Effect of formula supplemented with vitamin C and hawthorn beverage rich in flavonoids on blood pressure and oxidative stress in heat-exposed workers: a cluster-randomized controlled trial

doi: 10.6133/apjcn.202406/PP.0015
Published online: June 2024

Running title: Vit C and hawthorn drink prevent heat-exposed bp rise

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ABSTRACT

Background and Objectives: There is no evidence on rich-antioxidants diet in preventing hypertension in heat-exposed workers. We aimed to evaluate the effects of formula supplemented with vitamin C (VC) and hawthorn beverage on reducing blood pressure (BP) and oxidative stress levels in heat-exposed workers. **Methods and Study Design:** In the 40-day cluster-randomized controlled trial, four heat-exposed shift-teams were enrolled and randomly assigned to the intervention and control groups. The intervention group was given one VC tablet with 130.0 mg VC and a 500 mL hawthorn beverage with 278.7 mg flavonoids per day, the control group was given 500 mL of slightly salted water per day, and both groups were given healthy diet education. BP and creatinine-corrected urinary 8-isoprostane-prostaglandin F2α (8-iso-PGF2α/Cr) concentrations were assessed at baseline, Day 17 (only BP) and Day 41, respectively. **Results:** Compared with the control group, the systolic BP (SBP), diastolic BP (DBP), and log10-transformed 8-iso-PGF2α/Cr in the intervention group decreased by 7.41 mmHg, 7.93 mmHg and 0.232, respectively, from baseline to day 41 (all $p<0.05$). When considering baseline BP levels, compared with the control group, DBP in the intervention group was reduced by 5.46 mmHg ($p<0.05$) among participants with lower baseline BP, and SBP and DBP experienced reductions of 9.74 and 9.22 mmHg among participants with higher baseline BP (both $p<0.05$). **Conclusions:** The intervention involving the supplementation of VC and hawthorn beverage rich in flavonoids to heat-exposed workers had prevented the elevated BP caused by heat exposure and this might through its inhibition of oxidative stress.

Key Words: vitamin C, hawthorn flavonoids, blood pressure, 8-iso-PGF2α, heat exposure

INTRODUCTION

Hypertension is a key risk factor for cardiovascular diseases and caused one-fifth of all deaths (approximately 10.8 million) worldwide in 2019.[1] In China, the prevalence of hypertension among adults has steadily increased in the past three decades, reaching 27.5% (approximately 300 million people) in 2018.[2] As a severe global public health concern, preventing hypertension has become an important task. From the perspective of prevention strategies and measures for cardiovascular disease, high-risk prevention strategies are more effective for people at high risk.[3] Heat-exposed workers are prone to hypertension, which is associated with vitamin C (VC) deficiency.[4,5] The increased metabolic rate and oxidation reactions caused by heat exposure, along with the excessive loss of water-soluble antioxidants through sweat, can disrupt the balance between oxidation and antioxidation, directly impacting cardiac systolic function.[6] Increasing foods (drinks) rich in antioxidants is a feasible solution for preventing hypertension caused by heat exposure.

Oxidative stress, due to the excessive production of oxygen free radicals and a decrease in antioxidant capacity, is linked to the development of cardiovascular damage.[7] A case–control study indicated that natural antioxidants found in fruits, vegetables, and drinks (tea and coffee) could reduce the incidence of hypertension.[8] Employing whole foods instead of isolated components as supplements in interventions is more reasonable and feasible. Therefore, high-quality vegetable and fruit diets or those fortified with nutrients are typically used in intervention trials.[9,10] An RCT reported that an 8-week high- vegetable and fruit juices intervention decreased the concentration of 8-iso-PGF2α in the urine, which is a biomarker of lipid peroxidation.[11] These beneficial effects are attributed to VC and flavonoids, which are abundant in vegetables and fruits and act as ideal antioxidants, playing an important role in maintaining intracellular redox homeostasis.[12] Recent RCTs have shown that diets naturally rich in antioxidants, such as orange juice rich in VC or diets rich in polyphenols, could reduce oxidative stress and improve elevated BP.[13,14] However, there is insufficient evidence that foods (drinks) rich in antioxidants can prevent elevated BP in high-risk populations.

Previous studies have shown that VC could induce the activity of the transcription factor Nrf2, which was involved in the antioxidant protection against the effects of oxidative damage in rats.[15] However, VC supplementation could not completely inhibit the oxidative stress caused by heat exposure and prevent BP levels from increasing in rats; instead, the combination of VC with flavonoids in hawthorn extract supplements could reduce BP levels.[16] There is a growing interest in the effect of consuming dark-colour fruits on oxidative stress.[17-20] Hawthorn, which is rich in flavonoids compared with other drupes, has a greater oxygen radical absorbance capacity[21] and can reduce BP levels.[22] Since 2000, heatstroke prevention by providing steel workers with ice lollies or dried hawthorn to soak in drinking water has been common among Chinese steel enterprises. Soaking dried hawthorn in drinking water is not convenient for workers to drink; using hawthorn juice may be a more suitable measure for heat-exposed workers experiencing oxidative stress.

Therefore, a cluster-RCT design was applied to evaluate the ability of a daily formula supplemented with VC and hawthorn beverage rich in flavanols to prevent an increase in BP, giving a 5-shift-work-cycle (40-day) intervention during the summer of the year. The effect on inhibiting oxidative stress, as measured by urinary isoprostane levels, was also evaluated.

A slightly salted gas water, commonly consumed by heat-exposed workers, was given to the control group. To date, no study has evaluated the effect of antioxidant drink supplementation on BP levels in heat-exposed workers.

MATERIALS AND METHODS

Study design

This study was a cluster-RCT at the continuous casting workshop of a steel company in Hebei Province, North China, from July to August 2023. The study adhered to the Declaration of Helsinki and was approved by the Ethics Committee of North China University of Science and Technology (approval no. 2023064). This trial was registered with the Chinese Clinical Trial Registry (No. ChiCTR2400080611), and all participants provided written informed consent before the start of the trial.

Study participants

All four shift teams from the workshop were selected, each consisting of 40 to 50 steel workers. Steel workers exposed to heat (wet bulb globe temperature: 28.5 - 35.5 ℃) were invited to participate in the trial; they were eligible for heat exposure post and lived in dormitories during workdays. The inclusion criteria were as follows: (1) male, 22-55 years old steelworkers; (2) service length ≥1 year. The exclusion criteria were as follows: (1) participated in other intervention trials in the past 3 months; (2) took anti-hypertensive drugs; (3) consumed dietary supplements; (4) had prediabetes or diabetes; (5) had other cardiovascular diseases; (6) had gastrointestinal disease in the past 1 month; (7) planned to take a vacation; (8) refused to participate in the trial.

The sample size was estimated by using the formula for a cluster-RCT. Based on data from a previous study targeting cardiovascular health following VC supplementation,[23] assuming a standard deviation (SD) of systolic BP (SBP) of 10 mmHg, with an intraclass correlation coefficient of 0.01, we estimated that a sample size of 124 participants would provide 80% power (with a two-sided alpha=0.05) to detect a difference in mean SBP ≥5 mmHg between the intervention and control groups, allowing for a 10% loss-to-follow-up rate of participants. Eligible participants completed and met all the investigations within the study period, and a total of 132 qualified data were included in the analysis. A summary of the study process based on the CONSORT flow diagram (2010) is presented in Figure 1.

Randomization

Participants were provided with a 500 mL bottle of slightly salted gas water during an 8-day run-in period. After that, the four eligible shift teams (clusters) were randomly assigned to the intervention and control groups by a random numbers table in a 1:1 ratio. Due to the nature of the intervention, blinding of the participants was infeasible.

Intervention programs

In the antioxidant formula supplement intervention group, each participant was given one VC tablet with two cans of hawthorn beverage per workday in a 5-shift work cycles (2-day shifts + 2-night shifts + 4-rest days/cycle, total of 40 days). The VC Chew Tablet was manufactured by By health Co., Ltd., Zhuhai, China; each tablet contained 130.0 mg of VC. The Boiled Hawthorn Drink was manufactured by Liyuan Food Co., Ltd., Tangshan, China; each can had 250 mL, containing less than 1 mg of VC and 139.4 mg of flavonoids (Supplementary Table 1). Briefly, the VC tablet was requested to be chewed before the shift, and the hawthorn beverage was requested to be consumed during work breaks.

For the control group, each participant was given a bottle of slightly salted gas water daily during the workdays in the workshop. The slightly salted gas water, as a common heatstroke prevention drink at the worksite, was manufactured by Beishuang Drink Water Co., Ltd., Tangshan, China; each bottle had 500 mL and contained 0.5 g of salt. The reason to use slightly salted gas water as a control was based on the requirement for heatstroke prevention drinks on site. In addition, the sweat loss of salt among heat-exposed workers (sweat excretion during work is 3.18 L, and the sodium concentration in sweat is 1173.1 mg/L) was also considered.[5] The daily total calculated sodium intake of the control group was 5811 mg/d (183 mg in slightly salted gas water and 5629 mg in the diet), accounted for 89.4% of the maximum recommended intake (6500 mg/d) for heat-exposed workers,[24] and was not significantly different from that of the intervention group (5629 mg/d) (p=0.058).

Each beverage bottle was marked with a different number. A trained doctoral candidate, who collaborated with shift team leaders, was responsible for distributing the VC tablet and hawthorn beverage or slightly salted gas water to the participants every day. Participants were requested to upload photos of used tablets, empty cans or bottles to an online document, based on which the candidate recorded the daily consumption of each participant. In order to enhance the compliance of individuals, a brief guidance on healthy drinking habit was provided to all participants in both groups on the first day of each work cycle through cell phone messages. The guidance content included the appropriate drinking frequency, quantity and beverage temperature for heat-exposed workers, and the advantage of drinking hawthorn beverage (sent to participants in the intervention group) or slightly salted gas water (sent to participants in the control group). The participants were asked about any side effects of the supplements on the last day of each shift cycle.

Outcomes

Our primary outcome was the difference in net changes in SBP or diastolic BP (DBP) between the intervention and control groups from baseline to the end of the 5-shift-work-cycle intervention period. The secondary outcome was the net change in the urine 8-iso-PGF2α concentration.

Data collection and follow-up

BP measurement and fasting urine sample collection were carried out on the first day of the intervention (baseline), on the day after the 2-shift-work-cycle intervention (day 17) (BP measurement only), and on the day after the 5-shift-work-cycle intervention (day 41), in the morning before the start of the shift, in exactly the same way for all participants by trained researchers.

Before randomization, the characteristics of each participant, such as age, length of service, type of work, smoking status, alcohol drinking status, tea drinking status, physical activity, sleeping time, and personal and family history of illness, were collected via face-to-face interviews using structured questionnaires. Body height and weight were also measured, and the participants' general dietary intake was assessed before randomization.

BP measurements

The SBP and DBP of the right upper arm were measured three times using a fully automatic electronic sphygmomanometer (HEM-7301-IT, Omron Healthcare Co., Ltd., Kyoto, Japan) with an interval of 1 to 2 min after the participants quietly sitting in an upright position for 5 min. The first reading was taken but discarded. If the difference between the last two readings was >5 mmHg, the measurements were taken again and the mean of the last two recorded values was used for analysis. In addition, the participants needed to leave their working environment for more than 12 h to have their BP measured.

Urine collection and 8-iso-PGF2α concentration assessment

Midstream urine samples (15 mL) were collected from the participants from the first void in the morning following an 8-h period without drinking or intake. The study participants were adequately trained on proper collection and storage procedures. The urine samples were placed in a foam box with ice packs and transported to the laboratory at North China University of Science and Technology. Urine samples were separated at a low temperature, fractionated into aliquots, and stored at -80°C.

For the analysis of 8-iso-PGF2α, one aliquot was thawed on ice immediately before the assay, centrifuged, and the resulting supernatant was diluted 20 times. The concentrations of 8-iso-PGF2α were measured using an 8-isoprostane enzyme immunoassay kit (Cayman Chemicals Co., Ann Arbor, MI, USA). The assay was conducted manually, and the results were read in triplicate with a spectrophotometer. Concentrations of creatinine were measured using a sarcosine oxidase kit (Jiancheng Bio. Co., Nanjing, China). The concentrations of creatinine-corrected 8-isoprostane-prostaglandin F2α (8-iso-PGF2α/Cr) in urine were expressed as μg/mol.

Anthropometric examinations

The body height and weight of each participant were assessed using a height measuring scale (C200, Shunjia Liming Medical Technology Co., Ltd., Jiangsu Changzhou, China) and an electronic weight scale (EB5636H, Senssun Weighing Apparatus Group Ltd., Guangdong Zhongshan, China). The precision of body height was 0.1 cm, and the body weight was 0.01 kg, with the participants wearing light clothing (work clothes were required to be removed) and no shoes.

Assessments of workday dietary intake

Workday dietary intake was assessed using a combination of 24-h dietary recall over a 2-day period (including one day shift and one night shift). Models and photographs of food portions were provided to assist in estimating food intake. Participants' food intake during the day shift was recorded by face-to-face inquiry, and the intake during the night shift was recorded by participants using an online questionnaire designed and distributed by the researcher. After the dietary data were classified and sorted, the VC contents in various foods were converted and manually calculated by the researcher according to the China Food Composition,[25] and the flavonoid contents were converted based on the corresponding literature.[26-31] The workday dietary intakes were calculated as the average values of the 2-day food records.

Statistical analysis

The main analysis of intervention effect evaluation followed the intention-to-treat analysis (ITT). All the subjects completed our trial, and no missing value imputation was applied. Continuous variables were presented as the mean ± SD or as median (P25, P75), and categorical variables were expressed as numbers (percentages). Baseline characteristics were compared between the intervention and control groups to evaluate their comparability using Student's t-test for normally distributed data, the Mann–Whitney U test for skewed distributed or ordinal categorical data, and Pearson chi–square tests for unordered categorical variables.

Linear mixed model analysis with a random intercept at the participant level was used to examine the effectiveness of the antioxidant formula intervention on BP levels and 8-iso-PGF2α/Cr concentrations between groups, time and their interactions. The shift team level cluster was not included in the model because the intraclass correlation coefficient was less than 5%. The model was adjusted for age, body mass index, service length, type of work, etc. The environmental temperature on the day of BP measurement was further adjusted when comparing the differences in SBP and DBP. Mean estimation was conducted with a 95% confidence interval (CI). The models utilized a variance component model to account for repeated measures within subjects. Comparisons between the intervention and control groups were conducted at all three time points (baseline, day 17and day 41) for SBP and DBP and at two time points (baseline and day 41) for 8-iso-PGF2α/Cr. A Bonferroni adjustment was used to correct for multiple comparisons. For 8-iso-PGF2α/Cr with a skewed distribution, a log10 transformation was conducted to achieve a normal distribution before analysis. In addition, we conducted post hoc subgroup analyses considering baseline BP status. Participants were stratified into a lower BP group if their baseline SBP was <130 mmHg and DBP was <85 mmHg and a higher BP group if their baseline SBP was ≥130 mmHg or DBP was ≥85 mmHg.

The analyses were performed using SPSS software version 23.0 (IBM Corp: Armonk, NY, USA). The statistical significance level was defined as $p<0.05$.

RESULTS

Characteristics of participant and intervention adherence

Among the 132 workers who participated in the trial, no one voluntarily withdrew during the study period, as shown in Figure 1. The average ages of the participants in the intervention and control groups were 39.4±5.2 years and 40.4±6.1 years, respectively. The baseline characteristics were not significantly different between the intervention and control groups, as depicted in Table 1 (p>0.05). The overall compliance in the intervention group was 93.6% (Supplementary Table 2).

Dietary intake assessment

Supplementary Table 3 and 4 display the general mean or median daily intakes of food groups and nutrients of VC and flavonoids from their workday diet in the both intervention and control groups. There were no significant differences in the dietary intake of major food groups (e.g., cereals, soybeans, animal foods, vegetables, fruits, nuts, drinks), VC or flavonoids from each relevant source food between the intervention and control groups (all p>0.05). In addition, the total energy intake in the intervention and control group were 2269±570 kcal/d and 2398±659 kcal/d, respectively; there was no significant difference between the two groups (p=0.231).

During the study period, as shown in Table 2, the intervention group had significantly greater median daily total intake of VC (181 mg vs. 77.9mg) and flavonoids (382 mg vs. 129 mg) compared with the control group due to supplementation (both p<0.001).

Effects of VC and hawthorn beverage intervention on BP

Figure 2 shows the changes in the mean SBP and DBP at three time points in the intervention and control groups. There was no significant difference between the intervention and control groups in terms of SBP or DBP at baseline (both p>0.05). According to the time point comparisons, in the intervention group, there was a significant decrease in SBP and DBP from baseline to day 17 and from day 17 to day 41 (all p<0.05), except for SBP from baseline to day 17 (p=0.155). In the control group, there was a significant increase in SBP from baseline to day 41 (p=0.023), but a significant change was not observed in SBP or DBP at other time points (all p>0.05). After examining the effect of the intervention using LMM controlled for covariates, the mean difference between the intervention group and the control group from baseline to day 41 was -7.41 (-11.0 to -3.86) mmHg for SBP and -7.93 (-10.9 to -4.96) mmHg for DBP (Table 3).

The study participants in the two groups were further categorized into lower and higher BP subgroups based on baseline BP levels (SBP/DBP < or ≥130/85 mmHg). The response of participants in each subgroup to the intervention was assessed using the LMM adjusted for baseline values of confounding factors. A dramatic difference in response was observed depending on the baseline BP levels analysed. Interestingly, participants with lower baseline BP in the intervention group showed a significant increase in the mean level of SBP and DBP on day 41 compared to baseline (both $p<0.05$); however, there was no significant change in SBP or DBP in the intervention group on day 41 compared to baseline (both $p>0.05$). In participants with higher baseline BP levels, the mean SBP significantly decreased on day 41 in the intervention group compared to that at baseline and day 17 (both $p<0.05$); conversely, no significant change was observed in the control group (both $p>0.05$). Similarly, the mean DBP significantly decreased on day 41 compared to that at baseline and day 17 in the intervention group (both $p<0.05$), while no significant change was noted in the control group (both $p>0.05$). Thus, regarding the effect of the antioxidant formula on different BP levels from baseline to day 41, although no significant effect was observed on SBP ($p=0.190$), a restrained increase in DBP was noted among the participants with lower baseline BP levels ($p=0.004$). In contrast, a more substantial reduction was observed in both SBP and DBP in participants with higher baseline BP levels (both $p<0.01$) (Table 3).

Effects of VC and hawthorn beverage intervention on oxidative stress biomarkers

The raw Cr-corrected urine 8-iso-PGF2α concentrations of the intervention group and control group at baseline and day 41 were 91.7 (74.9, 122.6), 85.1 (50.9, 134.6), 94.7 (72.6, 139.0) and 152.4 (106.7, 215.0), respectively. After log10 transformation, there was no significant difference in the urine 8-iso-PGF2α/Cr concentration between the intervention and control groups at baseline ($p=0.095$). There was no significant change in the urine 8-iso-PGF2α/Cr concentration observed in the intervention group ($p=0.221$), while a significant increase in this value was noted in the control group ($p<0.001$) from baseline to day 41. After adjustment for confounders, the mean difference between the intervention and control groups was -0.211 (-0.328 to -0.094) (Figure 3 and Table 3).

DISCUSSION

Principal findings

The results of this study indicated that supplementation with a formula of 130 mg/d VC plus 500 mL/d hawthorn beverage (containing 279 mg flavonoids) over 5 shift work cycles during the hottest period in summer significantly prevented BP increase in heat-exposed workers. In addition, it significantly decreased urine 8-iso-PGF2α concentration, which might be the beneficial effects of this antioxidant formula. There were no side effects reported.

Formula of VC and hawthorn beverage improved BP

The findings on BP improvement are in disagreement with most non acute antioxidation interventions.[8] Although BP is commonly decreased in high-risk cardiovascular health interventions, studies exploring the preventive functions of VC and flavonoids (dietary) in preventing BP elevation in populations experiencing a high oxidative stress state need more evidence.[13,14] A trend of magnitude in BP levels was observed following the antioxidant formula interventions in the current study; particularly, compared with heat-exposed workers in the control group who experienced elevated BP levels (although the change in DBP was not significant), the BP levels of workers in the intervention group significantly decreased during the intervention. This finding was also partly supported in a previous antioxidant intervention trial that divers prophylactically ingesting VC (2 g/day) effectively abrogated peripheral vascular dysfunction following exposure to 60% O_2.[32] However, the difference lies in the intervention dosages and total daily intakes of VC (130.0 mg & 186.5 mg) and hawthorn beverage flavonoids (278.7 mg & 400.2 mg), which are more similar to the needs of diets naturally rich in antioxidants for this special population. Thus, these findings support the beneficial effect of VC combined with flavonoid supplementation on BP control in heat-exposed workers.

In addition, since it was reported that middle-aged Chinese individuals with BP levels above 130/85 mmHg had a greater risk of hypertension after 10 years than did those with lower BP levels,[33] we conducted post hoc subgroup analyses considering baseline BP status. Among participants with lower baseline BP levels (SBP <130 and DBP <85 mmHg), BP levels in the intervention group did not change from baseline, whereas those in the control group increased. Conversely, among participants with higher baseline BP levels (SBP ≥130 or DBP ≥85 mmHg), BP levels in the control group did not change from baseline, whereas those in the intervention group decreased. It was speculated that the ventricular adjustments of participants with higher BP may not be as easily affected by heat exposure (e.g., cardiovascular adaptations to temperature-sensitive mechanisms), meaning that their BP may not continue to rise.[34] These results indicated that nutritional formula supplementation of VC and hawthorn beverage for heat-exposed workers had a positive influence on preventing a normal increase in BP or reducing higher BP. Currently, there are limited data on antioxidant/dietary preventative interventions for BP. A recent crossover RCT evaluating the impact of blueberry consumption showed no effect on SBP or DBP in healthy adults.[35] This suggested that antioxidant supplements do not affect normal BP in the absence of risk factors that could elevate it. Our study was consistent with an RCT in healthy adults undergoing intense physical training, in which compared to participants consuming carbohydrate-based control beverage, participants receiving 10 mL/kg/day purple grape juice supplementation (phenolics content 1821 mg/L) for 28 days experienced a decrease in SBP.[18] This difference in preventing the increase in DBP in our study may be associated with the lower baseline BP. In contrast, intervention with VC and hawthorn beverage rich in flavonoids targeting populations with higher BP levels resulted in a reduction in BP. This finding was consistent with other reports indicating that hawthorn extract had a significant decreasing effect on BP in patients with stage I hypertension.[36] Previous treatment interventions had shown that consuming VC or juice rich in flavonoids/polyphenols can reduce hypertension in individuals with other CVD risk factors, and these effects may be attributed to the potent antioxidant properties of hawthorn flavonoids or the synergistic action of VC at a safe dosage,[37] which were more responsive and efficient at enhancing antihypertensive capabilities by combating high oxidative stress. Our findings highlighted the importance of sufficient VC intake combined with hawthorn beverage consumption for heat-exposed workers who had limited access to antioxidant-rich foods or who were at high CVD risk.

Formula of VC and hawthorn beverage reduced oxidative stress

The beneficial effects of VC and hawthorn beverage/flavonoid intervention on oxidative stress were supported by membrane lipid peroxidation-related biomarkers, such as decreased urinary isoprostane excretion. This was also observed in a previous study where the concentration of peroxide products decreased after healthy subjects with habitually low intake of fruits and vegetables consumed antioxidant and polyphenol-rich blackcurrant juice.[17] The results indicated that supplemental VC and hawthorn beverage/flavonoids led to apparent changes in oxidation resistance and biomarker formation. The antioxidant intervention group showed significantly lower BP levels than did the control group after the trial, which was consistent with the improvement in BP observed. Previous studies have shown the beneficial effects of VC or flavonoid-rich food on oxidative stress and oxidative lipid damage markers in individuals at high risk for cardiovascular issues. For example, chronic VC treatment can prevent oxidative stress-induced dysregulation of BP by reducing serum 8-iso-PGF,[38] and 4 weeks of red wine consumption might decrease urine 8-iso-PGF2α concentrations in healthy men with an elevated body mass index, indicating a high risk of obesity.[39]

8-iso-PGF2α is considered a biomarker of lipid peroxidation, representing an end product of peroxide derived from free radicals attacking arachidonic acid on the cell membrane.[40] It is commonly used to reflect the overall level of oxidative stress in the body and to evaluate the impact of intervention measures on oxidative damage. 8-iso-PGF2α is present in plasma at low concentrations, rapidly excreted, and accumulated in urine without the influence of other factors.[41] In addition, the method used for collecting urine samples is easily accepted and can be carried out on site, which can address the limitations of using blood samples. The detection of lower 8-iso-PGF2α excretion in urine was consistent with the finding of decreased peroxide products indicated by a significant reduction in serum malondialdehyde concentrations in high-risk cardiovascular populations following a heart-healthy diet.[42]

Oxidative stress interference (OSI) aims to regulate oxidative stress effectively during the critical period, preventing the body from undergoing pathological changes.[43] Previous experimental studies have shown that the effect of antioxidants in the treatment of cardiovascular diseases is not ideal.[44] It is necessary to explore a more suitable antioxidant formula for reducing the oxidative stress caused by external factors. In our current trial, we observed that the formula of VC and hawthorn beverage rich in flavonoids could effectively inhibit oxidative stress in heat-exposed workers, which was supported by the significant decrease in the urine 8-iso-PGF2α concentration in the antioxidant formula group compared to that in the control group at the end of the intervention. An RCT demonstrated that the combination of antioxidant supplements significantly reduced oxidative stress in patients with cardiometabolic risk compared to a high-vitamin diet.[45] Therefore, daily supplementation of 130 mg of VC and 400 mg flavonoids rich in hawthorn beverage may be sufficient to prevent oxidative stress in heat-exposed workers during the hottest period in summer, helping to maintain BP stability.

Nutritional factors in the VC and hawthorn beverage formula and antioxidation effects

Previous RCTs have shown that dietary supplementation is the most natural intervention method for preventing and improving hypertension in high-risk populations.[46] The effect of the fruity VC tablet and hawthorn beverage may be attributed to the active nutritional ingredients contained in the formula.[47,48] Water-soluble VC and flavonoids have superior antioxidant properties. They can penetrate cell membranes and reach mitochondria to inhibit peroxidation reactions, thereby reducing the formation of peroxide products and protecting the lipid membrane of vascular cells from damage.[49] The remarkable improvement in SBP and DBP in the antioxidant formula intervention group indicated that by drinking beverage rich in flavonoids and taking VC tablets (diet + additional supplement), an adequate VC intake of 186.5 mg/d, accounting for 103.6% of heat-exposed workers' RNI of 180 mg/d, along with a flavonoid intake of 400.2 mg/d, which is within the range from the general population's SPL of 200 mg/d to UL of 800 mg/d, could meet the requirements for optimal antioxidant ability to combat oxidative stress induced by heat-exposure. This highlights the importance of adequate antioxidant supplementation and a well-functioning antioxidant network for mutual regeneration among heat-exposed workers. In addition, hawthorn in beverage provides a relatively rich source of high-quality flavonoids, similar to those found in blueberries, which could help improve cardiovascular health.[41] This drink supplement pattern was favourable for heat-exposed workers because of the need for plenty of water and the appetite for a strong fruit acid taste. Moreover, considering the challenge of achieving optimal nutrient intake from fruits and vegetables due to unrestricted cooking methods and its barriers to intervention effects on BP, as indicated in a randomized trial,[50] we added VC chewable tablets in formula to fortify the low VC content in hawthorn beverage, which need to be boiled during processing, to meet the recommended intake requirements for heat-exposed workers. Together with flavonoids, the formula met the need for anti-peroxidation and protected cardiovascular health. In addition, the hawthorn beverage provides a relatively low source of potassium, contributing to only 4.3% of the recommended intake, although sufficient potassium intake could help improve cardiovascular health.[51]

Strengths and limitations of this study

This study has several strengths. First, participants in two groups from four shift work groups were selected through cluster randomization to balance potential confounding bias and enhance the statistical power of this trial. Second, the study was conducted on healthy participants who had an increased risk of high BP due to oxidative stress induced by heat exposure. This is a key priority for preventing CVD. Next, the high adherence (100.0%) to the formula of VC tablet and hawthorn beverage supplementation in the intervention group and slightly salted gas water in the control group reduced the likelihood of false negatives for the primary study outcome. Finally, intervention products were distributed daily only to the shift group on duty to prevent contamination of the intervention.

Some limitations should be noted. First, participant recruitment was conducted at a single site, which might introduce bias due to demographic characteristics and could limit the generalizability of the results. However, this study lays the groundwork for future research on the preventive efficacy of antioxidant supplementation on BP increase in a larger sample of heat-exposed populations at various sites. Second, we could not provide beverage with the same salt and sugar content for both groups or opt for sugar-free drinks due to the varying ingredients in packaged foods. Slightly salted gas water was utilized as a control based on heat-exposed workers' daily requirements, taking into account their sweat loss. The total sodium intake was below the maximum recommended intake, with no significant difference between the two groups. Third, general workday dietary intake was only assessed once at baseline, and no assessment was performed after the intervention. However, the participants were shift workers living in dormitories during workdays and eating at factory canteens most of the time. Their dietary habits were unlikely to change during the 40-day intervention.

Conclusions

In conclusion, this study showed that daily supplementation with VC and hawthorn beverage decreased BP and urine 8-iso-PGF2α concentrations in heat-exposed workers, suggesting the positive impact on preventing BP increase, inhibiting lipid peroxidation so as to inhibit oxidative stress. Our findings highlight the importance of consuming adequate ideal antioxidants, such as VC and flavonoid beverage, especially for populations exposed to intense heat. It is suggested that controlling the increase in BP contributes to the hypertension-protective effect of the antioxidant formula during the hottest period in summer. Further replication in long-term intervention studies is needed.

ACKNOWLEDGEMENTS

We sincerely thank all the co-investigators and researchers for their participation in this study. We also greatly thank the cooperative individuals from the steel company for their help with the study.

CONFLICT OF INTEREST AND FUNDING DISCLOSURE

The authors declare no conflicts of interest.

This study was supported by the North China University of Science and Technology postgraduate funding and the National Natural Science Foundation Joint Fund Project for Regional Innovation and Development (No. u22A20364).

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Table 1. Baseline characteristics of the study participants†

Variables	Overall (n = 132)	Intervention group (n = 64)	Control group (n = 68)	*p* value
Age (years)				
25-34	19 (14.4)	11 (17.2)	8 (11.8)	0.189
35-44	87 (65.9)	43 (67.2)	44 (64.7)	
45-54	26 (19.7)	10 (15.6)	16 (23.5)	
Service length (years)				
5-14	17 (12.9)	8 (12.5)	9 (13.2)	0.480
15-24	104 (78.8)	49 (76.6)	55 (80.9)	
25-35	11 (8.30)	7 (10.9)	4 (5.90)	
Work types				
Captain	13 (9.80)	6 (9.40)	7 (10.3)	0.916
Large ladle operator	28 (21.2)	13 (20.3)	15 (22.1)	
Steel caster operator	10 (7.50)	4 (6.30)	6 (8.80)	
Second operator	81 (61.5)	41 (64.0)	40 (58.8)	
BMI (kg/m^2)				
Underweight (<18.5)	2 (1.50)	1 (1.60)	1 (1.50)	0.582
Normal (18.5-23.9)	45 (34.1)	23 (35.9)	22 (32.4)	
Overweight (24.0-27.9)	60 (45.5)	29 (45.3)	31 (45.6)	
Obesity (≥ 28.0)	25 (18.9)	11 (17.2)	14 (20.5)	
Current smoker (Yes)	76 (57.6)	39 (60.9)	37 (54.4)	0.084
Current alcohol drinker (Yes)	69 (52.3)	34 (53.1)	35 (51.5)	0.385
Current tea drinker (Yes)	49 (37.1)	29 (45.3)	20 (29.4)	0.059
Regular exercise (Yes)	15 (11.4)	7 (10.9)	8 (11.8)	0.881
Sleeping time ≥6 (h/d)	88 (66.7)	43 (67.2)	45 (66.2)	0.902
SBP (mmHg)	128±14.2	128±14.4	128±14.0	0.884
DBP (mmHg)	85.7±11.4	86.8±11.5	84.6±11.2	0.277
8-iso-PGF2α/Cr(μg/mol)	87.9 (62.9, 123)	91.7 (74.9, 123)	85.1 (50.9, 135)	0.203

BMI: body mass index.
†Continuous data with a normal distribution are expressed as mean ± SD, other continuous variables with a skewed distribution are shown as median (P_{25}, P_{75}), and ordinal and unordered categorical data are expressed as n (%).
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Table 2. The average daily VC and flavonoids intake during the intervention†

Nutrients	Intervention group (n = 64)	Control group (n = 68)	*p* value
VC (mg/d)			
Hawthorn beverage (Supp.)	<2.0	—	—
VC tablet (Supp.)	117 (117, 124)	—	—
Dietary intake	56.5 (35.9, 87.5)	77.9 (45.0, 98.9)	0.076
Total	181 (157, 208)	77.9 (45.0, 98.9)	<0.001
Flavonoids (mg/d)			
Hawthorn beverage (Supp.)	251 (251, 265)	—	—
Dietary intake	125 (98.4, 167)	138 (99.9, 262)	0.246
Total	385 (362, 421)	138 (99.9, 262)	<0.001

Supp.: supplement.
†All continuous data with skewed distributions are expressed as median (P_{25}, P_{75}), and the Mann–Whitney *U* test was applied to compare the differences between groups.

Table 3. Effects of supplementation with VC and hawthorn beverage rich in flavonoids on blood pressure†

Outcome	Intervention group		Control group		Intervention versus Control group	
	n	Mean differences‡ (95% CI)	n	Mean differences‡ (95% CI)	Mean differences§ (95% CI)	*p* value
SBP (mmHg)						
All	64	-4.55 (-7.91, -1.19)**	68	2.86 (0.296, 5.42)*	-7.41 (-11.0, -3.86)	<0.001
Lower BP	26	1.80 (-2.60, 6.19)	31	4.89 (1.52, 8.27)**	-3.10 (-7.74, 1.55)	0.190
Higher BP	38	-8.62 (-13.1, -4.18)***	37	1.13 (-2.20, 4.45)	-9.74 (-14.4, -5.10)	<0.001
DBP (mmHg)						
All	64	-5.89 (-8.69, -3.08)***	68	2.04 (-0.110, 4.19)	-7.93 (-10.9, -4.96)	<0.001
Lower BP	26	-1.65 (-5.17, 1.87)	31	3.861 (1.20, 6.42)**	-5.46 (-9.16, -1.77)	0.004
Higher BP	38	-8.69 (-12.6, -4.78)***	37	0.529 (-2.21, 3.26)	-9.22 (-13.4, -5.07)	<0.001
lg 8-iso-PGF2α/Cr	64	0.051 (-0.031, 0.133)	68	0.283 (0.204, 0.362)	-0.232 (-0.346, -0.118)	<0.001

SBP: systolic blood pressure; DBP: diastolic blood pressure; Lower BP: SBP <130 and DBP <85 mmHg; Higher BP: SBP ≥130 or DBP ≥85 mmHg; CI: confidence interval.

†All comparisons were analysed separately using linear mixed models, and *p* values were adjusted for age, body mass index, service length, type of work, smoking status, alcohol consumption, tea drinking habit, intake days of the salt gas water or antioxidant intervention ingredients and environmental temperature on the day of BP measurement.

‡Comparison of changes from the first day before the intervention started (baseline) to the day after the 5-shift-work-cycle intervention (day 41) within the group.

‡Comparison of changes from the first day before the intervention started (baseline) to the day after the 5-shift-work-cycle intervention (day 41) between the intervention and control groups; negative values support the effect of the intervention on lowering BP or 8-iso-PGF2α/Cr.

**p*<0.05, ***p*<0.01, ****p*<0.001 compared within groups using paired sample *t*-test.

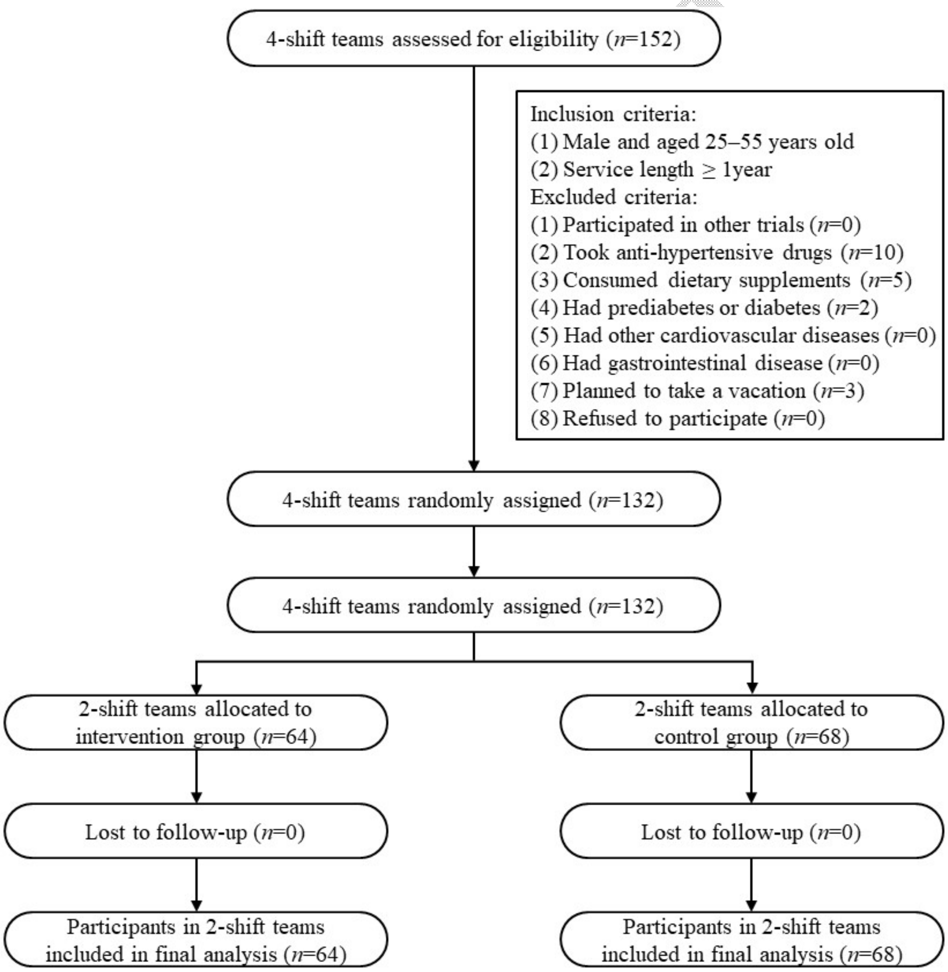

Figure 1. Flow diagram of the recruitment and follow-up of study participants

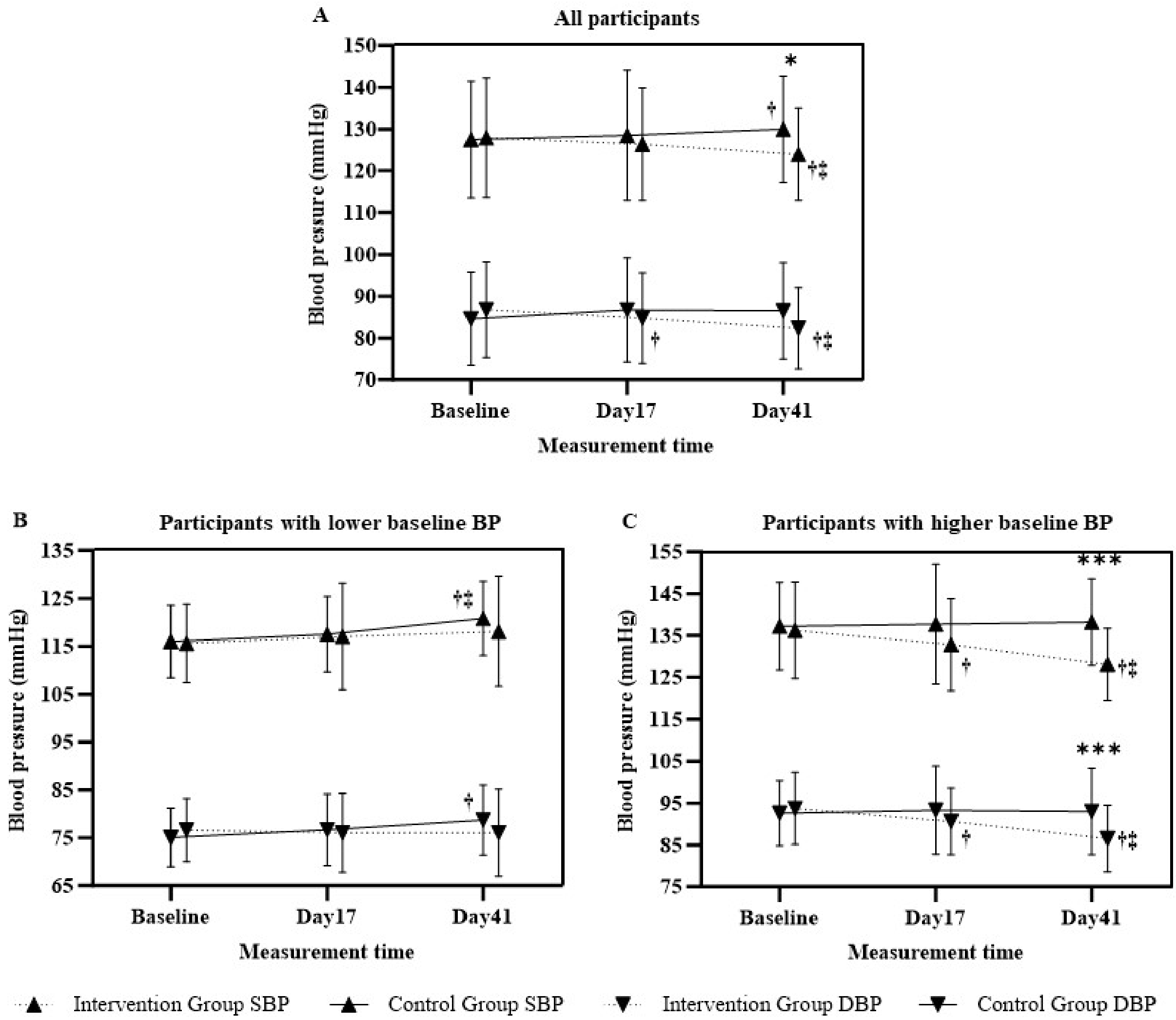

Figure 2. Mean blood pressure during the intervention according to assigned group. (A) In all participants. (B) Participants with lower baseline BP levels (SBP <130 and DBP <85 mmHg). (C) Participants with higher baseline BP levels (SBP ≥130 or DBP ≥85 mmHg). The error bars represent the standard error of the means. Baseline: on the first day before the intervention started; Day 17: after the 2-shift-work-cycle intervention; Day 41: after the 5-shift-work-cycle intervention; SBP: systolic blood pressure; DBP: diastolic blood pressure. $^{\dagger}p<0.05$ compared to baseline using paired sample *t*-test; $^{\ddagger}p<0.05$ compared to day 17 using paired sample *t*-test; $^{*}p<0.05$, $^{***}p<0.001$ compared between groups using independent sample *t*-test.

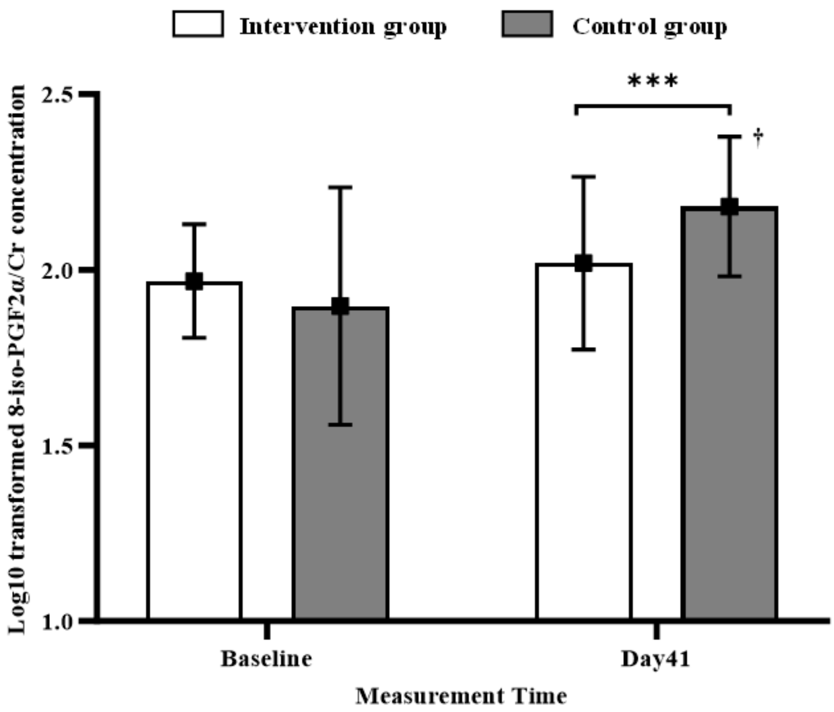

Figure 3. Mean urinary 8-iso-PGF2α/Cr concentration during the intervention according to the assigned groups. The 8-iso-PGF2α/Cr concentration was log10 transformed. The error bars represent the standard error of the means. Baseline: on the first day before the intervention started; Day 41: after the 5-shift-work-cycle intervention; 8-iso-PGF2α/Cr: creatinine-corrected 8-isoprostane-prostaglandin F2α. [†]p<0.05compared to baseline within group using paired sample *t*-test; *** p<0.001 compared between groups using independent sample *t*-test.

Supplementary Tables

Supplementary Table 1. Main nutrient compositions of the intervention and control groups

Components of foods	Intervention group		Control group
	VC Chew Tablet (per 1 tablet/1 g)	Hawthorn (dried) Beverage (per 2 cans/500 ml)	Slightly Salted Gas Water (per 1 bottle/500 ml)
VC (mg)	130.0[†]	<2.00[‡]	–
Flavonoids(mg)	–	278.7[§]	–
Sodium (mg)	–	0.00[¶]	200.0[¶]
Potassium (mg)	–	137.3[††]	–
Total Carbohydrates (g)	–	57.2[¶]	12.5[¶]
From Hawthorn (g)	–	23.5[††]	–
From Sugars (g)	–	33.7[††]	12.5[¶]

[†]VC Chew Tablet information from By-health Co., Ltd.
[‡]Analysed by the researcher at North China University of Science and Technology, applying the 2,6-dichlorphenol indophenol method.
[§]Analysed by the Beijing Nutrition Analysis Centre, applying the spectrophotography method (UV-1780 spectrophotometer; Shimadzu, Japan), using rutin as a standard sample.
[¶]From nutrition label information on food package.
[††] Converted by the researcher with China Food Composition information.

Supplementary Table 2. Intervention adherence of the participants[†]

	Overall (n = 132)	Intervention group (n = 64)	Control group (n = 68)
Participants who adhered 90%~94% of the study period	69 (52.3)	33 (51.6)	36 (52.9)
Participants who adhered 95%~99% of the study period	19 (14.4)	16 (25.0)	3 (4.40)
Participants who adhered 100% of the study period	44 (33.3)	15 (23.5)	29 (42.7)
Overall compliance (%)	94.1	93.6	94.6

Overall compliance: The ratio of the total number of adherence days to the total number of intervention days.
[†]Values are n (%), unless otherwise indicated.

Supplementary Table 3. Baseline workday food groups intake of participants†

Food groups	Intervention group (n = 64)	Control group (n = 68)	p value
Cereals (g/d)			
Rice/Wheat	331±81.7		0.076
Coarse cereals/beans	0.00 (0.00, 11.9)	0.00 (0.00, 23.1)	0.393
Starch product	0.00 (0.00, 10.0)	0.00 (0.00, 0.00)	0.210
Total	347±81.0		0.069
Soybean (g/d)	12.5 (0.00, 20.0)	10.0 (0.00, 12.5)	0.055
Animal foods (g/d)			
Livestock	75.0 (37.5, 113)	75.0 (50.0, 122)	0.341
Poultry	25.0 (0.00, 50.0)	0.00 (0.00, 50.0)	0.106
Eggs	76.3 (50.0, 97.5)	65.0 (21.3, 110)	0.281
Aquaculture	0.00 (0.00, 5.00)	0.00 (0.00, 50.0)	0.115
Dairy	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	0.976
Total	225±103	229±100	0.816
Vegetables (g/d)			
Leafy vegetables	50.0 (0.00, 100)	75.0 (0.00, 125)	0.115
Dark root/stem	62.5 (25.0, 150)	62.5 (25.0, 150)	0.921
Others	100 (75.0, 186)	125 (75.0, 200)	0.250
Total	288 (216, 400)	333 (250, 400)	0.241
Edible Fungus	12.5 (0.00, 28.8)	3.80 (0.00, 25.0)	0.325
Fruits (g/d)	0.00 (0.00, 100)	25.0 (0.00, 100)	0.165
Nuts (g/d)	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	0.586
Drinks (ml/d)			
Pure water	2500 (1410, 2750)	2500 (1880, 2750)	0.625
Tea water	250 (0.00, 1230)	0.00 (0.00, 930)	0.247
Fruit beverage	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	0.710
Carbonate beverage	0.00 (0.00, 200)	0.00 (0.00, 0.00)	0.091
Total	2800 (2500, 3200)	2800 (2300, 3300)	0.516
Ice lolly (g/d)	0.00 (0.00, 90.0)	0.00 (0.00, 60.0)	0.283
Oil (g)	33.2±11.8	33.4±10.8	0.924
Salt (g)	13.0±4.10	14.1±4.80	0.185
Sugar (g)	0.00 (0.00, 3.00)	0.00 (0.00, 4.50)	0.785

†Continuous data with a normal distribution are expressed as the mean ± SD, and two independent t-test was applied to compare the two groups. Other continuous variables with a skewed distribution are shown as median (P_{25}, P_{75}), and the Mann–Whitney U test was used to compare the differences.

Supplementary Table 4. Baseline workday VC and flavonoids intake of the participants†

Nutrients	Intervention group (n = 64)	Control group (n = 68)	p value
VC (mg/d)			
Vegetables	50.0 (31.4, 81.2)	67.1 (43.8, 89.9)	0.056
Fruits	0.00 (0.00, 12.0)	1.50 (0.00, 12.0)	0.151
Other beverage	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	0.393
Total	56.5 (35.9, 87.5)	77.9 (45.0, 98.9)	0.076
Flavonoids (mg/d)	12.5 (0.00, 20.0)		
Vegetables	21.9 (11.4, 26.6)	18.8 (13.4, 26.2)	0.827
Fruits	0.00 (0.00, 8.80)	3.0 (0.00, 8.80)	0.130
Cereals	50.8 (37.9, 72.5)	58.5 (44.6, 97.7)	0.054
Soybean	25.6 (0.00, 41.0)	20.5 (0.00, 25.6)	0.055
Nuts	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	0.524
Teas	12.0 (0.00, 16.1)	0.00 (0.00, 17.3)	0.523
Total	125 (98.4, 167)	138 (99.9, 262)	0.246

†All continuous data with skewed distributions are expressed as median (P_{25}, P_{75}), and the Mann–Whitney U test was applied to compare the differences between groups.