Cardiovascular Benefits of Vitamin C Supplementation Against Particulate Air Pollution in Healthy Adults: A Double-Blind Randomized Crossover Trial

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Abstract

Background: Accumulating evidence indicates that ambient air pollution exposure is associated with the adverse effects of cardiovascular diseases (CVDs). Evidence on the health benefits of vitamin C supplementation in highly polluted areas has not been evaluated.

Objectives: We aims to evaluate whether dietary vitamin C supplementation can improve cardiovascular health linked to PM exposure.

Methods: A randomized double-blind crossover trial was performed in 58 health young adults in Shijiazhuang, China in 2018. All subjects were randomly assigned to vitamin C supplementation or placebo group for a week alternating with a two-week washout period. We measured blood pressure (BP), blood lipid, biomarkers of oxidative and biomarkers of inflammation. Linear mixed-effect model was applied to evaluate the effect of vitamin C supplementation on health outcomes.

Results: During the intervention periods, the average concentration of $PM_{2.5}$ and PM_{10} was 164.91μg/m^3 and 327.05μg/m^3, respective. Vitamin C supplementation was significantly associated with decrease in several inflammatory indicators and blood pressure, including 19.47% in interleukin-6 (IL-6), 17.30% in tumor necrosis factor-a (TNF-α), 34.01% in C-reactive protein (CRP), 3.37% in systolic blood pressure (SBP) and 6.03% in pulse pressure (PP). Further, glutathione peroxidase (GSH-Px) was significantly increased by 7.15%. The effect of nutritional intervention on other indicators were beneficial, but there was no statistical difference. Gender-subgroup analysis showed that vitamin C supplementation significantly reduced SBP by 3.31%, PP by 4.94%, IL-6 by 20.97%, TNF-α by 27.85% and CRP by 38.5% in males, and significantly reduced SBP by 3.65%, PP by 8.12%, IL-6 by 17.35% and CRP by 29.15% in females. In contrast, vitamin C supplementation significantly increased APOB by 6.28% and GSH-Px by 14.47% in female participants only.

Conclusion: This study indicates that vitamin C supplementation may protect cardiovascular system against particulate matter (PM) exposure among healthy young adults in China.

Clinical trial registration information: Identifier: ChiCTR2100051371. Registered 19 October 2018, https://www.chictr.org.cn.

1. Introduction

Epidemiological studies have showed that short-term and long-term exposure to ambient fine particulate matter (PM) is closely related to the morbidity and mortality of cardiovascular diseases (CVDs) (Liu et al. 2019b; Zhang et al. 2021). With the rapid development of economy, air pollution is a serious environmental problem globally, especially in developing countries. As the largest developing country, China has received attention from all over the world for its rapid economic growth and its severities of air pollution. According to World Health Organization (WHO), more than 90% of the world's population live in places exceeding the health-based limits (Bai et al. 2018; She et al. 2019).

Although the most effective way is to control environmental pollutant emissions, the needs of damage-reduction strategy is more urgent on a personal level. In addition to efforts to air pollution-related hazards, lifestyle intervention may be a practical protective strategy for the prevention of diseases. Recent some studies have shown that short-term and long-term use of air purifiers have significant cardiovascular benefits(Chuang et al. 2017; Chen et al. 2015). In many cases, however, these exposure-reduction strategy is difficult to achieve (e.g., outdoors). Therefore, other practical approaches to mitigate the impact of air pollution on human health are urgently needed. Dietary supplementation may present another promising approach to modulate the adverse effects of ambient PM exposure.

Although the precise molecular mechanisms of air pollution-related cardiovascular disease not well understood, inflammation and oxidative stress has been confirmed to two predominant underlying pathways(Aryal et al. 2021). Ambient air pollution exposure can cause serious inflammatory responses by releasing inflammatory cytokines, such as interleukin-6 (IL-6), tumor necrosis factor-a (TNF-α) and C-reactive protein (CRP). Meanwhile, the release of pro-inflammatory cytokines is usually accompanied by strong oxidative stress(Roy et al. 2014). The major source of oxidative stress may result from excessive generation of reactive oxygen species (ROS) or PM-mediated impaired mitochondrial function(Vogel et al. 2020). Additionally, activation of inflammatory cells also could induce an increase in production of ROS and reactive nitrogen species (RNS) and cause oxidative DNA damage(González-Flecha 2004; Risom et al. 2005). Excess ROS/RNS results in an imbalance of the oxidation/anti-oxidation system, and then causes CVDs. It is therefore reasonable to speculate that measures targeting these biological pathways or mechanisms may modulate the adverse effect related to air pollution exposure.

Several studies have shown the subclinical cardiovascular benefits linked to PM exposure derived from dietary supplementation (e. g. fish oil, vitamin E) (Becerra et al. 2016; Lin et al. 2019; Tong et al. 2015). These dietary supplements have been reported to have activities of anti-inflammation and antioxidant, as well as myocardium protecting and improvement of endothelial function. Vitamin C is widely used as an antioxidant and a free radical scavenging dietary supplement, which has been proven to have an immediate effect on improving blood lipids, blood pressure (BP) and endothelial function(Boonthongkaew et al. 2021; Mason et al. 2021). However, there are no studies exploring the role of vitamin C on the adverse cardiovascular effect of air pollutant exposure.

Therefore, we designed a randomized double-blind crossover study to evaluate the potential cardiovascular benefits associated with using vitamin C supplementation among healthy young adults in Shijiazhuang, China. We assessed cardiovascular function and circulating biomarkers that had been proved linking to CVD caused by air pollution. We also explored the health response to the vitamin C supplementation in men and women separately.

2. Materials And Methods

2.1 Study design and Participants

The study protocol was approved by the Ethics Committee of Hebei Medical University and registered in the Chinese Clinical Trial Registry (ChiCTR2100051371). The study was conducted according to the ethical principles of the Declaration of Helsinki. All participants provided written informed consent at enrollment. We initially recruited 58 healthy college students at Hebei medical university, located in the urban center of Shijiazhuang, China. We excluded students who had a history of tobacco smoking (never smoking), regular alcohol drinking, clinically diagnosed chronic diseases (e. g. asthma, rhinitis, and others) and recently infections. Experimental stage was the period from December 2018 to January 2019. All eligible participants in a double-blind fashion were randomized into two groups and daily supplementation of vitamin C (The main component was L-ascorbic acid) or placebo (The main component was citric acid) for one week with a 2-week washout period, and were monitored throughout the study period. During the experiment, all participants can freely move on campus. To be specific, we conducted a baseline survey of all participants on October 20th, including age, sex, height, weight, BP measurements, and blood sample collection. During the experiment, we asked all participants to record daily activities, daily concentration of $PM_{2.5}$, daily concentration of PM_{10}, temperature and relative humidity in the classroom, dormitory, canteen, and playground. In order to assess Vitamin C dietary level, we also asked all participants to use weighting method to record a three-days dietary intake (a weekend and two week days) at each intervention. BP and blood samples were collected at the end of each intervention period at approximately the same time (the second morning, 7:00 am). Because all participants lived in campus dormitory and studied in campus, they were seldom exposed to environments tobacco smoke because smoking was banned in all public places on the campus. The entire study was completed within one season to avoid potential confounders resulting from long-term trends of health outcomes.

2.2 Vitamin C supplements

Participants in the intervention group were instructed to oral 4 pills (2000 mg) vitamin C everyday throughout the study period. The main ingredients are L-ascorbic acid and sodium L-ascorbate, sorbitol, magnesium stearate, aspartame, lemon yellow aluminum lake, sweet orange flavor and other accessories. Participants in the placebo group received 4 pills placebo every day. The placebo bill is identical, and each capsule contained citric acid and the same accessories. The participants and study staff were blind to group assignment.

2.3 Exposure assessment

The air pollution levels including $PM_{2.5}$ and PM_{10} were intermittently monitored throughout the whole study period using a direct reading air pollution monitor (OSEN-1A $PM_{2.5}$ monitor) which light source based on the laser diode. Before the interventions, all devices were corrected. In order to obtain real-time particle measurements, we asked participants to measure particle concentration at the location of daily activity (i.e., dormitories, classrooms, canteens and playgrounds). We also recorded the environmental data ($PM_{2.5}$, PM_{10}) from the nearby monitoring stations located 2.5 km upwind from the study site during the experiment on the website of the Environmental Protection Department (EPA) of Hebei Province (hbepb.hebei.gov.cn) to ensure the accuracy of the air pollution monitor. The temperature and relative humidity were recorded on the website of Hebei meteorological service (he.cma.gov.cn/) at the study period. We recorded these environmental data on an hourly basis and used the average as the uniform exposure level for all subjects at each interventional period.

2.4 Health measurements

At baseline, we collected basic demographic information (such as age and gender). we also measured weight and height to calculate the body mass index (BMI) of all participants. After each intervention period, peripheral venous blood samples were collected to measure health indicators that have shown associations with air pollution exposure in previous studies(Lin et al. 2019).

2.4.1 Blood pressure

After sitting in a quiet room at least 15 min, left upper arm BP was measured by OMRON HEM-7121 autonomic sphygmomanometer at 3 times within 2-min intervals between measurement. The average of the second and third measurements was recorded as the final outcomes. Pulse pressure (PP) was calculated as the difference between the mean SBP and DBP. If the difference was higher than 5 mm Hg among measurements, a new measurement was arranged.

2.4.2 Circulating Biomarkers

Morning fasting blood were collected by using vacuum collection tubes (5ml) for each participant (between 7 am and 8 am) before and after vitamin C intervention. Within 30 min, serum and plasma were separated and immediately stored at -80°C until analysis. We collected blood samples at the same time to minimize the influence of the circadian rhythm.

We selected 15 circulating biomarkers for quantitative analyses: (1) 4 oxidative stress biomarkers, including catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MDA) and glutathione peroxidase (GSH-Px). (2) 4 inflammation biomarkers, including IL-6, TNFα, CRP and Interleukin 8 (IL-8). (3) 6 blood lipid biomarkers, including Triglyceride (TC), Total cholesterol (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), apolipoprotein A (APOA) and apolipoprotein B (APOB). (4) blood glucose (GLU). Blood lipid biomarkers and GLU were measured by automatic analyzer (Beckman AU5800; Beckman Coulter) at The NO.1 hospital of Hebei medical university. Oxidative stress biomarkers were measured were measured using assay kits (Nanjing Jiancheng Bioengineering Institute, China) and inflammation biomarkers were measured using enzyme-linked immunosorbent assay (Shanghai Enzyme-linked Biotechnology Co., Ltd.). All tests were performed in strict accordance with the manufacturer's instructions.

2.5 Statistical analyses

To account for the repeated measurement of health endpoints under the two experimental scenarios, we applied liner mixed-effect models to compare the change of biomarkers(Δbiomarker) before and after vitamin C intervention refer to the previously published study(Cui et al. 2018). This model account for repeated measurements taken from the same subject and allowed each subject to act as their own references. We incorporated random intercepts for subjects to account for the correlation between repeated measures per person. We calculated Δbiomarker (the post-intervention biomarker level minus the pre-intervention biomarker level) and used it in mixed-effects models to compared results between intervention group and placebo group within the same participants. The absolute difference between Δbiomarker associated with vitamin C supplementation and Δbiomarker associated with placebo group was quantified as β. The percentage difference between Δbiomarker associated with vitamin C supplementation and Δbiomarker associated with placebo group was calculated by dividing the absolute difference with the mean of all measurements of this biomarker and multiplying that by 100%(Cui et al. 2018). All model adjusted for the following variables as fixed-effect covariates: age, gender, body mass index, mean temperature and mean relative humidity. As crossover study was completed within one month, we did not consider the temporal trends of the health endpoints. Furthermore, gaseous air pollutants did not confound the analyses, as their concentrations were exactly same between the 2 groups due to this 1:1 crossover design. In addition, we also conducted gender-stratified analysis and explored effect modification by gender.

To test the reliability of our results, we additionally performed sensitivity analysis. First, the associations between vitamin C supplementation and health indicators were estimated with adjustment for the concentration of $PM_{2.5}$ and PM_{10}, respectively (Table S1; Table S2). Second, we adjusted both PM2.5 and PM10 as fixed-effect covariates (Table S3). In addition, to control the potential confounding of dietary vitamin C intake, we further adjusted for the average daily intake of VC (Table S4).

All statistical tests were two-sided with an alpha of 0.05. All analyses were performed using the "nlme" package of R software (Version 4.0.4; R Development Core Team).

3. Results

3.1 Baseline description

We explored eligibility for 65 participants, among whom 58 were eligible and completed the scheduled follow-ups (Figure S1). Five participants did not meet inclusion criteria and two participants drooped out of the study due to personal reason. 24 females and 34 males with a mean age of 20.1 years and an average BMI of 22.1 kg/m^2 completed the entire study. The general characteristics of participants are presented in Table 1.

Table 1
Baseline characteristics of the study participants

Parameters	Mean (SD)
Gender (Male/Female)	34/24
Age, year	20.1 (3.0)
Hight	169.6 (9.7)
Weight	63.8 (12.3)
Body mass index	22.1 (3.6)
Waistline	76.7 (10.4)
Hipline	96.5 (8.7)

3.2 Environmental data

Table 2 presents the PM concentrations and meteorological parameters during the study. The mean daily $PM_{2.5}$ concentrations were 164.91 μg/m³ at the living environment and 327.05 μg/m³ for PM_{10}. The $PM_{2.5}$ data recorded on the website of Environmental Protection Department of Hebei Province were 159.93μg/m³ and PM_{10} were 255.57μg/m³, which is consistent with the trend of monitoring data (Figure S2). During the study period, the mean outdoor temperature ang relative humidity were 1.45℃ and 40.47%, respectively (Table 2).

Table 2 The Time-Varying $PM_{2.5}$, PM_{10} Concentration of EPA data and monitor recording data.

Parameters	Mean	SD	Min	Median	Max
Monitor data					
$PM_{2.5}$ (μg/m^3)	164.91	98.24	41.33	132.70	451.46
PM_{10} (μg/m^3)	327.05	233.66	134.03	243.47	884.54
EPA data					
$PM_{2.5}$ (μg/m^3)	159.93	81.23	48.00	139.50	349.00
PM_{10}(μg/m^3)	255.57	99.02	117.00	232.00	480.00
Temperature (°C)	1.45	2.92	-3.5	0.94	7.1
Humidity (%)	40.47	11.10	20.1	41.41	82.6

$PM_{2.5}$ = particulate matter with an aerodynamic diameter < 2.5 mm. PM_{10} = particulate matter with an aerodynamic diameter < 2.5 mm. Max = maximum; Min = minimum.

3.3 Blood pressure

The mixed-effect linear model demonstrated that vitamin C supplementation was associated with a 3.37% [95% CI: -5.22%, -1.52%] decrease in SBP and a 6.03% [95% CI: -9.53%, -2.52%] decrease in PP compared with placebo group (Figure1, Table 3).

Table 3

Percentage difference in changes of health indicators between the vitamin C group and the placebo group.

Health Endpoints	**Change**	**95%CI**	**P**
Blood pressure			
SBP	-3.37	(- 5.22,-1.52)	0.001
DBP	-1.58	(- 3.83,0.68)	0.168
PP	-6.03	(- 9.53,-2.52)	0.001
Blood lipid			
GLU	0.88	(-0.92,2.68)	0.331
TG	-0.58	(-9.28,8.12)	0.894
TC	1.25	(-1.71,4.22)	0.400
HDL	2.19	(-0.67,5.04)	0.130
LDL	1.05	(-2.21,4.31)	0.520
APOA	1.57	(-1.88,5.01)	0.366
APOB	1.10	(-2.05,4.25)	0.485
Oxidative stress			
CAT	-15.45	(- 38.97,8.07)	0.194
SOD	-0.96	(-4.45,2.53)	0.584
MDA	-4.74	(-14.01,4.53)	0.310
GSH-PX	7.15	(3.19,11.11)	0.001
Inflammation			
IL-6	-19.47	(-25.97,-12.97)	<0.001
TNF-α	-17.30	(-26.11,-8.49)	<0.001
CRP	-34.01	(-48.64,-19.38)	<0.001
IL-8	-2.40	(-12.72,7.93)	0.644

The change was calculated by taking the absolute difference between the change of health indicators (Δbiomarker) in vitamin C supplementation group and the Δbiomarker in placebo group, dividing that by the average of this biomarker among all participants, and multiplying by 100%. This β is the absolute differences between the change of health indicators (Δbiomarker) vitamin C supplementation group and the Δbiomarker in placebo group.

Definition of abbreviations: SBP = systolic blood pressure; DBP = diastolic blood pressure; PP = pulse pressure; GLU = glucose; TG = total cholesterol; TC = triglyceride; LDL-C =low-density lipoprotein cholesterol, HDL-C = high-density lipoprotein cholesterol; APOA = apolipoprotein A; APOB = apolipoprotein B; CAT = catalase; SOD = superoxide dismutase; MDA = malondialdehyde; GSH-Px = glutathione peroxidase; IL-6 = interleukin 6; TNF-α = tumor necrosis factor α;CRP = C-reactive protein; IL-8 = interleukin 8.

3.4 Circulating biomarkers

The changes in indicators of blood lipid were not significantly different between vitamin C group and placebo group. Similarly, no statistically significant differences were found for the following biomarkers after vitamin C intervention: plasma GLU, serum CAT, serum MDA, serum SOD and serum IL-8. Compared with the placebo group, vitamin C supplementation resulted in a 7.15% [95% CI: 3.19%, 11.11%] significant increase in GSH-Px concentration. Additionally, vitamin C supplementation resulted in a 19.47% [95% CI: -25.97%, -12.97%] lower in IL-6 levels, a 17.30% [95% CI: -26.11%, -8.49%] lower in TNF-α levels and a 34.01% [95% CI: -48.64%, -19.38%] lower in CRP levels (Figure1, Table 3).

The impact of Vitamin C supplementation was measured separately for men and women participants in gender-stratified analyses. Vitamin C supplementation was associated with a decrease in SBP, PP, IL-6 and CRP in both males and females. However, compared to placebo group, vitamin C supplementation was associated with a 6.28% [95% CI: 0.29%, 12.27%] increase in APOB levels and a 11.47% [95% CI: 7.81%, 21.12%] increase in GSH-Px activity in female participants only. In contrast, vitamin C was associated with a 27.85% [95% CI: -36.69%, -16.02%] decrease in TNF-α levels in male participants only. The interaction term between gender and vitamin C supplementation indicated non-significant effect modification for the above health indicators (Figure 2; Table 4).

Table 4
Percentage difference of health indicators between the vitamin C group and the placebo group, stratified by gender.

Health Endpoints	male			female			Gender × vitamin C interaction		
	change[a]	95% CI	P	change[a]	95% CI	P	change[b]	95% CI	P
Blood pressure									
SBP	-3.31	(-5.9, -0.71)	0.014	-3.65	(-6.44, -0.85)	0.013	0.77	(-4.19, 5.74)	0.756
DBP	-2.12	(-5.44, 1.20)	0.202	-0.95	(-4.04, 2.14)	0.531	0.68	(-4.56, 5.93)	0.795
PP	-4.94	(-9.08, -0.81)	0.021	-8.12	(-15.24, -1.01)	0.027	0.91	(-9.70, 11.51)	0.865
Blood lipid									
GLU	0.61	(-1.29, 2.51)	0.519	1.18	(-2.56, 4.92)	0.520	0.50	(-10.57, 11.57)	0.928
TG	-7.24	(-19.28, 4.81)	0.230	10.37	(-1.47, 22.21)	0.083	8.22	(-12.78, 29.23)	0.436
TC	-1.08	(-4.30, 2.14)	0.500	4.15	(-1.5, 9.81)	0.142	0.84	(-13.19, 14.88)	0.904
HDL	3.08	(-0.03, 6.19)	0.052	0.21	(-4.94, 5.37)	0.932	-2.97	(-16.54, 10.61)	0.663
LDL	-2.17	(-5.70, 1.37)	0.221	5.54	(-0.57, 11.66)	0.074	1.66	(-13.06, 16.38)	0.822
APOA	3.66	(-0.81, 8.12)	0.105	-0.99	(-6.77, 4.79)	0.726	-8.23	(-21.6, 5.15)	0.223
APOB	-2.46	(-5.70, 0.78)	0.131	6.28	(0.29, 12.27)	0.041	1.06	(-12.41, 14.54)	0.875
Oxidative stress									

[a]The change was calculated by taking the absolute difference between the change of health indicators (Δbiomarker) in vitamin C supplementation group and the Δbiomarker in placebo group, dividing that by the average of this biomarker among all participants, and multiplying by 100%. This β is the absolute differences between the change of health indicators (Δbiomarker) in vitamin C supplementation group and the Δbiomarker in placebo group.

[b]The change was calculated by β divided the average of this biomarker among all participants. This β is the coefficient of the gender by vitamin C interaction term, representing the difference between the health impact of vitamin C supplementation group (when compared to placebo group) in female participants and the health impact of vitamin C supplementation group(when compared to placebo group) in male participants.

Definition of abbreviations: SBP = systolic blood pressure; DBP = diastolic blood pressure; PP = pulse pressure; GLU = glucose; TG = Total cholesterol; TC = Triglyceride; LDL-C = Low-density lipoprotein cholesterol, HDL-C = high-density lipoprotein cholesterol; APOA = apolipoprotein A; APOB = apolipoprotein B; CAT = catalase; SOD = superoxide dismutase; MDA = malondialdehyde; GSH-Px = glutathione peroxidase; IL-6 = interleukin 6; TNF-α = tumor necrosis factor α;CRP = C-reactive protein; IL-8 = interleukin 8.

Health Endpoints	male			female			Gender × vitamin C interaction		
	change[a]	95% CI	P	change[a]	95% CI	P	change[b]	95% CI	P
CAT	-25.63	(-61.6, 10.35)	0.156	-0.14	(-31.33, 31.05)	0.993	11.04	(-25.57, 47.66)	0.548
SOD	2.40	(-0.76, 5.56)	0.132	-5.30	(-12.53, 1.93)	0.143	10.67	(2.42, 18.92)	0.012
MDA	-5.27	(-15.43, 4.90)	0.299	-1.46	(-20.62, 17.71)	0.876	12.38	(-14.02, 38.77)	0.351
GSH-PX	1.55	(-2.66, 5.76)	0.460	14.47	(7.81, 21.12)	0.0002	12.70	(-0.12, 25.52)	0.052
Inflammation									
IL-6	-20.92	(-30.86, -10.97)	<0.001	-17.35	(-25.5, -9.21)	<0.001	-9.74	(-39.34, 19.87)	0.513
TNF-α	-27.85	(-39.69, -16.02)	<0.001	-3.90	(-14.61, 6.8)	0.458	5.81	(-26.92, 38.54)	0.723
CRP	-38.5	(-58.65, -18.36)	0.001	-29.15	(-49.28, -9.01)	0.007	18.25	(-24.38, 60.88)	0.395
IL-8	-6.65	(-21.00, 7.69)	0.3517	1.88	(-14.3, 18.06)	0.812	-6.76	(-26.41, 12.88)	0.493

[a]The change was calculated by taking the absolute difference between the change of health indicators (Δbiomarker) in vitamin C supplementation group and the Δbiomarker in placebo group, dividing that by the average of this biomarker among all participants, and multiplying by 100%. This β is the absolute differences between the change of health indicators (Δbiomarker) in vitamin C supplementation group and the Δbiomarker in placebo group.

[b]The change was calculated by β divided the average of this biomarker among all participants. This β is the coefficient of the gender by vitamin C interaction term, representing the difference between the health impact of vitamin C supplementation group (when compared to placebo group) in female participants and the health impact of vitamin C supplementation group(when compared to placebo group) in male participants.

Definition of abbreviations: SBP = systolic blood pressure; DBP = diastolic blood pressure; PP = pulse pressure; GLU = glucose; TG = Total cholesterol; TC = Triglyceride; LDL-C = Low-density lipoprotein cholesterol, HDL-C = high-density lipoprotein cholesterol; APOA = apolipoprotein A; APOB = apolipoprotein B; CAT = catalase; SOD = superoxide dismutase; MDA = malondialdehyde; GSH-Px = glutathione peroxidase; IL-6 = interleukin 6; TNF-α = tumor necrosis factor α;CRP = C-reactive protein; IL-8 = interleukin 8.

3.5 Vitamin C intake

The mean daily dietary vitamin C intake in the intervention group and the placebo group was 45.66±65.29 mg, 57.66±57.58 mg, respective(Figure 3). There were no statistically significant differences throughout the study period (P=0.218).

4. Discussion

This randomized crossover trial was carried out in healthy young adults who lived in campus, we aimed to examine the potential cardiovascular impact of vitamin C supplementation against air pollution exposure, and potential molecular mechanism were explored. The crossover design with repeated measures is conducive to evaluate the effect of the intervention, and we took advantage of the fact that the participants lived and studied in the same building and had similar time-activity patterns. A series of markers of cardiovascular health in response to short-term air pollution exposure were assessed, and found that VC supplementation had beneficial effects on BP, oxidative stress biomarkers and inflammation biomarkers.

Epidemiological studies have established that increasing mortality of CVDs was associated with exposure to air pollution, and the most intuitive indicator of response is blood pressure(Brook et al. 2010). A recent study showed that very short-term exposure to PM has positive relationship with higher BP, and this effect occurred on the same hour of BP measurement(Hu et al. 2021). Although many methods (e.g. indoor air filtration, wearing respirators) have been conducted to avoid this adverse effect of PM(Shi et al. 2017), those are still unconvincing. In a study of 35 healthy college volunteers in Shanghai, China, SBP was decreased by 2.7% and DBP was decreased by 4.8% when the students lived in dormitories with air purification(Chen et al. 2015). However, another air purification-related intervention study indicated that air purification had no significant effect on BP(Dong et al. 2019). Therefore, there is an urgent need for a more effective therapy to decrease the adverse effects of PM exposure on BP. In our study, we found that vitamin C supplementation lead a notable decrease in SBP and PP comparing with placebo group. This discovery is consistent with previously published studies. A meta-analysis, enrolled in 29 studies, conducted from 1982 to 2010, included 1407 participants reported that the dose of vitamin C supplementation ranged from 60 to 4000mg per day could significantly reduce SBP and DBP(Juraschek et al. 2012). Vitamin C has potential hypotensive effect may relate to its antioxidant activity through increasing nitric oxide (NO) synthesis and bioavailability. Vitamin C is thought to scavenge superoxide, and it can reduce the reactivity of NO with superoxide and inhibit the formation of peroxynitrite(Mason et al. 2021). It has also been shown that Vitamin C can preserve concentrations of the endothelial NO synthase cofactor tetrahydrobiopterin, in turn, the production of NO is maintained by the NO synthase of endothelial cells(Huang et al. 2000).

Inflammation is thought to an important pathological mechanism of adverse cardiovascular effects caused by air pollution. Vitamin C is a water-soluble vitamin and its anti-inflammatory activity has been widely proved(Akolkar et al. 2017; Ang et al. 2018). Ellulu et al found(Ellulu et al. 2015) dietary vitamin C supplement had potential effects in reducing the inflammatory state, mediated through hs-CRP, IL-6, in hypertensive and/or diabetic obese patients. Also, He et al(He et al. 2021) proved that the animals were fed vitamin C could reduce the levels of inflammatory factors, such as IL -1β, IL-6 and TNF-α. However, no such treatment-related benefits were found in a previous study among healthy adults when the patient is exposed to high level of $PM_{2.5}$. In our study, multiple biomarkers relevant to inflammation, such as IL-6, TNF-a, CRP and IL-8, were detected. Our results showed vitamin C supplementation lead a notable decrease in IL-6, TNF-a and CRP compared with the placebo group. Such potential anti-inflammatory effect of vitamin C against air pollution exposure is consistent with in vitro and in vivo experimental studies(Su et al. 2013; Zhang et al. 2018).

Oxidative stress represents another important pathophysiological mechanism of how air pollutants jeopardize cardiovascular health. Our study suggests that vitamin C supplementation was association with a significant increase in GSH-Px in response to air pollution exposure, probably by reducing the occurrence of oxidative stress and increasing antioxidant enzyme activity. Our results are consistent with the previously published studies(Eatemadyboroujeni et al. 2021). Previous experiments in animal model and cultured cells showed that vitamin C led to a notable increase in GSH-Px and SOD activities in response to PM exposure, accompanied by reduced levels of MDA(Liu et al. 2019a; Frikke-Schmidt et al. 2011). Similarly, Previous studies have shown that individuals with high vitamin C concentrations have stronger antioxidant capacity than individuals with low vitamin C concentrations(Paschalis et al. 2016). The protective effect of vitamin C observed in those study is biologically plausible. Vitamin C, act as an electron donor for the enzymes present in the human body and free-radical scavenger(Wilson 2005), can replace glucose in many chemical reactions and prevent the non-enzymatic glycosylation of proteins(Afkhami-Ardekani M 2003). It has been reported that oxidative stress could lead to the release of inflammatory cytokines, such as IL-8 and NO(Qiu et al. 2015). The beneficial effects of vitamin C may relate to its effect on the release of NO to protect the human body from oxidative stress damage, and then play an important role in disease prevention. Our findings yielded consistent results that VC supplementation alleviated oxidative stress caused by exposure to PM air pollution, which may decrease incidence of adverse cardiovascular events.

Accumulating evidence indicated that the effect of PM on circulating biomarkers were generally stronger among male participants than female participants(Wang et al. 2015). Previous two studies have shown that the inflammation caused by short-term and long-term exposure to PM are more pronounced in men(Allen et al. 2011; Hoffmann B 2009). In this study, limited evidence on the effectiveness of vitamin C interventions for individuals of different genders are inconsistent. Stratification analyzes indicated that vitamin C supplementation resulted in a significant reduction in APOB and GSH-Px in females but not in males. On the contrary, vitamin C supplementation resulted in a significant reduction in TNF-α in males but not in females. The results described above suggest that dietary vitamin C supplementation may have more obvious benefits on inflammation in male and have more obvious benefits on blood lipid and oxidative stress in female, while there was no statistically significant effect modification by gender. Similarly, Allen et al. (Allen et al. 2011) observed that healthy male living in woodsmoke-impacted community affected the community’s vascular endothelial function and systemic inflammation were more pronounced than healthy female. It has been reported that female and male individuals have different microvasculature and dynamics in balancing vasoconstriction and vasodilation(Joyner et al. 2016; Cui X 2018). additionally, different hormone levels may also account for the effects of the intervention differ between men and women.

Our study has certain advantages. First, this study is, to our knowledge, first evidence of vitamin C supplementation on cardiovascular health among free-living population over a reasonably long follow-up period. Compared with exposure to a control environment, our findings may be more applicable to real-world situations. Second, we obtain personal exposure data of PM in all public places on the campus, thus our environmental pollution data can more accurately reflect the individual PM exposure level. Finally, the biomarkers we evaluated were chosen that have been implicated as markers of possible mechanism that reflect cardiovascular risk induced by air pollution exposure. This allows us to have a better understanding of the potential cardiovascular benefits of VC supplementation linked with PM exposure.

There are also several limitations to this study. First, our study only recruited healthy young adults rather than those are more sensitive to ambient air pollution (e. g. patients with CVD), which may be better to balance various factors. However, healthy young adults may be less susceptible to air pollution-linked adverse health effects and underestimate the treatment benefits of vitamin C supplementation. Our results should be extrapolated to other populations carefully. Therefore, future research should be verified in populations who are more sensitive to adverse effects in order to fully reveal the protective effect of vitamin C. Second, although we use a three-day diet record method to calculate dietary Vitamin C intake, we cannot rule out the possible role of assimilate ingested other nutrients to the results. However, because of randomly grouped volunteers have a regular diet at university canteens, this potential confounding might not be substantial. Third, since the sample size of our study is small, this limitation affects the precision and statistical power. Finally, the short-term nature of our study might lead us to underestimate or miss some potential lagged health benefits attributable to vitamin C supplementation.

5. Conclusion

In summary, this study suggested that dietary vitamin C supplementation may produce cardiovascular benefits in decrease BP, reduce oxidative stress levels and inflammation levels. Our study provides preliminary evidence that vitamin C supplementation is a simple and effective way to protect the cardiovascular health against air pollution in areas with very high air pollution levels. Although these findings are still need to verify with further investigation, our study provides novel support for the prevention of adverse cardiovascular effect of air pollutant exposure.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of Hebei Medical University and registered in the Chinese Clinical Trial Registry (ChiCTR2100051371).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

There was no conflict of interest.

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Authors' contributions

Jingyi Ren: Conceptualization, Methodology, Software, Formal analysis, Resources, Writing- original draft, Writing-review & editing, Project administration, Jufeng Liang: Formal analysis, Investigation, Data curation, Writing-original draft, Jiaqi Wang: Formal analysis, Investigation, Data curation, Visualization, Bowen Yin: Methodology, Software, Investigation, Fan Zhang: Software, Investigation, Xiang Li: Software, Investigation, Siqi Zhu: Validation, Investigation, Hao Tian: Investigation, Qiqi Cui: Investigation, Jianshi, Song: Investigation, Gang liu: Conceptualization, Methodology, Resources, Wenhua Ling: Conceptualization, Resources, Yuxia Ma: Conceptualization, Methodology, Investigation, Writing-review & editing, Funding acquisition.

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Figures

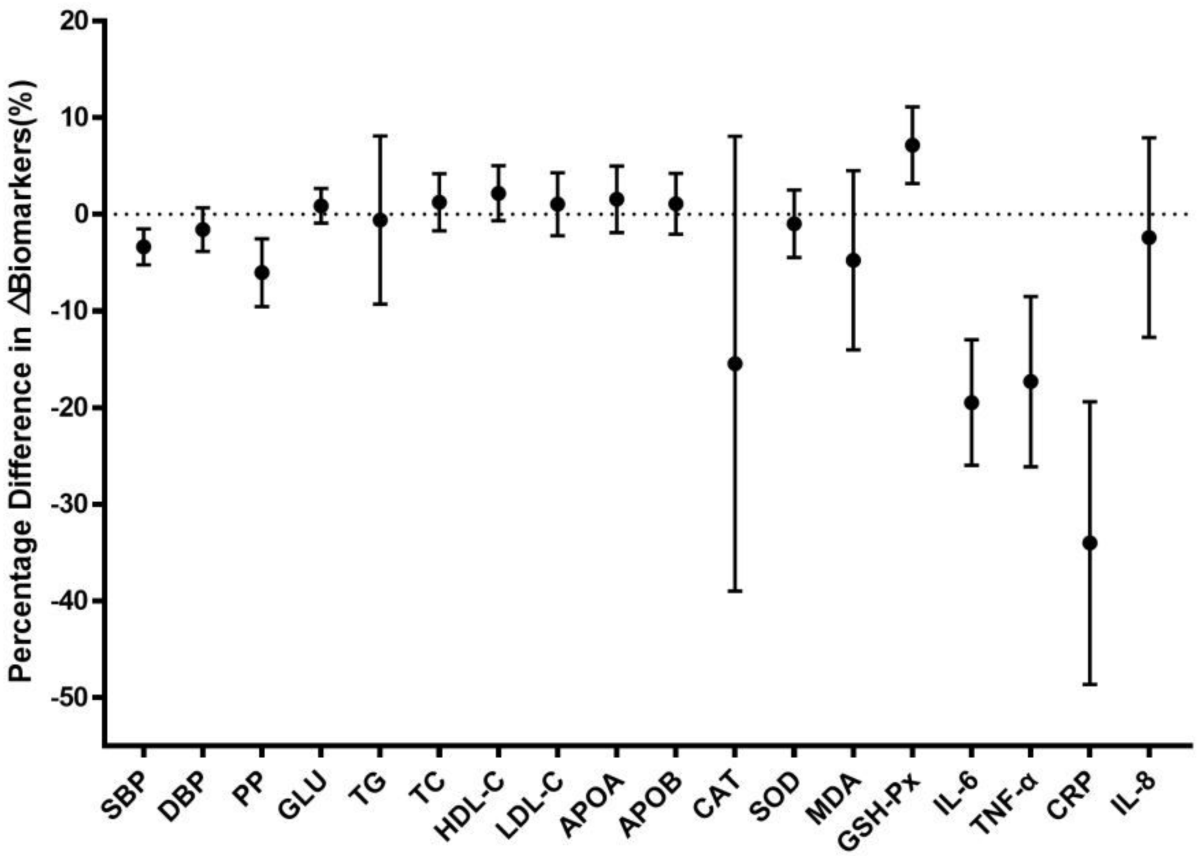

Figure 1

Percentage difference in changes with 95% confidence intervals in health biomarkers comparing vitamin C group and the placebo group. Definition of abbreviations: SBP = systolic blood pressure; DBP = diastolic blood pressure; PP = pulse pressure; GLU = glucose; TG = Total cholesterol; TC = Triglyceride; LDL-C = Low-density lipoprotein cholesterol, HDL-C = high-density lipoprotein cholesterol; APOA = apolipoprotein A; APOB = apolipoprotein B; CAT = catalase; SOD = superoxide dismutase; MDA = malondialdehyde; GSH-Px = glutathione peroxidase; IL-6 = interleukin 6; TNF-α = tumor necrosis factor α;CRP = C-reactive protein; IL-8 =interleukin 8.

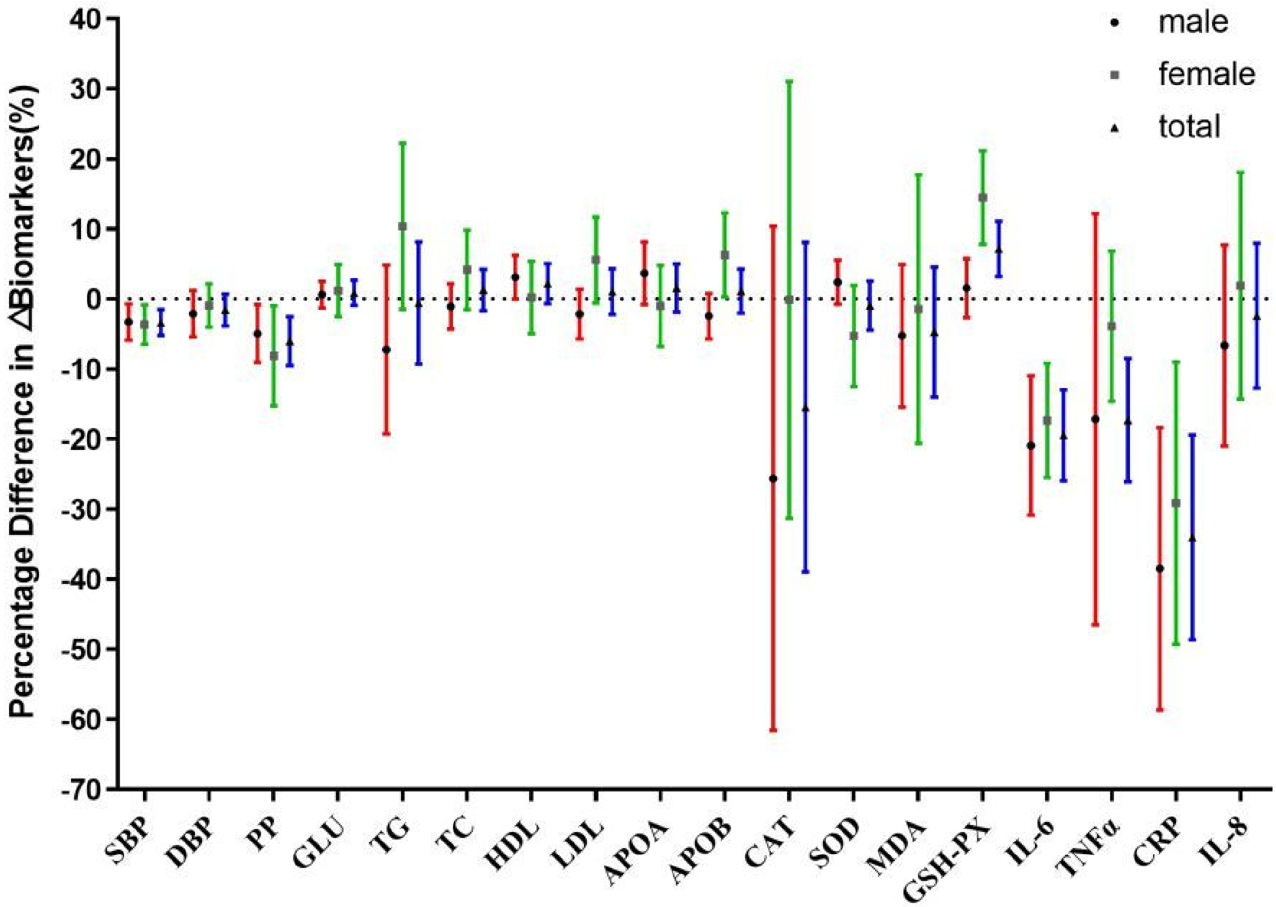

Figure 2

Percentage difference in changes with 95% confidence intervals in health biomarkers between vitamin C group and the placebo group in female, male and all participants. Definition of abbreviations: SBP = systolic blood pressure; DBP = diastolic blood pressure; PP = pulse pressure; GLU = glucose; TG = Total cholesterol; TC = Triglyceride; LDL-C = Low-density lipoprotein cholesterol, HDL-C = high-density lipoprotein cholesterol; APOA = apolipoprotein A; APOB = apolipoprotein B; CAT = catalase; SOD = superoxide dismutase; MDA = malondialdehyde; GSH-Px = glutathione peroxidase; IL-6 = interleukin 6; TNF-α = tumor necrosis factor α;CRP = C-reactive protein; IL-8 = interleukin 8.

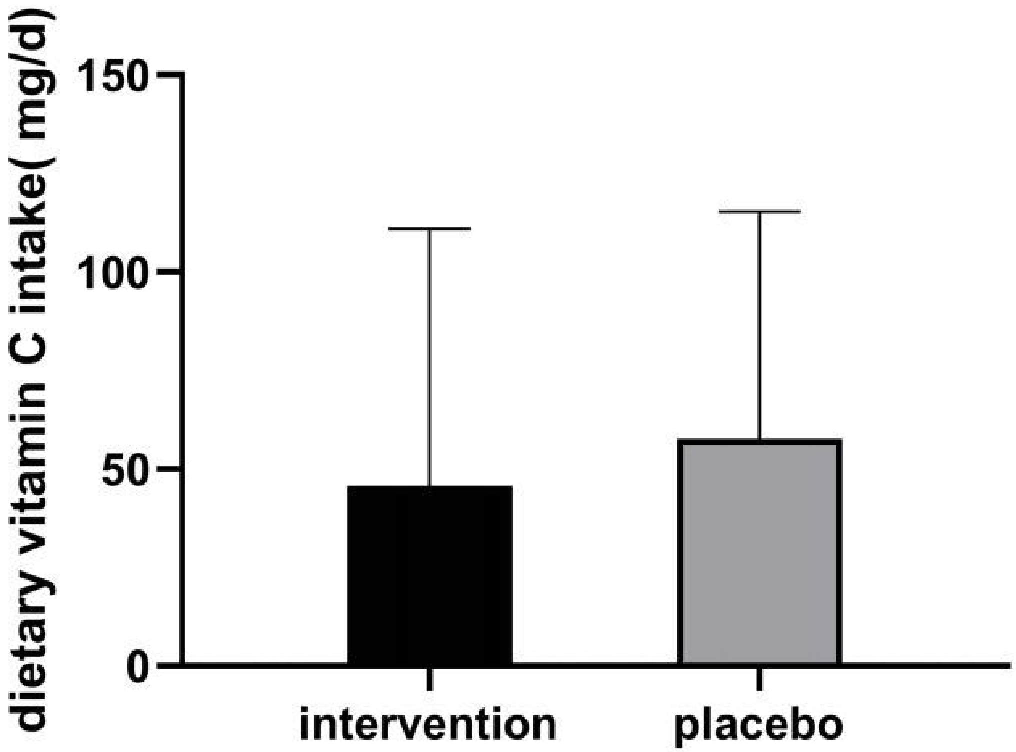

Figure 3

The estimated daily dietary intake of Vitamin C throughout the study period. Values are presented as the mean ± SD.

Supplementary Files

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