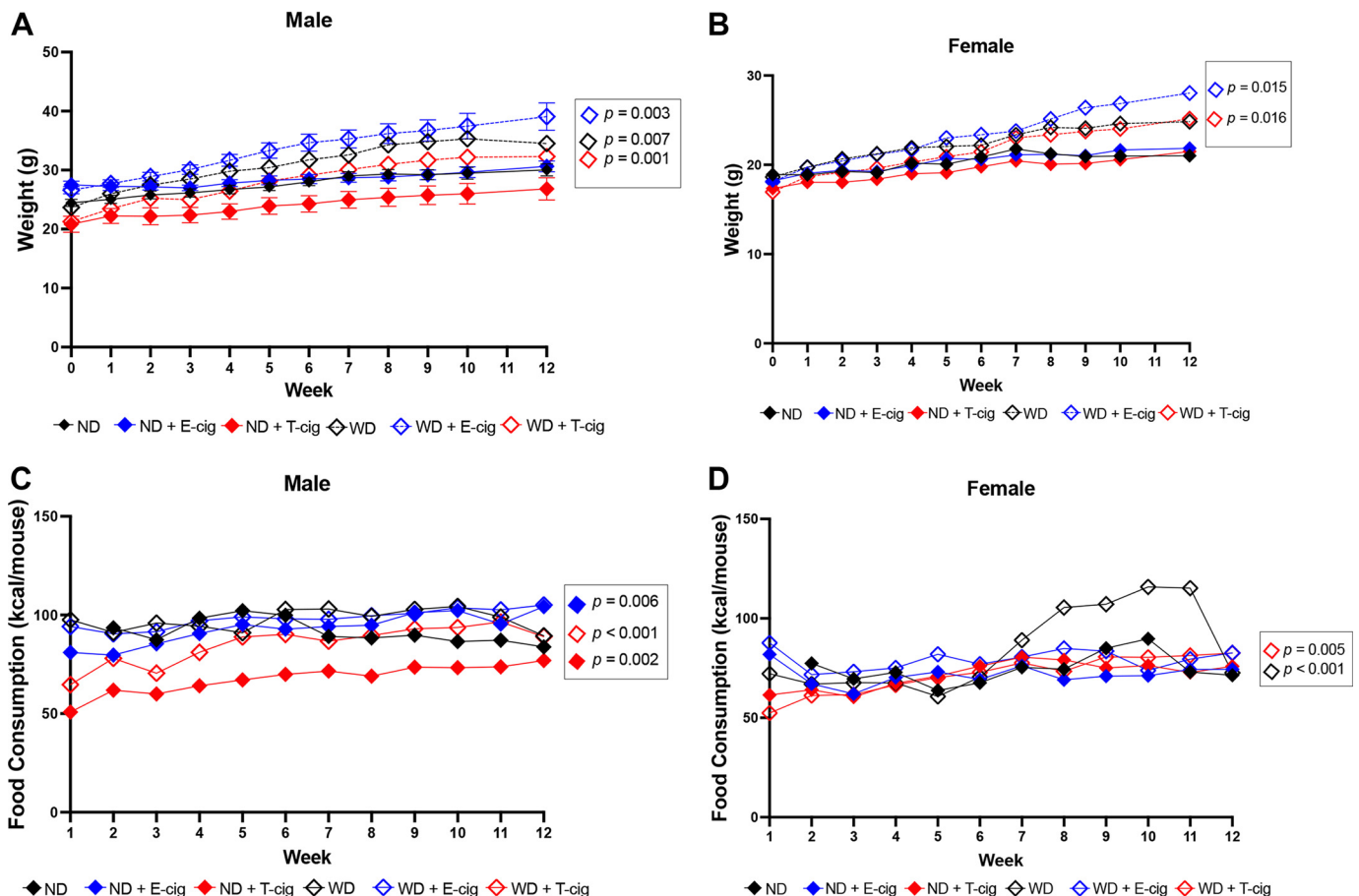
## RESULTS

### Nicotine Dose Verification

To determine that the mice were exposed appropriately to yield levels of nicotine comparable to human smokers, dose verification tests were performed in separate E-cig and T-cig groups. Nicotine levels (in ng/mL; mean  $\pm$  SE) were  $47 \pm 7$  for E-cig,  $55 \pm 8$  for T-cig groups ( $P = 0.51$ ). The plasma nicotine levels were similar to those seen in human moderate to heavy smokers (16, 27, 28). The levels in the control group were undetectable ( $< 2.5$  ng/mL nicotine) (16).

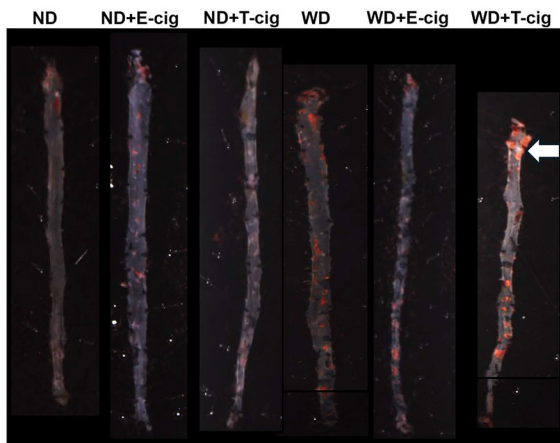
### Effects on Body Weight and Caloric Intake

The weekly body weights of males and females over the duration of the study are shown in Fig. 2 (A and B). The mice of both sexes on WD weighed more than their ND counterparts at week 12. The slopes of weekly weights in the three-way ANOVA model reflect the trends of weight changes with time during the 12-wk study period, independent of initial body weight. Slope analysis revealed that all the ND fed mouse cohorts had no significant weight change during the 12-wk study period (slope not significantly different from 0).

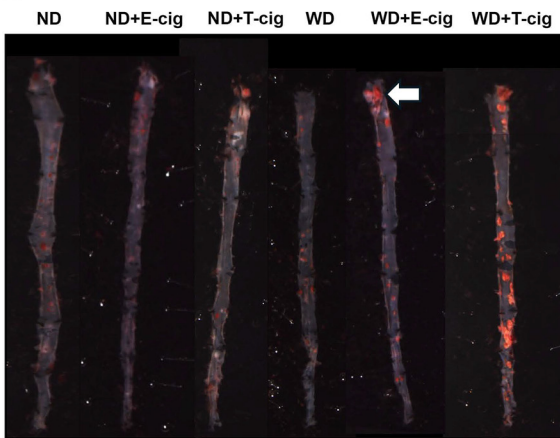


**Figure 2.** Body weight and caloric intake. Weekly weights and caloric intake from all 12 cohorts are plotted for male mice (A and C) and female mice (B and D) for the 12-wk study. Changes in body weight and caloric intake over time were estimated by slopes, using three-way repeated measures ANOVA [factors: 1) diet, 2) treatments (air, E-cigs, and T-cigs), and 3) time], with repeated measures on one factor—time taken as a continuous variable. The ANOVA models provide  $P$  values for every estimated slope of the cohort group data. Significant weight gain and increase in caloric intake (positive slopes,  $P \leq 0.05$ ) are indicated with the corresponding color-coded symbol adjacent to the line. E-cig, electronic cigarette; ND, normal diet; T-cig, tobacco cigarette; WD, Western diet.

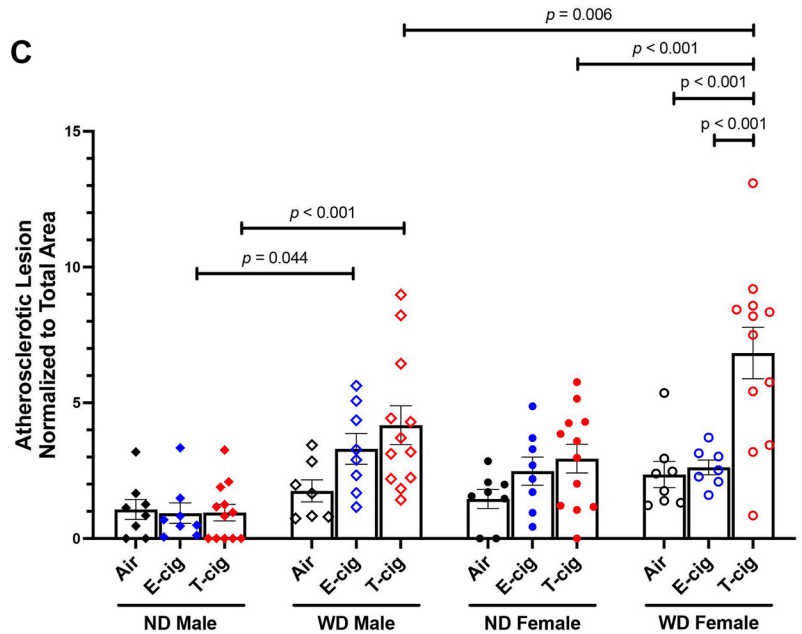
## A Males



## B Females



## C



**Figure 3.** Aortic atherosclerotic lesions. Representative images, showing lesions in male (A) and female (B) cohorts exposed to control, E-cigs, or T-cigs on both diets. Arrows indicate representative lesion areas. C: quantitative analysis of the lesion area. Data were analyzed with three-way ANOVA [factors: 1) diet, 2) sex, and 3) treatments] followed by multiple pairwise comparisons with the Sidak method. E-cig, electronic cigarette; ND, normal diet; T-cig, tobacco cigarette; WD, Western diet.

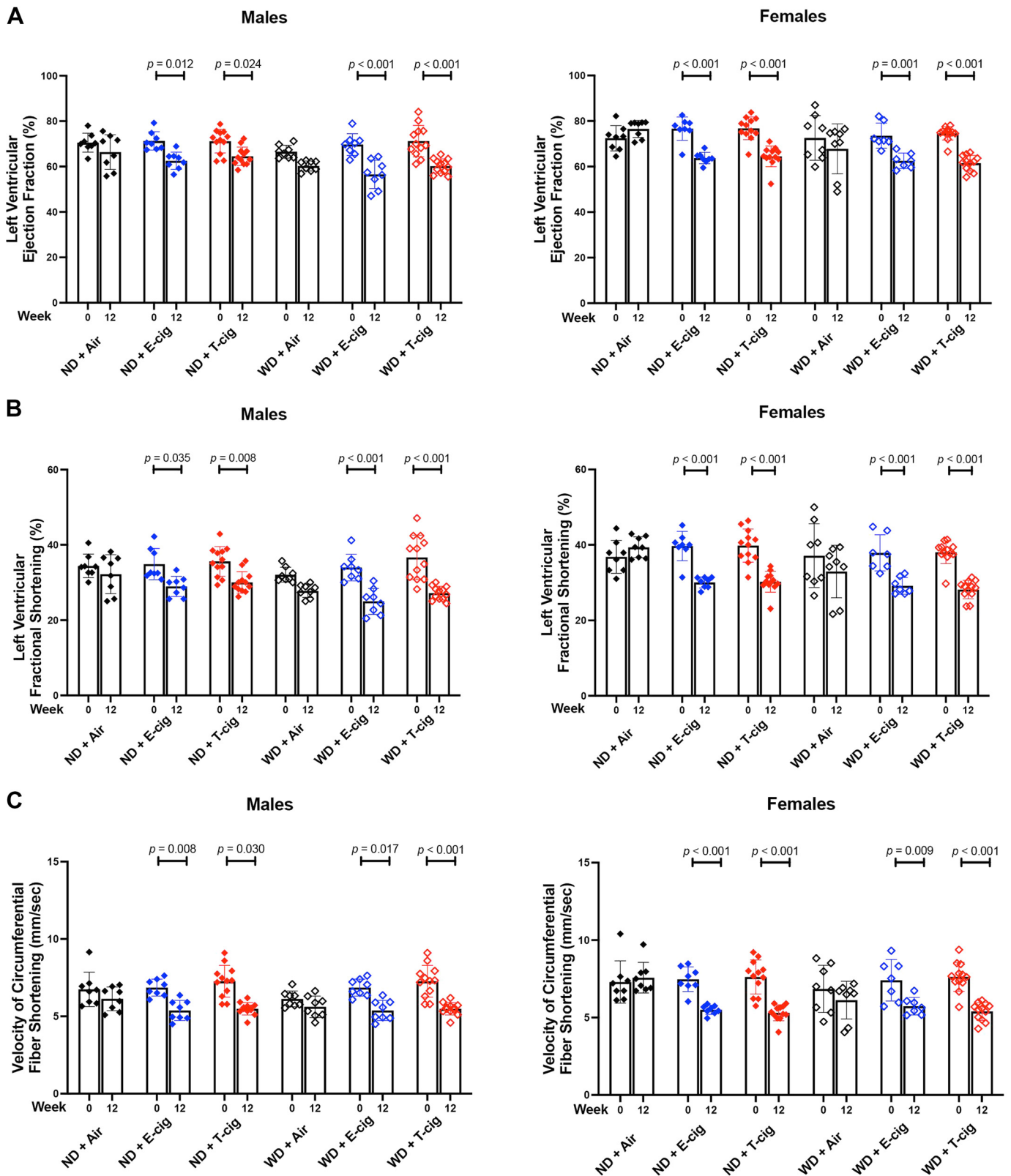
However, five of the six WD-fed cohorts had significant weight gain (positive slope) over the period (slopes: male WD air = 0.97,  $P = 0.007$ ; WD E-cig = 1.08,  $P = 0.003$ ; WD T-cig = 0.95,  $P = 0.001$ ; female WD E-cig = 0.82,  $P = 0.015$ ; WD T-cig = 0.66,  $P = 0.016$ ). The WD female (air) group trended higher, but did not have a significant slope increase (0.519;  $P = 0.123$ ) even though they had a greater weight at the end than their ND counterparts. The slope findings were reinforced by calculating the delta % weight change from week 0 to 12 for each cohort in Supplemental Fig. S1 (see <https://doi.org/10.6084/m9.figshare.30015274>). All the cohorts with significant slope increases had more than a 7% weight increase, whereas the insignificant cohorts had less, regardless of sex or exposure. All WD-fed cohorts weighed significantly more than their ND counterparts. The female WD E-cig was significantly greater than the control WD air. Finally, three-way ANOVA revealed significant sex differences in three cohort pairs with the same diet (ND air, WD air, and WD T-cig; Supplemental Fig. S1).

The weekly caloric intake of males and females, in terms of kilocalories/mouse/week, is shown in Fig. 2, C and D. Caloric intake was determined from the actual food intake

by weight multiplied by caloric value per unit weight (gram) of the two food sources each week. In males, the caloric intake increased (significant positive slopes) in the cohorts of ND E-cigs (slope = 1.99,  $P = 0.006$ ), ND T-cig (slope = 1.9,  $P = 0.002$ ), and WD T-cigs (slope = 2.29,  $P < 0.001$ ) during the study period, whereas the ND air, WD air, and WD E-cigs cohorts had no significant change. The differences in slopes of ND E-cigs versus ND air and ND T-cigs versus ND air were significant by pairwise comparisons. In females, WD-fed air control and WD T-cig-treated mice showed significant caloric intake increases (slopes: WD air = 3.95,  $P < 0.001$ ; WD T-cigs = 2.54,  $P = 0.005$ ), whereas the E-cig-treated cohort was not significant. All 3 ND fed female cohorts showed no significant changes in slope. There were also no significant differences (pairwise comparisons) between all 6 female cohorts in caloric intake.

## Effects on Atherosclerotic Lesions

The effects of E-cig and T-cig on atherosclerotic lesions were assessed in the aortas of both male and female mice by en face analysis. Representative images of aortas are shown in Fig. 3, A and B and quantitative data in Fig. 3C. On ND,



**Figure 4.** Echocardiographic analyses. A–C: show the indices of left ventricular (LV) structure and function. D: shows the LV mass. Data were analyzed with four-way repeated measure ANOVA [factors: 1) diet, 2) sex, 3) treatments, and 4) time] repeated measures on one factor-time (before and after 12-wk aerosol exposure) followed by multiple pairwise comparisons with the Sidak method. E-cig, electronic cigarette; ND, normal diet; T-cig, tobacco cigarette; WD, Western diet.

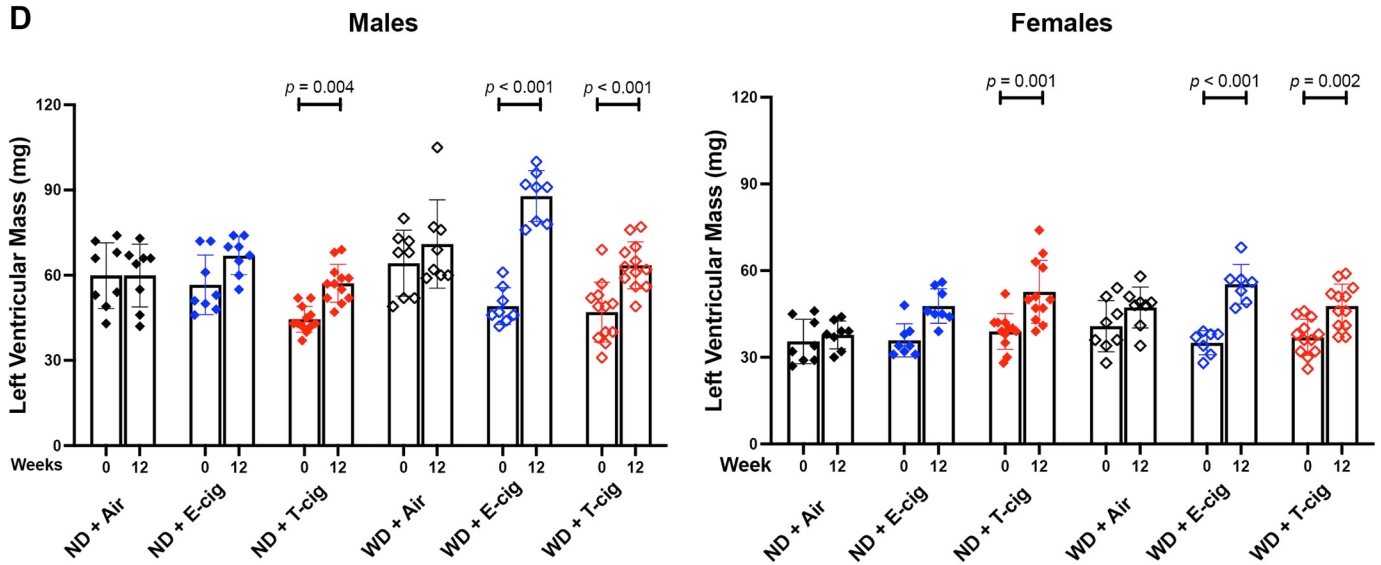


Figure 4. Continued

E-cig or T-cig exposure did not promote atherosclerotic lesions in both males and females. In contrast, on WD, T-cig-exposed male and female mice had greater lesions than their ND counterparts, whereas E-cig effect was observed only in male mice. Between the exposure sources, T-cig induced greater lesions than those exposed to air (control) or E-cig in females on WD ( $P < 0.001$ ). This effect was not observed in males on WD. Between the sexes, T-cig exposure combined with WD induced significantly greater lesions in females than males ( $P = 0.006$ ).

### Effects on Cardiac Structure and Function

The echocardiographic parameters for the male cohorts are listed in Tables 1 and 2 and the females in Tables 3 and 4. E-cig or T-cig exposure significantly reduced all three indices of left ventricular function (LVEF, LVFS, and Vcf) similarly

on both diets in both sexes (Tables 1–4, Fig. 4, A–C). The functional deficit was greater in the mice on WD compared with ND in both sexes. In the control (air) cohorts, only ESD was significantly affected in both male and female mice on WD, but not on ND. In the E-cig or T-cig-exposed cohorts, morphometric parameters (PWT, EDD, and ESD) were similarly affected in male and female mice on WD, but only EDD and ESD on ND. Interestingly, in both sexes, T-cig, but not E-cig, exposure adversely affected VST in mice on WD. There were also significant increases in LV mass in both sexes from the T-cig-exposed cohorts on both diets (Fig. 4D). The E-cig-exposed cohorts fed WD also had significant increases in LV mass in both sexes. The E-cig cohorts on ND trended to have greater mass, but did not reach statistical significance. Interestingly, LV mass was greater in E-cig + WD male cohort than in E-cig + WD

Table 1. Male echocardiographic data: ND

| Diet + Exposure | ND + Air  |           |        | ND + E-Cig |           |       | ND + T-Cig |           |        |
|-----------------|-----------|-----------|--------|------------|-----------|-------|------------|-----------|--------|
| Time, wk        | 0         | 12        | P      | 0          | 12        | P     | 0          | 12        | P      |
| No. of mice     | 8         | 8         |        | 8          | 8         |       | 12         | 12        |        |
| VST, mm         | 0.49±0.01 | 0.47±0.01 | >0.999 | 0.45±0.01  | 0.48±0.01 | 0.384 | 0.42±0.01  | 0.48±0.01 | <0.001 |
| PWT, mm         | 0.45±0.01 | 0.44±0.02 | 1.000  | 0.44±0.01  | 0.47±0.01 | 0.335 | 0.42±0.01  | 0.48±0.01 | <0.001 |
| EDD, mm         | 4.00±0.10 | 4.05±0.12 | 1.000  | 4.01±0.12  | 4.20±0.06 | 0.888 | 3.71±0.07  | 3.87±0.07 | 0.863  |
| ESD, mm         | 2.63±0.08 | 2.75±0.12 | 0.994  | 2.61±0.10  | 2.99±0.07 | 0.062 | 2.39±0.07  | 2.70±0.06 | 0.056  |
| AoET, ms        | 51.5±1.7  | 52.4±1.4  | 1.000  | 48.3±0.9   | 50.9±1.2  | 0.854 | 49.1±1.08  | 48.9±0.8  | 1.000  |
| HR, beats/min   | 513±15    | 518±13    | 1.000  | 586±10     | 562±8     | 0.978 | 543±19     | 544±9     | 1.000  |
| LV%FS           | 34.4±1.1  | 32.2±1.8  | 0.976  | 34.9±1.5   | 28.9±1.0  | 0.035 | 35.6±1.1   | 30.0±0.8  | 0.008  |
| Vcf, mm/s       | 6.74±0.39 | 6.13±0.27 | 0.909  | 7.28±0.43  | 5.70±0.22 | 0.008 | 7.31±0.34  | 6.17±0.24 | 0.030  |
| LVEF, %         | 70.5±1.5  | 66.4±2.7  | 0.776  | 71.3±1.4   | 62.5±1.3  | 0.012 | 71.2±1.5   | 64.5±1.2  | 0.024  |
| LV mass, mg     | 59.8±4.1  | 59.8±3.9  | 1.000  | 56.5±3.8   | 66.9±2.3  | 0.191 | 44.5±1.3   | 57.3±2.0  | 0.004  |
| E, m/s          | 0.72±0.06 | 0.65±0.04 | 0.996  | 0.63±0.05  | 0.66±0.06 | 1.000 | 0.54±0.04  | 0.60±0.04 | 0.997  |
| A, m/s          | 0.41±0.03 | 0.38±0.03 | 1.000  | 0.34±0.03  | 0.39±0.04 | 0.997 | 0.30±0.02  | 0.37±0.03 | 0.736  |
| E/A             | 1.74±0.07 | 1.73±0.08 | 1.000  | 1.88±0.09  | 1.74±0.08 | 0.993 | 1.82±0.06  | 1.63±0.06 | 0.725  |

Data is expressed as means ± SE. A, late (atrial) filling velocity; AoET, aortic ejection time; E, early filling velocity; E-cig, electronic cigarette; EDD, end diastolic dimension; EF, ejection fraction; ESD, end systolic dimension; FS, fractional shortening; HR, heart rate; ND, normal diet; PWT, posterior wall thickness; T-cig, tobacco cigarette; VST, ventricular septal thickness; Vcf, velocity of circumferential fiber shortening. *P* values compare data between week 0 and week 12 within each cohort, using four-way repeated measure ANOVA [factors: 1) diet, 2) sex, 3) treatments, and 4) time].

**Table 2. Male echocardiographic data: WD**

| Diet + Exposure | WD + Air  |           |        | WD + E-Cig |           |        | WD + T-Cig |           |        |
|-----------------|-----------|-----------|--------|------------|-----------|--------|------------|-----------|--------|
|                 | 0         | 12        | P      | 0          | 12        | P      | 0          | 12        | P      |
| No. of mice     | 8         | 8         |        | 8          | 8         |        | 12         | 12        |        |
| VST, mm         | 0.51±0.02 | 0.49±0.01 | 0.995  | 0.44±0.01  | 0.54±0.01 | <0.001 | 0.43±0.01  | 0.49±0.01 | <0.001 |
| PWT, mm         | 0.50±0.02 | 0.49±0.02 | 0.999  | 0.44±0.01  | 0.53±0.02 | <0.001 | 0.43±0.01  | 0.49±0.01 | <0.001 |
| EDD, mm         | 3.94±0.08 | 4.24±0.11 | 0.284  | 3.78±0.09  | 4.55±0.04 | <0.001 | 3.69±0.10  | 4.03±0.07 | 0.034  |
| ESD, mm         | 2.68±0.06 | 3.06±0.09 | 0.0468 | 2.50±0.10  | 3.41±0.05 | <0.001 | 2.38±0.10  | 2.93±0.06 | <0.001 |
| AoET, ms        | 52.4±0.6  | 49.8±1.2  | 0.887  | 49.5±1.1   | 46.5±1.6  | 0.737  | 50.5±1.3   | 49.5±0.6  | 0.999  |
| HR, beats/min   | 519±12    | 555±16    | 0.750  | 560±22     | 582±18    | 0.986  | 522±14     | 553±15    | 0.466  |
| LV%FS           | 32.0±0.7  | 27.8±0.6  | 0.319  | 34.0±1.3   | 25.0±1.2  | <0.001 | 36.7±1.7   | 27.3±0.5  | <0.001 |
| Vcf, mm/s       | 6.13±0.18 | 5.61±0.25 | 0.974  | 6.86±0.19  | 5.38±0.23 | 0.017  | 7.27±0.28  | 5.50±0.13 | <0.001 |
| LVEF, %         | 66.5±1.0  | 60.3±0.8  | 0.191  | 69.7±1.7   | 56.6±2.2  | <0.001 | 71.2±1.9   | 60.2±0.9  | <0.001 |
| LV mass, mg     | 64.3±4.2  | 71.1±5.5  | 0.773  | 49.2±2.3   | 88.0±3.2  | <0.001 | 47.1±3.1   | 63.7±2.6  | <0.001 |
| E, m/s          | 0.78±0.06 | 0.83±0.08 | 0.999  | 0.66±0.05  | 0.62±0.07 | 1.000  | 0.61±0.04  | 0.62±0.04 | 1.000  |
| A, m/s          | 0.48±0.05 | 0.48±0.05 | 1.000  | 0.41±0.05  | 0.35±0.03 | 0.966  | 0.35±0.03  | 0.37±0.02 | 1.000  |
| E/A             | 1.68±0.11 | 1.76±0.10 | 0.999  | 1.70±0.15  | 1.75±0.09 | 1.000  | 1.79±0.06  | 1.69±0.12 | 0.999  |

Data is expressed as means ± SE. A, late (atrial) filling velocity; AoET, aortic ejection time; E, early filling velocity; E-cig, electronic cigarette; EDD, end diastolic dimension; EF, ejection fraction; ESD, end systolic dimension; FS, fractional shortening; HR, heart rate; ND, normal diet; PWT, posterior wall thickness; T-cig, tobacco cigarette; Vcf, velocity of circumferential fiber shortening; VST, ventricular septal thickness. *P* values compare data between week 0 and week 12 within each cohort, using four-way repeated measure ANOVA [factors: 1) diet, 2) sex, 3) treatments, and 4) time].

female mice (*P* = 0.02). In male WD-fed mice, there was a greater LV mass change in E-cig exposure versus T-cig exposure (88.0 vs. 63.7 mg, *P* < 0.001; Table 2). There were no significant differences between males and females in any other parameter or in any of the other standard echo parameters between the cohorts [aortic ejection time (AoET), heart rate (HR), E, A, and the E/A].

## DISCUSSION

We previously reported adverse effects of chronic intermittent E-cig exposure on cardiovascular parameters in *Apoe*<sup>-/-</sup> and wild-type male mice (18–20). In this study, we directly compared the effects of T-cig versus E-cig, WD versus ND on body weight, caloric intake, and cardiovascular health in both sexes. The principal findings of this study suggest that E-cigs are not a safer alternative to T-cigs, as they also induced significant reductions in heart function and

potentially elicited enlarged left ventricles, particularly in WD-fed mice. T-cig exposure fostered greater atherosclerotic lesions than E-cig in female mice on WD after the 12-wk exposure, whereas E-cig-exposed males on WD had greater lesions than their male counterparts on ND.

*Apoe*<sup>-/-</sup> mice were chosen for the present study, as they are proven to be susceptible to atherosclerosis and cardiac pathology upon cigarette smoke or E-cig exposure (18, 22). To match our previous work and that of others (29–34), the age of initial exposure was kept at 9–10 wk, which is also roughly equivalent to adolescent to young adult human smokers. We also included equal numbers of female mice since there were only a few previous studies that reported using both sexes (34–36) or only females (33, 37).

We designed our protocol for cigarette exposure to mimic human smoking or vaping behavior. Since humans smoke while awake, all mouse exposures were also performed in the dark during their active circadian period. Exposure duration,

**Table 3. Female echocardiographic data: ND**

| Diet + Exposure | ND + Air  |           |       | ND + E-Cig |           |        | ND + T-Cig |           |        |
|-----------------|-----------|-----------|-------|------------|-----------|--------|------------|-----------|--------|
|                 | 0         | 12        | P     | 0          | 12        | P      | 0          | 12        | P      |
| No. of mice     | 8         | 8         |       | 8          | 8         |        | 12         | 12        |        |
| VST, mm         | 0.44±0.01 | 0.43±0.01 | 0.990 | 0.41±0.01  | 0.44±0.01 | 0.891  | 0.44±0.01  | 0.49±0.01 | 0.003  |
| PWT, mm         | 0.44±0.01 | 0.43±0.01 | 0.999 | 0.43±0.01  | 0.45±0.01 | 0.996  | 0.46±0.01  | 0.48±0.01 | 0.780  |
| EDD, mm         | 3.10±0.12 | 3.30±0.10 | 0.837 | 3.23±0.08  | 3.67±0.09 | 0.014  | 3.22±0.07  | 3.61±0.08 | 0.006  |
| ESD, mm         | 1.96±0.10 | 2.00±0.06 | 1.000 | 1.95±0.08  | 2.57±0.07 | <0.001 | 1.94±0.07  | 2.52±0.07 | <0.001 |
| AoET, ms        | 51.0±1.3  | 52.4±1.8  | 0.999 | 53.2±0.8   | 54.6±0.6  | 0.999  | 52.7±1.3   | 57.0±0.8  | 0.053  |
| HR, beats/min   | 501±18    | 497±17    | 1.000 | 522±13     | 518±6     | 1.000  | 491±14     | 510±14    | 0.981  |
| LV%FS           | 36.8±1.5  | 39.4±1.0  | 0.930 | 39.7±1.4   | 30.0±0.5  | <0.001 | 39.8±1.3   | 30.2±0.8  | <0.001 |
| Vcf, mm/s       | 7.30±0.48 | 7.57±0.35 | 0.999 | 7.47±0.28  | 5.50±0.10 | <0.001 | 7.62±0.32  | 5.31±0.15 | <0.001 |
| LVEF, %         | 72.5±2.0  | 76.5±1.3  | 0.786 | 76.6±1.8   | 63.7±0.9  | <0.001 | 76.8±1.4   | 64.5±1.3  | <0.001 |
| LV mass, mg     | 35.6±2.7  | 38.0±1.7  | 1.000 | 35.8±2.0   | 47.6±2.1  | 0.070  | 38.9±1.8   | 52.6±3.1  | 0.001  |
| E, m/s          | 0.60±0.03 | 0.77±0.05 | 0.300 | 0.57±0.03  | 0.57±0.05 | 1.000  | 0.62±0.05  | 0.62±0.04 | 1.000  |
| A, m/s          | 0.40±0.03 | 0.47±0.03 | 0.893 | 0.32±0.02  | 0.33±0.03 | 1.000  | 0.36±0.03  | 0.36±0.03 | 1.000  |
| E/A             | 1.56±0.06 | 1.66±0.08 | 0.999 | 1.81±0.08  | 1.72±0.07 | 0.999  | 1.77±0.08  | 1.79±0.11 | 1.000  |

Data is expressed as means ± SE. A, late (atrial) filling velocity; AoET, aortic ejection time; E, early filling velocity; E-cig, electronic cigarette; EDD, end diastolic dimension; EF, ejection fraction; ESD, end systolic dimension; FS, fractional shortening; HR, heart rate; ND, normal diet; PWT, posterior wall thickness; T-cig, tobacco cigarette; Vcf, velocity of circumferential fiber shortening; VST, ventricular septal thickness. *P* values compare data between week 0 and week 12 within each cohort, using four-way repeated measure ANOVA [factors: 1) diet, 2) sex, 3) treatments, and 4) time].

**Table 4.** Female echocardiographic data: WD

| Diet + Exposure | WD + Air  |           |       | WD + E-Cig |           |        | WD + T-Cig |           |        |
|-----------------|-----------|-----------|-------|------------|-----------|--------|------------|-----------|--------|
|                 | 0         | 12        | P     | 0          | 12        | P      | 0          | 12        | P      |
| No. of mice     | 8         | 8         |       | 7          | 7         |        | 12         | 12        |        |
| VST, mm         | 0.44±0.01 | 0.44±0.01 | 1.000 | 0.43±0.01  | 0.48±0.02 | 0.163  | 0.44±0.01  | 0.46±0.01 | 0.735  |
| PWT, mm         | 0.47±0.01 | 0.45±0.01 | 0.962 | 0.41±0.01  | 0.48±0.01 | <0.001 | 0.42±0.01  | 0.48±0.01 | <0.001 |
| EDD, mm         | 3.28±0.11 | 3.65±0.12 | 0.070 | 3.19±0.09  | 3.77±0.06 | <0.001 | 3.24±0.09  | 3.60±0.08 | 0.016  |
| ESD, mm         | 2.08±0.15 | 2.46±0.17 | 0.046 | 1.99±0.10  | 2.67±0.04 | <0.001 | 2.01±0.08  | 2.59±0.08 | <0.001 |
| AoET, ms        | 54.6±2.5  | 53.7±0.7  | 1.000 | 51.7±1.8   | 50.9±1.0  | 1.000  | 50.2±1.1   | 52.4±1.1  | 0.862  |
| HR, beats/min   | 514±20    | 519±6     | 1.000 | 522±20     | 547±11    | 0.982  | 548±11     | 522±12    | 0.861  |
| LV%FS           | 37.1±3.0  | 32.9±2.5  | 0.353 | 37.9±1.8   | 29.1±0.8  | <0.001 | 38.0±0.9   | 28.1±0.7  | <0.001 |
| Vcf, mm/s       | 6.85±0.54 | 6.12±0.43 | 0.766 | 7.41±0.51  | 5.74±0.21 | 0.009  | 7.63±0.27  | 5.39±0.17 | <0.001 |
| LVEF, %         | 72.6±3.5  | 67.8±3.9  | 0.573 | 73.6±2.1   | 62.5±1.3  | 0.001  | 74.6±0.9   | 61.5±1.0  | <0.001 |
| LV mass, mg     | 40.8±3.1  | 47.3±2.5  | 0.813 | 34.9±1.6   | 55.1±2.7  | <0.001 | 37.0±1.8   | 50.3±2.7  | 0.002  |
| E, m/s          | 0.65±0.06 | 0.58±0.07 | 0.989 | 0.57±0.06  | 0.65±0.06 | 0.984  | 0.60±0.03  | 0.63±0.04 | 0.999  |
| A, m/s          | 0.33±0.04 | 0.36±0.05 | 0.999 | 0.34±0.05  | 0.37±0.04 | 1.000  | 0.38±0.04  | 0.37±0.03 | 1.000  |
| E/A             | 2.06±0.12 | 1.68±0.08 | 0.091 | 1.71±0.08  | 1.82±0.13 | 0.999  | 1.66±0.11  | 1.75±0.09 | 0.999  |

Data is expressed as means ± SE. A, late (atrial) filling velocity; AoET, aortic ejection time; E-cig, electronic cigarette; E, early filling velocity; EDD, end diastolic dimension; EF, ejection fraction; ESD, end systolic dimension; FS, fractional shortening; HR, heart rate; ND, normal diet; PWT, posterior wall thickness; T-cig, tobacco cigarette; Vcf, velocity of circumferential fiber shortening; VST, ventricular septal thickness. *P* values compare data between week 0 and week 12 within each cohort, using four-way repeated measure ANOVA [factors: 1) diet, 2) sex, and 3) treatments, and 4) time].

aerosol concentration, nicotine formulation (freebase or salt-based), and blood procurement methods are critical to obtaining consistent and meaningful dosing levels of nicotine (10, 16, 21, 38, 39). Therefore, in our study, cardiac puncture was used for blood collection to obtain enough quantity of plasma and to avoid contamination with tissue fluid. Plasma levels of nicotine confirmed via mass spec analysis that both E-cig and T-cig-treated mice received similar exposures in the same range as moderate to heavy human smokers (16, 27, 28).

We rigorously monitored each mouse's weight and food intake to ensure uniform growth and caloric intake for each cohort. WD was used in this study as it more closely mimics human dietary intake, and it was compared with ND. Thus, caloric intake was also analyzed to investigate its potential additive effects. The results show that all WD-fed cohorts exhibited significant weight gain via delta analysis and five of the six by slope analysis during the 12-wk study period whereas the corresponding ND-fed cohorts did not. Furthermore, there were sex differences in that three of the male cohorts had significantly greater weight gain than their female counterparts. Such differences could be related to sex hormone modulation of weight in addition to diet and nicotine exposure differences. Several other studies reported a range of weight gain data from none to significantly changed in E-cig and T-cig-exposed mice (29, 33, 34, 36, 38, 40). The differences between our study and the other studies likely lie in the choice of specific mouse models, specific diets, exposure protocols, and effective nicotine dosages. Relative to weight gain, the caloric intake in males increased (significant positive slopes) in the ND E-cigs, ND T-cigs, and WD T-cigs cohorts during the study period. The WD-fed air control and WD T-cig-treated female mice showed a significant increase in caloric intake, whereas the E-cig-treated cohort was not significant. All three female ND-fed cohorts showed no significant changes over the 12-wk study.

Although the increased propensity for the development of atherosclerotic lesions in T-cig smokers is well documented in humans (3, 22), the comparative effects of nicotine delivery via either T-cig or E-cig on lesion development are not

clear. In this study, we found sex- and diet-dependent effects of exposures to E-cigs or T-cigs. T-cig-exposed males and females on WD had greater lesions than their counterparts on ND, whereas E-cig effects were seen only in males. In addition, between the two exposure sources, T-cig appears to be more potent at inducing atherosclerosis than E-cig in females on WD. T-cig WD females had significantly greater lesion formation than their male counterparts (*P* = 0.006). It has been previously shown that factors such as mouse models, exposure systems, and durations, as well as nicotine formulations, can affect the magnitude of pathological changes (10, 22, 39). This study and our previous studies used E-cigs with a 2.4% freebase nicotine that has a lower effective nicotine concentration than exposures from newer nicotine-salt based E-cigs that have a more neutral pH (10). In the current study, we did not observe a significant increase in lesion formation from E-cig exposure than control, which contrasts with our prior study (18). This difference may be due to shorter cumulative E-cig-exposure time between the two studies (10 vs. 12 h daily over the 12 wk). In fact, Szostak et al. (37) reported that female *Apoe*<sup>-/-</sup> mice exposed to both T-cig and E-cig at 3 h/day, 5 days/wk for 6 mo developed atherosclerotic lesions, but only the T-cig-exposed mice were statistically greater than control. Also, in a different hyperlipidemic mouse model with twice daily exposures for 16 wk, Caruana et al. (36) reported that E-cig increased atherosclerosis in male, but not female mice, similar to our findings. Previously, that group found no increase in lesion size when exposed for a shorter 4-wk duration at a 5% nicotine salt concentration (40).

Echocardiographic findings are consistent with our earlier results seen in male mice (18–20). This current study extended the investigation to include female mice for E-cig exposures, as well as to both sexes exposed to T-cigs on both ND and WD diets. The most striking results are that mice exposed to E-cigs had just as much ventricular dysfunction as mice exposed to T-cigs. All three functional indices (LVEF, LVFS, and Vcf) were reduced by E-cig or T-cig exposure in both sexes on both diets. In addition, the LV mass, an index of hypertrophy, increased in the 4 cohorts exposed to

T-cigs on both diets in both sexes. Only the ND + E-cig mice of both sexes did not have significant increases. The LV mass data suggest a hypertrophic response in left ventricles to nicotine exposures in those six cohorts. The heart rates and ejection times during the imaging were not significantly different and within a normal physiological range. This ensures that the echo data are valid representations of morphometry and function under physiological conditions. The heart chamber dimensions were consistent with aging and exposures, showing some significant changes. There were no significant differences in the E, A, E/A ratios, suggesting that there was no induction of diastolic dysfunction with this exposure protocol. Overall, the echocardiographic analysis suggests that cardiac function is significantly altered within just 3 mo, independent of sex and diet, with levels of nicotine exposure similar to those of moderate to heavy human smokers/vapers.

Prior long-term studies from other investigators have shown variable results of cardiac structure and function that are likely due to differences in exposure levels, durations, mouse models, and diets. Most studies were with C57BL/6 male mice, others with *Apoe*<sup>-/-</sup> or FVB mice, and very few with female only, or both male and female mice (33–37). The exact exposure protocols also varied widely, and not all the studies directly assessed nicotine levels to permit comparisons. Among the earlier reports, Olfert et al. (33) noted a significant reduction in ventricular function after an 8-mo exposure of female mice with T-cigs, which is consistent with our findings. However, they only found a trend in ventricular function change and a modest increase in LV mass with E-cigs, probably due to a different exposure protocol and lower measured nicotine levels with E-cigs than in our study. Similarly, El-Mahdy et al. (38) also reported significant functional reductions with T-cigs, but not in generally lower dose E-cig exposures, in their 60-wk long study on male mice. They also noted a significant increase in LV mass with both T-cigs and E-cigs. In another comparison study, only in female *Apoe*<sup>-/-</sup> mice with exposures to T-cig or E-cig, Szostak et al. (37) reported significant reductions in ventricular function with T-cig, but not with E-cigs at 2-, 4-, and 6-mo time points, in contrast to our results. On the other hand, Neczypor et al. (35) reported fractional shortening and E/A ratio decrease in adolescent male, but not in female FVB mice, in contrast to our findings. Recently, in a 6-mo E-cig/T-cig comparison study in male C57BL6 mice, Dai et al. (29) reported adverse cardiovascular and pulmonary structural effects but no changes in heart function, contrary to our findings. In shorter-duration studies with 4-wk exposure, Fried et al. (31) found no significant changes in ventricular function with bubbled nicotine exposure in male mice. Recently, Whitehead et al. (34) reported functional alterations in E/A ratios, blood pressures, weights, and heart rates with a continuous exposure protocol in both male and female C57BL/6 mice on a high-fat diet. Except for E/A ratios, their results are consistent with our overall findings that nicotine exposures, along with a diet containing higher fat content, contribute to cardiovascular dysfunction.

As previously noted, nicotine is the primary active ingredient in both E-cigs and T-cigs that induces a variety of damaging changes to cardiovascular (and other) systems. This is largely due to its triggering a panoply of detrimental responses leading to a proinflammatory phenotype

and oxidative stress, as detailed previously in a review by our group (13). That does not rule out other contributing factors besides nicotine that potentially contribute to the observed detrimental alterations in this and other studies. Such factors in tobacco smoke or E-cig aerosols include particulate matter, heavy metals, residues from chemicals or vehicles postheating, flavorings, and other unknowns. In addition, the role of hormones (estrogen and testosterone) can certainly play a role in the sex differences reported here. Clearly, future E-cig studies should continue to analyze both sexes and to characterize the physical properties, such as particle size distributions, and chemical constituents of the aerosol from the chosen devices. It is particularly important since newer mod-pod devices use an effectively higher concentration of nicotine salts (10), which are more addictive and potentially more damaging.

In summary, our present study clearly suggests that electronic cigarettes (E-cigs) are not a safer alternative to conventional tobacco cigarettes (T-cigs) since they also reduced cardiac function and induced LV hypertrophy with 3 mo of daily exposures to tobacco smoke or E-cig aerosol in both male and female mice. In addition, Western diets, which contain higher fat content, tend to exacerbate cardiac dysfunction. Finally, it is worth noting that male and female mice respond differentially to the nicotine exposures by delivery sources (E-cig vs. T-cig). Further studies are needed to better understand the underlying sex- and diet-dependent mechanisms of nicotine exposure.

## DATA AVAILABILITY

Data will be made available upon reasonable request.

## SUPPLEMENTAL MATERIAL

Supplemental Fig. S1: <https://doi.org/10.6084/m9.figshare.30015274>.

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## DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

## AUTHOR CONTRIBUTIONS

C.J.L., M.C.J., Y.T., X.M.S., T.C.F., and K.P.R. conceived and designed research; C.J.L., M.C.J., J.C.R., Y.T., K.L., W.R.-T., T.Y., B.P., C.H., A.L., S.L.W., T.N., V.C., A.K., V.C.C., X.M.S., and K.P.R. performed experiments; C.J.L., M.C.J., J.C.R., Y.T., K.L., W.R.-T., C.H., T.N., X.M.S., and K.P.R. analyzed data; C.J.L., M.C.J., J.C.R.,

Y.T., K.L., X.M.S., T.C.F., and K.P.R. interpreted results of experiments; C.J.L., J.C.R., Y.T., X.M.S., and K.P.R. prepared figures; C.J.L., Y.T., X.M.S., and K.P.R. drafted manuscript; C.J.L., M.C.J., Y.T., X.M.S., T.C.F., and K.P.R. edited and revised manuscript; C.J.L., Y.T., X.M.S., T.C.F., and K.P.R. approved final version of manuscript.

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