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Chronic electronic cigarette use and atherosclerosis risk in young people: A cross-sectional study

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Abstract

Background: Little is known whether electronic cigarettes (ECIG) increase vulnerability to future atherosclerotic cardiovascular disease (CVD). We determined, using an *ex vivo* mechanistic atherogenesis assay, whether proatherogenic changes including monocyte trans-endothelial migration (MTEM) and monocyte derived foam cell formation (MDFCF) are increased in people who use ECIGs.

Methods: In a cross-sectional single-center study using plasma and peripheral blood mononuclear cells (PBMCs) from healthy participants who are non-smokers (NS) or with exclusive use of ECIGs or tobacco cigarettes (TCIGs), autologous PBMCs with patient plasma and pooled PBMCs from healthy NS with patient plasma were utilized to dissect patient-specific *ex vivo* proatherogenic circulating factors present in plasma and cellular factors present in monocytes. Our main outcomes were MTEM (% of blood monocyte cells that undergo TEM through a collagen gel) and MDFCF as determined by flow cytometry and the median fluorescence intensity (MFI) of the lipid-staining fluorochrome BODIPY in monocytes of participants in the setting of an *ex vivo* model of atherogenesis.

Results: Study participants (n=60) had median age of 24.0 years [interquartile range (IQR) 22.0–25.0 years] and 31 were females. MTEM was increased in people who exclusively used TCIGs (n=18) [median, IQR: 2.30 (1.29–2.82, p<0.001) and in people who exclusively used ECIGs (n=21) [median, IQR: 1.42 (0.96–1.91, p<0.01)] compared to NS controls (n=21) [median,

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Author Contributions

TK: Design of study, flow cytometry experiments, supervision of experiments, data analysis, interpretation of data, writing of manuscript (with HRM), revision of manuscript, financial support.

MS: Data collection, processing of biospecimens, performance of flow cytometry experiments

ES: Data collection, revision of the manuscript

IR: Data collection, revision of the manuscript

ET: Data collection, revision of the manuscript

HRM: Design of study, interpretation of data, writing of manuscript (with TK), revision of manuscript, financial support.

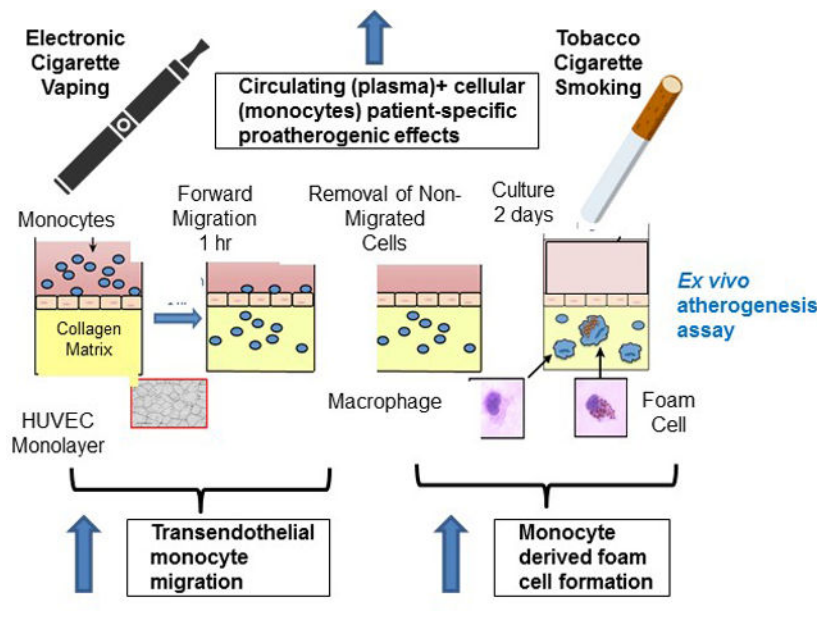
Disclosures

None

IQR: 1.05 (0.66–1.24)]. MDFCF was increased in people who exclusively used TCIGs [median, IQR: 2.01 (1.59–2.49, $p < 0.001$)] and in people who exclusively used ECIGs [median, IQR: 1.54 (1.10–1.86, $p < 0.001$)] compared to NS controls [median, IQR: 0.97 (0.86–1.22)]. Both MTEM and MDFCF were higher in TCIG smokers compared to ECIG users and in ECIG users who were former smokers vs ECIG users who were never smokers ($p < 0.05$ for all comparisons).

Conclusions: The finding of alterations in proatherogenic properties of blood monocytes and plasma in TCIG smokers compared to NS validates this assay as a strong *ex vivo* mechanistic tool with which to measure proatherogenic changes in people who use ECIGs. Similar yet significantly less severe alterations in proatherogenic properties of monocytes and plasma were detected in the blood from ECIGs users. Future studies are necessary to determine whether these findings are attributable to a residual effect of prior smoking or are a direct effect of current ECIG use.

Graphical Abstract



INTRODUCTION

Electronic cigarettes (ECIGs), which remain very popular^{1, 2}, have been available in the US for over 15 years, yet their contribution to cardiovascular risks has not been fully elucidated. In contrast, there is a large body of data informing our mechanistic understanding of atherosclerosis, a slowly progressive, inflammatory process in which circulating monocytes attach to endothelial cells, then migrate into the subintimal space, where they phagocytize oxidized lipids and become foam cells, generating the fatty streak. Foam cells then secrete pro-inflammatory cytokines, ultimately leading to the progressive process of inflammatory atherosclerosis that, unchecked, may present clinically as an acute coronary syndrome and even sudden death³. The role of tobacco cigarettes (TCIGs) in increasing oxidative stress and inflammation, thereby accelerating this process, is widely accepted⁴. Whether ECIGs, too, promote this process of monocyte trans-endothelial migration and foam cell formation is unknown.

We utilized our previously developed and validated *in vitro* model of early atherosclerotic events in which monocyte trans-endothelial migration and foam cell formation can be quantified^{5, 6}. In the present study, we first determined if this novel assay was able to detect enhanced monocyte trans-endothelial migration (MTEM) and foam cell formation in young people who exclusively smoke TCIGs. Then we turned to otherwise healthy young people who exclusively use ECIGs to determine if using ECIGs also elicits similar proatherogenic changes, portending increased cardiovascular risk.

METHODS

Data are available upon reasonable request to the corresponding author.

Study Participants

Healthy participants meeting eligibility criteria⁷ were enrolled: 1) TCIG smokers (exclusive TCIG smoking >1 year), 2) ECIG users (exclusive ECIG use >1 year), and non-smokers (NS) (neither TCIGs or ECIGs >1 year). The experimental protocol was approved by the Institutional Review Board at the University of California, Los Angeles, and written informed consent was obtained from each participant.

Experimental session

Blood was drawn for cotinine levels and for peripheral blood mononuclear cells (PBMC) collection, and processed as previously described^{7, 8}.

In vitro atherogenesis assay

Briefly, PBMCs were added to tumor necrosis factor -activated human umbilical vein endothelial cells monolayers on type I collagen gels to transmigrate over 1 hour in the presence of 10% v/v plasma in serum-free media and form foam cells over 48 hours as previously described^{5, 6}. To dissect circulating (present in plasma) and cellular (present in monocytes) patient-specific *ex vivo* proatherogenic effects we utilized autologous patient plasma and PBMCs. To dissect only circulating (present in plasma) patient-specific *ex vivo* proatherogenic effects we utilized autologous patient plasma and pooled PBMC from non-smokers (Figure 1A). Lipids present in the patient plasma drive *ex vivo* monocyte derived foam cell formation (MDFCF). Flow cytometry was utilized to determine the number of CD45+ CD11b+ macrophages inside the collagen gel (measure of MTEM) and the median fluorescence intensity (MFI) of BODIPY inside the transmigrated gel macrophages (measure of lipid content in macrophages and MDFCF). The gating strategy is shown in Figure 1B.

Statistical analysis

Kruskal—Wallis analysis of variance was used to compare the 3 groups and, if the results were statistically significant ($P < 0.05$), then the Mann—Whitney test was used to compare individual 2 groups. This study, largely exploratory, was not powered to detect effect sizes with adjustments for multiple comparisons. Our study is a small single-center study, and not powered to detect effect sizes with adjustment for multiple comparisons. Rather,

consistency, direction, and magnitude of the effect in conjunction with the nominal p values were considered in order to help distinguish true and false-positive findings⁹.

RESULTS

Baseline characteristics

Participants in the 3 groups (TCIG smokers vs ECIG users vs NS, were not different by age, median [interquartile range (IQR), (24.0 [23.0–24.8] vs 23.0 [22.0–25.0] vs 23.0 [21.0–26.0] years; $p=0.33$), sex (female/male/, 9/9 vs 12/9 vs 10/11; $p=0.85$), body mass index (BMI), median [IQR], (21.9 [19.8–24.1] vs 22.5 [21.1–24.0] vs 22.5 [20.2–24.1] kg/m^2 ; $p=0.49$), and all participants had at least some college education. Tobacco product use burden, as estimated by plasma cotinine, was not different between TCIG smokers vs ECIG users (plasma cotinine median [IQR] 72.0 [28.0–143.5] vs 56.0 [22.0–165.0] ng/ml , $p=0.58$).

TCIG smokers have increased proatherogenic MTEM

Using autologous PBMCs and plasma, compared to NS, TCIG smokers had ~110% mean increase in MTEM (Figure 1C, D).

To dissect whether the patient-specific *ex vivo* proatherogenic effects of TCIG smoking in our model of atherogenesis was secondary to circulating factors present in plasma or due to cellular factors present in monocytes, we then utilized patient specific plasma and pooled PBMCs from the NS controls (Approach B)(Figure 1A). There was no difference in MTEM between TCIG smokers and NS controls (Figure 1E), consistent with the notion that the *ex vivo* proatherogenic increased MTEM observed with autologous monocytes and plasma from TCIG smokers compared to NS was secondary to effects of TCIG smoking on monocytes rather than circulating factors.

ECIG users have increased proatherogenic MTEM

Using autologous patient-specific monocytes and plasma (Approach A), compared to the NS controls, ECIG users had ~40% mean increase in MTEM ($p<0.01$) (Figure 1C, D). This increase was significantly less than in the TCIG smokers ($p<0.01$) (Figure 1C, D).

To determine if this *ex vivo* proatherogenic finding in ECIG users was a residual effect from prior TCIG smoking, or attributable to current ECIG use, the ECIG group was divided into ECIG-former smokers and ECIG-never smokers. Current ECIG use, as judged by plasma cotinine levels, was similar in people who were (n=12), and were not (n=9), former smokers (plasma cotinine median[IQR] 78.0 [25.8–179.3] vs 27.0 [4.5–141.5] ng/ml , $p=0.34$, respectively). MTEM was significantly greater in ECIG-former smokers compared to ECIG-never smokers ($p<0.05$) (Figure 1F).

Healthy PBMCs and patient plasma were again used to dissect whether the patient-specific *ex vivo* proatherogenic effects of ECIG use in our model of atherogenesis was secondary to circulating factors present in plasma or due to cellular factors present in monocytes. We found no difference in MTEM between ECIG users and NS (Figure 1E), suggesting that MTEM in ECIG users was secondary to effects of ECIG use on monocytes rather than circulating factors.

TCIG smokers have increased proatherogenic MDFCF

Using autologous patient-specific monocytes and plasma, compared to NS, TCIG smokers had ~100% relative mean increase in the BODIPY MFI in CD11b+ transmigrated macrophages (Figure 2A, B). Using healthy PBMCs and patient plasma, we found TCIG smokers had ~67% mean increase in BODIPY MFI compared to NS (Figure 2C), suggesting that the increased MDFCF in TCIG smokers compared to NS was secondary to effects of TCIG smoking on both monocytes and circulating factors.

ECIG users have increased proatherogenic MDFCF

Compared to NS, ECIG users had ~50% mean increase in BODIPY MFI in CD11b+ transmigrated macrophages ($p < 0.05$) (Figure 2A, B). The mean BODIPY MFI in CD11b+ transmigrated macrophages was ~50% lower in ECIG users compared to TCIG smokers (Figure 2A, B). We then determined whether this intermediate level of MDFCF seen in ECIG users was a residual effect from prior TCIG smoking. MDFCF was significantly greater in ECIG-former smokers compared to ECIG-never smokers ($p < 0.05$) (Figure 2D).

Using healthy PBMCs and patient plasma, we found that ECIG users had a mean 34% increase in BODIPY MFI in CD11b+ transmigrated macrophages compared to NS (Figure 2C) ($p < 0.05$), suggesting that the *ex vivo* proatherogenic increased MDFCF observed in ECIG users compared to NS was secondary to effects of ECIG use on both monocytes and circulating factors.

DISCUSSION

To our knowledge, this is among the first studies in humans to confirm early alterations in proatherogenic properties of blood monocytes and plasma collected from otherwise healthy young people who smoke TCIGs compared to people who do not use tobacco products. Our findings in TCIG smokers compared to NS controls are consistent with the notion that this atherogenesis assay is a strong mechanistic tool to predict future cardiovascular risk in humans given established link of TCIGs with future cardiovascular disease⁹, and provides a rationale for its use in assessing proatherogenic changes in young people who use ECIGs. Similar yet significantly less severe alterations in proatherogenic properties were detected in blood monocytes and plasma collected in otherwise healthy young people who use ECIGs compared to people who do not use tobacco products. Both primary endpoints and key steps in early atherosclerosis, including monocyte trans-endothelial migration and foam cell formation, were significantly increased in people who use ECIGs compared to NS, but were significantly lower than in the TCIG smokers. Consistent with these data, we have previously reported an increase in established contributors to atherogenesis such as polyunsaturated fatty acids and oxidative biomarkers^{10, 11}, proatherogenic proteins of the toll like receptor 4-inflammasome-IL-6 axis in immune cell subtypes^{7, 12}, cellular oxidative stress^{8, 13} in otherwise healthy people who smoke TCIGs or use ECIGs compared to NS.

Since ECIG users and TCIG smokers have increased cellular oxidative stress compared to non-smokers^{8, 11}, and given that redox changes in biology of tissue macrophages may persist long term¹⁴, we then asked the question whether these intermediate levels MTEM

and foam cell formation seen in people who use ECIGs was a residual effect from prior smoking or was attributable to current ECIG use. Although all ECIG users who were former smokers had quit smoking more than 1 year prior to study enrollment, both proatherogenic processes were significantly greater in current ECIG users who were former smokers compared to ECIG users who were never smokers, consistent with a residual effect of prior TCIG smoking. These data suggest that exclusive ECIG use does not contribute to atherosclerosis, but this needs to be confirmed in a larger study.

The strengths of our study are the careful covariate phenotyping of our study population and established physiologically relevant model of atherogenesis able to detect key proatherogenic properties of monocytes in people who smoke TCIGs that could then be applied to people who use ECIGs. Although our study is a small study, we were able to find a consistent increase in MTEM and MDFCF in otherwise healthy young people who exclusively use either ECIGs or TCIGs compared to non-smokers. Additional larger studies that also include former TCIG smokers who do not use ECIGs will help if there is an additive or even synergistic effect between TCIG and ECIG use.

In conclusion, the finding that key proatherogenic properties of monocytes are elevated in otherwise healthy young people who use either TCIGs or ECIGs compared to non-smokers has public health implications. Our data provide urgency to messaging that ECIG use is not harmless, and that smoking cessation efforts must be redoubled.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Non-standard abbreviations and acronyms

ECIG	electronic cigarette
TCIG	tobacco cigarette
MTEM	monocyte trans-endothelial migration
NS	non-smokers
PBMC	peripheral blood mononuclear cells
IQR	interquartile range
MDFCF	monocyte derived foam cell formation
MFI	median fluorescence intensity

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Highlights

- Little is known whether electronic cigarettes (ECIG) increase vulnerability to future atherosclerotic cardiovascular disease.
- Using our *in vitro* model of early atherosclerotic events quantifying monocyte trans-endothelial migration (MTEM) and foam cell formation (MDFCF), we found that MTEM and MDFCF were increased in tobacco cigarette (TCIG) smokers and ECIG users compared to non-smoking (NS) controls.
- Both MTEM and MDFCF were higher in TCIG smokers compared to ECIG users and in ECIG users who were former smokers vs ECIG users who were never smokers.
- Future studies are necessary to determine whether these findings are attributable to a residual effect of prior smoking or are a direct effect of current ECIG use.

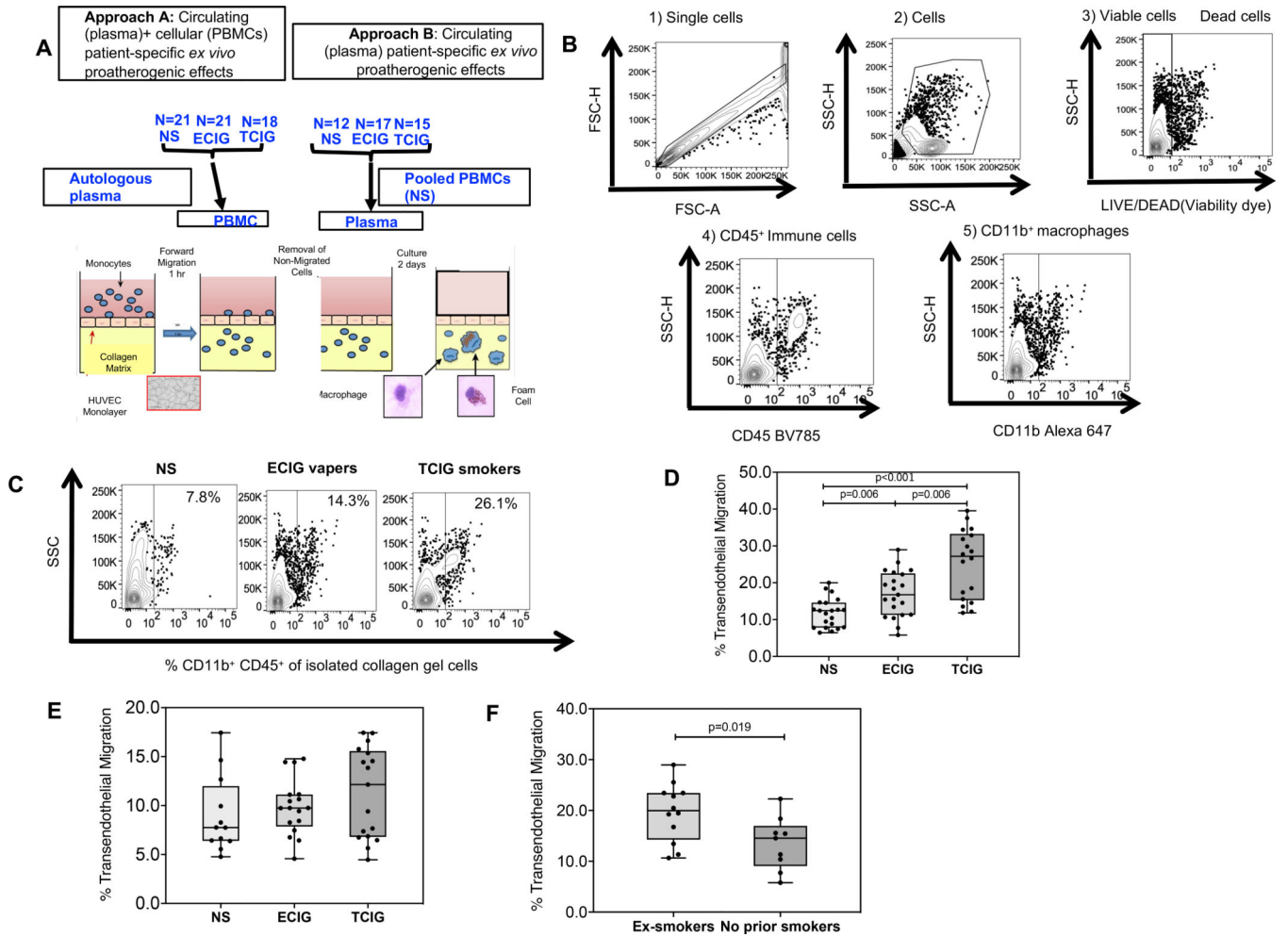


Figure 1: Transendothelial chemotaxis of monocytes among groups.

A. Optimal microscopy and flow cytometry was used to determine the percent of monocytes that underwent forward transendothelial migration (TEM) in collagen gel coated with HUVEC endothelial cells after 1 hour exposure to pooled plasma from healthy study participants as indicated in methods. The compared groups were nonsmokers (NS, light grey) (n=21), electronic-cigarette vapers (ECIG-vapers, grey)(n=21) and tobacco cigarette smokers (TCIG-smokers, dark grey)(n=18). Two different approaches were utilized to dissect the proatherogenic properties of circulating factors in plasma compared to cells.

B. Gating flow cytometry strategy in macrophages isolated from collagen gels.

C. Representative data of % transmigrated CD45+CD11b+ macrophages isolated from collagen gels among compared groups.

D. % TEM migration among compared groups (Approach A) expressed relatively to the mean of the non-smoker group. Autologous plasma and cells were used for the atherogenesis assay.

E. % TEM migration among compared groups (Approach B) expressed relatively to the mean of the non-smoker group. Patient plasma and pooled PBMCs from NS were used for the atherogenesis assay.

F. % TEM migration among ECIG groups who were ex smokers versus never smokers. Summary data are shown as box and whisker plots with minimum, median, interquartile range (IQR) and maximum values. Mann Whitney test was used to compare 2 groups.

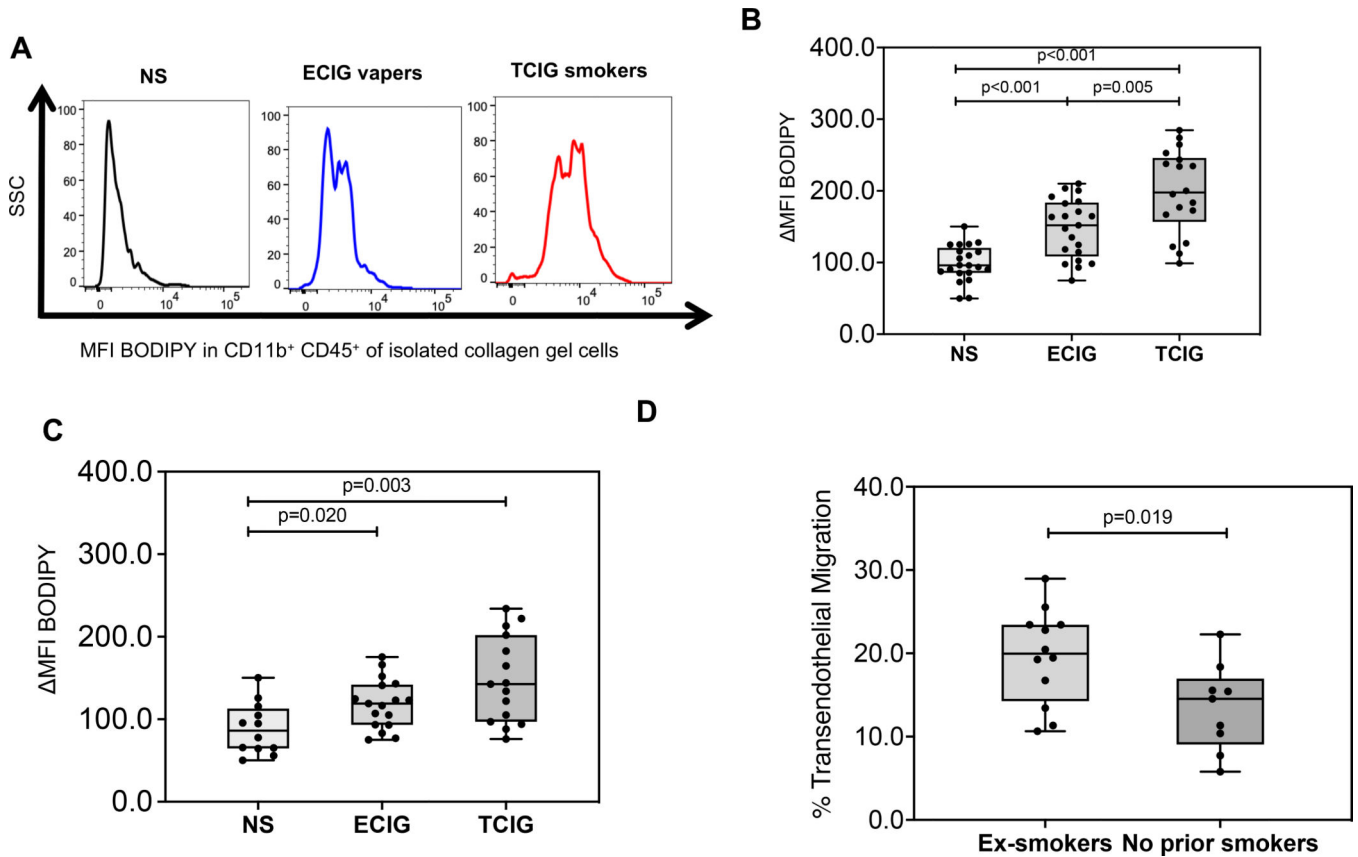


Figure 2: Monocyte derived foam cell formation among groups.

A. Flow cytometry was used to determine the median fluorescence intensity (MFI) of the lipid-staining fluorochrome BODIPY in monocyte derived CD11b⁺ macrophages that underwent TEM and were isolated from the collagen gel after 48-hour exposure to autologous plasma as indicated in methods. Fluorescence intensity of a positive cell population was compared to a negative cell population (fluorescence minus one negative control for staining) (MFI). The compared groups were nonsmokers (NS, light grey) (n=21), electronic-cigarette vapers (ECIG-vapers, grey) (n=21) and tobacco cigarette smokers (TCIG-smokers, dark grey) (n=18). **B.** MFI BODIPY among compared groups (Approach A) expressed relatively to the mean of the non-smoker group. **C.** MFI BODIPY among compared groups (Approach B) expressed relatively to the mean of the non-smoker group. Summary data are shown as box and whisker plots with minimum, median interquartile range (IQR) and maximum values. Mann Whitney test was used to compare 2 groups (*p < 0.05, **p < 0.01, ***p < 0.001).

Summary data (mean, SEM) are shown. Mann Whitney test was used to compare 2 groups.