Impact of chronic use of heat-not-burn cigarettes on oxidative stress, endothelial dysfunction and platelet activation: the SUR-VAPES Chronic Study

Lorenzo Loffredo ,[1] Roberto Carnevale,[2,3] Simona Battaglia,[1] Roberta Marti,[4] Stefano Pizzolo,[4] Simona Bartimoccia,[5] Cristina Nocella,[1] Vittoria Cammisotto,[6] Sebastiano Sciarretta,[7,8] Isotta Chimenti,[3,9] Elena De Falco,[3,9] Elena Cavarretta,[3,9] Mariangela Peruzzi,[3,9] Antonino Marullo,[9] Fabio Miraldi,[4] Francesco Violi,[1,3] Andrea Morelli,[4] Giuseppe Biondi-Zoccai ,[3,9] Giacomo Frati[8,9]

► Additional material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/thoraxjnl-2020-215900).

For numbered affiliations see end of article.

Correspondence to
Professor Lorenzo Loffredo, Department of Clinical Internal, Anesthesiologic and Cardiovascular Sciences, Sapienza University of Rome, Roma 00161, Italy; lorenzo.loffredo@uniroma1.it and Dr Roberto Carnevale, Department of Medical-Surgical Sciences and Biotechnologies, Sapienza University of Rome, 00185 Roma, Italy; roberto.carnevale@uniroma1.it

LL and RC contributed equally.

GB-Z and GF are co-senior authors.

Received 29 July 2020
Revised 14 December 2020
Accepted 17 December 2020

► http://dx.doi.org/10.1136/thoraxjnl-2020-216253
► http://dx.doi.org/10.1136/thoraxjnl-2020-216743

To cite: Loffredo L, Carnevale R, Battaglia S, *et al*. *Thorax* Epub ahead of print: [*please include* Day Month Year]. doi:10.1136/thoraxjnl-2020-215900

ABSTRACT

Tobacco habit still represents the leading preventable cause of morbidity and mortality worldwide. Heat-not-burn cigarettes (HNBCs) are considered as an alternative to traditional combustion cigarettes (TCCs) due to the lack of combustion and the absence of combustion-related specific toxicants. The aim of this observational study was to assess the effect of HNBC on endothelial function, oxidative stress and platelet activation in chronic adult TCC smokers and HNBC users. The results showed that both HNBC and TCC display an adverse phenotype in terms of endothelial function, oxidative stress and platelet activation. Future randomised studies are strongly warranted to confirm these data.

INTRODUCTION

Tobacco habit still represents the leading preventable cause of morbidity and mortality worldwide, responsible for over six million deaths each year.[1] Tobacco smoking significantly impacts on all phases of the atherothrombotic process, contributing to endothelial dysfunction, oxidative stress, platelet activation and plaque development.[2] Concerning a realistic clinical scenario, tobacco smoking increases the risk of coronary heart disease, cerebrovascular disease, peripheral artery disease, and abdominal aortic aneurysm development and progression. Accordingly, smoking cessation decreases subsequent cardiovascular and cerebrovascular events with their related mortality and morbidity.[3] Heat-not-burn cigarettes (HNBCs) are considered as a possible alternative to traditional combustion cigarettes (TCC) due to the lack of both combustion and absence of combustion-related specific toxicants.[4]

Recently, adverse acute effects of HNBC on vascular function, oxidative stress and platelet activation have been demonstrated,[5–9] with evident implications for atherosclerosis development and progression and, ultimately, vascular disease. Specifically, we found that HNBC had less impact, even if non-negligible, than TCC on oxidative stress, flow-mediated dilation (FMD) and platelet aggregation (PA). However, to date, no independent comparative data on the chronic impact of HNBC on vascular function, oxidative stress and platelet activation are available in smokers.

The aim of the The Sapienza University of Rome-Vascular Assessment of Proatherosclerotic Effects of Smoking chronic study was to assess endothelial dysfunction, oxidative stress and platelet activation in chronic HNBC users.

METHODS

All methods are reported in the online supplemental material.

RESULTS

The study was conducted in 20 chronic users of HNBC, 20 chronic smokers of TCC and 20 non-smokers (table 1 and online supplemental material). In order to evaluate endothelial dysfunction, we analysed FMD and nitric oxide (NO) bioavailability. Results showed that compared with non-smokers, both TCC smokers and HNBC users had a significant decrease of FMD and NO bioavailability (table 2 and online supplemental figure S1), without significant differences between them.

In order to evaluate the impact of chronic smoking on oxidative stress, we analysed the concentration of soluble Nox2-derived peptide (sNox2-dp), a marker of nicotinamide adenine dinucleotide phosphate oxidase oxidase activation, and the production of hydrogen peroxide (H_2O_2). We observed that, compared with non-smokers, TCC smokers and HNBC users had higher levels of sNox2-dp and H_2O_2 production (table 2 and online supplemental figure S2). Notably, H_2O_2 production was similarly high in TCC smokers and HNBC users, whereas sNox2-dp was less markedly increased in HNBC users in comparison to TCC smokers. Platelet function was evaluated by the analysis of PA, sCD40L and sP-selectin. Compared with non-smokers, a significant increase of PA, sCD40L and sP-selectin was observed in TCC smokers and HNBC users (table 2 and online supplemental figure S3), without significant differences between them.

Finally, we explored the correlation between various features analysed in the study, highlighting several potential associations and clustering features between different variables, ranging from PA and sP-selectin to FMD and NO (online supplemental tables S1, S2 and online supplemental figures S4, S5), and performed multivariable linear regression analysis to expand on the results of the bivariate analysis (online supplemental tables S3, S4).

Table 1 Clinical characteristics of non-smokers, TCC smokers and HNBC users

Characteristic	Non-smokers	TCC smokers	HNBC users	Overall P value*	P value for non-smokers versus TCC smokers†	P value for non-smokers versus HNBC users†	P value for TCC smokers vs HNBC users†
Subjects	20	20	20	–	–	–	–
Age (years)	28 (23–33)	27 (24– 30)	33 (28–44)	0.008	0.370	0.018	0.005
Female gender	11 (55%)	10 (50%)	12 (60%)	0.946	1	1	0.751
Height (cm)	166 (160–175)	170 (165–181)	175 (170–181)	0.039	0.175	0.008	0.362
Weight (kg)	66 (59–76)	64 (60–84)	65 (57–76)	0.874	0.818	0.755	0.616
Body mass index (kg/m^2)	23.8 (21.8–24.8)	22.4 (20.4–25.0)	21.2 (19.7–23.9)	0.143	0.449	0.053	0.224
Cigarettes per day	0 (0–0)	13 (10–15)	11 (10–17)	<0.001	<0.001	<0.001	0.901
Pack-year	0 (0–0)	3.47 (2.4–4.8)	5 (3.7–10)	<0.001	<0.001	<0.001	0.049
Systolic blood pressure (mm Hg)	120 (110–123)	120 (110–123)	113 (108–120)	0.346	0.834	0.246	0.164
Diastolic blood pressure (mm Hg)	75 (70–80)	78 (70–80)	76 (69–80)	0.917	0.738	0.956	0.690
Mean blood pressure (mm Hg)	91 (83–95)	90 (85–93)	89 (83–93)	0.730	0.956	0.569	0.422
Total cholesterol (mg/dL)	180 (170–188)	181 (173–191)	173 (159–180)	0.039	0.684	0.048	0.018

*Kruskall-Wallis test.
†Wilcoxon-Mann-Whitney rank-sum test.
HNBC, heat-not-burn cigarette; TCC, traditional combustion cigarette.

DISCUSSION

In this observational cross-sectional study, chronic HNBC use was associated with having reduced endothelial function, increased oxidative stress and platelet activation.

In particular, we found that sNox2-dp levels were increased in chronic HNBC users, confirming the persistent effect on Nox2 activation on oxidative stress as documented by increased serum levels of H_2O_2. The increased oxidative stress derived by Nox2 activation may reduce the bioavailability of NO and may contribute to atherosclerosis progression by endothelial dysfunction.

In fact, compared with controls, chronic HNBC users had reduced FMD and NO bioavailability, suggesting an unbalance between oxidative stress and NO responsible of endothelial dysfunction. Conversely, no significant difference was observed between HNBC users and TCC smokers. Another interesting result was the close association between cotinine and FMD. This result is in accordance with a previous study that found an association with the number of TCCs smoked, assessed by cotinine, and FMD modification.[10] Platelets play a critical role in the pathophysiology of cardiovascular diseases as they play a main role in pathological thrombus formation.[11] It is well known that TCC smoke enhances spontaneous PA ex vivo and PA in vitro and increases sP-selectin expression.[12]

We have previously demonstrated that biomarkers of platelet activation increased after acute use of a single HNBC device.[5] In this study, we showed that compared with non-smokers, chronic HNBC users had a significant increase of PA, sCD40L and sP-selectin circulating levels, compared with non-smokers, confirming the negative impact of platelet function.

The negative impact of HNBC on health may depend on the emission of HNBC products.[13] In particular, formaldehyde cyanohydrin (a toxic precursor of formaldehyde and cyanide) is released from the polymer filter at the lower temperatures achieved by the IQOS. Both in vivo (human clinical and rat) and in vitro studies suggest that HNBC could have a negative cardiovascular and cerebrovascular effect. In particular, a recent study showed negative effects of HNBC on a panel of cardiovascular assessments: heart rate, blood pressure, carotid–femoral pulse wave velocity and brachial–ankle pulse wave velocity.[13] In addition, cultured mouse mononuclear macrophages exposed to HNBC aerosols elicited increased oxidative stress, as indicated by elevated reactive oxygen species and depleted intracellular glutathione.[14]

This work has many limitations, including the lack of randomisation, risk of residual confounding and also the risk of type I error due to the many statistical tests performed with several study groups despite a small sample size. Another limitation of the study is represented by the fact that absence of dual smoking was self-reported. Lastly, the TCC smokers were younger and had a lower pack-year history compared with HNBC users, possibly due to the fact that HNBCs have been available commercially

Table 2 Endothelial function and laboratory characteristics of non-smokers, TCC smokers and HNBC users

Characteristic	Non-smokers	TCC smokers	HNBC users	Overall P*	P non-smokers vs TCC smokers†	P non-smokers vs HNBC users†	P TCC smokers vs HNBC users†
Subjects	20	20	20	–	–	–	–
FMD (%)	7.1 (2.8–11.5)	1.6 (0–3.9)	3.3 (2.4–6.0)	0.002	0.001	0.019	0.088
NO (µM)	41 (38–49)	10 (9–13)	10 (8–13)	<0.001	<0.001	<0.001	0.775
sNox2-dp (pg/mL)	19 (15–23)	46 (41–50)	40 (34–41)	<0.001	<0.001	<0.001	0.003
H_2O_2 (µM)	8.8 (7.2–11.9)	33.5 (19.5–52.7)	26.7 (21.9–33.8)	<0.001	<0.001	<0.001	0.433
sCD40L (ng/mL)	1.6 (1.1–2.1)	3.2 (2.5–4.4)	3.0 (2.5–3.3)	<0.001	<0.001	<0.001	0.433
sP-selectin (ng/mL)	3.0 (2.0–3.9)	9.2 (6.7–12.0)	8.1 (5.5–9.2)	<0.001	<0.001	<0.001	0.099
Platelet aggregation (%)	62 (58–70)	80 (77–80)	76 (70–80)	<0.001	<0.001	<0.001	0.186
Cotinine (ng/mL)	2 (2–3)	139 (130–148)	137 (103–163)	<0.001	<0.001	<0.001	0.818

*Kruskall-Wallis test.
†Wilcoxon-Mann-Whitney rank-sum test.
FMD, flow-mediated dilation; HNBC, heat-not-burn cigarette; H_2O_2, hydrogen peroxide; NO, nitric oxide; sNox2-dp, soluble Nox2-derived peptide; TCC, traditional combustion cigarette.

only recently in Italy, and indeed it is conceivable that previous chronic TCC smokers may have recently converted to HNBC use for health reasons.

Nonetheless, multivariable analysis supports the validity of our findings, and in particular that chronic HNBC users have increased oxidative stress generated by Nox2, endothelial dysfunction and platelet activation compared with non-smokers without significant differences compared with TCC smokers (online supplemental figure S6). If confirmed by other large studies, these findings could provide evidence to strongly discourage non-smokers to start using HNBC and to encourage TCC smokers to quit smoking.

Most experts in tobacco control recommend limiting or even banning the use of HNBC and other modified risk products (MRPs). Few of them, however, have a more pragmatic approach, allowing its use among chronic TCC smokers as a strategy to limit the adverse impact of TCC and promoting in a stepwise fashion eventual abstinence.[15] Yet, it is unclear whether individual responses to MRP differ among consumers. Our findings, although hypothesis-generating, may be important for an individualised choice of MRP.[16]

Nonetheless, prospective future randomised controlled trials are needed to analyse the relationship among endothelial function, oxidative stress and cardiovascular events in HNBC users.

Author affiliations
[1]Department of Clinical Internal, Anesthesiologic and Cardiovascular Sciences, Sapienza University of Rome, Roma, Lazio, Italy
[2]Department of Medical-Surgical Sciences and Biotechnologies, Sapienza University of Rome, Sapienza University of Rome, Roma, Italy
[3]Mediterranea Cardiocentro, Napoli, Italy
[4]Sapienza University of Rome, Roma, Lazio, Italy
[5]Department of Clinical Internal, Anesthesiological and Cardiovascular Sciences, Sapienza University of Rome, Roma, Lazio, Italy
[6]Department of General Surgery and Surgical Speciality Paride Stefanini, Sapienza University of Rome, Roma, Lazio, Italy
[7]Universita degli Studi di Roma La Sapienza, Roma, Italy
[8]IRCCS NeuroMed, Pozzilli, Italy
[9]Department of Medical-Surgical Sciences and Biotechnologies, Sapienza University of Rome, Roma, Lazio, Italy

Contributors LL and RC: full access to all of the data in the study, takes responsibility for the integrity of the data and the accuracy of the data analysis, and wrote the manuscript; SBat, RM and SP: patient recruitment; SBar, CN and VC experimental work, writing the manuscript; SS, IC, EDF, EC, MP, AM, FM, FV and AM: data analysis and interpretation, writing the manuscript, review and editing; GB-Z: formal analysis; GF: conceptualisation, supervision, writing the manuscript, review and editing.

Funding This study was supported by Sapienza University of Rome (grant 2018 Prot. RM118164264ED867), 'The Sapienza University of Rome-Vascular Assessment of Proatherosclerotic Effects of Smoking (SUR-VAPES) 4 trial' to GF and by Sapienza University of Rome (grant 2020 Prot. RM120172B3C38D48), 'Impact of chronic use of heat-not-burn cigarettes on oxidative stress, endhotelial dysfunction and platelet activation in adults and children' to LL.

Competing interests GB-Z has consulted for Cardionovum, InnovHeart, Meditrial and Replycare. All remaining authors declare no conflict of interest or financial disclosures.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

ORCID iDs
Lorenzo Loffredo http://orcid.org/0000-0002-6542-6235
Giuseppe Biondi-Zoccai http://orcid.org/0000-0001-6103-8510

REFERENCES

1 GBD 2015 Tobacco Collaborators. Smoking prevalence and attributable disease burden in 195 countries and territories, 1990-2015: a systematic analysis from the global burden of disease study 2015. *Lancet* 2017;389:1885–906.
2 Csordas A, Bernhard D. The biology behind the atherothrombotic effects of cigarette smoke. *Nat Rev Cardiol* 2013;10:219–30.
3 Kalkhoran S, Benowitz NL, Rigotti NA. Prevention and Treatment of Tobacco Use: JACC Health Promotion Series. *J Am Coll Cardiol* 2018;72:1030–45.
4 Ratajczak A, Jankowski P, Strus P, *et al*. Heat not burn tobacco product—a new global trend: impact of heat-not-burn tobacco products on public health, a systematic review. *Int J Environ Res Public Health* 2020;17:409.
5 Biondi-Zoccai G, Sciarretta S, Bullen C, *et al*. Acute effects of Heat-Not-Burn, electronic Vaping, and traditional tobacco combustion cigarettes: the Sapienza University of Rome-Vascular assessment of proatherosclerotic effects of smoking (SUR-VAPES) 2 randomized trial. *J Am Heart Assoc* 2019;8.
6 Schweitzer KS, Chen SX, Law S, *et al*. Endothelial disruptive proinflammatory effects of nicotine and e-cigarette vapor exposures. *Am J Physiol Lung Cell Mol Physiol* 2015;309:L175–87.
7 Caporale A, Langham MC, Guo W, *et al*. Acute effects of electronic cigarette aerosol inhalation on vascular function detected at quantitative MRI. *Radiology* 2019;293:97–106.
8 Haptonstall KP, Choroomi Y, Moheimani R, *et al*. Differential effects of tobacco cigarettes and electronic cigarettes on endothelial function in healthy young people. *Am J Physiol Heart Circ Physiol* 2020;319:H547–56.
9 Fried ND, Gardner JD. Heat-not-burn tobacco products: an emerging threat to cardiovascular health. *Am J Physiol Heart Circ Physiol* 2020;319:H1234–9.
10 Celermajer DS, Sorensen KE, Georgakopoulos D, *et al*. Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. *Circulation* 1993;88:2149–55.
11 Libby P. Inflammation in atherosclerosis. *Arterioscler Thromb Vasc Biol* 2012;32:2045–51.
12 Fusegawa Y, Goto S, Handa S, *et al*. Platelet spontaneous aggregation in platelet-rich plasma is increased in habitual smokers. *Thromb Res* 1999;93:271–8.
13 Ioakeimidis N, Emmanouil E, Terentes-Printzios D, *et al*. Acute effect of heat-not-burn versus standard cigarette smoking on arterial stiffness and wave reflections in young smokers. *Eur J Prev Cardiol* 2020:204748732091836.
14 Wang L, Liu X, Chen L, *et al*. Harmful chemicals of heat not burn product and its induced oxidative stress of macrophages at air-liquid interface: comparison with ultra-light cigarette. *Toxicol Lett* 2020;331:200–7.
15 Yeh JS, Bullen C, Glantz SA. Clinical decisions. e-cigarettes and smoking cessation. *N Engl J Med* 2016;374:2172–4.
16 Frati G, Carnevale R, Nocella C, *et al*. Profiling the acute effects of modified risk products: evidence from the SUR-VAPES (Sapienza University of Rome-Vascular assessment of proatherosclerotic effects of smoking) cluster study. *Curr Atheroscler Rep* 2020;22:8.