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Original Research

Influence of antibacterial mouthwash on post-exercise hypotension

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Abstract

Background: Acute exercise induces a blood pressure (BP) reduction that lasts minutes to hours, which is called post-exercise hypotension (PEH). Accumulating data suggest that oral bacteria play a role in BP regulation by contributing to nitric oxide production, implying that antibacterial mouthwash (AM) could affect PEH. Therefore, this study aimed to investigate the effects of an AM on post-exercise changes in BP and arterial stiffness index (ASI). **Methods**: Ten healthy young men completed two treadmill exercises at moderate intensity. After exercise, the subjects rinsed their mouth for 1 min with AM or nitrite-free water at 5, 35, 65, and 95 min during the recovery period. BP and ASI were assessed at 0, 10, 20, 30, 60, 90, and 120 min during the recovery period. Heart rate (HR) and blood lactate (BL) were also measured. **Results**: As expected, PEH occurred showing a decrease in the systolic, diastolic, and mean arterial pressures after exercise in the placebo group. The ASI also decreased after exercise in the placebo group. However, these BP-lowering effects of exercise were significantly diminished by AM, particularly at the later times of the recovery period. In addition, there was a difference between the treatments in ASI changes, but not in HR and BL changes. **Conclusion**: These findings demonstrate that AM usage after exercise can attenuate the beneficial effects of exercise on BP and the vasculature.

Keywords: post-exercise hypotension; antibacterial mouthwash; arterial stiffness

1. Introduction

Hypertension is the strongest risk factor for coronary heart disease and stroke [1], accounting for 45% of deaths associated with heart diseases and 51% of all stroke-related deaths [2]. Hence, hypertension has been an important therapeutic target for mortality and health issues associated with cardiovascular diseases (CVDs) [3,4].

As a non-pharmacological therapeutic approach, the blood pressure (BP)-reducing effect of regular exercise is well documented [5]. Transient BP reduction occurs after an acute bout of aerobic exercise, and this is called post-exercise hypotension (PEH). The physiological aspect of PEH is that when exercise is ceased, cardiac output drops to the resting level more rapidly than systemic vascular resistance recovers and this imbalance between these two physiological factors results in PEH [6]. Consequently, the BP-lowering benefits of regular exercise can be ultimately achieved from the accumulation of recurring PEH exerted by repeated exercise. Previous studies have shown that 30- to 60-min moderate-intensity dynamic exercise can induce PEH for minutes to hours with a BP reduction of 5 to 10 mmHg in normotensive and up to 20 mmHg in hypertensive subjects [7]. Several factors that influence PEH have been identified, such as exercise type and volume, temperature, and body position [6,7]. However, the underlying mechanisms of PEH are still not fully understood.

Nitric oxide (NO) is an autacoid that has been extensively studied in BP regulation because of its role in vasodilation and angiogenesis [8]. NO is a lipid-soluble gas produced in endothelial cells from the amino acid L-arginine by endothelial nitric oxide synthase (eNOS) [9]. Many studies have shown that exercise activates eNOS expression which leads to vasodilation via increased NO production [8], indicating the possibility of the eNOS pathway involvement in PEH regulation.

However, a recent study has demonstrated that PEH is not affected by endogenous NOS inhibitors [7]; hence, it is possible that another NO-producing pathway exists independent of the eNOS pathway. Accumulating evidence indicates that NO can also be formed by an oral nitrate (NO_3^-)/nitrite (NO_2^-) pathway, suggesting the important role of oral microflora in the bio-activation of NO [10]. NO_3^- and NO_2^- can be recycled by absorption and secretion via the salivary glands [11]. In humans, the salivary glands can absorb more than 25% of circulating NO_3^- and secrete them into the oral cavity with the saliva [12], where the NO_3^- can be further reduced to NO_2^- by various species of oral bacteria residing in the oral cavity via bacterial NO_3^- reductases [13]. Swallowed NO_2^- with saliva is rapidly absorbed into the blood circulation, where several enzymes in the circulation reduce NO_2^- to NO, increasing the bioavailability of NO [14,15]. These results indicate that the oral NO_3^-/ NO_2^- pathway which contributes to NO production can be influenced by the oral microbiome.

Mouthwash, also called mouthrinse or oral rinse, is an antiseptic liquid solution intended to reduce the microbial load in the oral cavity and commonly used for oral health

care. Since it not only complements brushing and flossing, but also masks bad breath, mouthwash is frequently used in everyday life to reduce or prevent breath odor after eating and/or exercise [16]. A recent study found that the removal of oral bacteria using mouthwash can attenuate the NO-dependent biological effects of dietary NO_3 on BP [17]. It was also reported that using antibacterial mouthwash (AM) for a few consecutive days inhibits oral NO_3^- to- NO_2^- reduction and results in increased resting systolic BP (SBP) [18]. Given the notion that the NO_3-reducing activity of oral bacteria can contribute to NO production, previous findings suggest the negative effect of AM on PEH regulation. To the best of our knowledge, only one study, by Cutler *et al*. [19] directly explored the effect of AM usage on PEH. The authors found that PEH was significantly attenuated at 60 and 120 min on using an AM containing 0.2% chlorhexidine and this was accompanied by a reduction in circulatory NO_2 [19], suggesting that NO_2 synthesis via oral bacteria could be one of the key mechanisms for triggering PEH.

Two common cationic antiseptics used in most mouthwash products are chlorhexidine and cetylpyridinium chloride (CPC). Chlorhexidine is generally considered more effective in reducing dental plaque formation [20]; however, it is the active ingredient in a prescription product marketed according to Food and Drug Administration approval via the new drug application route [21]. Moreover, it has considerable side effects, such as changes in taste and yellow or brown pigmentation on tooth surfaces [22,23]. Thus, the use of chlorhexidine for caries prevention is still controversial [24]. Meanwhile, CPC is the most common ingredient in commercially available over-the-counter products, and the degree of staining by CPC has been shown to be considerably lesser than that by chlorhexidine [25]. In the only existing study on the effect of AM usage on PEH [19], chlorhexidine-containing AM was used, which is not be generally used in the daily life of normal people. Furthermore, their findings have not been replicated so far. Therefore, this study aimed to investigate the effects of using a commercially available AM, mainly containing CPC, on post-exercise changes in cardiovascular physiological parameters including vascular stiffness during the recovery period after an acute bout of aerobic exercise.

2. Materials and methods

2.1 Subjects

Ten healthy male volunteers (age 20.5 $\pm$ 0.5 years) participated in this study. The sample size was anticipated to detect a mean difference of 2 mmHg in SBP at a 5% significance level with 85% power for the crossover study design (http://hedwig.mgh.harvard.edu/sample_size). Smoking, hypertension, body mass index >30 kg·m^{-2}, type I or II diabetes, antibiotic use within 1 month, and oral problems such as gingivitis or periodontitis were unnoted in any participant. During the pre-experiment meeting, all subjects were informed of the potential risks and discomforts, and they provided written informed consent. Then, subjects performed an incremental treadmill test to determine the individual running speed for the specific intensity. This study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of the local institute (SeoulTech-2020-0019).

2.2 Experimental protocol

The present study employed a randomized crossover design. The subjects visited the laboratory twice at an interval of 1 week. They were asked to fast overnight, but not to refrain from water before each visit. Subjects were also instructed not to engage in any exerting physical activity within 48 h before the laboratory visit.

The experimental procedure is illustrated in Fig. 1. On arrival at the laboratory at 10:00 AM, anthropometric measurements were obtained using a digital height and weight measuring machine (DS-103M, Dong-Sahn JENIX Co., Seoul, South Korea), and body composition was determined by the bioelectrical impedance analysis using Inbody 720 (Biospace Co., Seoul, South Korea), which is widely used for estimating body fat percentage, fat mass and fat-free mass [26]. The subjects then rested on a medical couch for 10 minutes, following baseline resting heart rate (HR), BP, blood lactate (BL), and arterial stiffness index (ASI) measurements.

Upon completion of pre-exercise (resting) measurements, subjects performed a total of 40 min of treadmill running at 0% inclination, consisting of 10-min warm-up and 30-min main exercise at 60–65% of age-predicted maximal HR. HR during exercise was monitored using Polar H10 (Polar, Finland).

After exercise, the subjects were seated immediately and remained in the laboratory under resting conditions on a medical couch for 120 min. Food and drink were forbidden during the recovery period. AM (Garglin, 0.05% CPC, no ethanol; Dong-A Pharmaceutical Co., Seoul, South Korea) or placebo (nitrite-free water) was provided to the participants at 5, 35, 65, and 95 min after exercise. They rinsed their mouth for 1 min on each occasion with 15 mL of AM or placebo.

BP, HR and ASI were measured at 0, 10, 20, 30, 60, 90 and 120 min and BL concentrations were obtained at 0, 15, 30, and 60 min after exercise. HR was measured on the right arm using the pulse palpation method and BP was measured on the left arm using a mercury sphygmomanometer 600 (Yamasu Japan, Chiba, Japan). HR and BP were simultaneously measured twice with a 1-min interval between measurements, and the average value was used. Next, BL levels were assessed using a small amount of blood taken from the fingertip of the right hand via a blood lactate analyzer (Lactate Pro 2, Kyoto, Japan). ASI was determined on the left arm by the second derivative wave of a photoplethysmogram (SDPTG) obtained using

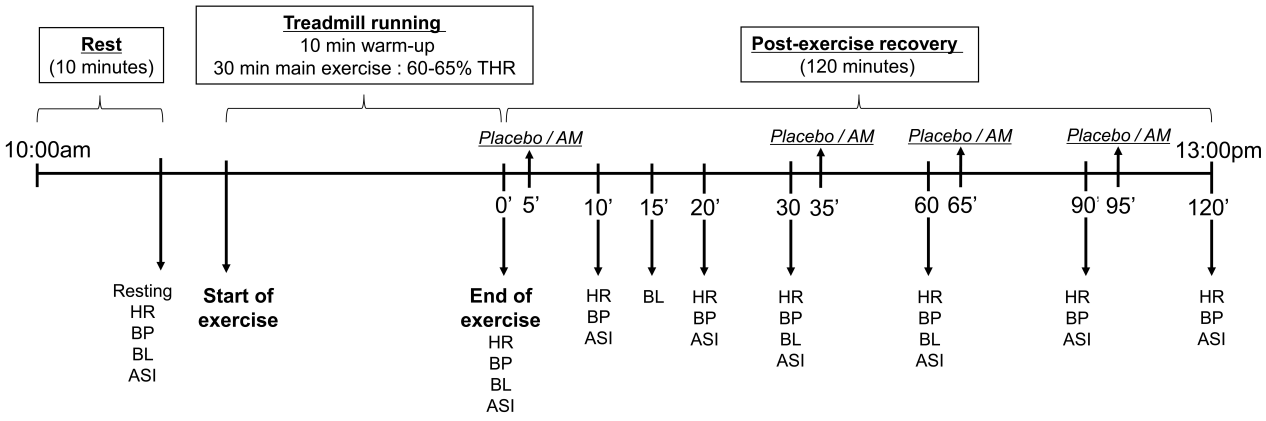

Fig. 1. Representative experimental procedure. HR, heart rate; BP, blood pressure; BL, blood lactate; ASI, arterial stiffness index; THR, target HR; AM, antibacterial mouthwash.

uBioClip v70 (Biosense creative Co., Seoul, South Korea) as previously used [27]. A sensor was placed on the tip of the index finger to measure the pulse wave in a capillary vessel for 1 min by a non-invasive method. SDPTG facilitates the analysis of wave indices, including b/a ratio, which can be used to identify increased arterial stiffness [28]. All measurements were performed by the same investigators.

2.3 Statistical analysis

Values are presented as mean ± standard error of the mean (SEM). Mean arterial pressure (MAP) was estimated as the sum of two thirds of diastolic BP (DBP) and one third of SBP. One-way Analysis of variance (ANOVA) with repeated measures was used to analyze the differences between treatments over the recovery period. The differences between pre-exercise and post-exercise or between two treatments at a specific time point were analyzed using paired *t*-test. All data were analyzed using SigmaPlot software 12.5 (Systat Software Inc., Palo Alto, CA, USA), and statistical significance was set at $p < 0.05$.

3. Results

The physical characteristics of the subjects are presented in Table 1. All pre-exercise measurements at rest were similar between the treatments (data not shown).

HR changes (post-exercise minus pre-exercise) during the post-exercise recovery period are shown in Fig. 2. HR significantly increased at 0, 10, 20, and 30 min after exercise in both groups compared to the resting HR. However, the exercise-induced increases in HR were not significantly different between the treatments.

Fig. 3 shows the changes in SBP, DBP, and MAP after exercise. In the placebo group, SBP and MAP significantly decreased until 120 min after exercise compared to pre-exercise. A significant reduction was found at every time point for SBP, at 30 and 60 min for DBP and at 30, 60, 90, and 120 min for MAP. These results in the placebo group indicated the occurrence of PEH as expected. In con-

Table 1. Physical characteristics of subjects (n = 10).

Variables	Mean ± SEM
Age (yrs)	20.5 ± 0.5
Weight (kg)	69.0 ± 2.0
Height (cm)	173.0 ± 2.4
BMI (kg·m^{-2})	23.0 ± 0.3
Body fat (%)	18.5 ± 1.3
Muscle mass (kg)	31.9 ± 1.2
Fat mass (kg)	12.7 ± 0.9
Resting HR (beats·min^{-1})	65.7 ± 2.1
Resting SBP (mmHg)	117.3 ± 2.2
Resting DBP (mmHg)	72.1 ± 1.2
Resting MAP (mmHg)	87.2 ± 1.6

BMI, body mass index; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; SEM, standard error of the mean.

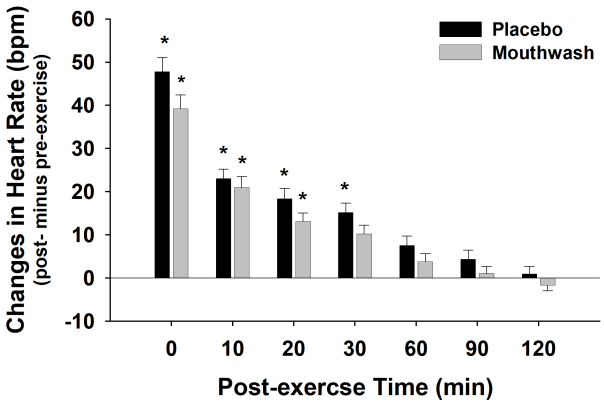

Fig. 2. Changes (post-exercise minus pre-exercise values) in heart rate during the recovery period (0 to 120 min). Values are presented as mean ± SEM. *, $p < 0.05$ compared with pre-exercise values. bpm, beats per minute.

trast, in the AM group, post-exercise decreases in BP were less versus the placebo group. Differences in BP reduction during the recovery period were significant at later time points between the treatments. Significant differences in post-exercise changes were found at 60, 90 and 120 min for SBP and at 30, 60, 90 and 120 min for both DBP and MAP. These results provide evidence that the BP-reducing effect of exercise can be blunted by AM use after exercise.

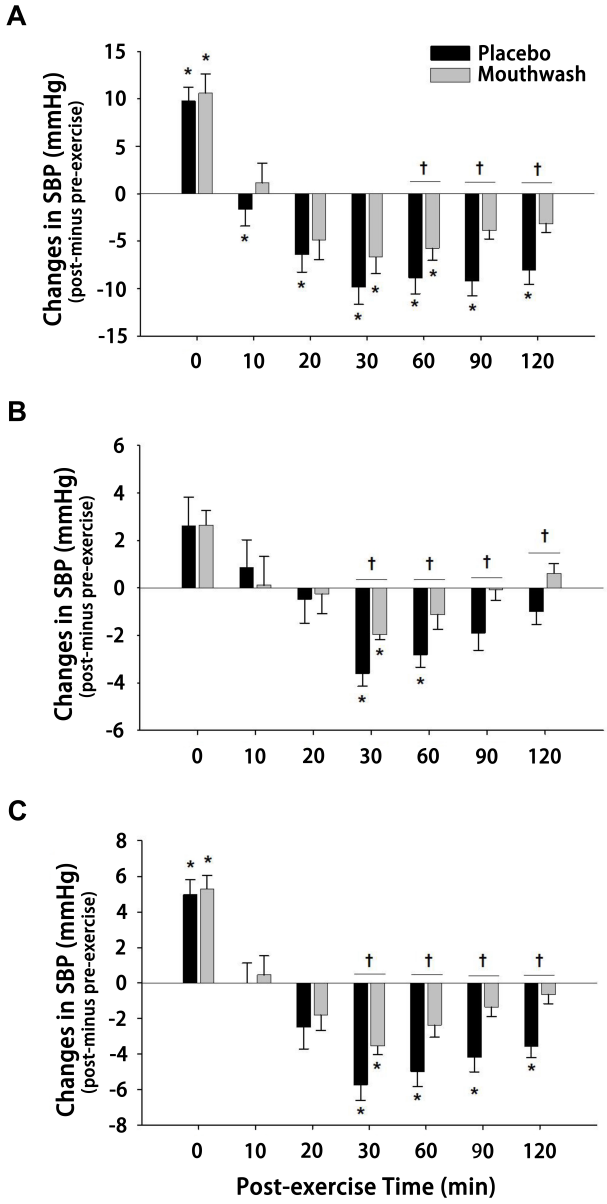

Fig. 3. Changes (post-exercise minus pre-exercise values) in (A) systolic blood pressure (SBP), (B) diastolic blood pressure (DBP) and (C) mean arterial pressure (MAP) during the recovery period (0 to 120 min). Values are presented as mean ± SEM. *, $p < 0.05$ compared with pre-exercise values. †, $p < 0.05$ between treatments.

Changes in ASI (b/a ratio) during the recovery period are shown in Fig. 4. In the placebo group, ASI at 0, 10, and 20 min after exercise was significantly decreased compared to the resting ASI. On the contrary, a significant reduction in post-exercise ASI was unnoted in the AM group. Moreover, at 120 min of the recovery period, a significant difference in ASI changes was found between treatments. This finding indicates that exercise can ameliorate arterial stiffness; however, this effect can be blocked by AM use after exercise.

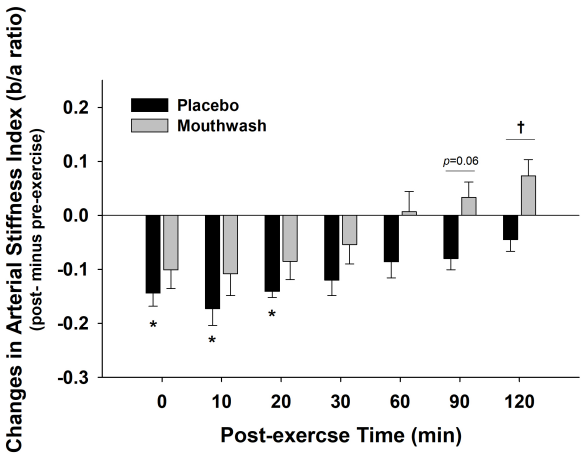

Fig. 4. Changes (post- exercise minus pre-exercise values) in the arterial stiffness index (b/a ratio) during the recovery period (0 to 120 min). Values are presented as mean ± SEM. *, $p < 0.05$ compared with pre-exercise values. †, $p < 0.05$ between treatments.

BL concentrations were measured as a marker of fatigue recovery after exercise (Table 2). In both groups, BL concentrations increased after exercise as expected, and this increase was restored 30 min after exercise. There was no difference in BL concentrations between the treatments during the recovery period.

Table 2. Blood lactate (mmol/L) measured before and after exercise.

Time (min)	Placebo	Mouthwash
At rest	1.8 ± 0.2	1.7 ± 0.2
0	6.6 ± 0.6*	6.1 ± 0.4*
15	4.3 ± 0.5*	4.4 ± 0.5*
30	2.5 ± 0.2	2.3 ± 0.3
60	2.0 ± 0.2	1.9 ± 0.1

* $p < 0.05$ compared with pre-exercise (at rest) values.

4. Discussion

Given that oral bacteria are involved in the systemic NO_3^-/ NO_2^- pathway and, ultimately, in NO production, this study investigated the effects of a commercially used AM on changes in BP and arterial stiffness after exercise. We found that changes in PEH and changes in arterial stiffness during the recovery period were significantly attenuated when the AM was used after exercise, providing evidence that AM usage immediately after acute exercise can diminish the beneficial effects of exercise on BP reduction.

Hypertension is a well-known strong risk factor for CVD [1]; thus, prevention or amelioration of increased BP is an important medical target for decreasing CVD-associated mortality and complications. Exercise has been strongly recommended as a non-pharmacological approach to BP regulation. Regular exercise can prevent ameliorate hypertension [5,6], and this can be achieved by the accumulation of transient BP reduction exerted by a single bout of exercise, which is called PEH. However, the magnitude and duration of PEH varies in the literature possibly due to the differences in sex, age, exercise modality, and disease status [29,30], indicating that the scope of research needed to fully clarify the causes, mechanisms and implications of PEH.

Several mechanisms have been proposed for PEH [30], including NO because of its vasodilatory effect on vascular smooth muscle. It has been established that NO is involved in PEH mainly via exercise-induced improvements in endothelial function by upregulating eNOS expression and phosphorylation [8]. However, recent evidence also suggests that there is another NO-producing pathway associated with the oral NO_3^-/ NO_2^- pathway [12]. Systemic NO_3^- concentrations are influenced by various NOS-independent factors, including dietary NO_3^- intake, saliva formation, bacterial NO_3^- synthesis within the bowel, denitrifying liver enzymes, inhalation of atmospheric gaseous NO, and renal function [31,32]. Koch *et al.* [32] reported that the tongue surface harbors facultative anaerobic bacteria that can rapidly reduce NO_3^- to NO_2^-, which can then be converted to NO in the stomach [33]. Recently, Cutler *et al.* [19] found that PEH occurred after interval exercise at moderate intensity and was accompanied by increased NO_2^- in the blood and increased muscle oxygenation, while the use of AM containing chlorhexidine after exercise induced a lack of change in circulatory NO_2^- and attenuated SBP reduction and muscle oxygenation. Our results are consistent with their findings in that an acute bout of aerobic exercise with moderate intensity induced PEH, which was also blunted by an AM containing a different antiseptic, CPC. Considering that both chlorhexidine and CPC can reduce microbial load in the oral cavity, our results also support the hypothesis that the systemic NO_3^-/ NO_2^- pathway via oral bacteria plays an important role in PEH regulation. However, we did not measure the levels of NO_2^- or microbiota in responses to exercise or AM. Thus, further studies incorporating direct measurements of NO_2^- and microbiota in oral cavity and/or blood with and without mouthwash are required to elucidate the mechanisms by which the oral microbiome contributes to PEH regulation via systemic NO_3^-/ NO_2^- pathway.

In the current study, the use of AM not only blunted PEH, but also prevented post-exercise decreases in arterial stiffness. Arterial stiffness is an independent risk factor for cardiovascular diseases [34], and studies have shown that regular exercise can alleviate vascular stiffness [35]. Given that some of the well-characterized factors influencing arterial stiffness include endothelium-derived substances such as NO [36], it can be assumed that exercise-induced changes in NO bioavailability would be one of the mechanisms for post-exercise changes in vascular stiffness [37], which is attributable to PEH. Many studies have found that exercise improves eNOS expression and activity, but whether the oral NO_3^-/ NO_2^- pathway is affected by exercise is unknown [31]. In this study, arterial stiffness was attenuated during the recovery period after exercise, whereas this exercise-induced attenuation was blunted by AM. This suggests that the NO_3^-/ NO_2^- pathway via oral bacteria also plays a role, at least partly, in post-exercise reductions in arterial stiffness. A previous study that showed NO_2^- has vasodilatory effects [33] further supports this notion. HR and BL increased after exercise as expected and AM did not influence the post-exercise changes in HR and BL. These suggest that AM-induced differences in post-exercise changes in BP and ASI in our result are neither associated with the central autonomic system, nor with the acid-base balance.

In the present study, SBP did not return to baseline (resting BP) until the end of experiment (120 min) during the recovery period. Therefore, for a better understanding of PEH, it is necessary to measure BP changes for longer than 120 min. Apart from this, our study has a few other limitations. As mentioned above, we did not measure salivary or plasma NO_2^-; therefore, we could not prove the underlying mechanism of our findings. Thus, further studies are required to determine whether the salivary or plasma NO_2^- level is changed in the setting of acute exercise and AM usage and whether these changes can be influenced by dietary NO_2^- supplementation. The subjects in this study were healthy young male individuals; therefore, additional studies are warranted before translating our findings to other populations, such as hypertensive patients and female subjects.

5. Conclusions

We found that an acute bout of moderate-intensity aerobic exercise induced PEH and resulted in decreased arterial stiffness in healthy young men, while the use of AM diminished these exercise-induced changes in BP and arterial stiffness. Therefore, these results indicate that AM use after exercise is contraindicated to maintain the BP-reducing effects of exercise.

Abbreviations

BP, blood pressure; PEH, post-exercise hypotension; AM, antibacterial mouthwash; ASI, arterial stiffness index; HR, heart rate; BL, blood lactate; CVD, cardiovascular disease; NO, nitric oxide; eNOS, endothelial nitric oxide synthase; NO_3^-, nitrate; NO_2^-, nitrite; SBP, systolic blood pressure; CPC, cetylpyridinium chloride; BMI, body mass index; SDPTG, the second derivative wave of photoplethysmogram; DBP, diastolic blood pressure; MAP, mean arterial pressure.

Author contributions

Conceptualization—YC, MK and SKK; methodology—YC, MK and SKK; data acquisition—YC and MK; data analysis—YC, MK and SKK; writing - original draft preparation, YC; writing - review and editing, SKK; funding acquisition—SKK. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

Prior to participating in the study, all subjects were informed of the potential risks and discomforts, and they provided written informed consent. This study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of Seoul National University of Science and Technology (SeoulTech-2020-0019).

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Conflict of interest

The authors declare no conflict of interest.

References

[1] O'Donnell MJ, Xavier D, Liu L, Zhang H, Chin SL, Rao-Melacini P, *et al*. Risk factors for ischaemic and intracerebral haemorrhagic stroke in 22 countries (the INTERSTROKE study): a case-control study. The Lancet. 2010; 376: 112–123.

[2] World Health Organization. A global brief on hypertension: silent killer, global public health crisis: World Health Day 2013. 2013. Available at: https://www.who.int/publications/i/item/a-global-brief-on-hypertension-silent-killer-global-public-health-crisis-world-health-day-2013 (Accessed: 19 October 2021).

[3] World Health Organization. Global status report on noncommunicable diseases 2014. 2014. Available at: https://apps.who.int/iris/handle/10665/148114 (Accessed: 19 October 2021).

[4] Yap YG, Duong T, Bland JM, Malik M, Torp-Pederson C, Køber L, *et al*. Prognostic value of blood pressure measured during hospitalization after acute myocardial infarction: an insight from survival trials. Journal of Hypertension. 2007; 25: 307–313.

[5] Sharman JE, La Gerche A, Coombes JS. Exercise and cardiovascular risk in patients with hypertension. American Journal of Hypertension. 2014; 28: 147–158.

[6] Chen C, Bonham AC. Postexercise hypotension: central mechanisms. Exercise and Sport Sciences Reviews. 2010; 38: 122–127.

[7] Halliwill JR. Mechanisms and Clinical Implications of Post-exercise Hypotension in Humans. Exercise and Sport Sciences Reviews. 2001; 29: 65–70.

[8] Green DJ, Maiorana A, O'Driscoll G, Taylor R. Effect of exercise training on endothelium-derived nitric oxide function in humans. The Journal of Physiology. 2005; 561: 1–25.

[9] Förstermann U, Sessa WC. Nitric oxide synthases: regulation and function. European Heart Journal. 2012; 33: 829–837d.

[10] Moretti C, Zhuge Z, Zhang G, Haworth SM, Paulo LL, Guimarães DD, *et al*. The obligatory role of host microbiota in bioactivation of dietary nitrate. Free Radical Biology and Medicine. 2019; 145: 342–348.

[11] Lundberg JO, Weitzberg E, Gladwin MT. The nitrate–nitrite–nitric oxide pathway in physiology and therapeutics. Nature Reviews Drug Discovery. 2008; 7: 156–167.

[12] Qin L, Liu X, Sun Q, Fan Z, Xia D, Ding G, *et al*. Sialin (SLC17a5) functions as a nitrate transporter in the plasma membrane. Proceedings of the National Academy of Sciences. 2012; 109: 13434–13439.

[13] Lundberg JO, Govoni M. Inorganic nitrate is a possible source for systemic generation of nitric oxide. Free Radical Biology and Medicine. 2004; 37: 395–400.

[14] Maron BA, Tang S, Loscalzo J. S-nitrosothiols and the S-nitrosoproteome of the cardiovascular system. Antioxidants & Redox Signaling. 2013; 18: 270–287.

[15] Divakaran S, Loscalzo J. The Role of Nitroglycerin and other Nitrogen Oxides in Cardiovascular Therapeutics. Journal of the American College of Cardiology. 2017; 70: 2393–2410.

[16] Rosenberg M. The science of bad breath. Scientific American. 2002; 286: 72–79.

[17] Govoni M, Jansson EA, Weitzberg E, Lundberg JO. The increase in plasma nitrite after a dietary nitrate load is markedly attenuated by an antibacterial mouthwash. Nitric Oxide. 2008; 19: 333–337.

[18] Bondonno CP, Liu AH, Croft KD, Considine MJ, Puddey IB, Woodman RJ, *et al*. Antibacterial mouthwash blunts oral nitrate reduction and increases blood pressure in treated hypertensive men and women. American Journal of Hypertension. 2014; 28: 572–575.

[19] Cutler C, Kiernan M, Willis JR, Gallardo-Alfaro L, Casas-Agustench P, White D, *et al*. Post-exercise hypotension and skeletal muscle oxygenation is regulated by nitrate-reducing activity of oral bacteria. Free Radical Biology and Medicine. 2019; 143: 252–259.

[20] Brookes ZLS, Bescos R, Belfield LA, Ali K, Roberts A. Current uses of chlorhexidine for management of oral disease: a narrative review. Journal of Dentistry. 2020; 103: 103497.

[21] Food and Drug Administration. Oral Health Care Drug Products for Over-the-Counter Human Use: tentative final monograph: notice of proposed rulemaking. Federal Register. 1988; 53: 2436–2461.

[22] Cortellini P, Labriola A, Zambelli R, Prato GP, Nieri M, Tonetti MS. Chlorhexidine with an anti discoloration system after periodontal flap surgery: a cross-over, randomized, triple-blind clinical trial. Journal of Clinical Periodontology. 2008; 35: 614–620.

[23] Mauland EK, Preus HR, Aass AM. Comparison of commercially available 0.2

[24] Fiorillo L. Chlorhexidine Gel Use in the Oral District: a Systematic Review. Gels. 2019; 5: 31.

[25] Sheen S, Addy M. An in vitro evaluation of the availability of cetylpyridinium chloride and chlorhexidine in some commercially available mouthrinse products. British Dental Journal. 2003; 194: 207–210.
[26] McLester CN, Nickerson BS, Kliszczewicz BM, McLester JR. Reliability and Agreement of Various InBody Body Composition Analyzers as Compared to Dual-Energy X-Ray Absorptiometry in Healthy Men and Women. Journal of Clinical Densitometry. 2020; 23: 443–450.
[27] Shin Y, Ham J, Cho H. Experimental study of thermal comfort based on driver physiological signals in cooling mode under summer conditions. Applied Sciences, 2021; 11: 845.
[28] Takazawa K, Tanaka N, Fujita M, Matsuoka O, Saiki T, Aikawa M, *et al*. Assessment of vasoactive agents and vascular aging by the second derivative of photoplethysmogram waveform. Hypertension. 1998; 32: 365–370.
[29] MacDonald JR. Potential causes, mechanisms, and implications of post exercise hypotension. Journal of Human Hypertension. 2002; 16: 225–236.
[30] Halliwill JR, Buck TM, Lacewell AN, Romero SA. Postexercise hypotension and sustained postexercise vasodilatation: what happens after we exercise? Experimental Physiology. 2013; 98: 7–18.
[31] Jones AM, Vanhatalo A, Seals DR, Rossman MJ, Piknova B, Jonvik KL. Dietary Nitrate and Nitric Oxide Metabolism: Mouth, Circulation, Skeletal Muscle, and Exercise Performance. Medicine & Science in Sports & Exercise. 2021; 53: 280–294.
[32] Koch CD, Gladwin MT, Freeman BA, Lundberg JO, Weitzberg E, Morris A. Enterosalivary nitrate metabolism and the microbiome: Intersection of microbial metabolism, nitric oxide and diet in cardiac and pulmonary vascular health. Free Radical Biology and Medicine. 2017; 105: 48–67.
[33] Miyoshi M, Kasahara E, Park A, Hiramoto K, Minamiyama Y, Takemura S, *et al*. Dietary nitrate inhibits stress-induced gastric mucosal injury in the rat. Free Radical Research. 2003; 37: 85–90.
[34] Kaess BM, Rong J, Larson MG, Hamburg NM, Vita JA, Levy D, *et al*. Aortic stiffness, blood pressure progression, and incident hypertension. JAMA. 2012; 308: 875–881.
[35] Doonan RJ, Mutter A, Egiziano G, Gomez Y, Daskalopoulou SS. Differences in arterial stiffness at rest and after acute exercise between young men and women. Hypertension Research. 2013; 36: 226–231.
[36] Bellien J, Favre J, Iacob M, Gao J, Thuillez C, Richard V, *et al*. Arterial stiffness is regulated by nitric oxide and endothelium-derived hyperpolarizing factor during changes in blood flow in humans. Hypertension. 2010; 55: 674–680.
[37] Hasegawa N, Fujie S, Horii N, Miyamoto-Mikami E, Tsuji K, Uchida M, *et al*. Effects of Different Exercise Modes on Arterial Stiffness and Nitric Oxide Synthesis. Medicine & Science in Sports & Exercise. 2018; 50: 1177–1185.