

# Electronic cigarette exposure on insulin sensitivity of ApoE gene knockout mice

Kai Lan<sup>1\*</sup>, Guangwei Zhang<sup>2,3,4,5\*</sup>, Lijuan Liu<sup>3</sup>, Ziwei Guo<sup>3</sup>, Xianyu Luo<sup>3</sup>, Hua Guan<sup>4</sup>, Qi Yu<sup>4</sup>, Enqi Liu<sup>4,5</sup>

## ABSTRACT

**INTRODUCTION** The current study aimed to investigate the effects of electronic cigarettes on insulin sensibility in ApoE gene knockout mice.

**METHODS** In total, 48 male ApoE gene knockout mice were randomly divided into four exposure groups: 1) electronic cigarette (e-cigarette) containing 12 mg/mL of nicotine, 2) e-cigarette without nicotine (0mg), 3) traditional cigarette (cigarette), and 4) fresh air (control). The first three groups were exposed to the associated smoke for 18 weeks. The body weight was recorded regularly in the four groups. After the last exposure, the concentrations of lipids, hs-CRP and TNF- $\alpha$  in serum were detected and the effect of electronic cigarettes on insulin tolerance was measured.

**RESULTS** The levels of serum lipid, hs-CRP and TNF- $\alpha$  in the e-cigarette, 0mg and cigarette groups were significantly increased compared with those in the control group ( $p < 0.05$ ). Also, the insulin tolerance in the e-cigarette, 0mg and cigarette groups was significantly decreased compared to that in the control group ( $p < 0.05$ ).

**CONCLUSIONS** Electronic cigarettes showed comparable effects to traditional cigarettes in influencing the metabolic functions in ApoE gene knockout mice.

## AFFILIATION

1 Department of Clinical Medicine, Xi'an Medical University, Xi'an, China

2 Department of Basic Medical Science, Xi'an Medical University, Xi'an, China

3 School of Public Health, Xi'an Medical University, Xi'an, China

4 Institute of Basic and Translational Medicine, Xi'an Medical University, Xi'an, China

5 Laboratory Animal Center, Xi'an Jiaotong University Health Science Center, Xi'an Jiaotong University, Xi'an, China

\*Co-first authors

## CORRESPONDENCE TO

Guangwei Zhang, Department of Basic Medical Science, Xi'an Medical University, Xi'an 710021, Shaanxi Province, China. E-mail: zgw\_1979@163.com

## KEYWORDS

electronic cigarette, smoke exposure, diabetes, inflammatory injury, insulin sensibility

Received: 7 March 2020

Revised: 6 June 2020

Accepted: 14 July 2020

## INTRODUCTION

The change of insulin sensibility is an important pathogenesis of type 2 diabetes. Although a number of studies have shown that cigarette smoking is closely related to metabolic diseases such as diabetes, it still needs further investigation to ascertain if smoking affects insulin sensibility<sup>1-3</sup>. With the emergence of electronic cigarettes (e-cigarettes) on the market as a substitute for traditional cigarettes, an

increasing large population has become consumers of e-cigarettes<sup>4,5</sup>. Therefore, it is of great importance to fully understand the potential hazards of e-cigarettes. Nevertheless, the information about potential hazards is insufficient, especially the effects on body metabolism. Many studies have indicated that the levels of blood lipid and chronic inflammatory indexes are closely related to insulin sensibility. ApoE gene knockout mice are frequently used to investigate

cardiovascular diseases; it is an ideal animal model to study the diseases related to the changes of blood lipids<sup>6</sup>. Therefore, the aim of the current study is to explore the effect of e-cigarette exposure on insulin sensibility based on the determination of serum lipids and chronic inflammatory indexes in order to clarify the potential hazards of e-cigarettes.

## METHODS

### Animals and smoking exposure

Forty-eight male ApoE knockout mice aged 6 weeks were purchased from the Experimental Animal Center of Xi'an Jiaotong University. According to the suggested formula containing 0.15% of cholesterol and 21% of fat (Wako, Japan), the diet used in the current study was prepared and supplied by Beijing Keao Xieli Feed Co, Ltd (Beijing, China). All the mice were randomly divided into four exposure groups: 1) e-cigarettes containing 12 mg/mL of nicotine (e-cigarette), 2) electronic cigarettes without nicotine (0 mg), 3) traditional cigarettes (cigarette), and 4) fresh air (control). We used a bionic simulation of human respiratory system smoking generating device (provided by the Central Laboratory of the Public Health College of Xi'an Medical College). A gas smoke generating device was docked with the intake port to ensure an airtight seal. The balloon device simulated the human respiratory system in its ability to absorb target gas, and the smoke was discharged from the outlet to the intake port. The e-cigarette group was given commercial electronic cigarettes with a 12 mg/mL nicotine content, while the 0mg group was given the same type of e-cigarette but without the nicotine content. The cigarette group was given ordinary commercial filter cigarettes with 12 mg tar and 0.8 mg nicotine content. The control group was given fresh air. Smoking exposure was conducted three times a day, 30 min each time, for a period of 18 weeks. Body weights were recorded once every two weeks. During the assay of blood glucose, the blood samples were collected from the tail vein. At the end of the experiment, the mice were sacrificed followed by collection of blood via cardiac puncture after anaesthesia. Blood was centrifuged at 2000 rpm at 4°C for 20 min and serum samples were stored at -80°C. All animal experiments were performed in accordance with the guidelines of the Xi'an Medical University (Shaanxi, China), and were approved by

the Ethics Committee of Xi'an Medical University (XYLS2018061). The present study was performed in accordance with the recommendations of the Council for International Organization of Medical Sciences. The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

### Detection of blood lipids and inflammatory indexes

The levels of total cholesterol, triglyceride, low-density lipoprotein and high-density lipoprotein were determined by automatic biochemical analyzer. Hypersensitive C-reactive protein and serum tumor necrosis factor-alpha were determined by ELISA (Wuhan Huamei Bioengineering Co., Ltd, Wuhan, China) according to the instructions.

### Effect of e-cigarette on insulin tolerance

The insulin dosage in the insulin tolerance test was 0.7 U/kg Insulin diluted with normal saline at a concentration of 0.7 U/mL. Blood glucose was measured at 0, 15, 30, 60, 90, 120 and 150 min after insulin injection in each group. Using Graph Pad Prism 6.0 software, taking time as abscissa and the blood glucose value measured at each time point as ordinate, a blood glucose curve was generated and the area under the curve calculated.

### Statistical analysis

Data were expressed as mean  $\pm$  SD. Differences between groups were evaluated by analysis of variance (ANOVA) with an unpaired t-test. Graph Pad Prism 6.0 was used for statistical analysis. A  $p < 0.05$  was considered to be statistically significant.

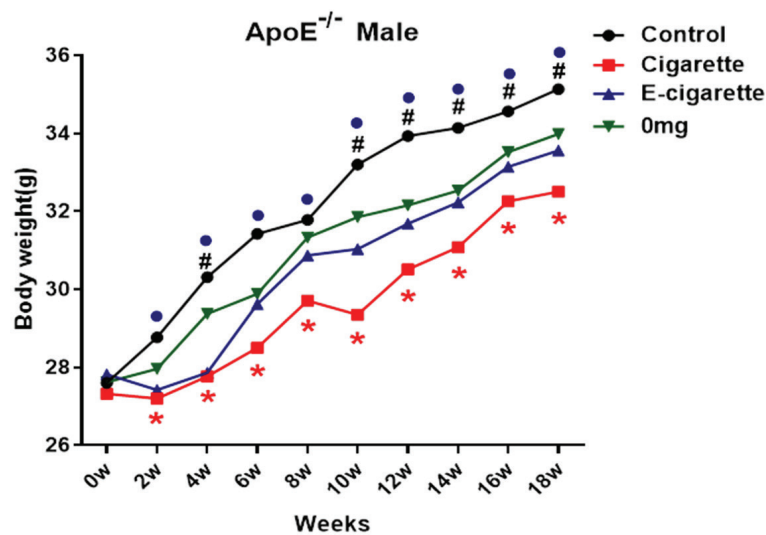
## RESULTS

### Effect of e-cigarette on body weight

After 18 weeks of exposure, changes in the body weight of mice in the e-cigarette, 0mg, and cigarette groups, were lower than in the control group, and differences were statistically significant ( $p < 0.05$ ; Figure 1).

### Effect of e-cigarette on blood lipids

After 18 weeks of smoking exposure, the concentrations of blood lipids in the exposure groups are shown in Table 1. The levels of total

Figure 1. Body weight changes of ApoE<sup>-/-</sup> mice during smoke exposure for 18 weeks (n=12)

Data are expressed as mean  $\pm$  SD. \* Cigarette vs control,  $p < 0.05$ . # E-cigarette vs control,  $p < 0.05$ . # 0mg vs control,  $p < 0.05$ .

**Table 1. Concentrations (mg/dL) of blood lipids in ApoE<sup>-/-</sup> mice in four tested groups after 18 weeks of smoke exposure (n=12)**

| Group       | TC                                | TG                                | LDL-C                           | HDL-C                         |
|-------------|-----------------------------------|-----------------------------------|---------------------------------|-------------------------------|
| E-cigarette | 749.31 $\pm$ 29.78 <sup>a,b</sup> | 623.71 $\pm$ 24.12 <sup>a,b</sup> | 453.59 $\pm$ 15.32 <sup>a</sup> | 26.47 $\pm$ 2.66 <sup>a</sup> |
| 0mg         | 731.97 $\pm$ 28.17 <sup>a,b</sup> | 614.68 $\pm$ 22.89 <sup>a,b</sup> | 451.56 $\pm$ 15.61 <sup>a</sup> | 27.07 $\pm$ 2.75 <sup>a</sup> |
| Cigarette   | 853.13 $\pm$ 37.93 <sup>a</sup>   | 705.34 $\pm$ 26.93 <sup>a</sup>   | 462.63 $\pm$ 15.83 <sup>a</sup> | 25.56 $\pm$ 2.87 <sup>a</sup> |
| Control     | 614.72 $\pm$ 21.87 <sup>b</sup>   | 418.57 $\pm$ 14.11 <sup>b</sup>   | 417.39 $\pm$ 13.27 <sup>b</sup> | 39.62 $\pm$ 3.12 <sup>b</sup> |

TC: total cholesterol; TG: triglyceride; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol. a Compared with the control group,  $p < 0.05$ . b Compared with the cigarette group,  $p < 0.05$ .

cholesterol, triglyceride and low-density lipoprotein were significantly increased in the e-cigarette, 0mg and cigarette groups, compared to the control group ( $p < 0.05$ ). Also, The HDL levels in the exposure groups were lower compared to the control group and differences were statistically significant ( $p < 0.05$ ).

#### Effect of e-cigarette on inflammatory indexes

After 18 weeks of exposure, the concentrations of hs-CRP and TNF- $\alpha$  in the exposure groups are summarized in Table 2. It was found that the traditional cigarette, the electronic cigarette with 12 mg/mL of nicotine and without nicotine, significantly elevated the levels of hs-CRP and TNF- $\alpha$  ( $p < 0.05$ ).

#### Effect of e-cigarette on insulin tolerance

The concentrations of blood glucose in the exposure

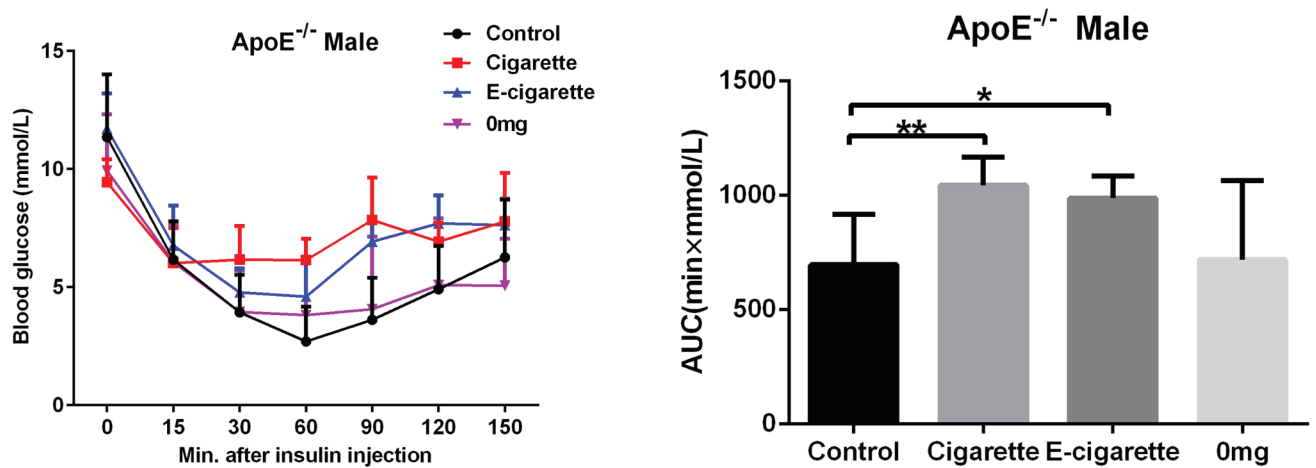
groups are plotted versus time in Figure 2 (left). For the control group versus cigarette group, the blood glucose number was statistically different at 60 and 90 min after insulin injection, while for the control group versus e-cigarette group, the blood glucose number

**Table 2. Concentrations (ng/mL) of serum hs-CRP and TNF- $\alpha$  in ApoE<sup>-/-</sup> mice in four tested groups after 18 weeks of smoke exposure (n=12)**

| Group       | hs-CRP                       | TNF- $\alpha$                  |
|-------------|------------------------------|--------------------------------|
| E-cigarette | 4.74 $\pm$ 0.59 <sup>a</sup> | 2.14 $\pm$ 0.27 <sup>a,b</sup> |
| 0mg group   | 4.65 $\pm$ 0.57 <sup>a</sup> | 1.95 $\pm$ 0.25 <sup>a,b</sup> |
| Cigarette   | 4.98 $\pm$ 0.61 <sup>a</sup> | 2.83 $\pm$ 0.31 <sup>a</sup>   |
| Control     | 3.21 $\pm$ 0.39 <sup>b</sup> | 1.73 $\pm$ 0.23 <sup>b</sup>   |

a Compared with the control group,  $p < 0.05$ . b Compared with the cigarette group,  $p < 0.05$ .

Figure 2. Effects of cigarette exposure on the insulin tolerance of ApoE<sup>-/-</sup> mice in four tested groups (n=12)



Data are expressed as mean  $\pm$  SD. Comparisons were conducted with control group: \* $p < 0.05$ ; \*\* $p < 0.01$ .

was statistically different at 90 and 120 min after insulin injection. The decline in the blood glucose curve in the cigarette group and the e-cigarette group was significantly slowed down. The areas under the curve (AUCs) were calculated and are plotted in Figure 2 (right). It was found that e-cigarette with 12 mg/mL nicotine and traditional cigarette significantly enhanced the AUCs, whereas e-cigarette without nicotine did not increase the AUC compared to the control group, indicating that insulin tolerance was significantly decreased by the e-cigarette with 12 mg/mL nicotine and by the traditional cigarette.

## DISCUSSION

A decrease in insulin sensitivity is the initial pathology of type 2 diabetes. It is found that the enlarged adipocytes attract macrophages and secrete inflammatory molecules, while the glucose utilization in the body is affected by blocking the insulin signal transduction in skeletal muscle via Jun N-terminal kinase. It was reported that traditional cigarettes cause dyslipidaemia, increase the inflammatory mediator and decrease insulin sensibility<sup>7</sup>. According to a large national study in China, the prevalence of diabetes in regular smokers is 15–30% higher than in non-smokers<sup>8</sup>. There is little research on the effect of e-cigarettes on the body metabolism.

Based on the results of the current study, it was found that both traditional cigarettes and electronic

cigarettes could affect blood levels of lipids and inflammatory factors. The dyslipidemia induced by smoking might be due to harmful substances in smoke stimulating the release of adrenaline from the adrenal cortex and so increasing the oxidative decomposition of adipose tissue. Therefore, the elevated free fatty acids stimulate the synthesis of triglycerides and low-density lipoproteins by hepatocytes and inhibit the synthesis of high-density lipoproteins by hepatic microsomes, which lead to dyslipidemia. Other studies have indicated that harmful substances in traditional smoke induce the activation and degranulation of mast cells in the respiratory tract, promoting the inflammation of airways<sup>9–11</sup>. Electronic cigarettes simulate the process of emission and inhaling of traditional cigarettes, using modern microelectronics technologies. Therefore, the combustion of cigarettes could be avoided with reduced emission of harmful substances such as tar, carbon monoxide etc.<sup>12,13</sup> As a result, electronic cigarettes have been launched in the market as a substitute to traditional cigarettes. Although the composition of electronic liquid is relatively simple and there is no combustion process, a number of harmful components are produced in the process of atomization at high temperature, including acrolein, formaldehyde hemiacetals, and specific nitrosamines in tobacco<sup>14,15</sup>. In the current study, the effect of e-cigarettes without nicotine on the body metabolism was investigated in addition to e-cigarettes

containing nicotine and traditional cigarettes. It was found that the levels of blood lipids and inflammatory factors were also significantly enhanced after exposure to e-cigarettes without nicotine. In the absence of nicotine, the physicochemical irritation of particles and alcohol compounds may affect the activity of repair proteins in lung tissue followed by damage to the respiratory system and induction of inflammation<sup>16,17</sup>. Hahn et al.<sup>18</sup> determined the contents of major components in 54 electronic liquids and found that 1,2-propanediol and glycerol were the most abundant solvents. Although they are considered to be safe solvents via oral administration, some chemical reactions might be induced in the process of atomization of the electronic liquid. Propylene glycol could change into propylene oxide, which is classified as a B2B carcinogen by the International Cancer Research Institute. Glycerol could change to acrolein, which stimulates the upper respiratory tract and promotes inflammation<sup>19,20</sup>.

A number of studies have indicated that the enhanced level of TNF- $\alpha$  promotes the phosphorylation of serine and threonine, triggering the degradation of insulin receptor substrates. The phosphorylation and the tyrosine kinase activity of insulin receptors is reduced, which induces the decrease of insulin sensibility<sup>21,22</sup>. According to the experiment of insulin tolerance, traditional cigarettes and electronic cigarettes with 12 mg/mL of nicotine significantly enhanced the insulin tolerance, whereas the electronic cigarette without nicotine showed no effect on the insulin tolerance compared to the fresh air control group. Other literature reported that nicotine and other alcohol compounds undergo complicated biological reactions in vivo and their metabolites cause DNA damage of repair proteins. Consequently, lesions on the respiratory tract and pulmonary functions cannot be efficiently repaired, resulting in repeated damage of the respiratory system<sup>23-25</sup>. The chronic toxic effect on the respiratory system by the generated metabolites would induce sustained and chronic damage to the respiratory system, causing damage of the vascular endothelial cells and increased coagulation of the blood vessels as well as reduced blood volume in skeletal muscles. Therefore, the insulin-mediated uptake of glucose is decreased, leading to reduced insulin sensibility<sup>26-28</sup>. Further experiments are planned to elucidate potential mechanisms.

## CONCLUSIONS

Traditional cigarettes and electronic cigarettes with/without nicotine increased the levels of blood lipids and chronic inflammation indexes and decreased the insulin sensibility in ApoE knockout mice, suggesting an effect of electronic cigarettes on body metabolism. The health hazards of electronic cigarettes should be carefully evaluated in order to supplement policies for public health.

## REFERENCES

1. The InterAct Consortium, Spijkerman AMW, van der A DL, et al. Smoking and long-term risk of type 2 diabetes: the EPIC- InterAct study in European populations. *Diabetes Care*. 2014;37(12):3164-3171. doi:10.2337/dc14-1020
2. Maziak W, Taleb ZB, Bahelah R, et al. The global epidemiology of waterpipe smoking. *Tob Control*. 2015;24:i3-i12. doi:10.1136/tobaccocontrol-2014-051903
3. Chen Z, Peto R, Zhou M, et al. Contrasting male and female trends in tobacco-attributed mortality in China: evidence from successive nationwide prospective cohort studies. *Lancet*. 2015;386:1447-1456. doi:10.1016/s0140-6736(15)00340-2
4. Zhang GW, Wang Z, Zhang K, et al. Safety Assessment of Electronic Cigarettes and Their Relationship with Cardiovascular Disease. *Int J Environ Res Public Health*. 2018;15:75. doi:10.3390/ijerph15010075
5. Bullen C, Howe C, Laugesen M, et al. Electronic cigarettes for smoking cessation: a randomised controlled trial. *Lancet*. 2013;382(9905):1629-1637. doi:10.1016/s0140-6736(13)61842-5
6. Titz B, Boué S, Phillips B, et al. Effects of Cigarette Smoke, Cessation, and Switching to Two Heat-Not-Burn Tobacco Products on Lung Lipid Metabolism in C57BL/6 and Apoe<sup>-/-</sup> Mice—An Integrative Systems Toxicology Analysis. *Toxicol Sci*. 2016;149(2):441-457. doi:10.1093/toxsci/kfv244
7. van Staden SR, Groenewald M, Engelbrecht R, Becker PJ, Hazelhurst LT. Car-boxyhaemoglobin levels, health and lifestyle perceptions in smokers converting from tobacco cigarettes to electronic cigarettes. *S Afr Med J*. 2013;103(11):865-868. doi:10.7196/samj.6887
8. Liu X, Bragg F, Yang L, et al. Smoking and smoking cessation in relation to risk of diabetes in Chinese men and women: a 9-year prospective study of 0.5 million people. *Lancet Public Health*. 2018;3(4):e167-e176. doi:10.1016/S2468-2667(18)30026-4
9. Erhardt L. Cigarette smoking: an undertreated risk factor for cardiovascular disease. *Atherosclerosis*. 2009;205(1):23-32. doi:10.1016/j.atherosclerosis.2009.01.007
10. Vardavas CI, Panagiotakos DB. The causal relationship between passive smoking and inflammation on the development of cardiovascular disease: a review of the

- evidence. *Inflamm Allergy Drug Targets*. 2009;8(5):328-333. doi:10.2174/1871528110908050328
11. Vansickel AR, Cobb CO, Weaver MF, Eissenberg TE. A clinical laboratory model for evaluating the acute effects of electronic 'cigarettes': Nicotine delivery profile and cardiovascular and subjective effects. *Cancer Epidemiol Biomarkers Prev*. 2010;19(8):1945-1953. doi:10.1158/1055-9965.epi-10-0288
  12. Canistro D, Vivarelli F, Cirillo S, et al. E-cigarettes induce toxicological effects that can raise the cancer risk. *Sci Rep*. 2017;7(1):2028. doi:10.1038/s41598-017-02317-8
  13. Mao J, Liu J, Pang X, et al. Nicotine induces the expression of C-reactive protein via MAPK-dependent signal pathway in U937 macrophages. *Mol Cells*. 2012;34(5):457-461. doi:10.1007/s10059-012-0205-0
  14. Xue J, Yang S, Seng S. Mechanisms of Cancer Induction by Tobacco-Specific NNK and NNN. *Cancers*. 2014;6(2):1138-1156. doi:10.3390/cancers6021138
  15. Flouris AD, Poulianiti KP, Chorti MS, et al. Acute effects of electronic and tobacco cigarette smoking on complete blood count. *Food Chem Toxicol*. 2012;50(10):3600-3603. doi:10.1016/j.fct.2012.07.025
  16. Kaiser MA, Villalba H, Prasad S, et al. Offsetting the impact of smoking and e-cigarette vaping on the cerebrovascular system and stroke injury: Is Metformin a viable countermeasure? *Redox Biol*. 2017;13:353-362. doi:10.1016/j.redox.2017.06.006
  17. Kuehn BM. Emerging Data Show E-Cigarettes May Pose Heart Risk. *Circulation*. 2017;136(2):232-233. doi:10.1161/circulationaha.117.029532
  18. Hahn J, Monakhova YB, Hengen J, et al. Electronic cigarettes: overview of chemical composition and exposure estimation. *Tob Induc Dis*. 2014;12(December). doi:10.1186/s12971-014-0023-6
  19. Bhatnagar A, Whitsel LP, Ribisl KM, et al. Electronic cigarettes: a policy statement from the American Heart Association. *Circulation*. 2014;130(16):1418-1436. doi:10.1161/cir.000000000000107
  20. Conklin DJ, Agarwal A, Sithu S. Chronic Inhaled Acrolein Depresses Circulating Endothelial Progenitor Cells and Promotes Atherosclerosis. *Circulation*. 2015;132(suppl 3):A15705.
  21. Vardavas CI, Anagnostopoulos N, Kougias M, Evangelopoulou V, Connolly GN, Behrakis PK. Short-term pulmonary effects of using an electronic cigarette: impact on respiratory flow resistance, impedance, and exhaled nitric oxide. *Chest*. 2012;141(6):1400-1406. doi:10.1378/chest.11-2443
  22. Goniewicz ML, Knysak J, Gawron M, et al. Levels of selected carcinogens and toxicants in vapour from electronic cigarettes. *Tob Control*. 2014;23(2):133-139. doi:10.1136/tobaccocontrol-2012-050859
  23. Lee J, Cooke JP. The role of nicotine in the pathogenesis of atherosclerosis. *Atherosclerosis*. 2011;215(2):281-283. doi:10.1016/j.atherosclerosis.2011.01.003
  24. Hutzler C, Paschke M, Kruschinski S, Henkler F, Hahn J, Luch A. Chemical hazards present in liquids and vapors of electronic cigarettes. *Arch Toxicol*. 2014;88(7):1295-1308. doi:10.1007/s00204-014-1294-7
  25. Goniewicz ML, Lee L. Electronic cigarettes are a source of thirdhand exposure to nicotine. *Nicotine Tob Res*. 2015;17:256-258. doi:10.1093/ntr/ntu152
  26. Lee J, Taneja V, Vassallo R. Cigarette smoking and inflammation: cellular and molecular mechanisms. *J Dent Res*. 2012;91(2):142-149. doi:10.1177/0022034511421200
  27. Dinakar C, O'Connor GT. The health effects of electronic cigarettes. *N Engl J Med*. 2016;375(14):1372-1381. doi:10.1056/nejmra1502466
  28. Grana R, Benowitz N, Glantz SA. E-Cigarettes: a scientific review. *Circulation*. 2014;129:1972-1986. doi:10.1161/circulationaha.114.007667

#### ACKNOWLEDGEMENTS

We thank the team members for their support and contribution to this study.

#### CONFLICTS OF INTEREST

The authors have each completed and submitted an ICMJE form for disclosure of potential conflicts of interest. The authors declare that they have no competing interests, financial or otherwise, related to the current work. All the authors report grants from Scientific Research Program of Shaanxi Education Department, and grants from Innovation and Entrepreneurship Training Program for College Students, during the conduct of the study.

#### FUNDING

The current study was supported by the Scientific Research Program of Shaanxi Education Department (Grant Nos. 19JS059 and 19JK0762) and Innovation and Entrepreneurship Training Program for College Students (Grant Nos. 201825001, 201825006, 121519009, and 121519011).

#### PROVENANCE AND PEER REVIEW

Not commissioned; externally peer reviewed.