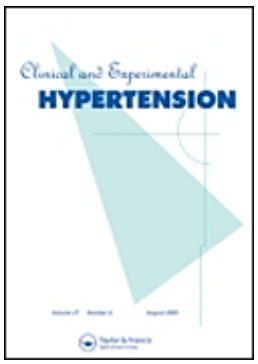

Effects of blackcurrant extract on arterial functions in older adults: A randomized, double-blind, placebo-controlled, crossover trial

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Complete List of Authors:	Okamoto, Takanobu; Nippon Sport Science University, Department of Exercise Physiology Hashimoto, Yuto; Nippon Sport Science University, Graduate School of Health and Sport Science Kobayashi, Ryota; Teikyo University of Science, Center for Fundamental Education Nakazato, Koichi; Nippon Sport Science University, Department of Exercise Physiology Willems, Mark ; University of Chichester, Institute of Sport
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Effects of blackcurrant extract on arterial functions in older adults: A randomized, double-blind, placebo-controlled, crossover trial

Running Head: Blackcurrant extract on arterial functions

Takanobu Okamoto, PhD, Yuto Hashimoto, MS, Ryota Kobayashi, PhD, Koichi Nakazato, PhD, Mark Elisabeth Theodorus Willems, PhD

Takanobu Okamoto, PhD, Yuto Hashimoto, MS, Koichi Nakazato, PhD

Department of Exercise Physiology, Nippon Sport Science University, Tokyo, Japan

Ryota Kobayashi, PhD

Center for Fundamental Education, Teikyo University of Science, Tokyo, Japan

Mark Elisabeth Theodorus Willems, PhD

Institute of Sport, University of Chichester, Chichester, United Kingdom

Corresponding author
Takanobu Okamoto
ORCID ID: 0000-0003-1978-2455
7-1-1, Fukasawa, Setagaya-ku, Tokyo, 158-8508, Japan
Tel: +81-3-5706-0966
Fax: +81-3-5706-0966
e-mail: tokamoto@nittai.ac.jp

ABSTRACT

Purpose: Blackcurrant extract mainly contains anthocyanins. Several reports suggest that anthocyanins have beneficial effect for cardiovascular functions. The aim of this study was to examine the effect of 7-day intake of New Zealand blackcurrant (NZBC) extract on arterial functions, e.g. arterial stiffness, and serum lipids.

Methods: A randomized, double-blind, placebo-controlled, crossover design study with a washout period of 28 days was conducted. Fourteen older adults participated in this study (age 73.3 ± 1.7 years). Participants took either a 7-day course of placebo or two capsules of NZBC extract (each 300 mg capsule contains 35% blackcurrant extract). Participants took one of the two trials first and then took the other after a washout period. Carotid-femoral pulse-wave velocity, an index of central arterial stiffness, and central blood pressure were measured at baseline and again at the end of the 7-day study period.

Results: Compared to baseline, carotid-femoral pulse-wave velocity (P = 0.03) and central blood pressure (P = 0.02) decreased significantly after the 7-day study period with NZBC intake. In addition, carotid-femoral pulse-wave velocity (P = 0.04) and central blood pressure (P = 0.001) in the NZBC intake trial decreased significantly more than in the placebo intake trial. No effects were observed on serum lipids.

Conclusion: These results suggest that short-term NZBC intake reduces central arterial stiffness and central blood pressure in older adults. Therefore, anthocyanin-rich blackcurrants

might be beneficial for maintaining or improving cardiovascular health as an alternative to pharmaceutical medications.

Key words: Anthocyanins, Pulse wave velocity, Augmentation index, Vascular function, Cardiovascular risk factors

Abbreviations

AIx, augmentation index; BP, blood pressure; cfPWV, carotid–femoral pulse-wave velocity; CVD, cardiovascular diseases; DBP, diastolic blood pressure; faPWV, femoral-ankle pulse-wave velocity; FG, fasting glucose; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; MBP, mean blood pressure; NZBC, New Zealand blackcurrant; PP, pulse pressure, SBP, systolic blood pressure; TG, triglycerides

INTRODUCTION

Mortality due to cardiovascular disease is a serious global problem (1), and many strategies are needed to prevent cardiovascular disease. An increase in central arterial stiffness as assessed by carotid-femoral pulse wave velocity (cfPWV) is a powerful predictor of future cardiovascular events, such as myocardial infarction or stroke, and all-cause mortality (2-4). In addition, central aortic blood pressure (BP) has greater prognostic capability than brachial BP, and is more strongly related to vascular hypertrophy, the extent of atherosclerosis, and other cardiovascular events (3-6). Therefore, minimizing increases in central arterial stiffness and central BP are important for the prevention of cardiovascular disease (CVD).

In previous randomized trials, it has been reported that consumption of polyphenols from foods or extracts significantly improved vascular health. In fact, polyphenol-rich foods such as berries, chocolate, or cocoa reduced brachial BP or central arterial stiffness and improved vascular endothelial function (7,8). Prospective studies investigating the association between flavonoid consumption and myocardial infarction risk in young and middle-aged adults showed that high intake of anthocyanins present in blueberries and strawberries is associated with a decreased risk of myocardial infarction (9). Anthocyanins are among the polyphenols that are part of the flavonoid family. Anthocyanins have potent antioxidant capacity and/or a powerful vasodilator effect by producing nitric oxide (10-13). It is known that short-term anthocyanin consumption significantly reduces arterial stiffness and improves

vascular endothelial function compared to placebo in young and middle-aged adults (14-17). In addition, according to a meta-analysis by Fairlie-Jones et al.(18) acute intake of anthocyanin significantly improved arterial stiffness in healthy young adults. On the other hand, blackcurrants include specific anthocyanins, consisting primarily of delphinidin-3-rutinoside, delphinidin-3-glucoside, cyanidin-3-rutinoside, and cyanidin-3-glucoside, which have numerous health benefits (19,20). Thus, intake of anthocyanin-rich blackcurrants may have a number of beneficial effects on cardiovascular health. However, to the best of our knowledge, the effects of short-term blackcurrant consumption on central arterial stiffness and central BP in older adults are unknown. More research is needed to determine various strategies to achieve cardioprotective effects in older adults.

The aim of this study was to examine the effect of 7-day intake of New Zealand blackcurrant (NZBC) extract on arterial functions, such as arterial stiffness and serum lipids. We hypothesized that short-term NZBC intake reduces central arterial stiffness and blood pressure in older adults.

METHODS

Study design

This was a double-blind, randomized, placebo-controlled, crossover study.

Participants

Fourteen volunteers (female: 8, male: 6) more than 65 years old were recruited from the area near Nippon Sport Science University in Japan. Figure 1 shows the flow diagram of enrollment and trial assignment of the participants. Inclusion criteria were: 1. non-smoking; 2. not obese (body mass index $\leq$30 kg/m^2); 3. $\geq$65 years of age; 4. no history of cardiovascular disease or diabetes; 5. no diseases or disorders affecting their daily life; 6. not taking medication that compromises the cardiovascular system, including antihyperlipidemic, antihypertensive, or antihyperglycemic medications; 7. not taking supplementation; 8. not taking habitual exercise; 9. postmenopausal women; and 10. not taking hormone replacement therapy. The inclusion criteria of each participant were confirmed by the authors during screening before the experiment. The number of participants who met the inclusion criteria and who were willing to participate in this study totaled 14 (female: 8, male: 6). Written, informed consent was obtained from all participants after providing detailed explanations of the potential risks of the study. A health history questionnaire was completed to confirm health states. Table 1 shows the physical characteristics of the participants. The study protocol was approved by the ethics

committee of Nippon Sport Science University (Japan) (018-H087).

Schedule

Participants visited the laboratory four times at the same time in the morning (8:00 am). Figure 2 shows the experimental schedule. Before arrival, participants were instructed to avoid vigorous exercise for 48 hours, alcohol for 24 hours and caffeine-containing products on the day of testing and be fasted for at least 10–14 hours overnight.

At every visit, arterial stiffness and BP were measured and blood samples collected from the ulnar vein of the nondominant arm of each participant.

NZBC and placebo intake

Participants ingested 2 × 300 mg capsules (total 210 mg of anthocyanins) of concentrated NZBC extract or a visually identical placebo for 7 days. Each 300 mg NZBC capsule contained 105 mg of anthocyanins, consisting of 35–50% delphinidin-3-rutinoside, 5–20% delphinidin-3-glucoside, 30–45% cyanidin-3-rutinoside, and 3–10% cyanidin-3-glucoside (CurraNZ®, Health Currancy Ltd., Surrey, UK). Each placebo capsule contained 300 mg microcrystalline cellulose. Two capsules were consumed with breakfast for the first 6 days. On the final morning of the supplementation period, participants were instructed to take the supplement or placebo without breakfast, with the last intake about 2 h before an

experimental visit. The two experimental conditions (NZBC extract and placebo) were separated by a 4-week washout period (21). Intake of beverages such as green tea and/or dietary supplements that included polyphenols were prohibited during the study. No restrictions were placed on food intake, but all participants were instructed to maintain their normal diet and lifestyle throughout the study period.

Body composition

The body composition of the participants was determined by bioelectric impedance using a body-composition analyzer (InBody770, Biospace Co Ltd, Seoul, South Korea).

Arterial stiffness

cfPWV and femoral–ankle PWV (faPWV), which reflect aortic and leg arterial stiffness, respectively, were measured. Carotid and femoral artery-pressure waveforms were obtained for 30 seconds using arterial applanation tonometry incorporating form PWV/ABI micropiezoresistive transducers (Omron-Colin Co Ltd, Tokyo, Japan), which comprise 15 aligned pressure-sensitive elements that can identify carotid and femoral pulse traces, arranged side by side and attached to the left common carotid and femoral arteries, respectively. A vascular testing device (form PWV/ABI; Omron-Colin) was used to measure simultaneously electrocardiograms, bilateral brachial and ankle BP, and carotid-arterial and femoral-arterial

pulse waves (PWs). cfPWV and faPWV were calculated by dividing the distance between the two arterial recording sites by the transit time, which is determined based on the time delay between the proximal and distal "foot" waveforms. faPWV is calculated based on the transit time between the femoral artery site and the ankle site. Next, time delays were obtained from between the right brachial and posttibial arteries, the carotid and femoral arteries (Tcf), and the femoral and posttibial arteries. A nonelastic tape measure was then used to make duplicate random zero-length measurements over the body surface in order to determine the path length from the carotid to the femoral artery (Dcf). cfPWV was calculated using the following equation:

$$cfPWV = Dcf/Tcf$$

The investigator who performed the PWV measurements was blinded to the condition assignment of the participants.

Central and brachial BPs and the augmentation index (AIx)

Arterial pulse waveforms of the left radial artery for estimating central BP were measured non-invasively by an automated tonometric system (HEM-9000AI, Omron Healthcare Co., Ltd.). Central BP and the AIx were measured as previously described (22,23).

Brachial BP was measured with an oscillometric manometer, and radial pulse waveforms were recorded non-invasively using an applanation tonometer. Signals of the radial arterial pressure wave were low-pass-filtered, first at a cut-off frequency of 105 Hz to remove high-frequency noise and then at 25 Hz to extract pressure waveforms. Inflection points or peaks that corresponded to first (early) and second (late) systolic BP were automatically identified using the fourth derivative wave as the second and third zero crossing points, respectively. Late SBP in the radial artery was used as an estimate of the central SBP and was calculated using the following equation:

Late SBP= (P2/PP) x (SBP-DBP) + DBP

In addition, the radial AIx was calculated using the following equation:

AIx(%)= (P2/PP) x 100

where P2 is the pressure difference between the peak systolic pressure and an early inflection point that indicates the beginning upstroke of the reflected pressure wave, and PP is the pulse pressure.

The AIx was adjusted for a heart rate of 75 bpm (AIx@75).

In addition, mean brachial BP (MBP) and brachial pulse pressure (PP) were calculated using the following equations:

MBP = [DBP + (SBP – DBP) / 3].

PP = SBP – DBP

Serum lipids profile and glucose

Whole blood was drawn into serum separator tubes, allowed to clot and centrifuged at 4,000 RCF for 15 minutes. Triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and fasting glucose (FG) in blood samples were measured using standard enzymatic techniques. The intra- and interassay coefficients of variance was less than 5%.

Statistics

All data are expressed as means ± standard error. Statistical analyses were performed using statistical software (SPSS ver. 24; SPSS, Inc., Chicago, IL, USA). The assumption of a normal distribution for all data was verified using the Kolmogorov–Smirnov test, and all data were normally distributed. Data were analyzed using two-way analysis of variance (trials × periods) with repeated measures. When the main effect or interaction was significant, the paired t-test was used to identify significant differences among the mean values. Statistical significance was set at $P < 0.05$. In addition, The interpretation of p as $0.05 > p \leq 0.1$ was according to guidelines by Curran-Everett and Benos (24).

RESULTS

Figure 3 shows changes in cfPWV before and after both NZBC and placebo. The cfPWV at baseline did not differ significantly between NZBC and placebo. cfPWV decreased significantly after NZBC intake compared with baseline values (P = 0.03). In contrast, the cfPWV did not differ significantly between before and after placebo intake. Significant differences in cfPWV were observed between the NZBC and placebo after intervention (P = 0.04).

Figure 4 shows changes in central BP before and after both NZBC and placebo. The central BP at baseline did not differ significantly between NZBC and placebo. Central BP decreased significantly after NZBC intake compared with baseline values (P = 0.02). In contrast, the central BP did not differ significantly before and after placebo intake. Significant differences in central BP were observed between the NZBC and placebo after intervention (P = 0.001).

Table 2 shows changes in brachial SBP, DBP, MBP, PP, AIx, and faPWV before and after NZBC and placebo. The brachial SBP, DBP, MBP, PP, AIx, and faPWV at baseline did not differ significantly between NZBC and placebo. Brachial SBP (P = 0.03), DBP (P = 0.02), MBP (P = 0.01), and AIx (P = 0.03) decreased significantly after NZBC intake compared with baseline values. PP had a tendency to decrease after NZBC intake compared with baseline values (P = 0.09). In contrast, brachial SBP, DBP, MBP, PP and AIx did not differ significantly

between before and after placebo intake. Significant differences in brachial SBP (P = 0.001), MBP (P = 0.01), PP (P =0.01), and AIx (P =0.01) were observed between the NZBC and placebo trials after intervention (P = 0.001). There was a trend for DBP to be lower with NZBC trial compared with placebo trial after intervention (p = 0.07). On the other hand, faPWV did not differ significantly between before and after both NZBC and placebo. In addition, no significant differences in faPWV were observed between the NZBC and placebo trials after intervention.

Table 3 shows changes in serum lipids profile before and after both NZBC and placebo. No significant differences were seen between NZBC and placebo in serum concentrations of TG, HDL-C, LDL-C, or FG at baseline. No significant changes were observed between the two trials in serum concentrations of TG, HDL-C, LDL-C, or FG after both NZBC and placebo.

DISCUSSION

The key finding of this study was that 7 days intake of anthocyanin-rich NZBC in older adults decreased cfPWV and cSBP with no changes in the placebo condition. These results suggest that short-term intake of NZBC would decrease central arterial stiffness and BP in older adults.

An increase in central arterial stiffness and/or central BP has been shown to be independently associated with future cardiovascular events, such as myocardial infarction or stroke, and all-cause mortality (25,26). Therefore, intake of NZBC may be an important type of non-pharmacological therapy to enhance cardiovascular health in older adults.

The present findings show for the first time that anthocyanin-rich foods such as NZBC reduce arterial stiffness in older adults. Anthocyanins are flavonoids, all of which are phenolic compounds (i.e., polyphenols). Recent studies have shown that ingestion of anthocyanins-rich foods, including black currants, blueberries, grapes, and purple potatoes, have the beneficial effects of reducing arterial stiffness in both young and middle-aged adults, and growing evidence highlights that specific flavonoids from plant bioactive compounds present in fruits improve vascular function (27,28). Some previous studies that examined the associations between anthocyanin intake and arterial stiffness focused predominantly on young and middle-aged participants. In our recent study, it was shown that intake of polyphenol-rich cocoa reduced cfPWV in postmenopausal women (8). In addition, previous studies showed an

association between polyphenol-rich food intake and decreased aortic stiffness in healthy adults, independent of age (3,4,29,30). Consistent with previous studies (8,31), the present results suggest that intake of polyphenol-rich foods, i.e. anthocyanin-rich blackcurrant, improves central arterial stiffness. Thus, the present findings expand on previous research on the effects of polyphenol-rich foods on arterial stiffness in older adults.

In addition to the improvements in central arterial stiffness, 7-day intake of NZBC extract was shown, for the first time, to reduce central BP by average of 10 mmHg, which appears to be a better predictor of cardiovascular events than brachial BP (3-6). Wang et al. (32) observed central BP reductions of 6 mmHg that reduced cardiovascular mortality by approximately 25%. Thus, the present findings showed that NZBC intake reduced central BP, suggesting the effectiveness of anthocyanin-rich foods for reducing central BP.

In the previous meta-analyses of randomized controlled trials, consumption of flavonoid-rich foods (e.g., green tea, soy protein isolates, and cocoa or chocolate) was shown to reduce peripheral (brachial) BP (33). Consistent with a previous study, the present findings showed that intake of NZBC reduced brachial BP. Moreover, NZBC intake reduced the AIx. The central aortic pressure wave consists of a forward traveling wave generated by left ventricular ejection, followed by a later-arriving reflected wave from the periphery (34). In addition, as arterial stiffness increases, central BP increases can occur due to increased forward and reflected wave amplitudes and earlier return of the reflected wave to the proximal aorta.

Reduction in the AIx is associated with increased peripheral vasodilation (35), caused by decreased wave reflection at medium-sized muscular arteries (36). Therefore, the decreased AIx in the present study may have been caused by peripheral vasodilation. Moreover, the measurements of arterial stiffness obtained from non-invasive pressure waveforms suggested that decreased aortic stiffness is associated with reduced AIx (37). Thus, the present findings suggest that the changes in central arterial stiffness and BP after NZBC intake were primarily the result of changes in arterial distension. Aortic stiffness affects both early systolic cardiac load, through elevation of the forward pressure wave, and late systolic cardiac load, due to earlier return of the reflected pressure wave (38-42). Thus, the present findings suggested that the effect of NZBC intake was mediated by reductions in both early systolic and late systolic pressures, suggesting decreased aortic stiffness and arteriolar tone, respectively. Therefore, since decreased wave reflection decreases LV afterload and myocardial oxygen demand (43), NZBC intake appears to be effective for reducing cardiovascular risk in older adults.

In the present study, there were no differences in serum lipids and glucose after intake of NZBC or placebo. Alvarez-Suarez et al. (44) reported that one-month intake of anthocyanin-rich strawberry improved the lipid profile by significantly reducing TC, LDL-C, and TG levels compared to baseline levels, while no changes were seen in HDL cholesterol. In this respect, the present results differ from that of previous study. One possible reason for this difference may be differences in the subjects and intake duration among studies. Moreover, since TC,

LDL-C, and TG in most participants of the present study were within the standard ranges, their values might not have differed from baseline values. In addition, Tsang et al. (28) reported that intake of an anthocyanin-rich potato did not change glucose. This result is consistent with our findings. Thus, the present findings showed that NZBC intake did not change serum lipid profile and glucose in older adults.

In individuals at risk, medical and pharmacologic interventions can decrease cardiovascular mortality, but such interventions are costly and may have adverse effects (45,46). In contrast, in epidemiological studies, higher anthocyanin intake was associated with decreased arterial stiffness and BP (47,48). Increased arterial stiffness or BP is a major CVD risk factor (49), which, when decreased significantly, reduces the risk of CVD and death in various populations (50). Various fruits, vegetables, and beverages that are commonly consumed in the human diet can be rich in anthocyanins (51-53) Therefore, the present findings emphasize the importance of incorporating more anthocyanin-rich foods, including fruits and vegetables and berries such as NZBC, which promote cardiovascular health without side effects.

This study has some limitations. First, the short time frame of the study might have prevented observation of the benefits of longer-term consumption on arterial stiffness and BP. Second, vascular endothelial function, antioxidant capabilities, and inflammatory markers were not measured. Third, the results in this study population of healthy older adults might not be generalizable to CVD patients. Finally, there was no information on anthocyanin-derived

metabolites with 7-day NZBC intake. Nevertheless, to the best of our knowledge, the present data provide the first evidence that short-term intake of NZBC may contribute to reductions in central arterial stiffness and BP in older adults. Further long-term, randomized, interventional studies are needed to establish the role of NZBC in supporting cardiovascular health in older adults.

Conclusion

In conclusion, the findings of the present study indicate that short-term NZBC intake reduces central arterial stiffness and BP in older adults. These results suggest that habitual NZBC intake may be an effective way to prevent CVD. Therefore, anthocyanin-rich blackcurrants might be beneficial for maintaining or improving cardiovascular health as an alternative to pharmaceutical medications.

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Compliance with ethical standards

Conflict of interest

The authors have no conflict of interest to declare.

Statement of human and animal rights

This study was approved by the Ethics Committee of Nippon Sport Science University.

Informed consent

Informed consent was obtained from all participants.

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Figure legends

Figure 1. Consort flow diagram of the participants

Figure 2. Experimental schedule

Figure 3. Changes in cfPWV before and after both NZBC and placebo

*: significantly (P<0.05) different from before.

†: significantly (P<0.05) different from placebo.

cfPWV, carotid-femoral pulse wave velocity; NZBC, New Zealand blackcurrants

Figure 4. Changes in central BP before and after both NZBC and placebo

*: significantly (P<0.05) different from before.

††: significantly (P<0.01) different from placebo.

SBP, systolic blood pressure; NZBC, New Zealand blackcurrants

536 Table 1. Physical characteristics of the participants

Sex (F/M)	8/6
Age (years)	73.3±1.7
Height (cm)	159.9±2.5
Weight (kg)	55.6±2.9
Body mass index (kg/m^2)	21.6±0.7
Body fat (%)	25.7±1.7

537 Values are mean ± SEM.

538 Table 2. Brachial SBP, DBP, MBP, PP, AIx, and faPWV before and after NZBC and placebo

	NZBC		Placebo	
	Before	After	Before	After
Brachial SBP (mmHg)	142±5	133±3*,††	140±5	142±4
Brachial DBP (mmHg)	79±3	76±2*,‡	79±3	79±3
Brachial MBP (mmHg)	100±3	95±3**,††	98±3	100±3
Brachial PP (mmHg)	63±4	58±2#, †	60±3	63±3
AIx (%)	87±4	83±3*,†	89±3	86±3
faPWV (cm/sec)	1239±49	1186±50	1234±55	1214±44

539 Values are mean ± SEM. *: significantly (P<0.05) difference from before. **: significantly
540 (P<0.01) difference from before. #: denotes a strong trend for a different from baseline (p =
541 0.09). †: significantly (P<0.05) difference from the placebo. ††: significantly (P<0.01)
542 difference from the placebo. ‡: Denotes a strong trend for a different between NZBC and
543 placebo trials (p = 0.07).

544 SBP, systoloc blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; PP,
545 pulse pressure; AIx, augmentation index; faPWV, femoral-ankle pulse wave velocity; NZBC,
546 New Zealand blackcurrant

Table 3. Serum Lipids profile and glucose before and after NZBC and placebo

	NZBC		Placebo	
	Before	After	Before	After
TG (mg/dL)	88±12	85±8	95±10	93±10
HDL-C (mg/dL)	73±5	76±4	71±6	74±5
LDL-C (mg/dL)	124±10	120±8	127±7	127±5
FG (mg/dL)	91±4	91±3	92±4	91±4

Values are mean ± SEM. NZBC, New Zealand blackcurrant; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FG, fasting glucose

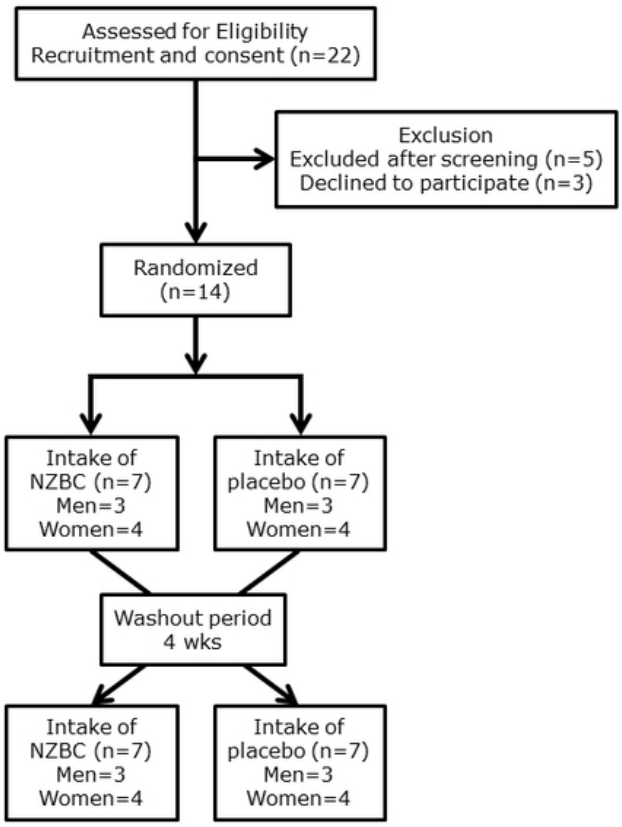

Fig. 1

Figure 1. Consort flow diagram of the participants

52x69mm (300 x 300 DPI)

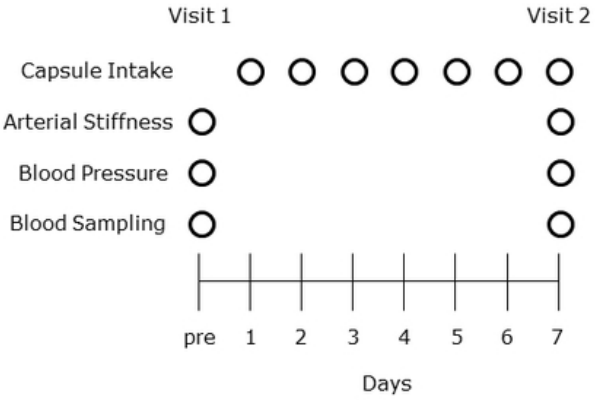

Fig. 2

Figure 2. Experimental schedule

52x69mm (300 x 300 DPI)

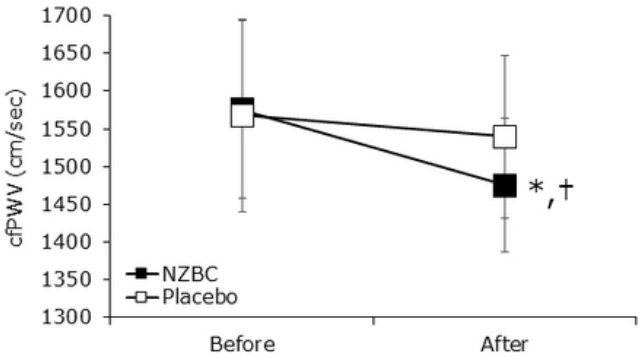

Fig. 3

Figure 3. Changes in cfPWV before and after both NZBC and placebo

52x69mm (300 x 300 DPI)

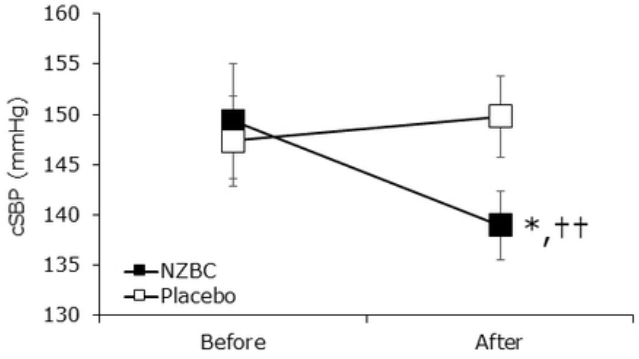

Fig. 4

Figure 4. Changes in central BP before and after both NZBC and placebo

52x69mm (300 x 300 DPI)