Title

Electronic and Tobacco Cigarettes Alter Polyunsaturated Fatty Acids and Oxidative Biomarkers

Journal

Circulation Research, 129(5)

ISSN

0009-7330

Authors

Gupta, Rajat

Lin, Yan

Luna, Karla

et al.

Publication Date

2021-08-20

DOI

10.1161/circresaha.120.317828

Peer reviewed

HHS Public Access

Author manuscript

Circ Res. Author manuscript; available in PMC 2022 August 20.

Published in final edited form as:

Circ Res. 2021 August 20; 129(5): 514–526. doi:10.1161/CIRCRESAHA.120.317828.

Electronic and Tobacco Cigarettes Alter Polyunsaturated Fatty Acids and Oxidative Biomarkers

Rajat Gupta[1,2], **Yan Lin**[3], **Karla Luna**[1,4], **Anjali Logue**[1], **Alexander J. Yoon**[5], **Kacey P. Haptonstall**[1], **Roya Moheimani**[1], **Yasmine Choroomi**[1], **Kevin Nguyen**[1], **Elizabeth Tran**[1], **Yifang Zhu**[3], **Kym F. Faull**[5,6], **Theodoros Kelesidis**[7], **Jeffrey Gornbein**[8], **Holly R. Middlekauff**[1], **Jesus A. Araujo**[1,2,3,6]

[1]Division of Cardiology, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, California

[2]Molecular Toxicology Interdepartmental Program, University of California Los Angeles, Los Angeles, California

[3]Department of Environmental Health Sciences, Fielding School of Public Health, University of California Los Angeles, Los Angeles, California

[4]Department of Biology, College of Science and Math, California State University, Northridge, California

[5]Pasarow Mass Spectrometry Laboratory, Jane and Terry Semel Institute for Neuroscience & Human Behavior and Department of Psychiatry & Biobehavioral Sciences, David Geffen School of Medicine at the University of California Los Angeles, Los Angeles, California

[6]Molecular Biology Institute, University of California Los Angeles, Los Angeles, California

[7]Division of Infectious Diseases, Department of Medicine, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, California

[8]Departments of Medicine and Computational Medicine, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, California

Abstract

Rationale: Chronic electronic cigarette (EC) users exhibit a higher susceptibility of low-density lipoprotein (LDL) to undergo oxidation as compared to non-user controls. However, there is a paucity of data regarding EC effects on lipid peroxidation in the blood and their relationship to cardiovascular risk.

Corresponding author: Jesus A. Araujo, MD, PhD. Division of Cardiology, Department of Medicine, David Geffen School of Medicine, University of California Los Angeles, 10833 Le Conte Avenue, CHS 43-264, Los Angeles, CA 90095. P.O. Box 951679. Phone number (310) 825-3222, Fax number (310) 206-9133. JAraujo@mednet.ucla.edu.

DISCLOSURES

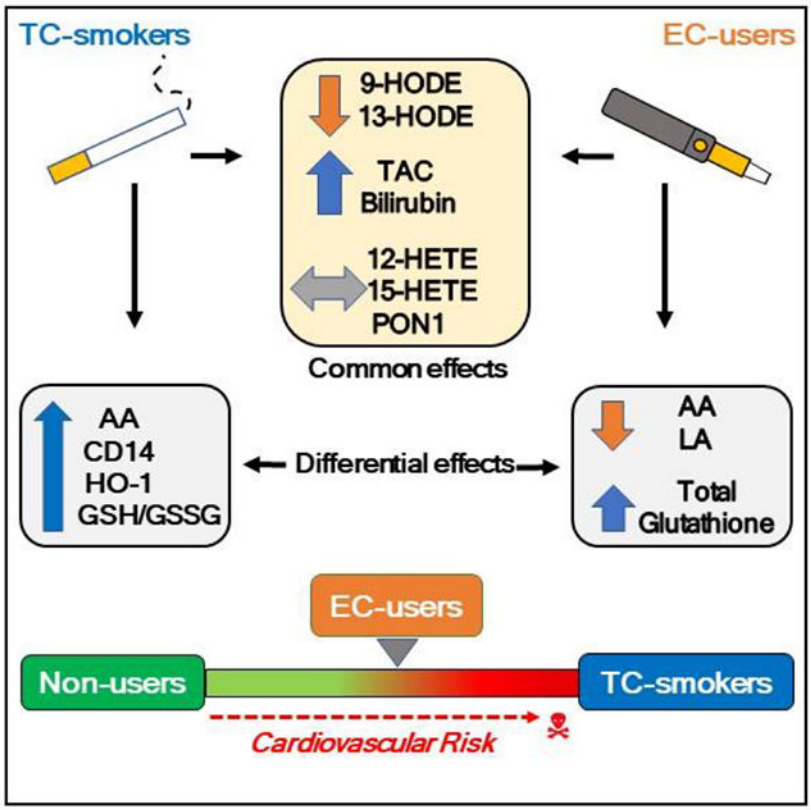
None

Objective: To test the hypothesis that chronic (≥1 year) EC use exerts intermediate effects on plasma lipid peroxidation and/or antioxidant defense compared to chronic tobacco cigarette (TC) smoking.

Methods and Results: We enrolled EC-users (n=32), TC-smokers (n=29) and non-users (n=45), with mean ages of 28.3, 27.8 and 27.4 years, respectively. Plasma concentrations of free polyunsaturated fatty acids and oxidized metabolites were assessed by mass spectrometry. Total antioxidant capacity (TAC), concentrations of glutathione, bilirubin, heme oxygenase-1 (HO-1), and functional activity of paraoxonase1 (PON1) were determined by colorimetric and enzymatic assays. Multivariable analysis was performed using classification models for segregating participants based on biomarker profiles. TC-smokers exhibited higher plasma arachidonic acid concentration. Instead, EC-users displayed lower plasma concentrations of both arachidonic acid and linoleic acid as compared to non-users and TC-smokers ($p<0.05$). Oxidized LA metabolites (9- and 13-hydroxyoctadecadienoic acid (HODE)) were lower in EC-users and TC-smokers as compared to non-users ($p<0.001$). Consistently, TAC and bilirubin were elevated in EC-users and TC-smokers as compared to non-users ($p<0.05$). Of interest, plasma HO-1 concentration was higher in TC-smokers as compared to non-users ($p=0.01$) with intermediate levels in EC-users. Multivariable analysis identified 5 biomarkers (13-HODE, LA, 9-HODE, 12-hydroxyeicosatetraenoic acid (HETE), AA) that discriminated EC-users from TC-smokers and non-users with an accuracy of 73.4%.

Conclusions: Chronic use of EC induces common (i.e. lower 9- and/or 13-HODEs and higher TAC and bilirubin) as well as differential effects (i.e. altered AA and LA concentrations) to those induced by TC, along with intermediate plasma HO-1 concentration, suggesting that EC, likewise TC smoke, could impact cardiovascular risk.

Graphical Abstract



Keywords

Electronic cigarettes; tobacco cigarettes; lipid peroxidation; total antioxidant capacity; polyunsaturated fatty acids; oxidized lipids; cardiovascular disease; smoking; antioxidants; vascular inflammation; biomarkers

INTRODUCTION

Cigarette smoking results in increased mortality, largely due to deleterious cardiovascular and cancer promoting effects via a variety of mechanisms, several of which involve the development of oxidative stress in target tissues[1]. Electronic cigarettes (EC), introduced in 2006 as a potentially safer alternative to tobacco cigarettes (TC), rapidly gained worldwide popularity, especially among young adults[2]. EC are battery-powered devices that contain the e-liquid in a reservoir that upon heating produces aerosols that are inhaled by the user. The absence of tobacco-related combustible constituents led to the notion that they may be less harmful than conventional TC. However, several studies have suggested that EC use is associated with oxidative stress[3, 4], inflammation[5, 6] and endothelial dysfunction[7]. While chronic use of EC alters lipid biosynthetic homeostasis and lung innate immunity processes[8], the potential of EC to induce adverse cardiovascular effects, which are well documented in TC-smokers[9, 10], remains largely unknown. Thus, it is important to determine whether EC use induces health effects that could lead to an increased risk of cardiovascular diseases.

Human studies have shown that TC smoking induces prooxidative effects in the lungs and systemic tissues in smokers by triggering and/or exacerbating the generation of reactive oxygen species (ROS), together with impaired intracellular antioxidant responses[7, 11]. Animal studies also indicate the ability of cigarette smoke to induce oxidative stress and lipotoxicity in the heart[12]. Interestingly, EC emissions generate ROS in concentrations comparable to TC smoke[13]. Thus, it has been reported that an EC aerosol extract induces ROS production in vascular endothelial cells in a concentration-dependent manner, resulting in increased cytotoxicity[14]. Furthermore, Carnevale et al showed that use of a single EC for a total duration of 9 puffs led to increased concentrations of soluble NADPH oxidase (Nox-2)-derived peptide, greater 8-iso-prostaglandin F2α production and decreased serum concentration of vitamin E[7]. Chronic TC-smokers with ≥10 cigarettes per day for ≥5 years exhibited elevated concentrations of 8-epi-prostaglandin $F_{2\alpha}$ as compared to non-smoker controls in the blood[15]. We have previously reported that chronic EC-users exhibit higher susceptibility for the oxidation of plasma low-density lipoproteins (LDL) as compared to non-smoking adults[3]. Therefore, it is possible that chronic EC use may exert prooxidative effects and/or lower antioxidant defense in the plasma that could further lead to release of proinflammatory cytokines, thereby resulting in increased cardiovascular risk among healthy young adults.

In the current study, we sought to investigate whether chronic EC-users exhibit intermediate effects on oxidative metabolism, in between non-users and TC-smokers, by assessing plasma concentrations of oxidized products of essential polyunsaturated fatty acids (PUFAs). Thus, we measured oxidized products of linoleic acid (LA) and arachidonic acid (AA), generated via the activity of lipoxygenases, which we have reported to be sensitive biomarkers of air pollution-induced lipid peroxidation[16]. In addition, we also assessed the effects of EC use and TC smoking on i) total plasma antioxidant capacity (TAC) and plasma concentrations of glutathione and bilirubin, as measures of systemic antioxidant reserve; ii) enzymatic activity of paraoxonase1 (PON1), an antioxidant enzyme associated with circulating high-density lipoproteins (HDL)[17], iii) biomarkers of innate immune response and chemokines, and iv) heme oxygenase-1 (HO-1), an intracellular enzyme that protects against oxidation[18], inflammation[19] and atherosclerosis[20], but leaks out into the blood in conditions of vascular injury[21, 22], thus serving as an indicator of cardiovascular risk[22–24].

METHODS

Data availability.

The authors declare that all supporting data including Major Resources Table are available within the article, and its online-only Data Supplement.

Study population.

Plasma samples were obtained between 2015 to 2018 from young healthy male and female study participants, ages 21-45 years, who had either chronically used EC (≥12 months, EC-users) or smoked TC (≥12 months, TC-smokers) or who had not used EC and/or smoked TC within the last year (non-users). See additional details including plasma collection and storage in the online-only Data Supplement.

Plasma free fatty acids and their oxidized metabolites.

Plasma free LA and AA, and their oxidized products 9- and 13-hydroxyoctadecadienoic acids (HODE) and 5-, 12- and 15- hydroxyeicosatetraenoic acids (HETE) were extracted and analyzed by liquid chromatography coupled to ion trap mass spectrometry (ITMS) as previously described[16]. See additional details including plasma lipid profile in the online-only Data Supplement.

Plasma antioxidants, HO-1, biomarkers of innate immunity and secretory phospholipase A_2,

Paraoxonase (PON) and arylesterase enzymatic activities as well as concentrations of TAC, total and conjugated (direct) bilirubin, glutathione, HO-1, biomarkers of innate immunity and secretory phospholipase A_2 ($sPLA_2$) were measured in the plasma, as described in the online-only Data Supplement.

Statistical methods.

Data were reported as means ± SEM or geometric means/medians with interquartile ranges (IQR) as specified. Examination of normal quantile plots and the Shapiro Wilk statistic were used to determine whether the distribution of a biomarker was normal in the original or log scale. See additional details in the online-only Data Supplement.

RESULTS

Baseline characteristics.

The study of 106 healthy participants included 32 EC-users, 29 TC-smokers and 45 non-users. Baseline characteristics of all participants (Table 1) show that the overall age and BMI were similar among the three groups ($p>0.05$). The ethnic distribution was also similar between the three groups ($p=0.732$) with most of the participants being Caucasians (62.2% non-users, 59.4% EC-users and 58.6% TC-smokers). 68.7% of EC-users were former smokers, which was expectedly higher than the 8.8% of non-users who were former smokers ($p=4.32 \times 10^{-8}$). Although both EC-users and TC-smokers included a higher proportion of males than females (71.9% and 65.5% respectively) in comparison to non-users (57.8%), there were no significant differences in overall sex distribution ($p=0.455$). Baseline cotinine concentrations were similar between TC-smokers and EC-users, consistent with a similar smoking burden.

Both EC use and TC smoking lowered plasma concentrations of oxidized free fatty acids.

Plasma lipid profiles denoted similar levels of total, unesterified, VLDL and HDL cholesterol, as well as triglycerides. However, while EC-users exhibited lower concentration of LDL cholesterol ($p=0.02$), TC-smokers displayed higher levels of free fatty acids (FFAs) as compared to non-users ($p=0.04$) (Supplemental table I). Plasma concentrations of all biomarkers are shown in Supplemental table II. Surprisingly, plasma concentrations of 9-HODE, 13-HODE and total HODEs were significantly lower in both EC-users and TC-smokers as compared to non-users, with similar levels between EC-users and TC-smokers (Figure 1A). On the other hand, there were no differences in the plasma concentrations of

12-HETE, 15-HETE or total HETEs (Figure 1B). To normalize the HODEs and HETEs by their parental PUFAs, we assessed the plasma concentrations of LA and AA, respectively. Unexpectedly, EC-users exhibited lower concentrations of both LA and AA while TC-smokers displayed higher concentration of AA as compared with the other groups (Figure 2); the latter consistent with the elevated FFAs observed in the lipid profiles (Supplemental table I). Expectedly, there were positive associations between the concentrations of 9-HODE ($p=0.006$), 13-HODE ($p=0.005$) and total HODEs ($p=0.004$) with the corresponding parental compound LA (Supplemental figure IA–C), as well as positive associations between the concentrations of 12-HETE ($p=0.086$), 15-HETE ($p=3.33x10^{-8}$) and total HETEs ($p=0.007$) with the parental compound AA (Supplemental figure IIA–C). We calculated the ratios of the concentrations of HODEs and HETEs to the concentrations of the corresponding LA and AA, which allowed us to determine whether changes in the oxidized metabolites were mostly due to the degree of lipid peroxidation or changes in the concentrations of the parental compounds. These ratios serve as surrogate estimates, although imperfect, of the degree of lipid peroxidation. Thus, TC-smokers exhibited significantly lower ratios of 9-HODE/LA, 13-HODE/LA, total HODEs/LA and 15-HETE/AA as compared to non-users and EC-users (Figure 3), consistent with lower lipid peroxidation. On the other hand, EC-users displayed similar ratios of HODEs/LA and HETEs/AA as compared to non-users, indicating that the lower concentration of HODEs were mostly due to lower LA concentration even if lower degree of lipid peroxidation could not be ruled out.

EC use and TC smoking promoted antioxidant homeostatic responses.

To determine potential mechanisms responsible for EC and TC effects on oxidative biomarkers, we assessed plasma TAC via a colorimetric assay. Consistently, plasma concentration of TAC was significantly higher in TC-smokers as compared to non-users ($p=1.03x10^{-4}$), suggestive of an elevated antioxidant defense, with intermediate levels in EC-users (Figure 4A). However, while adjustment for sex did not affect the observed differences between TC-smokers and non-users ($p=0.0003$) or between EC-users and TC-smokers ($p=0.03$), the differences between EC-users and non-users were no longer statistically significant ($p=0.18$) (Supplemental table III).

We then asked whether elevation in anti-oxidant defense involved higher functionality of PON1, an important anti-oxidant enzyme associated with HDL[17], which has been reported to be inhibited after chronic or subacute exposure to TC[25] or air pollution[16]. There were no significant differences in PON activity among the three groups (Supplemental figure IIIA). Since PON activity is known to be influenced by *PON1* single nucleotide polymorphisms (SNPs)[26], we also measured arylesterase activity which is not affected by genetic differences. Consistently, participants in all three groups displayed similar levels of arylesterase activity in the plasma or HDL fractions (Supplemental figure IIIB–C), indicating that effects on oxidized lipids were not due to elevations in PON1 functional activity.

We then determined plasma concentrations of bilirubin and glutathione using colorimetric assays. Importantly, total bilirubin levels were elevated in both EC-users ($p=0.009$) and TC-smokers ($p=0.008$) as compared to non-users, and unconjugated bilirubin was higher

among EC-users as well (p=0.006) (Figure 4B). Furthermore, both EC use and TC smoke influenced glutathione metabolism with effects on overall redox status. Indeed, EC-users exhibited higher total glutathione (p=0.006), GSH (p=0.06) and GSSG (p=0.1) concentrations without changes in the GSH/GSSG ratio, whereas TC smokers displayed elevated levels of GSH (p=0.05) and GSH/GSSG ratio (p=0.05) (Figure 4C–F). Altogether, our data indicates that both EC-users and TC-smokers exhibited compensatory antioxidant responses, with greater effects induced by TC smoke, especially on TAC, likely responsible for the inhibition in lipid peroxidation. On the other hand, EC-users exhibited intermediate effects on TAC and similar GSH/GSSG ratios to non-users, suggesting that those individuals had reached a state of redox homeostatic balance.

TC smoking but not EC use led to higher plasma HO-1 concentration and vascular inflammation.

We then asked whether antioxidant responses triggered by chronic EC use or TC smoke could have included the induction of HO-1, as evidenced by elevation in the plasma concentration of bilirubin, which is a byproduct of the catabolism of heme groups carried out by HO-1. We measured the plasma concentration of HO-1 which has been reported to leak into the blood after vascular inflammation or injury[22]. Indeed, our data indicates that HO-1 concentration was elevated in the plasma of TC-smokers as compared to non-users (p=0.01), with intermediate levels present among EC-users (Figure 5A), suggesting upregulation of HO-1 and subsequent leakage into the circulation. Interestingly, when HO-1 concentration was plotted against levels of 9-HODE, 13-HODE and TAC across all participants, we also observed that the spline curves for EC-users were intermediate between non-users and TC-smokers (Supplemental figure IV).

We further sought to determine whether chronic EC use or TC smoking could have induced vascular inflammation via Luminex immunoassays that included chemokines and/or biomarkers of innate immunity such as sCD14, sCD163, CCL2 and CX3CL1. TC-smokers but not EC-users exhibited elevated concentrations of sCD14 in comparison to non-users (p=0.006) (Figure 5B). In addition, plasma concentration of CCL2 associated with HO-1 among TC-smokers (p=0.009) but not among EC-users (p=0.09) or non-user (p=0.82) (Figure 5C). This data indicates that chronic TC smoking induced vascular proinflammatory effects that could have led to the release of HO-1 in the blood.

Biomarker profiles segregate EC-users from TC-smokers and non-users by multivariable analysis using classification models.

We used multivariable analyses to determine whether EC-users, TC-smokers and non-users could be segregated. The canonical scores from the nominal logistic regression model for discriminating between the 3 groups showed that there was a continuum with TC-smokers on the upper right of the plot (blue triangles), non-users on the lower left (green circles), and EC-users in the middle right (orange squares), with a small degree of overlap (Figure 6A). This model had a prediction accuracy of 94.4%.

We also used a classification tree model to generate a tree with 7 classification nodes with the use of 2 primary (i.e. 9-HODE and LA) and 3 derived (13-HODE/LA, 12-HETE/AA

and 9-HODE/LA) biomarkers, which allowed classification of individuals with a prediction accuracy of 73.4%. Importantly, this classification tree implied that the 5 most important biomarkers for discriminating modest associations between EC-users, TC-smokers and non-users were in the following order; 13-HODE/LA ratio, 9-HODE, 12-HETE/AA ratio, 9-HODE/LA ratio and LA (Figure 6B). For each branch of the tree, if the condition is true, one branches **left**; otherwise, if the condition is false, one branches **right**. Remarkably, only two biomarkers (13-HODE/LA and 9-HODE) were sufficient to discriminate between TC-smokers, generally on the right side of the tree, and non-users. The other three biomarkers (12-HETE/AA, 9-HODE/LA, LA) were required to discriminate between non-users, generally on the left side of the tree, from EC-users, in the middle.

DISCUSSION

To our knowledge, this is the first study to report alterations in PUFAs and their oxidized metabolites along with changes in systemic antioxidant defense, and inflammatory responses in otherwise healthy young adults who were habitual EC-users or TC-smokers as compared to non-users, with implications in the risk of cardiovascular disease (Figure 7).

We have previously reported that chronic EC use increased LDL susceptibility for oxidation[3]. To investigate EC effects on blood oxidation status, we measured plasma concentrations of 9- and 13-HODEs as well as 5-, 12- and 15-HETEs, derived at least in part, from lipoxygenase-mediated oxidation of LA and AA, respectively[27]. We and others have found that these compounds serve as exquisite biomarkers of air pollution-induced lipid peroxidation in animal models[28, 29], young healthy individuals[16] and human TC studies[30]. Surprisingly, both EC-users and TC-smokers displayed lower concentrations of 9- and 13-HODEs, with no differences in HETEs (Figure 1), which was in contrast to the data published in another study[30]. However, our data was consistent with previous studies that reported lower levels of 9- and 13-HODEs with no differences in the concentration of 15-HETE in the plasma[31] or 12-HETE in the saliva[32] of smokers vs non-smokers. In addition, effects on the concentrations of 9- and 13-HODEs were negatively associated with the plasma cotinine levels (Supplemental figure V). We also found that former TC smoking among EC-users did not affect these oxidative biomarkers differently in comparison to those who never smoked TCs (Supplemental figure VI).

We then normalized the concentrations of HODEs and HETEs by their respective parental PUFAs (LA and AA, respectively) to determine whether the lower concentration of oxidative biomarkers were due to lower degree of lipid peroxidation or lower concentration of the parental substrates. Interestingly, TC-smokers but not EC-users exhibited lower ratios of 9-, 13- and total HODEs/LA, and 15-HETE/AA (Figure 3), indicating lower lipid peroxidation in the former. On the other hand, EC-users displayed similar ratios as non-users, likely indicating that the lower concentration of oxidative biomarkers were largely if not exclusively, due to lower plasma concentrations of the parental substrates. Importantly, neither TC-smokers nor EC-users exhibited elevated ratios for any of the compounds which would have been indicative of higher lipid peroxidation, and in apparent contrast with a large number of studies that have documented the oxidative potential of TC[33, 34], and increasing evidence that EC also induces prooxidative effects in tissue

culture[14, 35], animal[36] and human studies[37]. We argued that chronic use of EC and TC may have induced antioxidant homeostatic responses triggered by the TC and EC prooxidative effects, leading to compensatory effects, especially prominent against the oxidation of LA in TC-smokers. Therefore, we assessed plasma TAC to gauge the cumulative antioxidant status, which includes antioxidants such as α- and γ-tocopherol, β-carotene, lycopene, bilirubin, ascorbic and uric acids[38, 39]. We found that plasma TAC was significantly elevated in TC-smokers and EC-users (Figure 4A), in support of our aforementioned hypothesis. While our results are opposite to the study of Bloomer et al, who reported lower concentrations of blood antioxidant capacity and increased malondialdehyde in 15 chronic TC-smokers vs. 13 non-smoker controls[40], they are consistent with the study of Charalabopoulos et al, who observed higher plasma TAC in healthy young chronic smokers vs non-smokers[41].

Considering that cigarette smoking has been reported to decrease plasma concentrations of vitamins C and E, β-carotene as well as enzymatic anti-oxidant activities for erythrocyte superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px)[42], we investigated whether TAC-elevated effects could have been due to changes in bilirubin, a powerful antioxidant, and/or glutathione metabolism. Indeed, both EC-users and TC-smokers exhibited elevated plasma concentrations of bilirubin (Figure 4B), and changes in glutathione metabolic parameters (Figure 4C–F). Thus, TC-smokers displayed higher concentration of reduced glutathione and GSH/GSSG ratio, consistent with significant elevation in TAC. Consistent with our data, smoking has been previously reported to exhibit significantly elevated blood concentration of reduced glutathione[43] and GSH/GSSG ratio[44] as compared to non-smokers, and according to the study of Ashfaq et al, plasma ratio of GSH/GSSG significantly associated with carotid intima media thickness (CIMT) and the Framingham risk score in healthy non-smoking individuals between the ages of 30 to 65 years[45]. Therefore, elevated ratio of GSH/GSSG in TC-smokers may predict an increased likelihood of sub-clinical atherosclerosis and cardiovascular risk, even though clinical cardiovascular outcomes were not directly assessed. Interestingly, while EC-users exhibited higher levels of total glutathione, their GSH/GSSG ratios were similar to non-users, suggesting that those individuals had reached a state of redox homeostatic balance.

We then investigated whether elevation in bilirubin concentrations were due to higher expression of HO-1, the rate-limiting enzyme in the catabolism of heme, resulting in the release of carbon monoxide, ferrous iron and biliverdin[21]; the latter further converted into bilirubin by biliverdin reductase[46]. Indeed, TC-smokers displayed elevated plasma HO-1 concentration, with intermediate levels present in EC-users (Figure 5A). Since HO-1 is an intracellular enzyme, it has been proposed that its presence in the blood denotes its release by leukocytes, monocytes/macrophages, smooth muscle cells or endothelial cells that are activated and possibly damaged by oxidative stress or inflammation[21]. Therefore, while HO-1 is deemed as an exquisite sensor for oxidative stress, elevated levels in the blood have also been proposed as a marker of vascular injury or inflammation[21, 22]. In support of this, HO-1 concentrations in the blood have been reported to be elevated in patients with non-alcoholic fatty liver disease[47], type-2 diabetes mellitus[48], Parkinson's disease[49], acute myocardial infarction[24] and coronary artery disease (CAD)[50], thereby serving as an indicator of cardiovascular risk.

We then asked whether changes in HO-1 could have been due to changes in the circulating inflammatory mediators by assessing biomarkers of innate immunity and chemokines that are associated with oxidative stress and cardiovascular disease burden[51–53]. Indeed, TC-smokers exhibited significantly elevated levels of CD14, with a lesser degree of induction in EC-users (Figure 5B), along with a positive association between HO-1 and CCL2 (Figure 5C) in TC-smokers, consistent with our previous study demonstrating a dose response increase in proinflammatory monocytes and cellular oxidative stress (COS), that was lowest in non-users, intermediate in EC-users, and highest among TC-smokers[4]. Thus, HO-1 associates with proinflammatory mediators[54–57] in TC-smokers, important in the development of CV events.

It was interesting that plasma concentrations of LA and AA were differentially affected in EC-users and TC-smokers, suggesting an alteration in lipid metabolism (Figure 2). Cigarette smoking has been inversely associated with serum concentrations of cholesterol esters and phospholipid AA[58]. One possibility to explain the higher concentration of AA in TC-smokers is that TC-smokers but not EC-users had elevated concentrations of $sPLA_2$[59], a key metabolic enzyme that hydrolyzes AA from phospholipids and releases the unoxidized form into the blood[60]. However, no significant differences were obtained in $sPLA_2$ concentrations in the plasma of our study participants (Supplemental figure VII). Another possibility is that elevated FFAs and AA in particular, could be related to lipase-mediated release of FFAs via lipolysis from adipose tissue[61]. While evaluation of intracellular lipases was not feasible in our study, we did observe higher concentration of plasma FFAs in TC-smokers (Supplemental table I). On the contrary, effects on PUFAs in EC-users were consistent with a previous study reporting lower concentration of AA in the brains of rats exposed to EC for 8 weeks[62]. In addition, the study of Canistro et al showed that rats exposed to EC for 4 consecutive weeks exhibited significant reduction in the plasma concentrations of a sum measure of various omega-6 PUFAs that included both AA and LA as compared to the non-exposed control group[63]. Importantly, we found that EC-users who had never smoked TCs also exhibited lower LA and AA concentrations in comparison to those who were former TC-smokers, indicating that these effects in chronic EC uses were not due to carryover effect due to TC smoking in the previous years (Supplemental figure VIII).

The use of multivariable analysis with a classification tree model segregated participants from all three groups, with an overall accuracy of 73.4%. The PLS-DA model also discriminated the 3 groups based on two canonical scores with TC-smokers on the left of the plot (blue triangles), non-users on the right (green circles), and EC-users in the middle (orange squares) (Supplemental figure IX). Scores predicted by the nominal logistic regression and PLS-DA models, when all variables were simultaneously considered, placed EC-users within the extremities of biomarker profiles of non-users and TC-smokers, with a classification accuracy of 94.4% and 66% respectively. These results were consistent with the distribution of participants stratified by HO-1 and 9-HODE, 13-HODE or TAC levels (Supplemental Figure IV), and other studies documenting EC effects that were less intense than TC effects induced in tissue culture[64, 65] and human studies[66]. While chronic EC use resulted in several effects that were similar to those induced by TC smoke, they were of smaller magnitude and accompanied by other effects that were different. Altogether, these effects segregated EC-users from non-users and TC-smokers (Figure 6).

Does chronic EC use or TC smoke augment cardiovascular risk among healthy young individuals? It is well known that TC smoking is the most prevalent, preventable risk factor for cardiovascular diseases resulting in increased morbidity and mortality[33, 67]. In addition to the increased GSH/GSSG ratio discussed above, TC-smokers exhibited a plasma HO-1 concentration that was 20% higher than in non-users, which fall within the same range of previous studies with elevations of 27.2%, 28.5% and 20.7% in patients with carotid atherosclerosis[23], CAD[50] and Parkinson's disease[49] respectively, as compared to healthy control individuals, supporting the notion that chronic TC smoke increased cardiovascular risk in our cohort of healthy young individuals. EC-users displayed a 5.3% higher concentration of HO-1 as compared to non-users, which was not statistically significant (Figure 5A). Future larger studies are required to determine whether these smaller elevations in HO-1 could indicate increased CV risk.

Limitations.

Our study depended on the reliability of each participant's self-reported questionnaire. These can be unreliable and prone to misstatements, underestimating the smoking status of an individual[3, 68]. Although 68% of EC-users were former cigarette smokers who reportedly had quit smoking at least 1 year before the study (Table 1), we cannot exclude the possibility that some of our participants occasionally consumed tobacco products. The longer duration of TC smoking as compared to EC use (Table 1) also has a potential to affect the biomarkers in our study that cannot be ignored. In addition, there are other factors beyond those recorded in our study (e.g. diet, lifestyle) that could have influenced some of the outcome variables such as blood concentrations of PUFAs[69]. Adjustment for the recorded factors did not alter the observed findings with the exception of sex, since after sex adjustment, the observed differences in plasma TAC between EC-users and non-users lost statistical significance ($p=0.18$), supporting a greater impact on plasma TAC in TC-smokers than EC-users. Furthermore, our measures of PUFAs and their oxidized metabolites were only circumscribed to their hydrolyzed free forms and did not include measures of the esterified forms, which would have allowed determination of their total (i.e. free + esterified) concentrations. While our multivariable analyses were helpful in segregating individuals based on their biomarker profiles, we do not have another dataset on which to validate these findings, which will be addressed in future studies. Furthermore, considering the exploratory nature of our study, we did not perform multiple comparisons adjustment within groups across all biomarkers to control for false positive results (Type-I error) in order to avoid increasing false negative results (Type II-error). Lastly, there was no data obtained for the daily exercise status or environment of residency across groups, that could have affected our study outcomes.

Conclusions.

Our results indicate that chronic use of EC and TC exerted a myriad of effects involving lipid metabolism, oxidative and inflammatory responses with important implications for cardiovascular risk. Some of the EC effects were similar to those exerted in TC-smokers although to a lesser degree, such as those affecting oxidative biomarkers derived from linoleic acid, total antioxidant capacity, heme oxygenase-1 and proinflammatory cytokines

like CD14, making them intermediate in between non-users and TC-smokers; while other effects were notably different such as those affecting levels of polyunsaturated fatty arachidonic and linoleic acids. Chronic use of EC and/or smoking of TC elicited antioxidant homeostatic responses that involved the HO-1/bilirubin axis and glutathione metabolism, and that in the case of EC users, resulted in a state of redox homeostatic balance. It is still to be determined whether elevated HO-1 among TC smokers is indicative of increased cardiovascular risk, and if years long usage of EC among young healthy individuals, and in individuals with other comorbidities could result in increased cardiovascular risk as well.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

We appreciate the contributions of Sarada Charugundla for conducting the lipid profile measurements and Henry Landers for assistance with data analysis. In addition, we also acknowledge the efforts of Fen Yin, May Bhetraratana, Benjamin Yang, Min Zhang, Nolan Rea, Hiromi Murata, and the Clinical and Translational Research Center (CTRC) at University of California Los Angeles for assistance with blood collection, processing and storage of plasma samples.

SOURCES OF FUNDING

This work was supported by the Tobacco-Related Disease Research Program (TRDRP) [23XT-0006H, 25IR-0024H, 28IR-0065 to H.R.M.], NIH National Center for Advancing Translational Science (NCATS) [UCLA CTSI Grant Number UL1TR001881], National Institute of Environmental Health Sciences (NIEHS) [R25ES025507 to Nola Kennedy] and in part by NIH [R01AG059501 and R03AG059462 to TK].

Nonstandard Abbreviations and Acronyms:

CCL2	C-C motif chemokine ligand 2
CX3CL1	C-X3-C motif chemokine ligand 1
EC	electronic cigarettes
GSH	reduced glutathione
GSSG	oxidized glutathione
HETE	hydroxyeicosatetraenoic acid
HODE	hydroxyoctadecadienoic acid
HO-1	heme oxygenase-1
PLS-DA	partial least square-discriminant analysis
PON1	paraoxonase1
PUFAs	polyunsaturated fatty acids
sCD14	soluble cluster of differentiation 14
sCD163	soluble cluster of differentiation 163

TAC	total antioxidant capacity
TC	tobacco cigarettes

REFERENCES

1. Ugur MG, Kutlu R and Kilinc I. The effects of smoking on vascular endothelial growth factor and inflammation markers: A case-control study. Clin Respir J. 2018;12:1912–1918. [PubMed: 29243879]
2. Singh T, Marynak K, Arrazola RA, Cox S, Rolle IV and King BA. Vital Signs: Exposure to Electronic Cigarette Advertising Among Middle School and High School Students - United States, 2014. MMWR Morb Mortal Wkly Rep. 2016;64:1403–8. [PubMed: 26741522]
3. Moheimani RS, Bhetraratana M, Yin F, Peters KM, Gornbein J, Araujo JA and Middlekauff HR. Increased Cardiac Sympathetic Activity and Oxidative Stress in Habitual Electronic Cigarette Users: Implications for Cardiovascular Risk. JAMA Cardiol. 2017;2:278–284. [PubMed: 28146259]
4. Kelesidis T, Tran E, Arastoo S, Lakhani K, Heymans R, Gornbein J and Middlekauff HR. Elevated Cellular Oxidative Stress in Circulating Immune Cells in Otherwise Healthy Young People Who Use Electronic Cigarettes in a Cross-Sectional Single-Center Study: Implications for Future Cardiovascular Risk. J Am Heart Assoc. 2020;9:e016983. [PubMed: 32896211]
5. Higham A, Rattray NJ, Dewhurst JA, Trivedi DK, Fowler SJ, Goodacre R and Singh D. Electronic cigarette exposure triggers neutrophil inflammatory responses. Respir Res. 2016;17:56. [PubMed: 27184092]
6. Boas Z, Gupta P, Moheimani RS, et al.Activation of the “Splenocardiac Axis” by electronic and tobacco cigarettes in otherwise healthy young adults. Physiol Rep. 2017;5.
7. Carnevale R, Sciarretta S, Violi F, et al.Acute Impact of Tobacco vs Electronic Cigarette Smoking on Oxidative Stress and Vascular Function. Chest. 2016;150:606–12. [PubMed: 27108682]
8. Madison MC, Landers CT, Gu BH, et al.Electronic cigarettes disrupt lung lipid homeostasis and innate immunity independent of nicotine. J Clin Invest. 2019;129:4290–4304. [PubMed: 31483291]
9. Bhat VM, Cole JW, Sorkin JD, Wozniak MA, Malarcher AM, Giles WH, Stern BJ and Kittner SJ. Dose-response relationship between cigarette smoking and risk of ischemic stroke in young women. Stroke. 2008;39:2439–43. [PubMed: 18703815]
10. Erhardt LCigarette smoking: an undertreated risk factor for cardiovascular disease. Atherosclerosis. 2009;205:23–32. [PubMed: 19217623]
11. Karademirci M, Kutlu R and Kilinc I. Relationship between smoking and total antioxidant status, total oxidant status, oxidative stress index, vit C, vit E. Clin Respir J. 2018;12:2006–2012. [PubMed: 29247592]
12. Santos PP, Oliveira F, Ferreira VC, et al.The role of lipotoxicity in smoke cardiomyopathy. PLoS One. 2014;9:e113739. [PubMed: 25462161]
13. Zhao J, Zhang Y, Sisler JD, Shaffer J, Leonard SS, Morris AM, Qian Y, Bello D and Demokritou P. Assessment of reactive oxygen species generated by electronic cigarettes using acellular and cellular approaches. J Hazard Mater. 2018;344:549–557. [PubMed: 29102637]
14. Anderson C, Majeste A, Hanus J and Wang S. E-Cigarette Aerosol Exposure Induces Reactive Oxygen Species, DNA Damage, and Cell Death in Vascular Endothelial Cells. Toxicol Sci. 2016;154:332–340. [PubMed: 27613717]
15. Ludicke F, Magnette J, Baker G and Weitkunat R. A Japanese cross-sectional multicentre study of biomarkers associated with cardiovascular disease in smokers and non-smokers. Biomarkers. 2015;20:411–21. [PubMed: 26616146]
16. Lin Y, Ramanathan G, Zhu Y, et al.Pro-Oxidative and Proinflammatory Effects After Traveling From Los Angeles to Beijing: A Biomarker-Based Natural Experiment. Circulation. 2019;140:1995–2004. [PubMed: 31744317]
17. Durrington PN, Mackness B and Mackness MI. Paraoxonase and atherosclerosis. Arterioscler Thromb Vasc Biol. 2001;21:473–80. [PubMed: 11304460]

18. Lawal A, Zhang M, Dittmar M, Lulla A and Araujo JA. Heme oxygenase-1 protects endothelial cells from the toxicity of air pollutant chemicals. Toxicol Appl Pharmacol. 2015;284:281–291. [PubMed: 25620054]
19. Romanoski CE, Che N, Yin F, et al.Network for activation of human endothelial cells by oxidized phospholipids: a critical role of heme oxygenase 1. Circ Res. 2011;109:e27–41. [PubMed: 21737788]
20. Araujo JA, Zhang M and Yin F. Heme oxygenase-1, oxidation, inflammation, and atherosclerosis. Front Pharmacol. 2012;3:119. [PubMed: 22833723]
21. Abraham NG and Kappas A. Pharmacological and clinical aspects of heme oxygenase. Pharmacol Rev. 2008;60:79–127. [PubMed: 18323402]
22. Idriss NK, Lip GY, Balakrishnan B, Jaumdally R, Boos CJ and Blann AD. Plasma haemoxygenase-1 in coronary artery disease. A comparison with angiogenin, matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-1 and vascular endothelial growth factor. Thromb Haemost. 2010;104:1029–37. [PubMed: 20838751]
23. Kishimoto Y, Sasaki K, Saita E, Niki H, Ohmori R, Kondo K and Momiyama Y. Plasma Heme Oxygenase-1 Levels and Carotid Atherosclerosis. Stroke. 2018;49:2230–2232. [PubMed: 30354985]
24. Novo G, Cappello F, Rizzo M, et al.Hsp60 and heme oxygenase-1 (Hsp32) in acute myocardial infarction. Transl Res. 2011;157:285–92. [PubMed: 21497776]
25. Reed RM, Borgan SM, Eberlein M, Goldklang M, Lewis J, Miller M, Navab M and Kim BS. Tobacco Smoke Exposure Reduces Paraoxonase Activity in a Murine Model. Int J Biomed Sci. 2017;13:20–25. [PubMed: 28533733]
26. Shunmoogam N, Naidoo P and Chilton R. Paraoxonase (PON)-1: a brief overview on genetics, structure, polymorphisms and clinical relevance. Vasc Health Risk Manag. 2018;14:137–143. [PubMed: 29950852]
27. Powell WS and Rokach J. Biosynthesis, biological effects, and receptors of hydroxyeicosatetraenoic acids (HETEs) and oxoeicosatetraenoic acids (oxo-ETEs) derived from arachidonic acid. Biochim Biophys Acta. 2015;1851:340–55. [PubMed: 25449650]
28. Yin F, Gupta R, Vergnes L, et al.Diesel Exhaust Induces Mitochondrial Dysfunction, Hyperlipidemia, and Liver Steatosis. Arterioscler Thromb Vasc Biol. 2019;39:1776–1786. [PubMed: 31340670]
29. Yin F, Lawal A, Ricks J, Fox JR, Larson T, Navab M, Fogelman AM, Rosenfeld ME and Araujo JA. Diesel exhaust induces systemic lipid peroxidation and development of dysfunctional pro-oxidant and pro-inflammatory high-density lipoprotein. Arterioscler Thromb Vasc Biol. 2013;33:1153–61. [PubMed: 23559632]
30. Seet RC, Lee CY, Loke WM, et al.Biomarkers of oxidative damage in cigarette smokers: which biomarkers might reflect acute versus chronic oxidative stress?Free Radic Biol Med. 2011;50:1787–93. [PubMed: 21420490]
31. Titz B, Luettich K, Leroy P, et al.Alterations in Serum Polyunsaturated Fatty Acids and Eicosanoids in Patients with Mild to Moderate Chronic Obstructive Pulmonary Disease (COPD). Int J Mol Sci. 2016;17.
32. Wu-Wang CY, Wang SL, Lim C, Milles M, Slomiany A and Slomiany BL. Cigarette smoking reduces human salivary eicosanoids. Prostaglandins Leukot Essent Fatty Acids. 1992;47:101–4. [PubMed: 1461919]
33. Ambrose JA and Barua RS. The pathophysiology of cigarette smoking and cardiovascular disease: an update. J Am Coll Cardiol. 2004;43:1731–7. [PubMed: 15145091]
34. Chow CK. Cigarette smoking and oxidative damage in the lung. Ann N Y Acad Sci. 1993;686:289–98. [PubMed: 8512254]
35. Ganapathy V, Manyanga J, Brame L, et al.Electronic cigarette aerosols suppress cellular antioxidant defenses and induce significant oxidative DNA damage. PLoS One. 2017;12:e0177780. [PubMed: 28542301]
36. Espinoza-Derout J, Hasan KM, Shao XM, et al.Chronic intermittent electronic cigarette exposure induces cardiac dysfunction and atherosclerosis in apolipoprotein-E knockout mice. Am J Physiol Heart Circ Physiol. 2019;317:H445–H459. [PubMed: 31172811]

37. Singh KP, Lawyer G, Muthumalage T, Maremanda KP, Khan NA, McDonough SR, Ye D, McIntosh S and Rahman I. Systemic biomarkers in electronic cigarette users: implications for noninvasive assessment of vaping-associated pulmonary injuries. ERJ Open Res. 2019;5.
38. Buico A, Cassino C, Ravera M, Betta PG and Osella D. Oxidative stress and total antioxidant capacity in human plasma. Redox Rep. 2009;14:125–31. [PubMed: 19490754]
39. Miller NJ, Rice-Evans C, Davies MJ, Gopinathan V and Milner A. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clin Sci (Lond). 1993;84:407–12. [PubMed: 8482045]
40. Bloomer RJ. Decreased blood antioxidant capacity and increased lipid peroxidation in young cigarette smokers compared to nonsmokers: Impact of dietary intake. Nutr J. 2007;6:39. [PubMed: 17996062]
41. Charalabopoulos K, Assimakopoulos D, Karkabounas S, Danielidis V, Kiortsis D and Evangelou A. Effects of cigarette smoking on the antioxidant defence in young healthy male volunteers. Int J Clin Pract. 2005;59:25–30. [PubMed: 15707460]
42. Zhou JF, Yan XF, Guo FZ, Sun NY, Qian ZJ and Ding DY. Effects of cigarette smoking and smoking cessation on plasma constituents and enzyme activities related to oxidative stress. Biomed Environ Sci. 2000;13:44–55. [PubMed: 10853840]
43. Bizon A and Milnerowicz H. [Effect of tobacco smoking on glutathione concentration in the blood]. Przegl Lek. 2012;69:809–11. [PubMed: 23421037]
44. Moriarty SE, Shah JH, Lynn M, Jiang S, Openo K, Jones DP and Sternberg P. Oxidation of glutathione and cysteine in human plasma associated with smoking. Free Radic Biol Med. 2003;35:1582–8. [PubMed: 14680681]
45. Ashfaq S, Abramson JL, Jones DP, Rhodes SD, Weintraub WS, Hooper WC, Vaccarino V, Harrison DG and Quyyumi AA. The relationship between plasma levels of oxidized and reduced thiols and early atherosclerosis in healthy adults. J Am Coll Cardiol. 2006;47:1005–11. [PubMed: 16516085]
46. Stocker R, Yamamoto Y, McDonagh AF, Glazer AN and Ames BN. Bilirubin is an antioxidant of possible physiological importance. Science. 1987;235:1043–6. [PubMed: 3029864]
47. Yuan XW, Li DD, Liu LD, Zhang Y, Zhao W, Cui LY, Yang Y and Nan YM. [Application of heme oxygenase 1 in the diagnosis of non-alcoholic fatty liver disease]. Zhonghua Gan Zang Bing Za Zhi. 2019;27:291–297. [PubMed: 31082341]
48. Bao W, Song F, Li X, et al.Plasma heme oxygenase-1 concentration is elevated in individuals with type 2 diabetes mellitus. PLoS One. 2010;5:e12371. [PubMed: 20811623]
49. Mateo I, Infante J, Sanchez-Juan P, Garcia-Gorostiaga I, Rodriguez-Rodriguez E, Vazquez-Higuera JL, Berciano J and Combarros O. Serum heme oxygenase-1 levels are increased in Parkinson's disease but not in Alzheimer's disease. Acta Neurol Scand. 2010;121:136–8. [PubMed: 19785642]
50. Kishimoto Y, Ibe S, Saita E, et al.Plasma Heme Oxygenase-1 Levels in Patients with Coronary and Peripheral Artery Diseases. Dis Markers. 2018;2018:6138124. [PubMed: 30159103]
51. Aristoteli LP, Moller HJ, Bailey B, Moestrup SK and Kritharides L. The monocytic lineage specific soluble CD163 is a plasma marker of coronary atherosclerosis. Atherosclerosis. 2006;184:342–7. [PubMed: 15979079]
52. Reiner AP, Lange EM, Jenny NS, et al.Soluble CD14: genomewide association analysis and relationship to cardiovascular risk and mortality in older adults. Arterioscler Thromb Vasc Biol. 2013;33:158–64. [PubMed: 23162014]
53. Saederup N, Chan L, Lira SA and Charo IF. Fractalkine deficiency markedly reduces macrophage accumulation and atherosclerotic lesion formation in CCR2−/− mice: evidence for independent chemokine functions in atherogenesis. Circulation. 2008;117:1642–8. [PubMed: 18165355]
54. Inui M, Ishida Y, Kimura A, Kuninaka Y, Mukaida N and Kondo T. Protective roles of CX3CR1-mediated signals in toxin A-induced enteritis through the induction of heme oxygenase-1 expression. J Immunol. 2011;186:423–31. [PubMed: 21131421]
55. Morita T, Imai T, Yamaguchi T, Sugiyama T, Katayama S and Yoshino G. Induction of heme oxygenase-1 in monocytes suppresses angiotensin II-elicited chemotactic activity through inhibition of CCR2: role of bilirubin and carbon monoxide generated by the enzyme. Antioxid Redox Signal. 2003;5:439–47. [PubMed: 13678532]

56. Seu L, Burt TD, Witte JS, Martin JN, Deeks SG and McCune JM. Variations in the heme oxygenase-1 microsatellite polymorphism are associated with plasma CD14 and viral load in HIV-infected African-Americans. Genes Immun. 2012;13:258–67. [PubMed: 22048453]
57. Thomsen JH, Etzerodt A, Svendsen P and Moestrup SK. The haptoglobin-CD163-heme oxygenase-1 pathway for hemoglobin scavenging. Oxid Med Cell Longev. 2013;2013:523652. [PubMed: 23781295]
58. Simon JA, Fong J, Bernert JT, Jr. and Browner WS. Relation of smoking and alcohol consumption to serum fatty acids. Am J Epidemiol. 1996;144:325–34. [PubMed: 8712189]
59. Thomson NC, Chaudhuri R, Spears M, et al.Arachidonic acid metabolites and enzyme transcripts in asthma are altered by cigarette smoking. Allergy. 2014;69:527–36. [PubMed: 24571371]
60. Balsinde J, Winstead MV and Dennis EA. Phospholipase A(2) regulation of arachidonic acid mobilization. FEBS Lett. 2002;531:2–6. [PubMed: 12401193]
61. Brunzell JD, Goldberg AP and Schwartz RS. Cigarettes smoking and adipose tissue lipoprotein lipase. Int J Obes. 1980;4:101–3. [PubMed: 7399800]
62. Cardenia V, Vivarelli F, Cirillo S, Paolini M, Canistro D and Rodriguez-Estrada MT. The effect of electronic-cigarettes aerosol on rat brain lipid profile. Biochimie. 2018;153:99–108. [PubMed: 30077815]
63. Canistro D, Vivarelli F, Cirillo S, et al.E-cigarettes induce toxicological effects that can raise the cancer risk. Sci Rep. 2017;7:2028. [PubMed: 28515485]
64. Husari A, Shihadeh A, Talih S, Hashem Y, El Sabban M and Zaatari G. Acute Exposure to Electronic and Combustible Cigarette Aerosols: Effects in an Animal Model and in Human Alveolar Cells. Nicotine Tob Res. 2016;18:613–9. [PubMed: 26272212]
65. Teasdale JE, Newby AC, Timpson NJ, Munafo MR and White SJ. Cigarette smoke but not electronic cigarette aerosol activates a stress response in human coronary artery endothelial cells in culture. Drug Alcohol Depend. 2016;163:256–60. [PubMed: 27137404]
66. Ikonomidis I, Vlastos D, Kourea K, et al.Electronic Cigarette Smoking Increases Arterial Stiffness and Oxidative Stress to a Lesser Extent Than a Single Conventional Cigarette: An Acute and Chronic Study. Circulation. 2018;137:303–306. [PubMed: 29335291]
67. Arnett DK, Blumenthal RS, Albert MA, et al.2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Circulation. 2019;140:e596–e646. [PubMed: 30879355]
68. Connor Gorber S, Schofield-Hurwitz S, Hardt J, Levasseur G and Tremblay M. The accuracy of self-reported smoking: a systematic review of the relationship between self-reported and cotinine-assessed smoking status. Nicotine Tob Res. 2009;11:12–24. [PubMed: 19246437]
69. Young AJ, Marriott BP, Champagne CM, Hawes MR, Montain SJ, Johannsen NM, Berry K and Hibbeln JR. Blood fatty acid changes in healthy young Americans in response to a 10-week diet that increased n-3 and reduced n-6 fatty acid consumption: a randomised controlled trial. Br J Nutr. 2017;117:1257–1269. [PubMed: 28534446]
70. Rumsey SC, Galeano NF, Arad Y and Deckelbaum RJ. Cryopreservation with sucrose maintains normal physical and biological properties of human plasma low density lipoproteins. J Lipid Res. 1992;33:1551–61. [PubMed: 1431579]
71. Hedrick CC, Castellani LW, Warden CH, Puppione DL and Lusis AJ. Influence of mouse apolipoprotein A-II on plasma lipoproteins in transgenic mice. J Biol Chem. 1993;268:20676–82. [PubMed: 8376417]
72. Warnick GR. Enzymatic methods for quantification of lipoprotein lipids. Methods Enzymol. 1986;129:101–23. [PubMed: 3724535]
73. Burstein M, Scholnick HR and Morfin R. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. J Lipid Res. 1970;11:583–95. [PubMed: 4100998]
74. Barlic J, Zhang Y, Foley JF and Murphy PM. Oxidized lipid-driven chemokine receptor switch, CCR2 to CX3CR1, mediates adhesion of human macrophages to coronary artery smooth muscle cells through a peroxisome proliferator-activated receptor gamma-dependent pathway. Circulation. 2006;114:807–19. [PubMed: 16908772]

75. Schlitt A, Heine GH, Blankenberg S, et al.CD14+CD16+ monocytes in coronary artery disease and their relationship to serum TNF-alpha levels. Thromb Haemost. 2004;92:419–24. [PubMed: 15269840]

NOVELTY AND SIGNIFICANCE

What Is Known?

- Electronic cigarettes (EC), introduced in 2006 as potentially safer alternatives to tobacco cigarettes (TC), have gained unprecedented popularity, especially among young adults, with little known about their long-term health effects.
- Chronic users of EC exhibit greater susceptibility for the oxidation of plasma low-density lipoprotein (LDL) in comparison to non-smoking individuals indicating increased oxidative stress.

What New Information Does This Article Contribute?

- Chronic use of EC and TC is associated with both similar as well as differential effects on polyunsaturated fatty acids, pro-oxidative and antioxidant biomarkers in the blood.
- EC-users display intermediate alterations in cardiovascular health metrics between non-users and TC-smokers.

With a growing popularity for EC use among younger adults, there is a gap in our understanding regarding EC effects on biomarkers of lipid peroxidation in the blood and their relationship to cardiovascular health. We asked whether chronic use (more than one year) of EC could potentially alter cardiovascular health metrics in healthy young adults using novel and sensitive biomarkers of lipid peroxidation. Thus, we recruited individuals who were either chronic users of EC, TC or non-users, for measurement of oxidized metabolites of polyunsaturated fatty acids and antioxidant capacity in their plasma. EC-users and TC-smokers exhibited lower levels of oxidized products of linoleic acid in comparison to non-users and the levels of antioxidant defense markers were also elevated likely due to antioxidant homeostatic responses. In addition, TC-smokers had increased concentrations of heme oxygenase-1 and cluster of differentiation-14, markers of vascular injury and inflammation, respectively, possibly reflecting increased cardiovascular injury. Lastly, our multivariable analysis demonstrated that EC-users exhibited biomarker levels that were overall intermediate between non-users and TC-smokers with a potential to be related to future adverse cardiovascular health effects. Therefore, our study indicates that long-term use of either EC or TC exerted health effects including alterations in lipid metabolism, oxidative and inflammatory responses, that are relevaent to cardiovascular health.

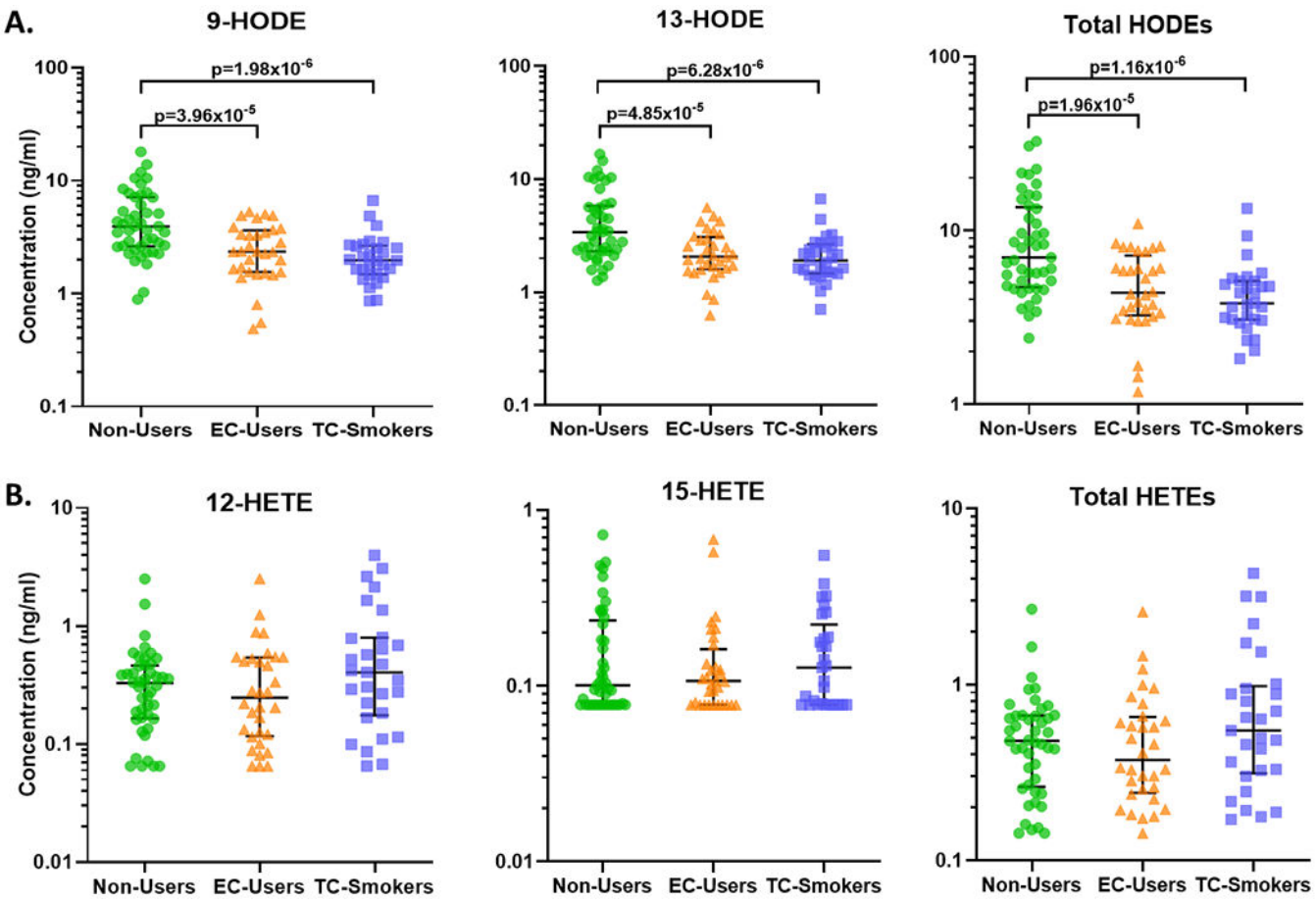


Figure 1. Plasma concentrations of oxidized metabolites of linoleic acid and arachidonic acid

Concentrations of (A) 9-HODE, 13-HODE and total HODEs (9-HODE + 13-HODE); and (B) 12-HETE, 15-HETE and total HETEs (12-HETE + 15-HETE) in the plasma of non-users (n=45), EC-users (n=32) and TC-smokers (n=29). The solid horizontal line indicates the median and error bars represent the interquartile range (IQR). Statistical analysis was performed using one-way ANOVA on the original or log scale as indicated followed by the post hoc t test using the Fisher's LSD criterion. EC = electronic cigarettes; HETE = hydroxyeicosatetraenoic acid; HODE = hydroxyoctadecadienoic acid; LSD = least significant differences; TC = tobacco cigarettes.

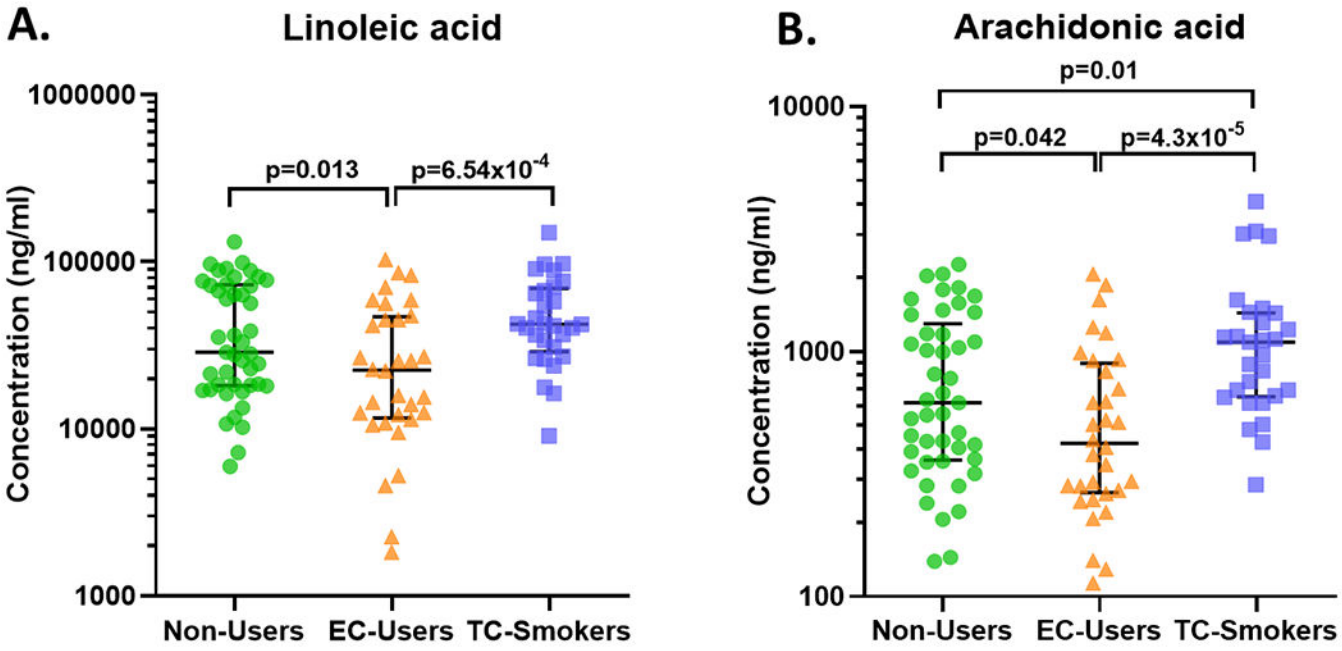


Figure 2. Plasma PUFAs.

Concentrations of (A) linoleic acid and (B) arachidonic acid in the plasma of non-users (n=45), EC-users (n=32) and TC-smokers (n=29). Figure representation, statistical analysis and other abbreviations are same as in Figure-1. PUFAs = polyunsaturated fatty acids.

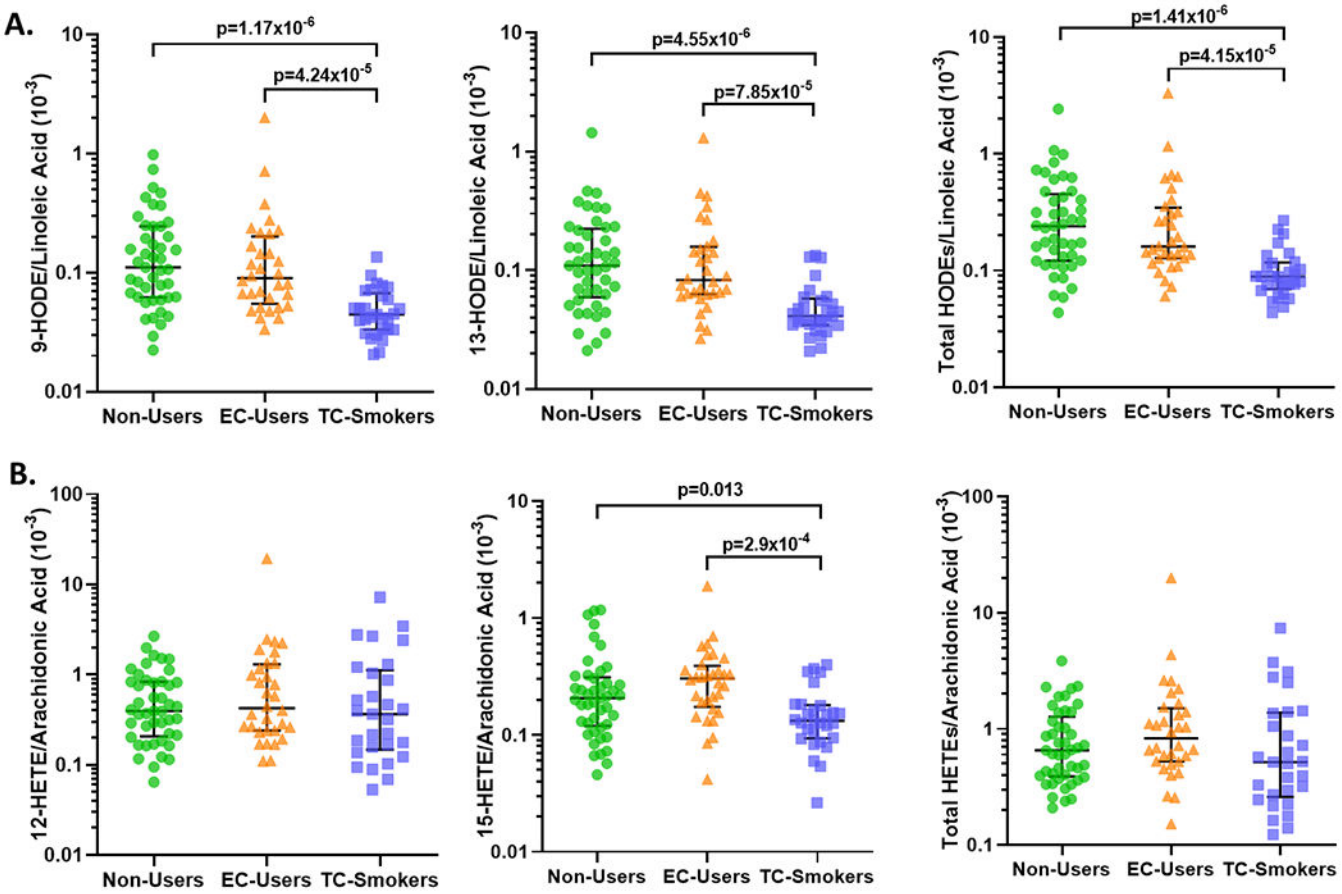


Figure 3. Plasma ratios of oxidized lipids to parental PUFAs.

Plasma levels of HODEs and HETEs normalized by linoleic acid and arachidonic acid, respectively, among non-users (n=45), EC-users (n=32) and TC-smokers (n=29). (A) 9-HODE/LA, 13-HODE/LA and Total HODEs/LA; and (B) 12-HETE/AA, 15-HETE/AA and Total HETEs/AA. Figure representation, statistical analysis and other abbreviations are same as in Figure-1. AA = arachidonic acid; LA = linoleic acid; PUFAs = polyunsaturated fatty acids.

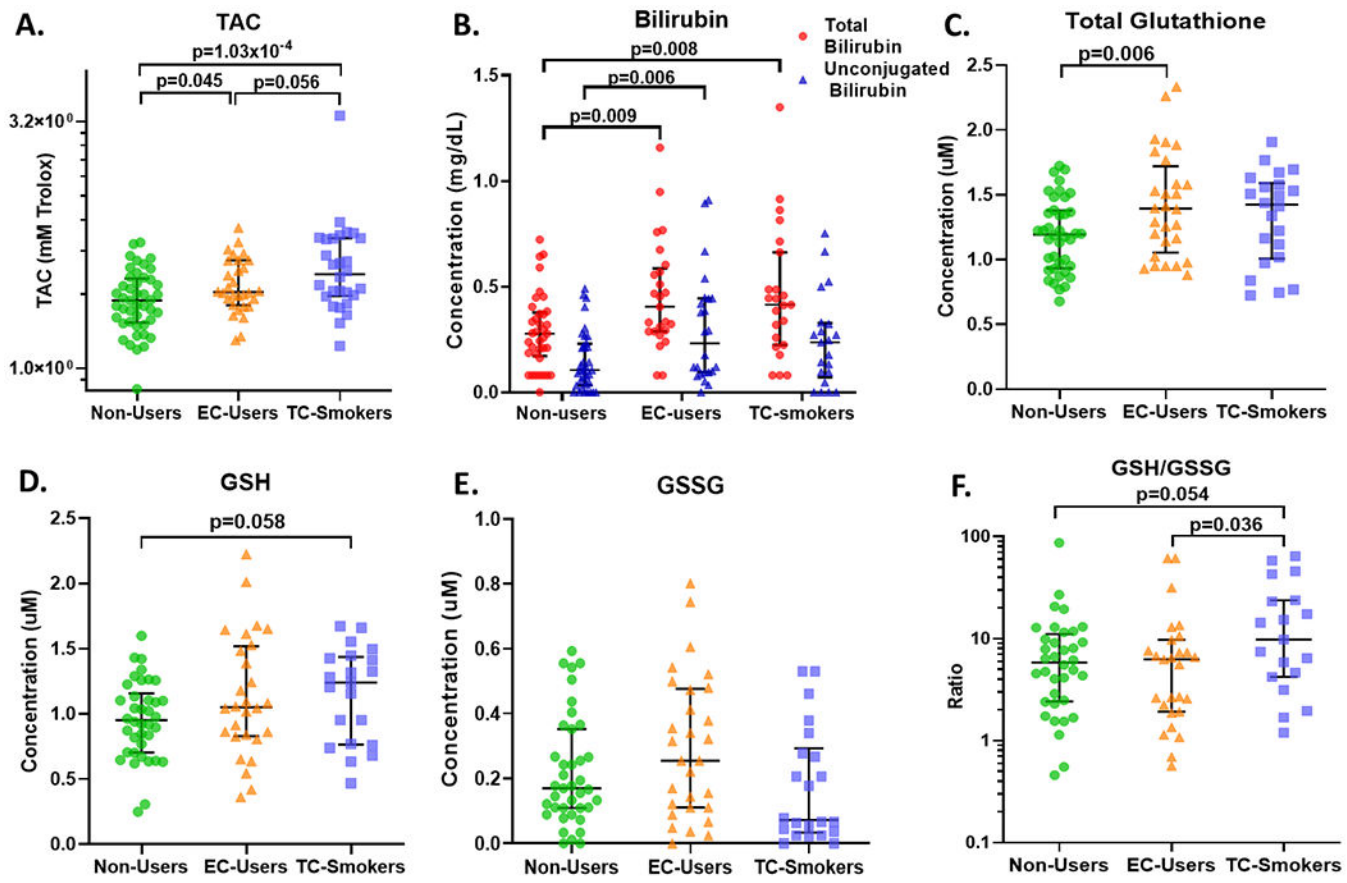


Figure 4. Plasma antioxidant status.

(A) Total antioxidant capacity (TAC) in non-users (n=45), EC-users (n=30) and TC-smokers (n=28); (B) total and unconjugated bilirubin in non-users (n=39), EC-users (n=25) and TC-smokers (n=23); (C) total glutathione, (D) reduced glutathione (GSH), (E) oxidized glutathione (GSSG) and (F) ratio of reduced to oxidized glutathione (GSH/GSSG) in non-users (n=40), EC-users (n=29) and TC-smokers (n=22). Figure representation, statistical analysis and other abbreviations are same as in Figure-1.

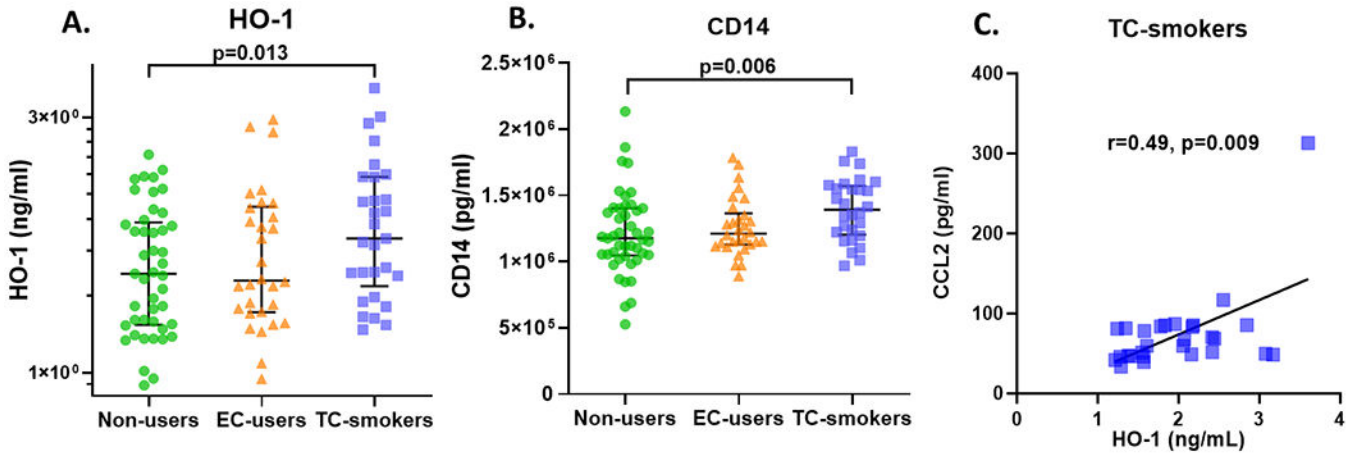


Figure 5. Vascular injury and inflammatory response in the plasma of smokers.

(A) Heme oxygenase-1 (HO-1), (B) cluster of differentiation 14 (CD14) in the plasma of non-users (n=45), EC-users (n=30) and TC-smokers (n=28), (C) association of C-C motif chemokine ligand 2 (CCL2) with HO-1 in TC-smokers. Spearman's correlation was computed along with linear regression. The correlation coefficient and p-value are indicated on the graph (panel C). Figure representation, statistical analysis and other abbreviations are same as in Figure-1.

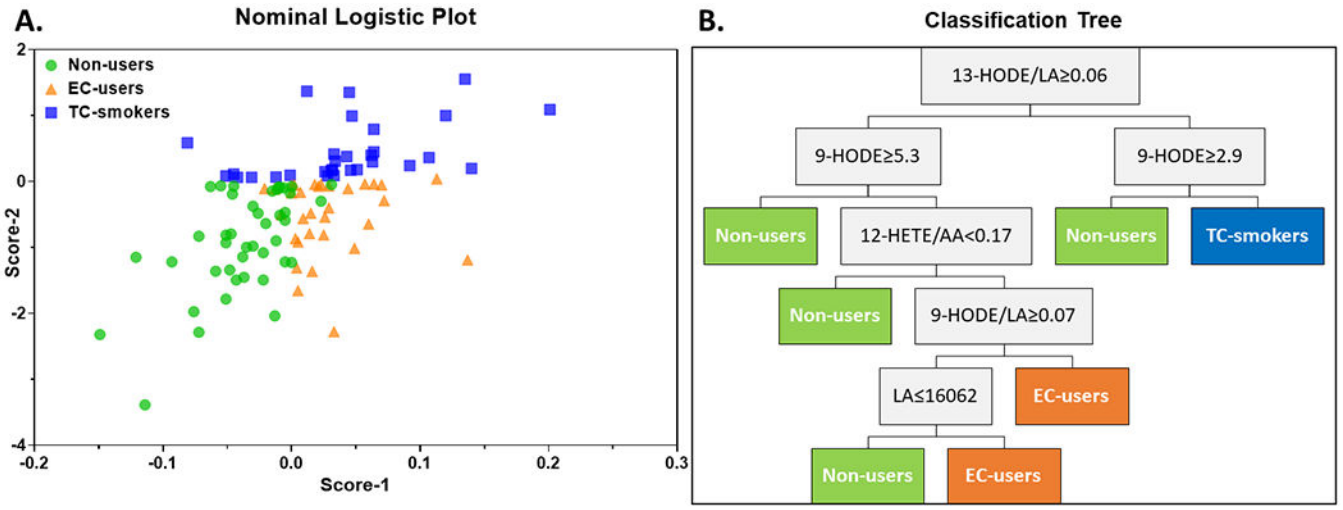


Figure 6. Multivariable analysis plots.

(A) Two-dimensional score plot of nominal multinomial logistic model *via* neural net based on multivariable analysis of 33 biomarkers considered simultaneously for discriminating between non-users (green circles, n=45), EC-users (orange triangles, n=32) and TC-smokers (blue squares, n=29). To improve the schematic representation, two data points for the non-users were excluded as outliers, which barely changed the prediction accuracy from 94.4% with inclusion to 94.3% with exclusion of the two outliers. (B) Classification tree separated EC-users (n=32) from non-users (n=45) and TC-smokers (n=29) based on 2 primary (i.e., 9-HODE and LA) and 3 derived (13-HODE/LA, 12-HETE/AA and 9-HODE/LA) variables out of all 18 predictor outcomes. For each branch of the tree, if the condition is true, one branches left; otherwise, if the condition is false, one branches right. AA = arachidonic acid; LA = linoleic acid; other abbreviations are same as in Figure-1.

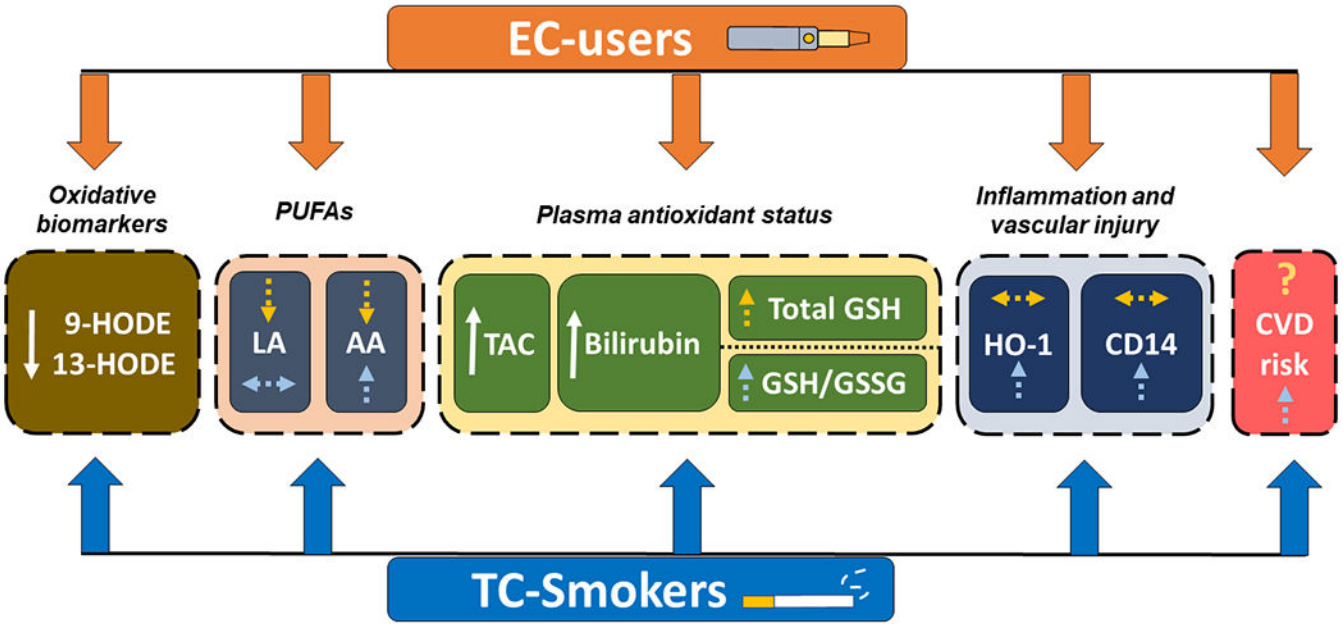


Figure 7. Schematic diagram.

Proposed model of cardiometabolic effects exerted by chronic EC-users and TC-smokers. White solid arrows indicate common effects, whereas orange and blue dotted arrows indicate differential effects due to EC use or TC smoking, respectively. Double headed arrows indicate no statistical differences. AA = arachidonic acid; CD14 = cluster of differentiation 14; CVD = cardiovascular disease; EC = electronic cigarette; GSH = reduced glutathione; GSSG = oxidized glutathione; HO-1 = heme oxygenase-1; HODE = hydroxyoctadecadienoic acid; LA = linoleic acid; PUFAs = polyunsaturated fatty acids; TAC = total antioxidant capacity; TC = tobacco cigarette; Total GSH = total glutathione.

Table 1.

Baseline characteristics of non-users, e-cigarette (EC) users and tobacco-cigarette (TC) smokers.

Characteristics	Non-users (n=45)	EC-users (n=32)	TC-smokers (n=29)	p-value
Age (years)	27.4 ± 0.8	28.3 ± 0.9	27.8 ± 1.1	0.79
Sex, No.				
Males	26	23	19	0.45
Females	19	9	10	
BMI	23.4 ± 0.4	24.8 ± 0.6	24.5 ± 0.5	0.11
Race/Ethnicity, No.				
African American	1	2	3	0.73
Asian	8	8	8	
Hispanic	5	2	1	
White (Non-Hispanic)	28	19	17	
Native Hawaiian	2	0	0	
Unknown	1	1	0	
Duration (years) of EC-use	NA	2 (1 – 3)#	NA	NA
Duration (years) of TC-smoking	NA	NA	8 (5 – 10)#	NA
Duration (years) of former TC-smoking*	NA	4 (3 – 7.25)#	NA	NA
Former Smoker, No.	4	22	NA	$4.32x10^{-8}$
Interval since quitting, y	2 (2 – 4)#	2.25 (1.5 – 4)#	NA	NA
Baseline Nicotine (ng/ml)	ND	4.7 (3.05 – 16.15)#	7.75 (4.85 – 11.93)#	0.42
Baseline Cotinine (ng/ml)	ND	89.8 (42.8 – 145.5)#	98 (52 – 205)#	0.68

Data are shown as mean ± SEM for parametrically distributed data or median (IQR)# for nonparametrically distributed data. Statistical analysis was performed using A) one-way ANOVA followed by the post hoc t test under the Fisher's LSD criterion for age and BMI data or B) Kruskal-Wallis test for baseline nicotine and cotinine data. The p values for comparing categorical data such as sex, race/ethnicity and former smoking status were computed using Fisher's exact test.

*EC-users had quit smoking for at least one year prior to the study onset.

Abbreviations: SEM, standard error of mean; IQR, interquartile range; BMI, body mass index; NA, not applicable; ND, not detected.