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Use of heated tobacco products (IQOS) causes an acute increase in arterial stiffness and platelet thrombus formation

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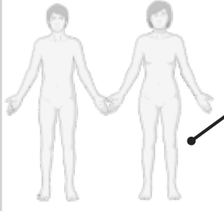
Author contributions

ML, JAB, LH, ST, AB, LH and GL did conceptualization. GL, EA, ML, AB, GM and ST did data collection. GL, LA, AB, ML contributed to data analysis, presentation and visualization. All authors partook in writing and critical review of the manuscript.

Brief use of heated tobacco products cause increased platelet thrombus formation and heightened arterial stiffness

Randomised crossover study



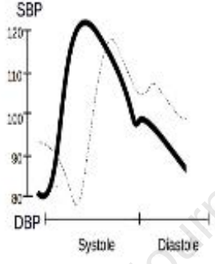
23 healthy particpants were included

Exposure to heated tobacco product

"No exposure" as control

Methods

Arterial stiffness



Pulse wave velocity

Pulse wave analysis

Total Thrombus analysis system

Platelet-thrombus formation

Fibrin rich thrombus formation

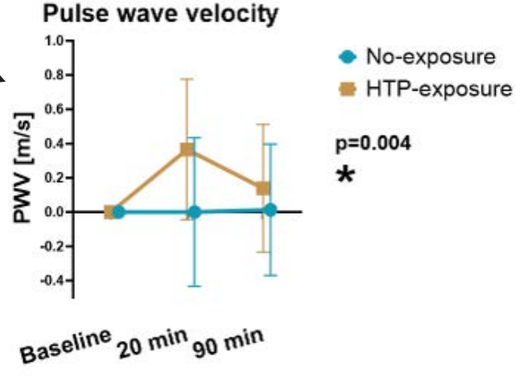
Results

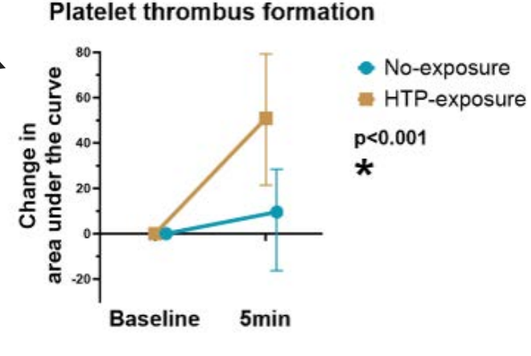
0.345 m/s

6.22%

16.5%

No effect





Use of heated tobacco products (IQOS) causes an acute increase in arterial stiffness and platelet thrombus formation

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Abstract:

Background and aims: Heated tobacco products (HTPs) are novel alternative tobacco products being promoted as an alternative to cigarettes. To evaluate the impact of HTP use on vascular function, we investigated the effects of a brief HTP usage on arterial stiffness and platelet thrombus formation in healthy volunteers.

Methods: In a randomised crossover study, twenty-four healthy young adults with occasional tobacco use smoked the HTP IQOS 3 Multi (Phillip Morris Int.) and "no-exposure" was used as a control, with a wash-out period of at least one week in-between. Arterial stiffness was assessed through pulse wave velocity and pulse wave analysis. Blood samples, collected at baseline and 5 minutes following exposure, were analysed with the Total-Thrombus-formation analysis system evaluating platelet and fibrin-rich thrombus formation tendency.

Results: HTP exposure caused immediate heightened pulse wave velocity (+0.365m/s, 95% CI: +0.188 to 0.543; p=0.004) and enhanced augmentation index corrected to heart rate (+6.22%, 95% CI: +2.33 to 10.11; p=0.003) compared to the no-exposure occasion. Similarly, blood pressure and heart rate transiently increased immediately following HTP inhalation. Platelet thrombus formation significantly increased following HTP exposure (area under the curve +59.5, 95% CI: +25.6 to 93.4; p<0.001) compared to no-exposure. No effect was seen on fibrin-rich thrombus formation following HTP-exposure.

Conclusions: Brief HTP use in healthy young adults had immediate adverse effects on vascular function resulting in increased arterial stiffness and platelet thrombus formation, known risk factors for the development of atherosclerosis. Further research is needed to address long term health impacts.

Keywords

Heated tobacco products
Arterial stiffness
Thrombosis
Tobacco
Nicotine
Platelets

1. Introduction

The World Health Organization (WHO) estimates that cigarette smoking is one of the leading causes of premature death worldwide, with an estimated 5-8 million lives lost annually due to tobacco usage (1). Heated tobacco products (HTPs) are novel nicotine delivery devices introduced in recent years. A variety of brands exists, the most common being: IQOS (Phillip Morris International), Glo (British American Tobacco), Pax (Pax lab) and Ploom (Japan Tobacco International). Sales of HTPs have risen sharply in the European union, with a reported 2,000% increase between 2018 and 2020 (2). HTPs usually consist of a prefabricated tobacco pod, also called heatstick or HEET, containing tobacco sheets, water, guar gum and cellulose fibres. This disposable tobacco pod is inserted into a battery-driven heating chamber and heated to about 350°C. During heating, an aerosol is created and, subsequently, inhaled. Compared to a conventional cigarette, where burning temperatures can exceed 850°C, HTPs manufacturers claim that the tobacco in HTPs is not combusted, only reaching the pyrolysis process, hence generating less quantities of toxic substances in the aerosol (3). However, this fact is disputed, as evidence exists that some degree of combustion occurs when using HTPs (4, 5). The use of HTPs as a smoking cessation product is under debate, as some studies highlight an increased risk for dual use with cigarettes and less likelihood of smoking cessation (6, 7).

There are limited data on short- and long-term health effects of HTP use. Whilst the HTP aerosol contains roughly 90-95% lower levels of toxic chemicals compared to cigarette smoke, more or less the same chemicals are still present (5, 8, 9), but there are limited data whether this translates to reduced health risks for users. Nicotine content in an IQOS heatstick is estimated to be around 70-80% of what is found in a regular cigarette (8). Furthermore, aerosols from HTP can spread in a room, enabling passive exposure of non-using individuals (9).

Vascular function can be assessed by several methods. Arterial stiffness, measured as pulse wave velocity and pulse wave analysis, is considered the gold standard method for assessment the arterial wall properties (10, 11). Structural arterial remodelling with gradual replacement of elastin with

collagen is the main contributor to arterial stiffening and is a consequence of either normal ageing or a pathological process (10, 11). Heightened arterial stiffness is strongly associated with smoking status as well as being recognized as a major factor in vascular aging and an independent risk factor for cardiovascular disease (11, 12). Assessment of thrombogenicity with the Total-Thrombus-formation analysis system (T-TAS) enables bedside evaluations of both platelet and fibrin-rich thrombus formation in whole blood under flow conditions. Evaluation of platelets and coagulation tendency is of importance, as it has emerged as an important link between inflammation, atherogenesis and thrombosis, contributing to atherothrombosis and cardiovascular disease (13, 14). The T-TAS method incorporates multiple pathways that mimic in-vivo conditions, including platelet activation and aggregation, rheological effects, von Willebrand factor-mediated adhesion, release of endogenous platelet agonists and activation of the coagulation system (15, 16). In addition, the method enables assessment of general platelet function, anti-platelet medication, bleeding risk during catheter interventions and differentiation of some haemostatic disorders (15, 17-20), as well as being a sensitive means of detecting traditional risk factors for cardiovascular disease (21).

Due to the limited knowledge about the cardiovascular effects of HTP use in humans, the aim of this study was to assess whether a brief HTP exposure would induce a negative impact on arterial stiffness and platelet-dependent and fibrin-rich thrombus formation in healthy volunteers.

2. Patients and methods

Initially, twenty-four healthy male and female occasional tobacco users (maximum 10 cigarettes or 10 pouches of Swedish snus per month) between 18-40 years of age were included in the study. Due to problems with blood sampling of study participants, resulting in loss of data for T-TAS analysis, an additional eight participants were included after additional ethical approval at a later date and underwent a modified study protocol in order to make up for the loss. The modified study protocol was identical to the original study protocol apart from exclusion of evaluation of arterial stiffness. The Written informed consent and a health declaration was obtained from all volunteers at inclusion. The study adhered to the 1975 Helsinki declaration and was approved by the Swedish Ethical Review Authority.

A randomised cross-over study design was used, participants were randomised and first exposed to either HTP usage or no exposure. Examinations took place on separate days with a washout period of at least one week, Figure S1 supplements. One week prior to participation, participants had to refrain from all nicotine products, anti-inflammatory medication and hard physical exercise. Intake of alcohol and caffeine was not allowed within 24 hours of the examination. Exclusion criteria were any chronic disease, ongoing infection or inflammatory symptoms during the last 7 days prior to participating, pregnancy and BMI above 30 kg/m^2. Participation started in the morning following an 8-hour fasting period. Vascular measurements were performed after 15 minutes of rest in a semi-supine position in a quiet, well-ventilated and temperature-controlled room at 21-23ºC.

All exposures were supervised by a research assistant. A HTP of the brand IQOS (IQOS 3 Multi, Phillip Morris International) was used. During HTP exposures, all subjects inhaled 28 puffs, one puff every 30 seconds for 14 minutes, and each puff lasting about 2-3 seconds. The tobacco pod used was commercially available (Sienna heatsstick, Philip Morris International) and contained 203.3 mg (25.6%) tobacco, 47.1 mg (5.9%) glycerol, 2.8 mg (0.347%) propylene glycol and produced an estimated nicotine delivery of 1.4 mg/heatstick when used (22, 23).

Venous blood was sampled from an antecubital vein at baseline following 15 minutes rest (Routine blood tests and T-TAS) and at 5 minutes (T-TAS) following exposures with no or minimal stasis. Routine blood tests assessed at baseline included a full blood cells count as well as electrolytes, serum-creatinine, serum-lipids and glucose levels.

2.1 Arterial stiffness

Blood pressure, pulse and arterial stiffness examinations were performed in a semi-supine position at baseline as well as 20 and 90 minutes after each exposure/no-exposure. Blood pressure was measured using a semi-automatic sphygmomanometer (Omron M7, Omron Healthcare Europe B.V., Hoofddorp, NL). Arterial stiffness was evaluated with two different methods, i.e. by pulse wave velocity (PWV) and pulse wave analysis measured through augmentation index (AIx). Pulse wave velocity, which measures the transit time of the pulse wave from the carotid to femoral artery, was assessed with the Vicorder™ system (Skidmore Medical, Bristol, UK) by placing inflatable cuffs around the neck and thigh. Pulse wave analysis (PWA) was performed using applanation tonometry (Millar Instruments, Texas, USA) of the radial artery. The registered pulse wave was analysed with SphygmoCor™ software (AtCor Medical, Sydney), which evaluates the retrograde pulse wave and provides an estimate of the central aortic pressure that correlates with the peripheral resistance in the vascular tree. Peripheral AIx is given by registering the retrograde pulse wave and dividing it by early systolic pressure, thereby providing the proportion of the total pulse pressure that the retrograde wave compromises. Augmentation index is inversely associated with heart rate and is mathematically adjusted to a normalized heart rate of 75 beats per minute and reported as the variable AIx75. All measurement were carried out according to instructions and quality criteria from SphygmoCor™ and Vicorder™.

2.2 T-TAS

The Total-Thrombus-formation analysis system (T-TAS®. Fujimoro Kogyo Co., Ltd., Japan) is a method to study thrombus formation in whole blood with a microchip flow chamber system. It is

155 designed to mimic in-vivo arterial conditions as closely as possible (15). Two different microchips
156 were used to study platelet- and fibrin-rich thrombus formation. The platelet-chip (PL-chip) consists of
157 25 artificial capillaries lined with type-1-collagen and provides a measurement of platelet-dependent
158 thrombus formation and primary haemostasis. The atheroma-chip (AR-chip) consists of one single
159 capillary lined with both type-1-collagen and tissue factor that stimulate primarily fibrin-rich thrombus
160 formation and give a measurement of platelet-dependent coagulation tendency.
161
162 All T-TAS measurements were performed according to recommendations from the manufacturer.
163 Different shear rates simulate the different flow conditions of the blood vessels. For the PL-chip a
164 flow rate of 18μL/min was used, corresponding to a shear rate of 1,500s^{-1}. In the PL-chip, 320μL of
165 whole blood was collected in a BAPA-canister (benzylsulfonyl-D-Arg-Pro-4- amidino benzylamide),
166 a thrombin and factor Xa inhibitor, before analysis. For AR-chip analysis, a flow rate of 10μL/min,
167 shear rate of 600s^{-1}, was used and 480μL citrated whole blood was recalcified and treated with corn-
168 trypsin inhibitor before analysis. Blood samples were analysed 1-3 hours after sampling. Flow analysis
169 was carried out until pressure reached 60kPA and 80kPa in the PL-chip and AR-chip respectively,
170 representing near occlusion of the microchip capillaries. Pressure build-up during microthrombi
171 formation was recorded on a graph, Figure S2 supplements. The recorded time-variables were T10
172 (the time in seconds to reach 10kPa) representing start of occlusion and formation of the clot together
173 with occlusion time (OT, in seconds, to reach 60/80kPa). Finally, area under the time – pressure curve
174 (AR-AUC, PL-AUC) was calculated as an estimation of total thrombogenicity. AR-AUC is a
175 measurement of the area under the curve for 30 minutes after start of perfusion in the AR-chip,
176 whereas PL-AUC is a measurement of the area under the curve for 5 minutes after start of perfusion in
177 the PL-chip. Increased thrombus formation in the capillaries causes earlier onset of T10, OT and an
178 increase in AUC.
179
180 **2.3 Statistical analysis**
181 Sample size analysis was based on previous studies evaluating arterial stiffness, measured through
182 pulse wave velocity, from electronic cigarette use (24). As it was estimated that 17 subjects would be

needed for detection of a 20% change in PWV in a repeated measures ANOVA with a power of 80% and a significance level of 0.05, it was planned to recruit twenty-four participants to account for drop-offs and technical difficulties (24). Statistical analysis was performed using SPSS 27.0.0.0 64-bit edition (IBM Corporation, NY, US) and GraphPad Prism 9.1.0 (GraphPad Software Inc., CA, US). Shapiro-Wilk test was used to test for normality. Parametric data were expressed as mean with standard deviation, while skewed data were reported as median and interquartile range. Repeated measures ANOVAs and paired T-tests were used to detect differences between groups, whereas Friedman's test and Wilcoxon signed-rank test were used for skewed data. P-values of <0.05 were considered statistically significant.

3. Results

Twenty-four healthy individuals were included in the initial data collection, one volunteer dropped out after inclusion. Due to loss of data, as explained in the method section, an additional 8 healthy individuals were included and underwent a slightly modified study protocol. Altogether, 23 individuals completed the arterial stiffness analysis and 22 the T-TAS analysis. The groups partially overlapped, and complete subject characteristics and baseline laboratory measurements are presented in Table 1 in the online supplement.

3.1 Arterial stiffness

Results for the 23 subjects included in the arterial stiffness analysis are presented in Figure 1 and Table 2 in the online supplement. HTP exposure caused a significant increase in arterial stiffness measured as PWV and AIx75 as well as increases in heart rate and diastolic blood pressure after 20 minutes, both compared to baseline and to the no-exposure occasion. These effects normalised within 90 minutes. A trend towards a significant increase in systolic blood pressure was observed 20 minutes after HTP exposure, also normalising after 90 minutes.

3.2 T-TAS

Results for the 22 subjects included in the T-TAS measurements are presented in Figure 2 and table 3 in the online supplements. Both PL-AUC and PL-OT changed significantly (Figure 2D and 2F) 5 minutes following exposure to HTP aerosol and differed significantly compared to the no-exposure group, indicating faster platelet-dependent thrombus formation and heightened platelet thrombogenicity. PL-T10 was not significantly altered following either HTP exposure or no-exposure (Figure 2 B). AR-AUC, AR-OT and AR-T10 did not change significantly following exposure to either HTP aerosol or no-exposure, indicating no effect on fibrin-rich thrombus formation (Figure 2 A, C, E).

4. Discussion

This study evaluated the effects of brief HTP usage on vascular function and blood thrombogenicity in healthy young individuals. We found that HTP exposure caused a transient increase in arterial stiffness measured through both PWV and AIx75, as well as increased heart rate and diastolic blood pressure. In addition, a brief HTP use was also associated with an immediate increase in the variables reflecting platelet-dependent thrombus formation in flowing whole blood.

Brief HTP smoking caused a transient increase in both PWV and AIx75 10 minutes following exposure that normalised after 90 minutes. PWV is one of the most widely used estimates of arterial stiffness. However, PWV is also influenced by acute hemodynamic changes, as shown in animal models and human studies (25-27). AIx75 is to a larger degree an indirect measurement and is moderately affected by arterial stiffness, also being influenced by ventricular contractility, the reflectance point and vascular smooth muscle tonus (28). Furthermore, it has been shown that vasoactive drugs can independently affect AIx75 without altering PWV (29). In the present study, it is unlikely that the observed increases in both PWV and AIx75 following brief HTP exposure would be caused by a structural change in arterial wall composition. Rather, the transient increases in PWV and AIx75 may be explained by several potential mechanisms following HTP exposure, such as nicotine-induced sympathetic activation causing endothelial dysfunction and impaired nitric oxide production; and an increase in the release of catecholamines, which bind to α1-adrenergic receptors on vascular smooth muscle fibres heightening arterial tonus (30-32). These short-term effects would likely increase the systemic vascular resistance, which in turn may contribute to the increased PWV (27). Whether the observed transitory increase in arterial stiffness following HTP exposure has clinical relevance cannot be answered by this short-term exposure study. However, the normalisation of arterial stiffness 90 minutes following the exposure is likely not the real-life case for common HTP users, as most tobacco users consume their product of choice multiple times daily. There are few studies into HTP consumption patterns, but one study showed that cigarette users who switched to IQOS heatsticks replaced 76-124% of their cigarettes with heatsticks, suggesting similar consumption

patterns (33). Hence, it can be argued that the observed transient increase in arterial stiffness following HTP smoking is clinically relevant, since long term users of HTPs consume their products multiple times per day, potentially leading to a chronic elevation of arterial stiffness. This notion is supported by studies evaluating effects of conventional cigarettes on arterial stiffness in chronic users (34). This is of importance as increased arterial stiffness is linked to and considered an independent risk factor for the development of arteriosclerosis, the major underlying cause of cardiovascular diseases (11-13).

One previous study by Franzen et al evaluated effects of short-term HTP exposure on arterial stiffness in humans. In contrast to the present study, Franzen et al evaluated conventional cigarette, e-cigarette, as well as HTP exposure and detected that all exposures increased arterial stiffness demonstrated by the variable AIx75, but saw only a trend of increased PWV following HTP and electronic cigarette exposure (35). The current study provides stronger data that HTP exposure increases arterial stiffness, as demonstrated by both heightened PWV and AIx75 in relation to the no-exposure control. Temporary heightened arterial stiffness measured through PWV and AIx75 has previously been demonstrated following brief electronic cigarette use with nicotine similar to the effects seen in the current study (24). Other studies have detected elevated PWV and AIx in chronic electronic cigarette users compared to non-smokers (36). As for cigarettes, heightened PWV and AIx75 following acute and chronic use is well established, both in non-smokers and smokers (36, 37). Hence, our findings of increased PWV and AIx75 following nicotine containing-HTP exposure agree with the result in previous studies investigating the effects of other tobacco products on the market.

The present study also demonstrated an immediate increase in platelet-dependent thrombus formation 5 minutes following HTP exposure, evaluated through the T-TAS PL-chip under flow conditions. No compelling effects of HTP exposure was seen on fibrin-rich thrombus formation, measured through the AR-chip, indicating that the HTP aerosol did not enhance fibrin-dependent thrombus formation. These results partially cohere with studies evaluating conventional cigarette exposure which was found to be associated with increased platelet aggregation and activation, however enhanced fibrin-rich thrombus formation has also been observed (38-40). The T-TAS method is relatively novel and

there are few studies evaluating vascular effects from tobacco exposure using it. However, we recently found that brief electronic cigarette use with nicotine, but not without, was associated with increased platelet thrombus formation and fibrin-rich thrombus formation measured through the T-TAS, indicating nicotine as the mediator substance (41). The same study showed that platelet and fibrin-rich thrombus formation increased 15 minutes after exposure to electronic cigarette with nicotine and normalised toward baseline values after 60 minutes (41). Assuming the mechanism is similar, this would indicate a short-lasting effect on platelet thrombus formation. However, as to why no effect is seen in the current study on fibrin-rich thrombus formation following HTP exposure compared to electronic cigarette with nicotine exposure might be due to several reasons such as difference in degree of exposure and unknown effects of other substances in the HTP aerosol. The increase in platelet thrombus formation following HTP exposure coheres to other studies on electronic cigarettes with nicotine, where heightened platelet activity was observed through an increase in systemic levels of circulating extracellular vesicles of platelet origin and increased levels of p-selectin (42, 43). Chronically increased platelet activation is linked to both inflammation, thrombosis and atherogenesis, all of which are involved in the atherosclerotic process leading to cardiovascular disease (44, 45).

Potential causative substances in the HTP aerosol that caused the increase in arterial stiffness and platelet thrombus formation include nicotine, reactive oxygen species, fine-particulate matters, and carbonyls (5, 8, 46, 47). Chronic indoor exposure to volatile organic compounds has been associated with increased arterial stiffness (47, 48), as is exposure to nicotine (32, 34). Nicotine is also known to cause heightened platelet activation and aggregation through release of endogenous epinephrine, which binds to alfa-2adrenergic receptors on platelets (49-51). Brief inhalation of electronic-cigarette aerosol with nicotine, but not without, has been shown to increase arterial stiffness and platelet thrombus formation (24, 41). The administration route of nicotine is of importance, as nicotine-patches cause less platelet activation than cigarette smoking and Swedish moist snus, indicating that bolus-like administration methods, such as inhalation and oral mucosal uptake, are more harmful (52, 53). To summarise, a nicotine-induced catecholamine release may be a potential pathway explaining both the increase in arterial stiffness as well as the platelet thrombus formation observed in the current study.

The role of nicotine in atherosclerosis has been debated. However, nicotine has been linked to endothelial dysfunction, enhanced inflammation and atherogenesis (34). Notwithstanding, as the HTP aerosol contains a complex mix of compounds, this makes it difficult to draw any definite conclusions. Further research is needed to determine what effects the different compounds in the HTP aerosol would have on arterial stiffness and haemostatic function.

4.1 Strengths and limitations

A limitation of the present study is its small size. Currently, there are no peer-reviewed studies on the mean daily HTP consumption, but it is likely considerably greater than the present exposure protocol of 28 puffs, at least if applying mean e-cigarette consumption for the average user which is estimated at 120-235 puffs daily (54). This makes it hard to determine to what degree this HTP exposure is applicable to the regular HTP consumer. Furthermore, this study included volunteers with lower nicotine tolerances that in certain cases caused nausea and/or/coughing, potentially reducing the exposure dose. As the present study included only young and healthy individuals, it may be difficult to extrapolate results to older individuals with, in some cases, already established cardiovascular disease. The T-TAS method displays significant heterogeneity with high interindividual variability, making it more suitable for the cross-over study design used here (21). However, the T-TAS method is relatively novel, with most studies to date being relatively small. Therefore, the method currently has no identified definite threshold value that correlate to increased thrombotic risk, although there are suggested reference intervals for the PL-chip analysis which correspond well to the data observed in the current study (21). The current study did not evaluate the duration of the acute increase in platelet thrombogenicity in humans following HTP exposure with additional measurements past 5 minutes postexposure, which needs to be assessed in future studies.

4.2 Conclusions

This study presents novel data that brief HTP use causes a transient increase in arterial stiffness as well as heightened platelet thrombogenicity in healthy young adults. This is an important finding as

both heightened arterial stiffness and platelet thrombogenicity are strongly associated with the development of atherosclerotic disease in humans (Fig. 3).

Conflicts of interest

No authors in this study declare any conflicting interests.

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Author contributions

ML, JAB, LH, ST, AB, LH and GL did conceptualization. GL, EA, ML, AB, GM and ST did data collection. GL, LA, AB, ML contributed to data analysis, presentation and visualization. All authors partook in writing and critical review of the manuscript.

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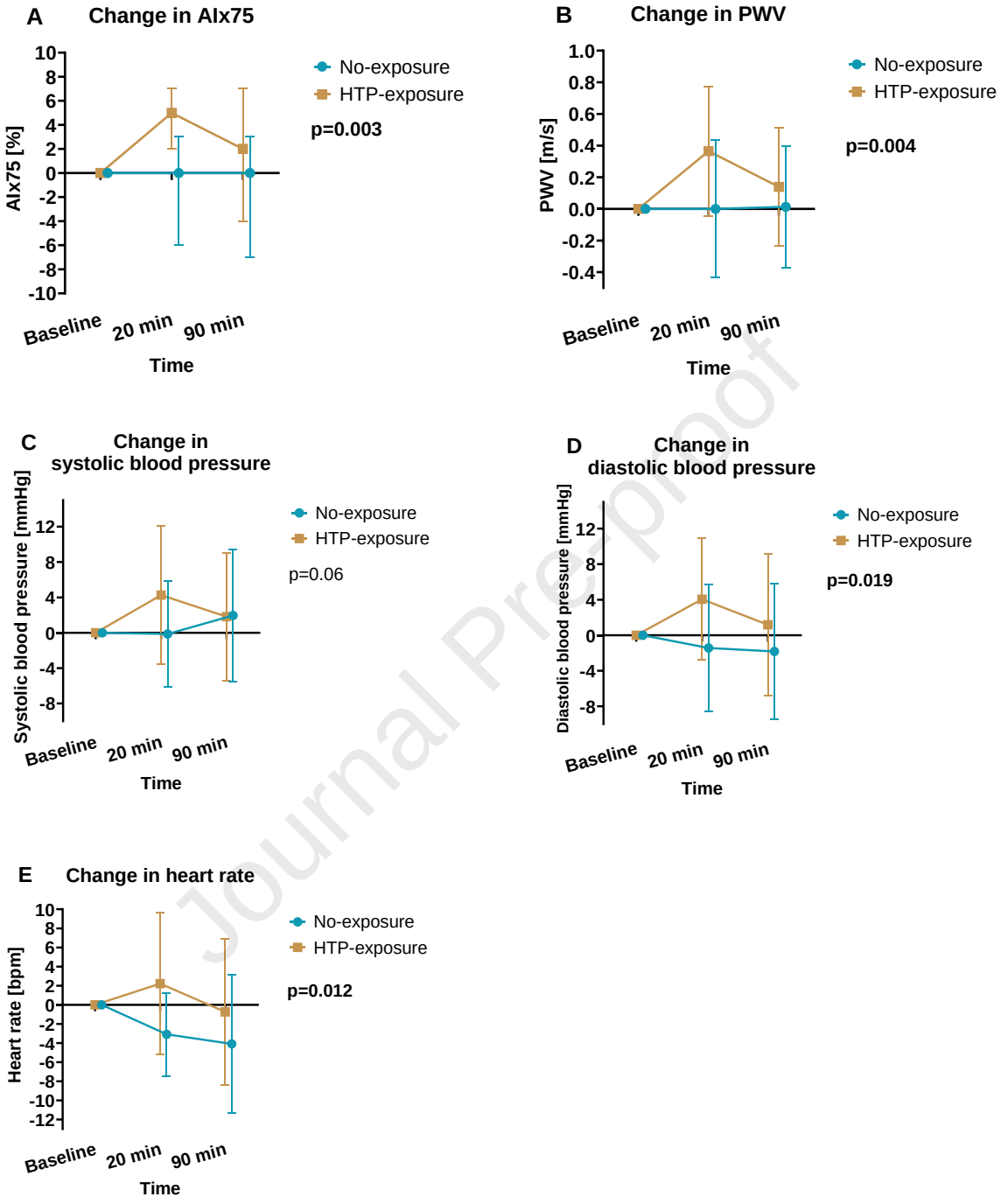
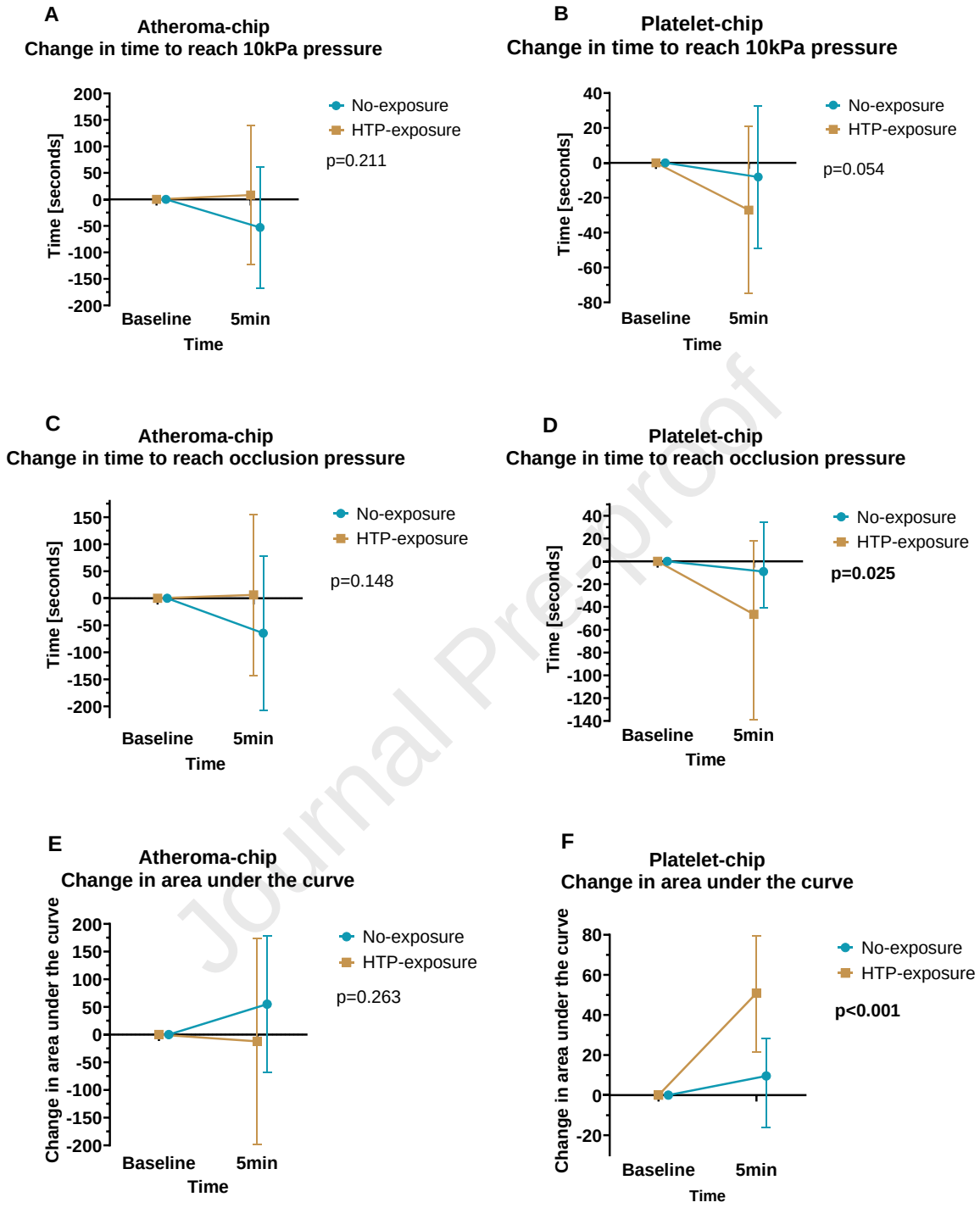
Figure 1: Changes in arterial stiffness, blood pressure and heart rate following HTP exposure and non-exposure.



(A) Augmentation Index corrected for heart rate (AIx75). (B) Pulse wave velocity (PWV). (C) Systolic blood pressure. (D) Diastolic blood pressure. (E) Heart rate in beats per minute (BPM). Graph A shows median values and interquartile range; p-value using Friedmans test. Graphs B-E show mean values and standard deviation; p-values are repeated measures ANOVAs, *p*-value below 0.05 is considered statistically significant and marked in bold text.

Figure 2. Changes in thrombotic tendency following HTP exposure and non-exposure.



(A) Atheroma-chip, time to reach 10kPa (AR-T10). (B) Platelet-chip, time to reach 10kPa (PL-T10). (C) Atheroma-chip, time to reach occlusion pressure (AR-OT) (D) Platelet-chip, time to reach occlusion pressure (PL-OT) € Atheroma-chip, area under the curve (AR-AUC) (F) Platelet-chip, area under the curve (PL-AUC).

Graphs B shows mean values and standard deviations; p-value is paired T-test. Graph A, C, D, E and F shows median values and interquartile ranges; p-value is reported for Wilcoxon's signed rank test, *p*-value below 0.05 is considered statistically significant and marked in bold text.

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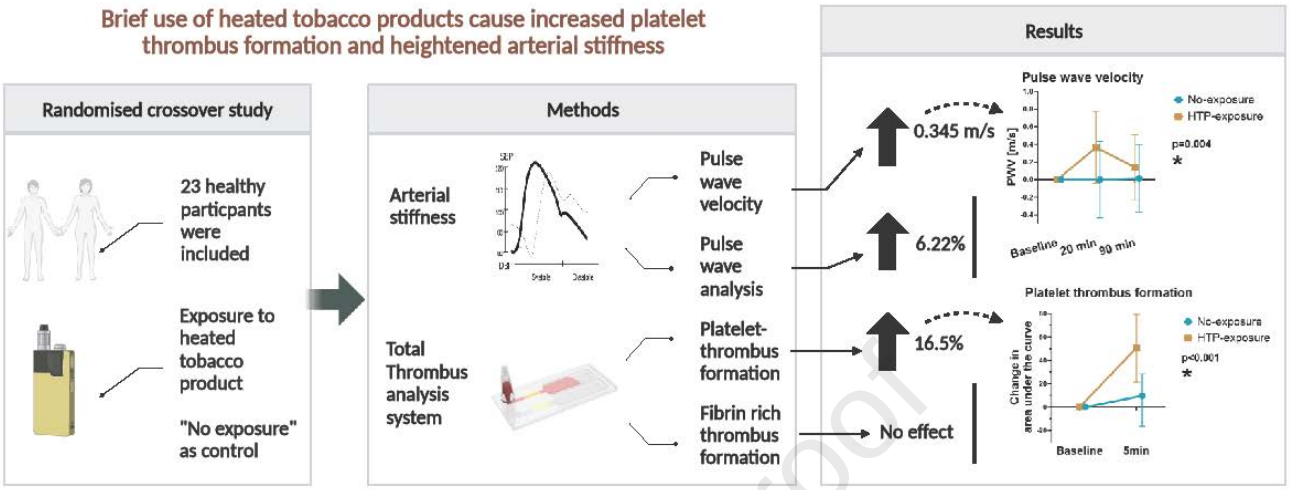

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Highlights

- Heated tobacco products are novel alternative tobacco products
- Brief use heated tobacco products had several adverse effects on vascular function
- A transient increase in arterial stiffness was observed following use
- Heightened platelet thrombus formation was seen immediately following use

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: