Nicotine and Tobacco Research, 2024, **XX**, 1–4
https://doi.org/10.1093/ntr/ntae008
Advance access publication 18 January 2024
Original Investigation

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Plasma Oxidative State Induced by Exercise in Young Heat-Not-Burn Cigarette Users

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Abstract

Introduction: Oxidative state, a risk factor for several diseases, is increased by habitual conventional cigarette (CC) smoking. Reports have demonstrated that heat-not-burn cigarettes (HNBCs), which have recently become popular among smokers, generate less oxidative state than CC in smokers with a long smoking history. However, no previous study has examined oxidative state in young HNBC users. Previously, we reported that exercise induces a greater oxidative state in young CC smokers than in never-smokers of similar age, but there was no difference in resting oxidative state. This study aimed to clarify the resting and exercise-induced oxidative states in young HNBC users, compared with those in never-smokers and CC users of similar age.

Methods: Healthy young never-smokers, HNBC users, and CC users were recruited, and they underwent the Wingate anaerobic test. Blood samples were collected before and after exercise, and the plasma hydroperoxide concentration, a marker of oxidative state, was measured.

Results: No significant differences in pre-exercise plasma hydroperoxide concentrations were detected among never-smokers, HNBC users, and CC users (n = 10 each). Plasma hydroperoxide concentration was significantly increased after exercise in all participants. The exercise induced a significant increase in plasma hydroperoxide concentration in HNBC users compared with that in never-smokers ($p < .005$), but it was significantly decreased compared with that in CC users ($p < .01$).

Conclusions: The use of HNBC increased exercise-induced plasma oxidative state compared with that in never-smokers, indicating that HNBC may lead to the risk of oxidative damage.

Implications: This study, for the first time, reports exercise-induced oxidative state in young HNBC users compared with never-smokers and CC users. The exercise-induced oxidative state in HNBC users was higher than that in never-smokers and lower than that in CC users. Our study suggests that the use of HNBCs increases the risk of acute oxidative damage.

Introduction

Habitual smoking of conventional cigarettes (CCs) increases oxidative state.[1] The increase in oxidative state caused by smoking CC is a risk factor for chronic obstructive pulmonary diseases, cardiovascular diseases, and cancers.[1–3]

Recently, heat-not-burn cigarettes (HNBCs) have become popular among smokers. HNBC is referred to as modified-risk tobacco product, as it releases fewer toxic chemicals such as acetaldehyde and carbon monoxide due to generation of less heat than that generated by CC.[4,5] Reports have demonstrated that HNBC generates a lower oxidative state than CC.[6,7] Sakaguchi et al. reported that the resting oxidative state in HNBC users with a long smoking history was lower than in CC users of similar age, and there was no difference compared with never-smokers of similar age.[8] However, no previous study has examined the oxidative state in young users of HNBC.

Previously, we reported that exercise induces greater oxidative state in young CC smokers, compared with never-smokers of similar age, but there was no difference in resting oxidative state.[9,10] We believe that exercise might be one of the methods that can clarify the oxidative state undetected in rest in young smokers. Therefore, this study aimed to investigate exercise-induced oxidative state in young HNBC users compared with that in never-smokers and CC users of similar ages.

Methods

Participants

Healthy young never-smokers, HNBC users, and CC users were recruited through poster announcements in the university. Participants were defined as follows: never-smokers refer to those who had never used either HNBC or CC; HNBC users refer to daily smokers using ≥5 HNBCs/day for >1 year and using HNBCs in ≥90% of total smoking opportunities within the last 1 year; CC users are daily smokers using ≥5 CCs/day for >1 year and have not used other tobacco products within the last 1 year. In this study, we set no limits on the type of HNBC products and brands used by the HNBC and CC participants because only a small number of HNBC and CC users participated. The number of years of smoking, daily cigarette consumption,

Received: November 7, 2022. Revised: December 12, 2023. Accepted: January 15 2024.

and cumulative cigarette consumption of HNBC and CC users were recorded. As age influences oxidative state, participants at ages 20–29 years were included in the experiment,[11] and those who exercise every day were excluded to avoid the effects of regular exercise on oxidative state.[12] Participants with metabolic, pulmonary, inflammatory, or orthopedic diseases, those willing to quit smoking, and those who were regularly taking antioxidant compounds (including vitamins) or anti-inflammatory medications were excluded.

Written informed consent was obtained after a full explanation of the purpose, content, and risks of the study. The study protocol was approved by the Ethical Committee for Clinical Research of Hiroshima University (approval number: C-333). This study was registered with the University Hospital Medical Information Network of Japan (http://www.umin.ac.jp/ctr/; registration number: UMIN000045860).

As there were no previous reports of similar studies, a preliminary study was conducted to determine the sample size. A sample size of eight was required to detect the effect of HNBC use on plasma oxidative state after exercise and to obtain the required α significance level of 0.05, statistical power of 0.8, and an effect size of 0.692 calculated during our preliminary study. We concluded that at least eight participants are required in each of the never-smoker, HNBC user, and CC user groups.

Procedures

Participants were required to refrain from eating and drinking, except water uptake, for 8 hours prior to the experiment to avoid the effects of diet on oxidative state[13]; they drank 100 mL water 10 minutes prior to the experiment to prevent dehydration during exercise. HNBC and CC users refrained from smoking cigarettes for 12 hours before the study to avoid the acute effects of cigarette smoking on oxidative state.[14]

Participants performed the Wingate anaerobic test[15] using a cycle ergometer (Power max VII; Combi, Tokyo, Japan). After a minute of no-load warm-up, participants pedaled for 30 seconds at a maximal speed against a constant force (0.09 kp/kg).[15]

Blood samples were collected from the fingertips using a single-use lancing device (Accu-Chek Safe-T-Pro Plus; Roche, Mannheim, Germany) and a tube (Microvette CB 300 LH; Sarstedt, Numbrecht, Germany) before and after the exercise test.

Measurements

Plasma hydroperoxide concentration was determined using the d-ROMs test kit (Diacron International, Grosseto, Italy) and a spectrophotometer (FREE Carpe Diem; Diacron International), as previously reported.[9] Plasma was obtained by centrifuging the collected blood at 6000 rpm for 2 minutes. Then, 20 μL of plasma and 20 μL of chromogen solution were added to 1.2 mL of acetate buffer solution (pH 4.8) and incubated at 37°C for 10 minutes, and optical measurements were made at 505 and 546 nm for 5 minutes.

Statistical Analyses

Data are presented as the mean ± standard deviation. Age, height, weight, body mass index (BMI), exercise frequency, exercise duration per week, pre-exercise plasma hydroperoxide concentration, and exercise-induced plasma hydroperoxide concentration were analyzed using Tukey's test. The number of years of smoking, daily cigarette consumption, and cumulative cigarette consumption of HNBC and CC users were analyzed using the Mann–Whitney U test. Paired t tests were performed to analyze pre- and post-exercise plasma hydroperoxide concentrations in never-smokers, HNBC users, and CC users. Statistical analysis was performed using IBM SPSS Statistics for Windows version 27.0 (IBM Japan Ltd, Tokyo, Japan), and $p < .05$ was considered statistically significant. The necessary sample size was estimated using G*Power version 3.1 (Franz Faul, University of Kiel, Kiel, Germany).

Results

Each of the never-smokers, HNBC user, and CC user groups had 10 male participants. All were students, clerical workers, medical personnel (never-smokers: 10 students; HNBC users: five students, three clerical workers, and two medical personnel; CC users: seven students, two clerical workers, and one medical personnel). The characteristics of the participants are listed in Table 1. There were no significant differences in mean age, height, weight, BMI, exercise frequency, and exercise duration per week among never-smokers, HNBC users, and CC users. There were no significant differences in the mean number of years of smoking, daily cigarette consumption, and cumulative cigarette consumption between HNBC and CC users.

Table 1. Characteristics of Participants

	Never-smokers (n = 10)	HNBC users (n = 10)	CC users (n = 10)
Age (years)	23.6 ± 1.7	24.2 ± 2.4	23.0 ± 2.0
Height (cm)	171.1 ± 4.6	169.9 ± 3.6	170.8 ± 5.7
Weight (kg)	66.7 ± 11.7	64.0 ± 7.3	66.2 ± 9.3
BMI (kg/m²)	22.7 ± 3.2	22.1 ± 2.2	22.7 ± 2.8
Exercise frequency (days per week)	1.0 ± 1.8	0.8 ± 1.2	1.6 ± 1.3
Exercise duration (hours per week)	1.0 ± 1.7	1.1 ± 1.3	2.7 ± 2.8
Number of years of smoking	NA	2.2 ± 0.6	5.0 ± 3.8
Daily cigarette consumption (heat sticks/cigarettes per day)	NA	10.5 ± 4.2	11.3 ± 4.2
Cumulative cigarette consumption (pack-years)	NA	1.2 ± 0.8	2.8 ± 2.7

Values are expressed as mean ± standard deviation. In HNBC users, smoking habit include only HNBC. They used very few CCs during the period. HNBC = heat-not-burn cigarette; CC = conventional cigarette; BMI = body mass index; NA = not available.

Table 2. Plasma Hydroperoxide Concentration at Pre- and Post-exercise

	Never-smokers (n = 10)	HNBC users (n = 10)	CC users (n = 10)
Pre-exercise (U.CARR)	226.9 ± 28.4	242.5 ± 26.2	234.6 ± 33.1
Post-exercise (U.CARR)	242.7 ± 30.8*	275.6 ± 23.0*	284.2 ± 33.6*

Values are expressed as mean ± standard deviation. HNBC = heat-not-burn cigarette; CC = conventional cigarette; U.CARR = Carratelli units. 1 U.CARR = 0.08 mg/100 mL H_2O_2.
*$p < .001$, compared with pre-exercise.

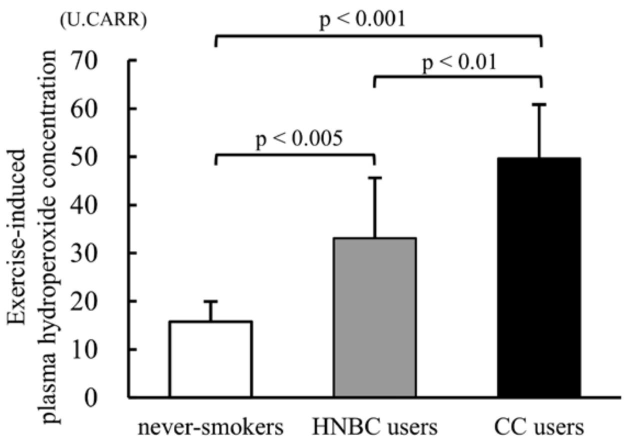

Figure 1. Exercise-induced plasma hydroperoxide concentration. Exercise-induced hydroperoxide concentration in plasma of never-smokers, HNBC users, and CC users. 1 U.CARR = 0.08 mg/100 mL H_2O_2. HNBC = heat-not-burn cigarette; CC = conventional cigarette; U.CARR = Carratelli units.

No statistical difference in pre-exercise plasma hydroperoxide concentrations was detected among never-smokers, HNBC users, and CC users (never-smokers vs. HNBC, p = .628; HNBC vs. CC, p = .919; CC vs. never-smokers, p = .847). The post-exercise plasma hydroperoxide concentration was significantly higher than the pre-exercise plasma hydroperoxide concentration in all groups (Table 2). The exercise-induced plasma hydroperoxide concentration in HNBC users was significantly higher than that in never-smokers ($p < .005$) and significantly lower than that in CC users ($p < .01$) (Figure 1). The exercise-induced plasma hydroperoxide concentration in the CC group was significantly higher than that in the never-smoker group ($p < .001$).

Discussion

This study demonstrated that the exercise-induced oxidative state in HNBC users was lower than that in CC users and higher than that in never-smokers.

This is the first report that compared the exercise-induced oxidative state in HNBC users with that in never-smokers and CC users. In this study, the plasma hydroperoxide concentration was induced by exercise in never-smokers, HNBC users, and CC users. Exercise may induce oxidative state in young HNBC users similar to other young persons; we have reported that exercise induces oxidative state in young never-smokers and CC users.[10] The exercise-induced oxidative state in HNBC users was lower than that in CC users. Biondi-Zoccai et al. observed in adults that the H_2O_2 and 8-iso-prostaglandin 2α-III levels in serum are influenced by a significant interaction between the type of tobacco device (ie, HNBC or CC) and the time relative to a single use (ie, before or after use) of tobacco.[6] They concluded that HNBC showed less acute effects on oxidative state than CC. In addition, Lüdicke et al. reported that smokers who had switched from CC to HNBC for 6 months had a lower resting 8-epi-prostaglandin F2α level in urine than that of the other smokers (who had continued using CC).[7] Differences in the reactive oxygen species (ROS) contents in HNBC and CC aerosols might have led to these results. In HNBC aerosols, particulate and gaseous ROS contents were reduced by 82% and 90%, respectively, compared with that present in CC aerosols.[16] In contrast, in this study, exercise-induced plasma hydroperoxide concentration in HNBC users was higher than that in never-smokers. The serum levels of H_2O_2 and 8-*iso*-prostaglandin 2α-III significantly increased immediately after a single use of HNBC.[6] These outcomes may be attributed to the exposure to HNBC aerosols. The oxidized and reduced glutathione ratio in human bronchial epithelial BEAS-2B cells was significantly increased after exposure to the HNBC aerosol.[17]

This study has some limitations. First, antioxidants was not measured in this study because we did not have sufficient volume of some of the samples. Oxidative stress is commonly determined on the basis of the enzymatic and nonenzymatic antioxidant profile. To elucidate the total exercise-induced oxidative stress in young HNBC users, further studies, including those on the complete antioxidant complete profile, are needed. Second, the type of HNBC products and brands used by the HNBC and CC participants could not be standardized. The amounts of oxidants in CC mainstream smoke differ by brands,[18] and according to a previous study on HNBC aerosols, the generation of carbonyl proteins differs from that of HNBC products.[19] These differences may affect exercise-induced oxidative state. Further studies are needed to clarify the effects of different brands of CC and HNBC products on exercise-induced oxidative state. Third, our findings cannot be generalized, as all the participants were men; further studies on a large number of smokers are needed.

In conclusion, this study suggests that exercise-induced oxidative state was lower in HNBC users than that in CC users, but it was greater than that in never-smokers; moreover, the use of HNBC may increase the risk of oxidative damage.

Funding

This work was supported by the Fundamental Budget for Education and Research.

Declaration of Interests

There is no conflict of interest.

Acknowledgments

We would like to thank Editage (www.editage.jp) for the English language editing.

Author Contributions

Aoi Takagi (Conceptualization [equal], Data curation [equal], Formal analysis [equal], Investigation [equal], Methodology [equal], Project administration [equal], Validation [equal], Visualization [equal], Writing—original draft [equal], Writing—review & editing [equal]), Hironobu Hamada (Conceptualization [equal], Project administration [equal], Supervision [equal], Writing—review & editing [equal]), Kiyokazu Sekikawa (Project administration [equal], Supervision [equal]), Haruchi Namba (Investigation [equal]), Hatsumi Ueoka (Validation [equal]), Yoshinobu Sato (Validation [equal]), Naoto Kanda (Validation [equal]), and Ryosuke Miyazaki (Investigation [equal])

Data Availability

All data generated or analyzed during this study are included in this published article.

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